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CRISPR: The rapidly evolving science of genome editing

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CRISPR: The rapidly evolving science of genome editing

CRISPR, a way of editing genomes adapted from nature, has captured the world's attention as scientists puzzle out how to exploit the tool to improve human health. Academic and industry labs around the world are exploring new uses for the technology, including as a sophisticated tool to better understand biology and as a potential therapy to repair, disable, or replace genes that cause disease.

Advances come with shortcomings: There have been technical obstacles to overcome, notably off-target edits — including what some studies suggest may be increased vulnerability to cancer. There are challenges with delivery of CRISPR to the right place in the body. And there are deep ethical concerns about permanently rewriting genomes not just for an individual but for generations to come. That fear became reality after a rogue scientist in China reported he'd edited human embryos, leading to the birth last year of twin girls whose genomes had been altered.

Meanwhile, CRISPR companies have cautiously begun testing specific gene-editing therapies in people. Others continue to refine the tool, hoping to transform it into a reliable workhorse of gene editing.

STAT reports on major developments as they occur and curates the scientific literature in its CRISPR Trackr. Here are select recent STAT stories charting discoveries, the businesses being built on them, and their potential impact on all our lives.

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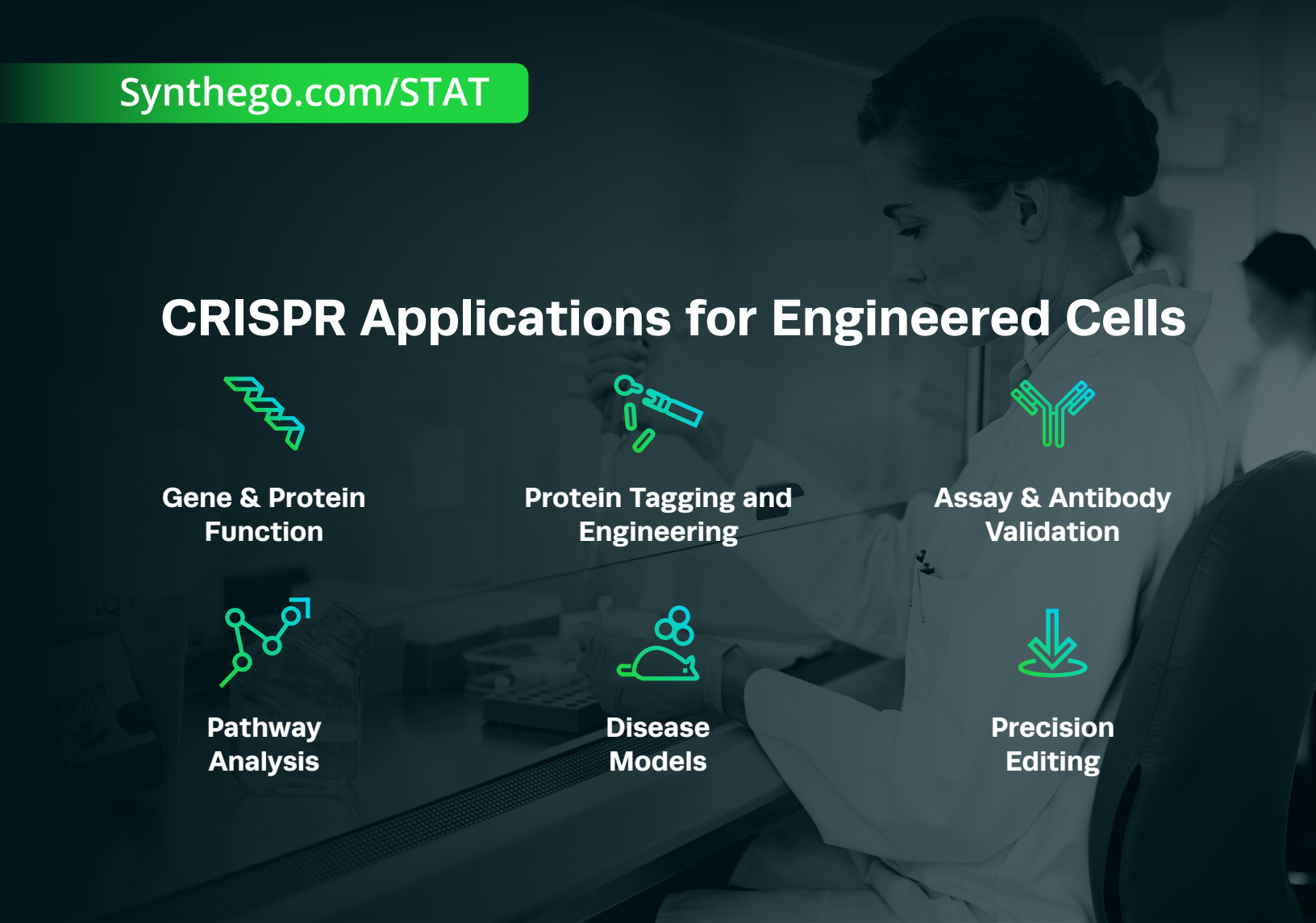

CRISPR Applications for Engineered Cells

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IN THE LAB

With nanobots and rare viruses, scientists work to solve CRISPR's delivery problem

By Sharon Begley @SXBEGLE | MAY 1, 2019

It was not your typical CRISPR experiment. The scientists had just injected a mouse's tail with magnetic nanoparticles bound to an exotic virus containing today's genome editor of choice. They then plopped the mouse belly-side down atop a block magnet about the size of a deck of cards, positioning it just so. Even with all their attention to detail, however, "we were never sure it would work," admits bioengineer Gang Bao of Rice University. "We figured, let's just see what happens."

What happened was that the magnetic field quickly steered the CRISPR-containing magnetic nanoparticles to the surface of the mouse's liver cells and kept the particles well away from the heart, lungs, brain, and other organs. The liver is a big target, which makes hitting it the biological version of hitting the proverbial barn door, but the ability to direct CRISPR to a target organ and only a target organ was a big step toward solving one of the toughest challenges for genome editing: precise delivery.

CRISPR is easy enough to design and produce that thousands of scientists are using it to identify the function of specific genes, create animal models of genetic disorders, and pursue CRISPR-based therapies for diseases as different as Duchenne muscular dystrophy and a form of congenital blindness. But the "easy" part stops at cells' outer boundaries. Getting CRISPR to the right cells, bypassing all others, and slipping it through their membranes is the opposite of easy.

"It's one of the major barriers in gene editing research, certainly for translational work, and a very active area of investigation," said Patrick Hsu of the Salk Institute, who studies whether CRISPR can, among other things, repair mutations in neurons that cause brain disorders. "You can make gene editing tools until you're blue in the face, but unless you get them to the right place, it doesn't matter."

That's because if scientists can't nail the delivery, CRISPR is as useless as the coolest 512GB phone purchased online during a FedEx and UPS strike. They therefore have to get inventive. From nanobots to rare viral strains and bespoke nanoparticles, they are developing delivery systems that, they hope, will one day help make CRISPR medicine a reality.

Which is how Bao's mice wound up on magnets. A nanomedicine expert, he knew that the classic way of carrying CRISPR into animals ("in vivo") is via living viruses, which excel at that. Two such bugs, lentiviruses (HIV is the best known) and adeno-associated viruses (AAV), have been genetics workhorses for years: Lentiviruses carry genes into T cells to produce cancer-fighting CAR-Ts, and Spark Therapeutics uses AAV for its blindness-curing Luxturna gene therapy.

Each has drawbacks when it comes to therapeutic genome editing, however. For one thing, viruses are small; some CRISPR assemblies won't fit inside them. For another, lentiviruses integrate into the genomes of the cells they enter, said Neville Sanjana of the New York Genome Center; at the wrong place, that could activate cancer-causing genes or silence cancer-fighting ones. AAV delivers its cargo outside the nucleus, which is safer, but is tricky to target to a particular organ even though different AAV "serotypes" infect one organ more than others, said Chris Nelson of the University of Arkansas. No one knows how the Food and Drug Administration will view a CRISPR therapy that edits DNA in unintended organs.

Some viruses have another significant downside: Once they deposit genes for CRISPR's components into a cell, those components last for years.

That includes a DNA-cutting enzyme (classically, Cas9). Since perpetual DNA-cutting scissors are unlikely to be useful, and might not be safe, "You probably don't want that," said bioengineer James Dahlman of the Georgia Institute of Technology.

Bao therefore reasoned that with hundreds of species of virus capable of infecting mammalian cells, some might be better than the existing workhorses. He settled on a little-known moth virus that happens to be great at penetrating mammalian cells, but only if it can outrun a component of blood called complement C3. Bao had a plan for that: A magnetic field might get the CRISPR-carrying virus to its target before the blood could destroy it.

The Rice scientists packaged the usual CRISPR components (a guide RNA and the DNA-cutting enzyme Cas9) into the viral particles. For their secret sauce, they mixed the viruses with nontoxic nanoparticles of iron oxide, which are magnetic, and injected the mixture into mice's tail veins.

Positioning the anesthetized animals on block magnets drew the iron oxide nanoparticles toward the liver cells, getting five times the CRISPR efficiency than with virus alone. But while the viruses-cum-nanoparticles punched through liver cells, they avoided the heart, lung, kidney, and other vital organs, Bao and his colleagues reported last year. The scientists were also able to steer CRISPR to the spleen, though not quite as accurately, and are now working on ways to generate more precise magnetic fields than the crude ones a block magnet produces.

"This is a critical issue for genome editing," Bao said. "If you use a virus, it tends to go everywhere. You need a second component, such as nanomagnets, to get CRISPR only where you want it."

Viruses' limited cargo space and other drawbacks have driven scientists to look outside of nature's offerings and make CRISPR carriers from scratch.

Nanoparticles, made of lipids or even gold, have advantages that are making them the go-to delivery vehicles for a growing number of both academic and commercial labs. Intellia Therapeutics, for instance, is banking on lipid nanoparticles for the genome editing therapies it is developing.

Whether made of downmarket lipids or luxury-market gold, nanoparticles are extremely roomy and penetrate cells well. What they do less well is break through the lining of blood vessels, which Massachusetts Institute of Technology bioengineer Sangeeta Bhatia calls "the biggest barrier to [them] getting into the [target] tissue." Her solution: nanorobots.

Bhatia and her colleagues created nickel-coated micropropellers the size of a single cell, they reported last week: These nanoparticles are drawn to a target by a magnet, and drag the particles along in their wake, at least in the artificial blood vessels where the scientists tested them. Although they didn't load the nanoparticles with CRISPR, Bhatia said, they're big enough to carry it.

Once a swarm of nanoparticles has punched their way out of blood vessels, they'd ideally penetrate only cells they're supposed to — liver, say, and not spleen. Trouble is, although properties such as size and electric charge affect what kind of cells nanoparticles penetrate, scientists don't know the exact list of properties that mean, "good at entering lung cells," for instance.

Georgia Tech's Dahlman is therefore developing a greased-lightning way to test hundreds of nanoparticles at a time for their ability to enter specific types of cells, he and his colleagues reported last year. He packages genes for a fluorescent molecule into hundreds of different kinds of nanoparticles and injects them all into mice. The red glow reveals where each kind went. The screening system is promising enough, Dahlman said, that he launched a company, GuideRx, to commercialize it.

For some hoped-for CRISPR therapies, the genome editor doesn't need to be injected into patients' bodies in vivo, as will be required for blindness, Duchenne muscular dystrophy, and many other diseases. Instead, for blood disorders such as sickle cell and beta thalassemia, stem cells removed from the body ("ex vivo") could be edited and then reinfused.

That opens up two additional delivery modes. One is brute force microinjection, literally jabbing CRISPR's guide RNA and Cas9 enzyme (or genes that make them) into cells. If AAV is the Honda Ridgeline of delivery vehicles (small cargo capacity), microinjection is the Chevy Silverado: its capacity seems limitless. That keeps microinjection in the running for ex vivo uses requiring a repair gene, which can be huge. (Microinjection is also the go-to method for editing embryos, which requires getting CRISPR into only a single cell.)

The other brute-force method, electroporation, zaps cells with high-voltage currents. That opens nanometer-sized pores, allowing guide RNA and Cas9 or other enzyme to slip in. It, too, works in single-cell embryos and cells removed from the body, but zapping a living patient is definitely not in the cards.

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UP NEXT

CRISPR edits lung-disease gene in utero, hitting only the affected organ in a mouse study

By Sharon Begley

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IN THE LAB

CRISPR edits lung-disease gene in utero, hitting only the affected organ in a mouse study

By Sharon Begley @SXBEGLE | APRIL 17, 2019

Companies that hope to treat severe inherited diseases via CRISPR genome editing are already testing the technique in adults, while push-the-envelope types are arguing for repairing defective genes much earlier — in IVF embryos so new they're still in a lab dish (the "CRISPR babies" route). Now scientists in Philadelphia have taken preliminary steps toward a possible middle way: They injected CRISPR into the amniotic fluid of pregnant mice, editing a lung-disease-causing gene in a small number of mouse fetuses, they reported on Wednesday.

In utero editing offers advantages for at least some diseases, said Dr. William Peranteau, of Children's Hospital of Philadelphia, a pediatric and fetal surgeon who co-led the study. Some mutations wreak havoc so early in development that editing genes even in a newborn would be too late: Mutations in a gene called SFTPC, whose mouse version Peranteau and his colleagues edited, cripple developing lungs so disastrously "that these kids are going to die at birth," he said. Also, the fetal immune system is less likely than an adult's or even a child's to attack the CRISPR molecules or the virus that carries them.

Editing a single-cell IVF embryo, on the other hand, alters genes in every cell, which might be excessive. Such "germline" editing is also heritable, which some ethicists deem unacceptable. And embryo editing isn't possible if conception occurs naturally, rather than in a Petri dish.

Nor would it repair mutations that arise during the nine months of gestation (which can often be detected via tests of fetal DNA) rather than being present at conception.

"It's wonderful that the field of in utero therapy is moving forward," said Graça Almeida-Porada of Wake Forest University, an expert on fetal therapy who was not involved in the study, published in Science Translational Medicine. "For many genetic disorders, there's not a lot that can be offered to the patient [after birth], so it's important to develop novel therapies that provide a chance at life." The only option for newborns with the SFTPC mutation is a lung transplant, which is rarely performed because so few tiny lungs are available for donation.

The SFTPC gene makes a protein in pulmonary surfactant. Secreted by the lungs' alveolar cells, surfactant in humans as well as mice relaxes the surface tension in lungs so they don't collapse with every breath. The scientists injected CRISPR into the amniotic fluid of 87 mouse fetuses on day 16 of their 20-day gestation, analogous to the third trimester in humans.

Mice can live without SFTPC, since surfactant is composed of many ingredients, said developmental biologist Edward Morrisey of the University of Pennsylvania's Perelman School of Medicine, who co-led the study. Mutant SFTPC, however, is lethal. He and his colleagues therefore used a form of CRISPR that deletes the gene, which is relatively easy, rather than repairing it, which is harder.

Although a key challenge of CRISPR is getting it to affected cells and organs while sparing healthy ones — to cure a liver disease, it doesn't help to CRISPR heart cells — a fetus' lungs literally inhale it from the amniotic fluid. Few other cells picked up the genome editor.

"What's exciting about this paper is that they showed specific targeting to just the affected organ, and even to specific cells within the lung," said Almeida-Porada.

Every mouse born with the mutation died of lung failure within hours. In fetuses where the gene edit succeeded (20 percent of them), seven survived for more than 24 hours.

Five survived past seven days, behaving and breathing normally and hitting their growth milestones.

Mortality of 92 percent seems disappointing, to say the least. On the other hand, the scientists emphasized that "this is just a proof-of-concept study to show you can [in some cases] edit a gene in utero," as Peranteau said. Although it will be years before such genetic surgery is ready to try in human fetuses, "we were psyched to see any survive, since normally none do." Almeida-Porada agreed, calling even the low rate of editing and minimal survival "a great accomplishment" for a first step.

Wake Forest's Christopher Porada, also an expert in fetal therapy (and Almeida-Porada's husband), said the study "has implications for other genetic disorders" where it would be safer and possibly more effective to correct a gene before it makes fetal development go off the rails but only in affected organs. There is no need to repair the gene that cause Duchenne muscular dystrophy anywhere but muscles, for instance.

Last year, the Philadelphia team used another approach to edit mouse genes in utero. They injected CRISPR into pregnant mice's vitelline vein, which drains blood from the yolk sac, and didn't achieve organ-specific targeting as this experiment did.

Peranteau and his colleagues are working to increase the percentage of successful edits and the rate of survival, and are optimistic on both counts. The virus they chose to carry CRISPR into cells, called an adeno-associated virus, can cause lung inflammation and other problems; when it was used in normal mouse fetuses, those with healthy SFTPC, 75 percent died. A safer virus would be needed if the therapy ever moves into human studies.

In animals with longer gestation times, including people, it should be possible to get editing efficiencies much higher than the 20 percent seen in the mice, Morrisey said: The longer a fetus stays in amniotic fluid spiked with CRISPR, the greater the chance of editing its cells.

One crucial difference between people and mice would make editing SFTPC in human fetuses more difficult, however. People, unlike mice, need that gene, so CRISPR would have to repair it, not delete it, a much stiffer challenge.

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CRISPR-Chip: A powerful tool for gene detection in minutes

By Meenakshi Prabhune, Ph.D.,
Science Writer and Journalist

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CRISPR-Chip: A powerful tool for gene detection in minutes

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The field of genome engineering is recently abuzz with CRISPR updates. CRISPR technology is a two-component system that allows precise recognition and editing of genomic sequences. Researchers are harnessing its potential of editing genetic mutations for developing curative therapies, but CRISPR's potential in diagnostics is only recently being explored.

Now, Kiana Aran, a professor at the Keck Graduate Institute in Claremont, California, and her team have developed CRISPR-Chip, an electronic biosensor that uses CRISPR to detect specific genes in genomic DNA. Their findings were published today in Nature Biomedical Engineering.

"This is the first time that the CRISPR complex has been combined with electronics for detection mechanism," says Dr. Aran.

THE NEED FOR CRISPR-CHIP

The CRISPR system involves two components: the guide RNA is complementary to the target sequence and recognizes it, and the Cas nuclease cuts at this site. While most researchers focus on the editing function of CRISPR, Dr. Aran wanted to explore alternative directions.

"Everyone is thinking about the therapeutic application of CRISPR, but sometimes we forget that the first thing that CRISPR does is searching," says Dr. Aran.

Traditional diagnostic methods for nucleic acid detection depend on heavy instrumentation and time-consuming assays. Recently, CRISPR has been used for diagnostic kits — SHERLOCK, HOLMES, and Mammoth Biosciences' kits being prime examples. However, their approach of using Cas13a and Cas12a nucleases for diagnostic detection requires amplification of signal for a clear readout.

Aran wondered if she could devise an even simpler gene-detection system that requires minimal reagents, effort, and time.

A GRAPHENE-BASED ELECTRONIC PLATFORM COMBINED WITH CRISPR: HOW IT WORKS

Dr. Aran used her electrical engineering background and biomedical engineering expertise to solve this problem. She combined the powerful search potential of CRISPR with a graphene-based field effect transistor (gFET) platform for sensitive readout to develop CRISPR-Chip.

The principle of CRISPR-Chip is simple. The gFET platform comprises a graphene layer that connects source and the drain electrodes. Aran and her team immobilized ribonucleoproteins (RNPs) — complexes of dead Cas9 protein conjugated with single guide RNAs — on the graphene layer. Dead Cas9 (dCas9) is catalytically inactive, i.e. it recognizes but does not cut the target DNA. The graphene platform is sensitive to the adsorption and interaction of charged molecules at its surface. Therefore, when a voltage is applied across the surface, binding of target DNA to the RNPs translates to a change in electrical current, which makes for a simple readout.

CRISPR-CHIP DETECTS TARGET GENES IN GENOMIC DNA

The researchers first tested their system on PCR amplicons, which are less complex to deal with than genomic DNA. RNPs with guide RNA specific for the gene coding for blue fluorescent protein (*bfp*) were immobilized on the platform. As a negative control, RNPs with guide RNA targeting a scramble (nonspecific) sequence were used. The team observed a significantly larger signal with *bfp*-specific amplicons than with the nonspecific functionalized RNPs. This result indicates that CRISPR-Chip's signal output is specific for genes complementary to the immobilized RNP complex.

Confident about their platform after the preliminary test, the researchers performed a similar experiment with genomic DNA extracted from HEK cells. They used two types of DNA: one containing the *bfp* sequence and a control group lacking the sequence. Once again, CRISPR-Chip showed significantly enhanced signal on exposure to the target genomic DNA, relative to control samples lacking the target sequence.

This was a big deal, because they realized their system could detect DNA without amplification. Plus, their assay time was just 15 minutes.

"Graphene has been used before to develop systems for detecting DNA or RNA, but for every one of them you needed to amplify your target significantly in order to develop a very specific probe," Dr. Aran explains.

DEMONSTRATION OF CRISPR-CHIP SUCCESS IN CLINICAL SAMPLES

Dr. Aran's ultimate goal of using this platform in diagnostics warranted a sample measurement showing the ability of this system for the same. Therefore, the team tested samples with two commonly deleted exons (exon 3 and 51) in patients suffering from Duchenne Muscular Dystrophy (DMD). Samples from healthy patients were used as a control.

CRISPR-Chip functionalized with RNPs specific to either exon showed significantly enhanced signal output after exposure to genomic samples containing the target exons, relative to DMD samples carrying exon deletions.

Dr. Aran notes that she used Synthego's single guide RNAs for their experiments.

"We are so happy with the Synthego's guides that we for all of my other projects are starting to order from Synthego. For my muscular dystrophy samples, we also have ordered guides from Synthego."

OVERCOMING CHALLENGES AND FUTURE STEPS

The CRISPR-Chip system with its fast detection time and low limit of detection might sound simple, but it is not devoid of challenges.

"Developing this platform requires a very good understanding of nanotechnology and electronics too," says Dr. Aran. The team had to not only optimize CRISPR guides and RNPs, but they also needed heavy optimization on the electronics side, according to Dr. Aran. For instance, they needed to make sure that they were not absorbing the salts from buffers in their biological solution.

Dr. Aran plans to exercise similar caution when moving forward with her ambitious projects using this platform. Apart from further optimization for diagnostic applications, there are multiple ways to expand this tool in the future for better understanding CRISPR binding. "We can get a lot more information which is actually very useful for pharma companies using CRISPR for therapeutics because we can actually monitor the binding efficiency of their guides and their complex with target DNA," says Dr. Aran. "So with our sensor and the immobilized CRISPR atop of it, we can actually do a lot of quality control testing, which is the next thing that we are going to do with the sensors."

ORIGINAL PUBLICATION

Hajian, Reza, et al. "Detection of unamplified target genes via CRISPR–Cas9 immobilized on a graphene field-effect transistor." Nature Biomedical Engineering (2019): 1.

Do you want to learn more about CRISPR? If so, check out Synthego's free CRISPR 101 eBook.

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UP NEXT

CRISPR is ascending again, after scientists find 'elegant' fix for cancer worry

By Sharon Begley

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IN THE LAB

CRISPR is ascending again, after scientists find 'elegant' fix for cancer worry

By Sharon Begley @SXBEGLE | MARCH 21, 2019

If the genome-editing system CRISPR-Cas9 is biology's precise, disciplined, Swiss army knife, its march toward the clinic is more like the roller coaster from hell. One moment it's riding high with the promise of curing devastating genetic diseases, then its prospects plummet after the discovery of previously unsuspected risks, and the next moment it turns out those risks are either overblown or avoidable. Buy another ticket and get back on board.

On Thursday, scientists led by one of the world's foremost gene therapy experts reported a way around one of the more worrisome obstacles: that CRISPR'd cells might be prone to becoming cancerous, as two 2018 studies suggested.

"The CRISPR field has this cycle of discoveries-obstacles-resolution," said genetics researcher Gaétan Burgio of Australian National University. On the cancer worry, "we are now on the resolution side."

The cancer concern arose when separate teams of scientists, at Novartis and Sweden's Karolinska Institute, found that many cells whose DNA is successfully edited (to, say, cure sickle cell disease) self-destruct, go into a sort of suspended animation, or revert to their original, disease-causing genetic state. Neither dead cells nor unedited (still disease-causing) cells, needless to say, would work as a therapy.

But, these teams found, cells that survive and retain the DNA edits — exactly what you want for therapy — do so only because they've also lost what biologists call "the guardian of the genome": a gene called p53, which protects healthy cells from transforming into malignant ones.

Put another way, a successfully CRISPR'd cell could also be a p53-less cell and therefore a cancer-prone cell.

In the new study, published in Cell Stem Cell, scientists in Italy made two key discoveries.

First, if CRISPR makes a very precise cut at a single spot in the genome (which is what it's supposed to do but doesn't always), then the damage is too minimal to activate the DNA repair and p53 pathways more than briefly, said Luigi Naldini of Milan's San Raffaele Telethon Institute for Gene Therapy, who co-led the study.

With the p53 pathway basically under control, CRISPR'd cells (the scientists used blood-making cells called hematopoietic stem cells) survived and proliferated. The cells were also able to set up house in bone marrow, Naldini and his colleagues found in mice. Such proliferation and "engraftment" is what you'd need for edited stem cells to cure sickle cell, other blood disorders, or immunodeficiency diseases.

"The most important finding from this part of our study is that if you make a double-stranded break at only one site in the DNA, the damage is finite and constrained, and so is the cellular response," said study co-leader Raffaella Di Micco of the San Raffaele institute. The DNA-repair/p53 pathway kicks in so briefly and half-heartedly that the cell neither self-destructs nor undoes the CRISPR edit. Instead, it hangs on to its edit and proliferates normally, which is what CRISPR'd cells must do after being infused into patients to cure inherited blood and immune diseases. And contrary to last year's alarming studies, the p53 pathway remains intact, ready to stop the cell from becoming cancerous in the future.

"There are now very powerful tools to design your guide RNAs to be very specific, almost insuring that you hit only one target," Di Micco said.

Sometimes, however, there was no way to avoid awakening the slumbering p53 monster. When the scientists used a virus called AAV to deliver both highly specific DNA-cutting enzymes and a repair gene — a possible strategy for curing such disorders as immunodeficiencies and possibly cystic fibrosis — the cell acted as if its DNA had been cut to ribbons. It activated DNA damage response mechanisms, including the p53 pathway, said co-author Pietro Genovese.

That might have led to the worrisome scenario painted by last year's p53 studies.

But in what Di Micco called "the crucial part of our story," she and her colleagues added a little something to their CRISPR package: a molecule called GSE56, which blocks the p53 response. They got everything into the hematopoietic stem cells via a tiny doorway they created with an electric shock.

Temporarily suppressing p53 "was a way of communicating to the cell, 'this DNA damage response is not a problem, be quiet for the genome edit, then be active again, proliferate, and engraft [where you should]," Genovese said. This kept the cell from self-destructing, undoing the CRISPR edits, or refusing to grow and proliferate normally — any one of which could leave too few vigorous, CRISPR'd cells to treat a disease.

Although the "guardian of the genome" was offline for a time (less than 48 hours), the cells did not accumulate random, unintended mutations. They did, however, retain their DNA edits and their health, able to proliferate normally when returned to lab mice standing in for patients.

"We think that transient inhibition of p53 could be a viable strategy" for therapeutic genome editing to treat blood diseases such as sickle cell and thalassemias, especially when large quantities of cells (removed from patients, edited, and infused back into them) are likely to be required, Di Micco said.

If she is right, then the CRISPR-cancer worry would join other roller coaster plunges that were followed by a return to optimism. The fear that CRISPR would hit lots of genes it isn't supposed to ("off-target effects") has largely gone away, as has the concern that people's immune system might attack the main CRISPR enzyme, Cas9.

Scientists not involved in the new p53 study heaped praise on it.

Burgio called it "excellent, elegant, and comprehensive," emphasizing the importance of the discovery that p53 can be temporarily, and safely, inactivated. That "is indeed likely to alleviate or resolve the p53 issue" raised by last year's studies, he said.

Going even further, genome scientist Fyodor Urnov of the independent Altius Institute for Biomedical Sciences in Seattle said the study "shows convincingly that p53 is fully manageable, thank you very much." Naldini, who spearheaded the development of Europe's first approved gene therapy, for severe immunodeficiency disease, "has a reputation for solving technical challenges [in genetic therapy] and he's done it again," Urnov added. "Last year's rumors of CRISPR's death were greatly exaggerated."

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UP NEXT

For CRISPR enzymes, the gold rush is on

By Sharon Begley

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IN THE LAB

For CRISPR enzymes, the gold rush is on

By Sharon Begley @SXBEGLE | FEBRUARY 4, 2019

Young scientists collaborating with CRISPR impresario Jennifer Doudna of the University of California, Berkeley, generally don't envision mucking about with groundwater teeming with all manner of microscopic beasts. But there they were, analyzing water samples from the abandoned Iron Mountain gold and silver mine in northern California, from an old uranium mine in Colorado, and from a frigid geyser in Utah, in each case running a "metagenomic analysis," sequencing the genome of every one of the aquatic residents.

It's like panning for biological gold, and the result was an 18-karat treasure, Doudna and her colleagues announced on Monday: a CRISPR protein different from any previously known, able to edit human genomes like a charm, and with properties that could make it a workhorse of therapeutic editing.

"Many labs are busily looking through [genomic] databases for CRISPR proteins, but it's rare to find one that's useful for genome editing," said Doudna. The new enzyme, described in a paper in Nature and called CasX for now (it will likely be called Cas12e, but the Cas nomenclature is a hot mess), not only edits human genes in cells growing in lab dishes, she and her colleagues found. It also has properties that could allow it to enter human cells more readily than the commonly used CRISPR-Cas9 and replace disease-causing segments of DNA with healthy chunks.

"Having an expanded toolbox is of great use" to would-be genome editors, said protein engineer Ben Kleinstiver of the Center for Genomic Medicine at Massachusetts General Hospital, who was not involved in the CasX work.

Each new CRISPR enzyme "enables researchers to do different things because they have unique functionalities."

It's 1849 for CRISPR enzymes, and fortunes are up for grabs.

Startup Arbor Biotechnologies has raised more than $15 million on the promise of discovering new CRISPR enzymes and other valuable biomolecules. Last month Arbor and Vertex Pharmaceuticals (VRTX) announced that they had begun a collaboration (financial terms undisclosed) focused on discovering novel CRISPR enzymes, aimed at genome-editing therapies for cystic fibrosis and four other diseases to be chosen later. Doudna and four of her co-authors have filed for a CasX patent, as have the discoverers of every other new Cas enzyme.

In addition to making CRISPR-based therapies more efficient and versatile, the enzyme gold rush could provide workarounds to the CRISPR patent dispute, which involves only Cas9: Each new enzyme offers a way to do genome editing without worrying about who owns the rights to CRISPR-Cas9.

Since about 2012, scientists using the CRISPR genome-editing system had been contentedly pairing the Cas9 enzyme with a guide RNA (the RNA acts like a bloodhound, finding a specific target in a cell's genome, while the enzyme cuts the DNA, either snipping out a disease-causing region or replacing it with healthy DNA as well). But in 2015 scientists at the National Institutes of Health and the Broad Institute discovered another bacterial enzyme that works with CRISPR. They named it Cpf1 (it's now Cas12a) and reported that it cut DNA in a way that could make it an even better genome editor than Cas9.

Since then, scientists have discovered a plethora of CRISPR enzymes in bacteria that make yogurt (Streptococcus thermophilus), colonize human noses (Neisseria lactamica), kill people (Legionella pneumophila), live in soils (Alicyclobacillus acidoterrestris), or inhabit the seas (Oleiphilus species), among other sources. Last month, Arbor announced the discovery of four more: Cas12c, Cas12g (which edits RNA, not DNA), Cas12h, and Cas12i.

For a CRISPR system to become a therapy, it has to edit genomes, get into target cells, edit only what it's supposed to, and do it efficiently. "All of the CRISPR systems harnessed for genome editing to date show differences across these four properties," said Arbor co-founder David Scott. "Having a diversity of enzymes increases the chances of having the right tool for different applications."

The CRISPR enzyme gold rush doesn't end with mere discovery, however. Like the Forty-Niners who turned their raw nuggets into gems, these prospectors go beyond what nature coughs up. Last month, for instance, the Broad's Feng Zhang and his team reported that they had induced mutations in Cas12b, which in its natural form doesn't work well in human cells. With the mutations, it does, even making it superior to the original Cas9 on some measures.

CasX, which was discovered in groundwater from the Colorado site in the metagenomics research led by Berkeley's Jill Banfield, shows similar promise. For starters, it's small, at 980 amino acids (Arbor's 12h is the smallest known, at 870 to 933 amino acids, while the original Cas9 has 1,368). That's important for future therapies because CRISPR is usually ferried into target cells with a virus, "and there's only so much you can fit in," said MGH's Kleinstiver. If the enzyme takes up less space, there's more room for the rest of CRISPR, including more repair DNA. "In general," said Arbor's Scott, "smaller enzymes enable more delivery options to enhance genome editing," such as nanoparticles rather than viruses.

The small size of CasX gives it what Doudna calls "interesting properties for things like delivery into cells."

Its guide RNA is so large relative to the enzyme that it changes the surface of the CRISPR package in a way that makes it more electrically charged. That, Doudna said, which could ease its passage into cells — one of the trickiest challenges to CRISPR therapies now in development.

When the Berkeley scientists tested how well CasX edited human cells in lab dishes, they got editing efficiencies of about 34 percent, comparable to how well both CRISPR-Cas9 and CRISPR-Cas12a did when they were first discovered. But by tweaking how they ferried it into cells, they raised that to as high as 55 percent.

CasX has another property that seems arcane but may prove invaluable for CRISPR therapies. It cuts DNA in a way that leaves what's called a staggered double-stranded break: One of the double helix's complementary strands overhangs the other by about 10 nucleotides. (Cas12a and 12b also make staggered cuts.)

"That could be useful for triggering certain kinds of DNA repair," Doudna said. In particular, it might promote what's called homology-directed repair, meaning the genome accepts repair DNA rather than reacting to CRISPR by patching the cut willy-nilly. Repair DNA will likely be needed for at least some therapeutic uses. "The next breakthrough in genome editing is going to be figuring out how to favor homology-directed repair," Doudna said.

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UP NEXT

A pencil, not a pair of scissors: CRISPR pioneers' new company bets on base editing to cure disease

By Sharon Begley

STAT

BIOTECH

A pencil, not a pair of scissors: CRISPR pioneers' new company bets on base editing to cure disease

By Sharon Begley @SXBEGLE | MAY 14, 2018

Months after its dozen scientists began working in secret on what's been called "the most clever CRISPR gadget" so far, the latest company hoping to deploy genome-editing to cure diseases came out of stealth mode on Monday.

Beam Therapeutics, which registered as a corporation in Massachusetts in March and has been doing experiments since last year, is debuting as CRISPR companies are popping up like dandelions, but right out of the gate Beam stands out in a crowded field. Its three founders are among the world's leading CRISPR'ers, Editas Medicine (EDIT) has an equity stake, and in addition to the $13 million it's raised (from Arch Venture Partners and F-Prime Capital Partners), it has commitments for another $85 million.

The name "Beam"? BE stands for base editing, the CRISPR technology it aims to turn into therapies, said co-founder David Liu of Harvard, who invented base editing in 2016. "Beam" evokes the precision of a laser beam, he said, and co-founder Dr. J. Keith Joung of Massachusetts General Hospital pointed out that the "AM" could mean "and more," referring to other CRISPR discoveries they hope to harness. The third co-founder is Feng Zhang of the Broad Institute, one of the first scientists to get CRISPR to edit genes in mammalian cells.

"We see base editing as a new wave of therapeutic modalities," CEO (and Arch partner) John Evans told STAT.

Liu's base editor neatly changes one nucleotide to another without breaking the double helix, as the original CRISPR-Cas9 genome-editing system does. Breaking DNA can spark what Harvard biologist George Church called "genome vandalism," with random nucleotides being haphazardly inserted and deleted as the double helix tries to patch the break, like a carpenter frantically throwing any handy chunk of trash into a hole in a plaster wall.

"We view base editing like a pencil in contrast to CRISPR-Cas9, which is more like a scissors," Liu told STAT, "For some jobs scissors are the best tool, but for others a pencil is."

The main "others" are diseases that are caused by a one-letter misspelling in a gene, called a point mutation. He declined to list them — "people will assume that's what we're going after" — but they include cystic fibrosis, Tay-Sachs, sickle cell, neurofibromatosis, and some cancers. All told, some 33,000 specific point mutations have been linked to inherited diseases, Liu said, raising hopes that CRISPR base editing might one day prevent or treat them.

Liu's original base editor, essentially an atom rearranger that he calls a "programmable molecular machine," changes a C to a T and then the C's original partner, a G, to an A. But many other inherited diseases have different misspellings. The improved base editor, unveiled in 2017, changes an A to a G and the T that had been paired with the A into a C that pairs with the new G. The base pair AT thus becomes GC.

Mutations in which a G has changed to an A have been linked to focal epilepsy, Duchenne muscular dystrophy, and Parkinson's disease.

The CRISPR part includes a molecule that carries the whole atom rearranger to a target on the genome.

Beam will choose which diseases to target based on whether correcting a point mutation can reverse or at least ameliorate the condition, and on whether it's relatively straightforward to reach the target tissue. Since it got up and running last year, Liu said, it has been generating data for about a dozen possible disease programs.

The company also aims to develop therapeutic technologies based on Zhang's RNA editor, which he reported last year. Called REPAIR (RNA Editing for Programmable A to I Replacement), it's a version of CRISPR that uses the Cas13 cutting enzyme, not the original Cas9, and targets not the DNA of the genome but the messenger molecules that carry the genome's instructions to a cell's protein-making machinery. REPAIR can make A-to-G changes.

There are some diseases where it will likely be prudent to avoid making a permanent change to the genome, as traditional CRISPR (including base editing) does, Zhang said.

"RNA editing is reversible, and there are some diseases where transient reversal [of a mutation] could be therapeutic, such as regeneration of the liver or increasing bone density," he said. In the latter, a permanent genetic change to, say, take the brakes off bone formation could cause runaway growth, but dinging RNA (which has a half-life in human cells of about 10 hours) should produce a change that lasts only long enough to be therapeutic. (If CRISPR hangs around the cell for a long time, however — something scientists are trying to determine — it might produce RNA editing that lasts longer than desired.)

Harvard, which filed for a patent on Liu's original base editor in 2014 and 2016 and on the improved version last October, has granted an exclusive license to Beam to use the invention to develop and commercialize technologies to treat human disease, for an upfront payment of (unspecified) several million dollars. Academic labs that want to use the base editors for nonprofit research don't have to pay a licensing fee.

Beam also negotiated a sublicense from Editas to use the underlying CRISPR-Cas9 technology (patents that are still the subject of a court battle) for base editing. Editas will be eligible for royalties on any therapies Beam develops using the sublicensed inventions.

A Harvard spokeswoman did not directly answer whether the university had shopped around Liu's inventions (the patents have not been issued yet), but said, "our practice is to license to the party or parties most committed and most capable of developing a technology to its fullest potential. The decision to license this platform to Beam entailed a thorough analysis, as we sought a partner with a dedicated focus, the right scientific/technical expertise, and sufficient capital. ... We do believe that in the field of human therapeutics, where R&D takes an immense investment of resources, a degree of exclusivity is necessary to make it feasible to bring new therapies to the clinic."

Why start a new company rather than getting Editas, of which Liu, Zhang, and Joung are co-founders, to plunge into base editing? "What's clear from the size of this round is that base editing is a new modality and will require a fair amount of independent funding," Evans said.

"We continue to have a great relationship with Editas," Liu said, "but this arrangement has the best chance to benefit patients and to maximize societal benefits."

Beam's agreement with Harvard lets other companies propose using base editing to treat a disease, Harvard said in a statement, if Beam passes on it. And Beam has to hit certain development milestones to retain exclusive rights to the technology; Harvard said the specifics are confidential.

Liu also founded Ensemble Therapeutics, in 2004, which raised nearly $40 million from Flagship and Arch, among others. Despite entering into partnerships with Novartis and Alexion, among other pharmaceutical companies, it closed last August without commercializing a drug.

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UP NEXT

As calls mount to ban embryo editing with CRISPR, families hit by inherited diseases say, not so fast

By Sharon Begley

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IN THE LAB

As calls mount to ban embryo editing with CRISPR, families hit by inherited diseases say, not so fast

By Sharon Begley @SXBEGLE | APRIL 17, 2019

Neena Nizar's earliest memory is of her father tying her to an ironing board. His beloved toddler, who seemed fine when she was born, had something very, very wrong with her: Neena's bones bent and curved and she wasn't growing normally, so his engineer's mind desperately seized on the ironing-board solution.

But the problem — which some doctors diagnosed as polio and others as rickets or "we have no idea" — was even worse than bones that wouldn't stay straight. They also broke down faster than they grew, with weak cartilage where strong bone should be. By the time other little girls were skipping and running and kicking balls, she was in pain and could barely get around. "I had to be carried into school, and I had rods in my hips and metal clamps to hold my bones in place," said Nizar, who was born in Dubai. "Growing up, that was beyond hard. It was horrible."

So when Nizar, now 41 and living in Nebraska, hears scientists' emphatic calls to prohibit "embryo editing" of disease-causing genes, her reaction is shaped by decades of her own suffering — compounded by that of the two sons who inherited her devastating mutation.

"It's easy to get on your high horse when you're not in our position," she said. "If editing an IVF embryo is the best option to mitigate the pain that a child would otherwise suffer, then give us the choice."

In 2012, scientists showed that CRISPR, an ancient bacterial immune system, can edit DNA much the way "find and replace" edits a document, setting off a race to refine the tool for human gene therapies. Barely three years after, leaders in the field convened a private meeting in California's Napa Valley to discuss their concerns about the possible use of CRISPR in IVF embryos, concluding that it should not be done, at least not yet.

Changing a single DNA "letter" in the genome of a very early embryo has the potential to correct a genetic defect not only in any resulting baby but also in all of that baby's descendants. That, warn opponents of such "germline editing," would change the human gene pool, a step they worry could have unforeseen and irreversible consequences. They also argue that known carriers of genetic diseases could have embryos screened for harmful mutations before being used in IVF.

The opponents have largely dominated public discussion of this use of CRISPR, especially after a Chinese scientist announced last November that he had changed the genome of two IVF embryos and produced the world's first "CRISPR babies." Worldwide condemnation was instantaneous, and since then a group of prominent CRISPR experts called for a global moratorium on using CRISPR for reproduction — research stopping short of a pregnancy is OK, they say.

Dr. Francis Collins, director of the National Institutes of Health, endorsed that call. Asked whether he would always feel that way, Collins told STAT through a spokeswoman that "'always' is a really big word," but that for now, he "can't imagine a circumstance where he would feel differently." NIH is prohibited from funding research that edits human embryos.

Watching all this have been people with a special interest in embryo editing: those who carry genetic mutations that can cause severe disease. They wonder whether experts who denounce embryo editing have any understanding of what millions of people with such inherited diseases — especially ones that have plagued their families for generations — suffer.

"Patients and their parents will be the ones pushing for research and eventually clinical trials" of embryo editing, said bioethicist Jeantine Lunshof of the Massachusetts Institute of Technology, who has been the in-lab ethicist for Harvard biologist George Church, who was one of the first scientists to edit human cells with CRISPR. Assuming the procedure is shown to be safe, "desperate parents who just lost a child [to a genetic disease] are going to say, 'For our next pregnancy, we want this.'"

Despite dozens of surgeries and consultations with far-flung specialists, Nizar's disease stumped every expert her father found. So when she and her husband decided to start a family, despite doctors telling her that her legs would crumble under the weight of a fetus, she had no idea she carried a devastating mutation — especially when her son Arshaan came into the world in 2008 at a robust 9 pounds, seemed perfectly normal, and walked at 1. Whatever crippling disease she had, Nizar felt, it stopped with her.

But when she was 32 and pregnant with her second son, a scan revealed skeletal and other abnormalities in the fetus. At about the same time, Arshaan, then 2, began to regress. It was as if time were taking him backwards to the hell his mother suffered as a child: His bones curved, and his walk became a waddle.

Finally, a geneticist diagnosed Nizar, Arshaan, and the soon-to-be little brother. All three have Jansen type metaphyseal chondrodysplasia. Caused by a mutation in a gene called PTH1R (parathyroid hormone-related peptide receptor), which controls the differentiation of bone and cartilage, its chief symptoms are the arms and legs having cartilage where they should have bone, making them abnormally short, weak, and painful.

To correct their curved bones, Arshaan and his brother, Jahan, 8, have had surgeries every year since they were diagnosed, "getting pins and rods to keep their bones straight," said Nizar, founder of the Nebraska-based nonprofit Jansen's Foundation. "But it's like working with putty because the 'bone' is mostly cartilage." As the limb grows it bends again, necessitating additional surgeries.

For unknown reasons, the second generation to carry the Jansen's mutation usually develops more severe disease than the first. The boys often have to crawl on hands and knees to get around and miss out on simple things like playing at the park, not to mention anything as physical as contact sports. Their pain never stops, and they don't understand what the "smiley face" on the pain scale means.

"The third generation might be even worse," Nizar said. "We don't know." If her boys grow up and want to be fathers, she said, embryo editing should be an option. "You're not changing who they are, but fixing a defect that causes agony and pain," she said. "Having options is a personal right. No one knows the path you walk except yourself."

"Let them say we're playing god," she added. "This is not about something frivolous like changing eye color to make a designer baby. It's about a child's suffering."

Another argument against germline editing is that many inherited diseases can be corrected later, once a child is born, including sickle cell disease and Duchenne muscular dystrophy. It is not clear whether that approach will be as successful as germline editing might be, especially in a disease that leaves lasting damage, as sickle cell does, or seemingly irreversible damage, as some neurological conditions do. What is indisputable, however, is that such a correction would not be inherited, so the next generation would have to undergo the therapy, too.

> "You're not changing who they are, but fixing a defect that causes agony and pain. Having options is a personal right. No one knows the path you walk except yourself."
>
> NEENA NIZAR, WHO HAS JANSEN TYPE METAPHYSEAL CHONDRODYSPLASIA

Opponents of embryo editing argue that parents can have a healthy IVF embryo without it, by having their IVF embryos (each cycle yields eight or so) tested for a particular mutation and implanting only healthy ones. The odds of having at least one unaffected embryo vary with the genetics of a disease, however, including whether the mutation is dominant or recessive, and carried by one parent or both. In general, the odds of getting an unaffected IVF embryo range from 25 percent to 75 percent, but "a substantial percentage (27%) of [such] cycles produce no viable, disease-free embryos for transfer," three experts in genetic medicine wrote in the New England Journal of Medicine last month. If both parents have the same recessive genetic disease, then all their embryos would be affected as well.

Andrea Taylor understands losing odds. She and her husband both carry mutations in a gene called SLC2A10. The mutation makes arteries elongate, twist, and turn, leaving vital organs starved for oxygen and forming aneurysms. This arterial tortuosity syndrome kills 40 percent of the children who inherit it by age 5, but because it is recessive, neither Taylor nor her husband have it or had any reason to suspect they were carriers. The chance that they'd meet, marry, and have children was hundreds of million to one; the chance that their sons would have it was 25 percent.

Their first, Aaron, is healthy. But Aiden lost the genetic lottery. Born in 2008, he inherited one ATS-causing gene from each parent. His life has been a series of operations, including open heart surgery to reconstruct his pulmonary arteries and three heart catheterizations.

Taylor, president and founder of the Arkansas-based ATS advocacy and awareness nonprofit A Twist of Fate, knows the philosophical objections to germline editing. "It's hard for me to believe that the people saying this went through anything like what our families have, with a child tethered to a bed and the light blinking" to signal yet another medical emergency, she said. "It's hard to reconcile the philosophical arguments against changing the human gene pool with what a child suffers. If there were a safe way to do it, a million times over I would do it, and every mom I know would do it, too."

The "playing God" objection leaves her cold, said Taylor, who describes herself as a person of faith. "God gave us the knowledge and the ability to do this," she said. "We would not have been created the way we were, with intelligence and the ability to make scientific discoveries, if we weren't meant to do it. If you could fix something like this in a child from the very beginning, why would you not try?"

Monica Weldon, too, struggles to square the "playing God" objections with her son's suffering. He was born with SYNGAP syndrome, the result of a mutation in a gene called SYNGAP1. The DNA misspelling causes abnormalities in neuronal growth and synapse function, leading to developmental delays, intellectual disability, and other neurological symptoms.

It starts slowly, said Weldon, who founded the Texas-based research and advocacy group Bridge the Gap. As a newborn, her son Beckett seemed as normal as his twin sister, Pyper. But while Pyper hit developmental milestones such as rolling over and sitting up and babbling, Beckett was "more like a limp noodle," Weldon said, and hardly babbled.

Weldon compares the notion of embryo editing to prenatal surgery. Correcting spina bifida and other abnormalities in fetuses, once considered cowboy medicine, is now mainstream. "If someone wants to try to fix a gene to save their baby, they should have that option," she said. "Obviously you have to move cautiously, but saying absolutely not, no, never … I don't think you can say that unless you understand the patient experience."

The "altering the human gene pool" concern also puzzles families — as well as some experts. CRISPR doesn't introduce, say, fish genes into tomatoes, as old-line recombinant DNA does. It changes a disease-causing version of a gene into a healthy, far more common form. "It's hard to see how giving someone the form of a gene that 6 billion other people have is changing the human gene pool," Lunshof said.

In 2009 Diana Daus's mother died of Huntington's. The fatal, incurable disease destroys brain neurons, cruelly tearing away at a person's physical and mental capacity, usually starting between ages 30 and 50, until there is nothing left. Daus's brother, too, has Huntington's, which is caused by repeats of the CAG nucleotide triplet; the repeats produce an abnormally long protein that accumulates in and kills neurons. The mutation is dominant; a single copy causes Huntington's even if the copy from the other parent is normal.

Daus, an adjunct professor at the City University of New York, has devoted her adult life to developing occupational therapies for people with Huntington's. Her mother, she said, would have opposed embryo editing. "She was a devout Catholic who believed that whatever God gave us, it was the decision of a greater power," and one that people shouldn't question, let alone undo, Daus said.

She disagrees: "I personally would be in favor of using any available technology so families would not have to pass down" the Huntington's mutation.

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UP NEXT

The CRISPR shocker: How genome-editing scientist He Jiankui rose from obscurity to stun the world

By Sharon Begley *and* Andrew Joseph

STAT

DECEMBER 17, 2018

SPECIAL REPORT

The CRISPR shocker: How genome-editing scientist He Jiankui rose from obscurity to stun the world

By Sharon Begley @SXBEGLE *and* Andrew Joseph @DREWQJOSEPH

One of the world's most celebrated biologists, Jennifer Doudna is not easily rattled. But she was struggling to process what she had just heard. Moments before, she met with the researcher whose bombshell had shaken the world of medicine like nothing since the birth of the first test tube baby 40 years earlier. As she walked up from the lobby of Hong Kong's Le Méridien Cyberport hotel, the University of California, Berkeley, biochemist was shaking her head ... as if that would jostle her thoughts into a place where everything made sense again.

It was the last Monday in November, the day news broke that a little-known scientist in China named He Jiankui claimed he had created what instantly became known as the world's first "CRISPR babies": twin girls who came into existence as IVF embryos and whose genomes had been changed by the revolutionary DNA editor called CRISPR. It was something everyone in the burgeoning, multibillion-dollar field of genome editing knew would come one day, but which nevertheless shook even experts with its timing, its secrecy, and PR trappings that made the rollout of Beyonce's "Lemonade" look amateurish.

Doudna, who co-led a 2012 study showing that a weird bacterial immune system called CRISPR could edit DNA as niftily as Word edits documents, and hundreds of other experts were in Hong Kong for the International Human Genome Editing Summit. He Jiankui, who was scheduled to speak at the summit on Wednesday, had asked to meet privately with Doudna, one of the summit's organizers. In his presentation, He had planned to talk about the ethics of embryo editing and his experiments on mouse, monkey, and human embryos, with nary a hint that two of those embryos were now living, breathing, baby girls whom He, in an astonishing YouTube birth announcement, called Nana and Lulu. Was that okay?, he asked Doudna as they sat in the lobby.

Um, Doudna replied, you've dropped this shocking news on the world, right before our summit, and you're not planning to mention it? He seemed surprised that she expected him to but agreed to have dinner with her and other members of the summit organizing committee that evening to talk it out.

"His demeanor was an odd combination of hubris and naivete," she recalled in an interview. "He was very confident in his work, and totally not understanding what an explosion he had caused" — one that, some scientists feared, could derail hopes for using CRISPR to prevent some of the most devastating diseases lurking in the double helix.

In the three weeks since the remarkable announcement about Nana and Lulu, STAT has pieced together the story of the years leading up to that fateful Monday. With details reported for the first time, it describes the many times He met with and spoke before some of the world's leading genome-editing experts, the low opinion they had of his research, and the hints he dropped about his grandiose aspirations. It is based on interviews in Hong Kong and with experts on four continents, with scientists and others who have crossed paths with He, as well as on documents and published accounts. He did not reply to requests for an interview.

NOBEL DREAMS, SECOND-RATE SCIENCE

The tensions between He and Doudna and other scientists came to a head that night in Hong Kong. But it was the culmination of acts marked by crowning ambition that others had seen in him for years.

He, whose lab is at Southern (sometimes translated "South") University of Science and Technology in the tech-booming city of Shenzhen, had sought to insert himself into the CRISPR elite. But they viewed his science as second-rate. He had hours-long discussions with a leading bioethicist who warned him against creating "CRISPR babies" — yet never revealed that the discussion was far more than academic. He confided in at least two U.S. scientists about his plan, but ignored their arguments that he was making a potentially disastrous mistake. He studied recommended ethical guidelines for embryo editing — but flouted them. He claimed he had been transparent about working toward pregnancies with CRISPR'd embryos — yet never breathed a word about those plans in his talks at science meetings and stalled for months before listing his experiment on an official Chinese registry of clinical trials.

For a driven and fame-seeking scientist who had set his star on changing the world, heeding doubters and sticklers wasn't part of the plan.

He believed he would be hailed for his scientific first, especially in his homeland, as someone who did for China what the Sputnik engineers did for the old Soviet Union. In conversations with scientists and others, he brought up Dr. Robert Edwards, part of the team who created the world's first test tube baby, won the 2010 Nobel prize for it, and brought joy to millions of otherwise infertile couples.

No wonder He seemed stunned that Monday, as worldwide condemnation of his work grew and even the stars of the CRISPR firmament weren't applauding him. Over the hastily arranged Cantonese buffet dinner at Le Méridien, Doudna and three other summit organizers peppered him with technical questions (How many embryos did you try to edit with CRISPR? How many succeeded? How did you decide which embryos to implant?

What tests did you run to see if the editing worked as planned?) and challenged the ethics of the experiment (Why did you pick the gene CCR5, which is involved in HIV infection, to edit? Did the parents understand the risks to their potential child? How do you know?).

After just over an hour, He had enough, participants told STAT. He pulled some cash out of his pocket, threw it on the table, and stormed off. Fearful of his safety, he left the Méridien and checked into another hotel. His dinner companions were left wondering if he would even show up for his scheduled talk at the summit on Wednesday.

FORGING CRISPR CONNECTIONS

In retrospect, He had been hiding in plain sight. Although he has been a shooting star in Chinese science for about five years, ever since he returned to the country of his birth after graduate and postdoctoral stints in the U.S., he is not an alumnus of any of the world's leading CRISPR labs. He had written no important CRISPR papers before his shocking announcement. (He still hasn't: The CRISPR babies experiment remains unpublished, and a study editing mouse, monkey, and human embryos without starting pregnancies has been rejected.) He was on no one's radar screen.

He tried to make up for it.

In April 2016, he wrote to Feng Zhang of the Broad Institute of MIT and Harvard. After Doudna's 2012 study showing that CRISPR can edit DNA in a test tube, Zhang and his colleagues got it to do so inside living cells, including human cells growing in a lab dish. That made him one of the world's best-known CRISPR scientists.

He identified himself as CEO of a Shenzhen-based DNA sequencing company called Direct Genomics and requested a tour of Zhang's lab in Cambridge, Mass. The visit never happened. But He kept trying to make CRISPR connections.

In late 2016, on a trip to the Bay Area, He emailed biologist Mark DeWitt of Berkeley's Innovative Genomics Institute. Could DeWitt meet for coffee? They did, laying the groundwork for months of discussions.

Then He aimed higher, contacting Doudna "out of the blue," she said. He would be in the Bay Area in early 2017, he said; perhaps they could meet.

The email landed at an opportune time. Doudna and Stanford bioethicist William Hurlbut had just received a grant of more than $215,000 from the Templeton Foundation to study "The Challenge and Opportunity of Gene Editing: a Project for Reflection, Deliberation, and Education." To kick it off, they were holding a workshop at Berkeley for nearly 20 scientists, ethicists, and historians in January 2017. None, Doudna realized, were from outside the U.S.

Maybe we should invite him, Doudna proposed to Hurlbut. They did. He came. On the workshop's second day, in a session called "Evolution and Human Development," He presented work on using CRISPR to edit mouse, monkey, and human embryos (without pregnancies). His talk did not leave much of an impression, "and I don't think it was received very well," Doudna said.

That was partly because He was, in a sense, two years late. In 2015, scientists at Sun Yat-sen University in Guangzhou used CRISPR to edit the gene whose abnormality causes the often-fatal blood disease beta thalassemia. Their experiment, which also sent shock waves around the world, used nonviable IVF embryos. He, too, was using nonviable embryos. It didn't seem like he was moving the science forward.

Worse, another attendee recalled, scientists said He's "science was sloppy and the application unnecessary." One biologist challenged He on technical details of his work, especially how he analyzed the edited genomes for the unintended edits called off-target effects, a critical safety concern.

Other scholars who attended were struck by what Harvard's Sheila Jasanoff called He's "great smoothness." Although He did not explicitly discuss his ethical views, Jasanoff said, he "clearly did not have deep misgivings about plugging ahead with gene editing, and I sensed no exposure to the sorts of ethical debates our guys are routinely involved in."

To CRISPR's leaders, "He wasn't seen as a major player," Doudna said. Having published no papers on CRISPR editing didn't help; neither did presenting research that didn't seem to move beyond what others had reported.

Nor did his academic pedigree. He, 34, grew up the son of rice farmers in an impoverished county in Hunan province, in southeastern China. According to Chinese media reports, he built a simple lab at home and in high school became obsessed with physics, earning an undergraduate degree in that discipline from the respected University of Science and Technology of China, in Hefei, in 2006. Flush with a scholarship to study in the U.S., he began pursuing a Ph.D. in physics and astronomy at Rice University. According to a 2010 article from the Rice news office, it was the only graduate school that accepted him.

He was a star there, the news office said, specializing in mathematical modeling and computer simulations of biological systems. As president of the Rice Chinese Students and Scholars Association, he organized "a steady stream of events for a community of more than 400." He made time for life outside the lab: "I love to play soccer," he said then. "Oh, my God, Rice has six soccer fields! That's awesome."

He earned his degree in less than four years (his thesis was on how CRISPR evolved), published three papers that the news office described as "of tremendous significance," and in 2011 began a one-year postdoctoral fellowship at Stanford with bioengineer Stephen Quake. As he was finishing up at Rice, He expressed gratitude to his Ph.D. adviser, physicist Michael Deem, for encouraging him to apply his training broadly: "I did not restrict my work to conventional physics," he said. "Instead, I applied the techniques and methods in physics to the study of biology and the economy."

The news office quoted Deem as calling He "a very high-impact student," adding, "I am sure he will be highly successful in his career." Eight years later, Deem was in He's lab in China for work that would indeed make an impact, though perhaps not the kind he had in mind.

China also had its eye on He. In April 2011, the city of Shenzhen, across the border from Hong Kong, launched its "Peacock Program" to attract scientists, setting them up in spanking-new labs and staking them to generous research budgets and salaries. He was a peacock. After Stanford, He became, at age 28, the youngest associate professor at the city's Southern University.

His focus: gene sequencing. Academia was too small a playground. In 2012, He founded Direct Genomics, which builds DNA-sequencing machines based on technology Quake developed. Its "GenoCare Analyzer," for clinical diagnostics, reached the market last year, only the second Chinese-made sequencer in commercial use. "We're a new generation of entrepreneurs," He told a reporter in 2015. Government health officials, he said, "really hope our Chinese brand could be used in hospitals." By 2017, Direct Genomics would raise 200 million yuan ($30 million) from investors and 40 million yuan in subsidies from the Peacock Program.

'I KNEW WHERE HE WAS HEADING'

Although He failed to wow the high-powered attendees at the Berkeley workshop, he was bold about asking for advice. That winter He and DeWitt communicated several times, with He asking about the best way to analyze edited genomes for unintended, off-target alterations.

But now something new entered the discussions. He told DeWitt he was planning to start pregnancies with CRISPR'd human embryos. DeWitt was aghast, he told STAT, and argued that there was no justification for such an experiment. The technology simply wasn't ready to use on babies-to-be.

The Berkeley workshop opened another door for He. He struck up a friendship with Hurlbut, and over the last two years had "several long conversations, like four or five hours long, about science and ethics," Hurlbut said. He went to the Bay Area with some frequency, and made his goal clear.

"I knew where he was heading," Hurlbut said. "I tried to give him a sense of the practical and moral implications," including ethical objections to research on human embryos. He pushed back; wasn't it only a fringe group in the U.S. that adamantly opposes that?, he asked; if CRISPR can be used to prevent a dreaded genetic disorder in a baby who would otherwise inherit it, why should we hold a one-cell embryo in the same ethical regard as a suffering child?

"My overall feeling," Hurlbut said, "was that he's a well-meaning person who wants his efforts to count for good."

In July 2017 He gave an updated version of his Berkeley talk, at a meeting on "Genome Engineering: The CRISPR-Cas Revolution" at Cold Spring Harbor Laboratory on New York's Long Island. The data from his experiments in mouse, monkey, and human embryos included ways to improve CRISPR's efficiency and measurements of its accuracy. He had injected CRISPR into the first human embryo, he said, on Nov. 10, 2016, doing two or three each month (though four that December). He reminded his audience of the many ways embryo editing could fail, including off-target edits and mosaicism (when only some of an organism's cells are edited, creating a genetic patchwork with unknown implications for health).

"My overall feeling was that he's a well-meaning person who wants his efforts to count for good."

WILLIAM HURLBUT, STANFORD BIOETHICIST

Continuing in a cautionary vein, He concluded with the tragic case of Jesse Gelsinger, whose 1999 death in a gene therapy clinical trial set that field back years. That, He said, should be a warning to anyone hoping to turn CRISPR into a tool of medicine.

As at Berkeley, the talk left scientists unimpressed. "It just didn't stand out," said Doudna, who co-organized the meeting.

Viewing He as less than a heavy hitter in the genome-editing world, many skipped his talk. Computational biologist Max Haeussler of UC Santa Cruz, who shared a double room with He and did attend, is struck in retrospect at He's discussing how dangerous editing human embryos is. "I found this remark already strange back then," he said. "Everyone in the room knew that it's out of the question to edit human embryos. Why mention that it's dangerous?"

In the room they shared, He and Haeussler talked shop, including how to detect off-target edits and how deeply He probed to find any. One red flag was that He was doing what's called "short read sequencing," meaning he sequenced short segments of DNA. That can miss big rearrangements along a chromosome. It's similar to proofreading a document a sentence or two at a time. You'll find garbles like, "Once time upon," but not grossly out-of-place passages such as Cinderella losing her glass slipper before she goes to the prince's ball. Later, in Hong Kong, He would say that large scrambles like that are not a big problem if there is no important gene nearby.

He did not breathe a word of any plans to establish CRISPR pregnancies. That was about to change.

SHOCKING SECRET BEGINS TO SLIP OUT

Two months after the Cold Spring Harbor meeting, Berkeley's DeWitt had received a stunning email from He: The Chinese scientist planned to conduct a clinical trial creating the first genome-edited children, the soon-to-be "CRISPR babies." He was enrolling patients, He told DeWitt, and had received ethics committee approval.

Yet in apparent violation of Chinese regulations, He did not list the trial on China's official trials registry until Nov. 8, 2018, which is thought to be the birthdate of Nana and Lulu.

DeWitt didn't know what to do with this information. Since He asked for confidentiality, "I let it be," DeWitt said.

He was back in Berkeley in January of this year, meeting DeWitt for dinner and reporting that his trial was on track. Since Nana and Lulu were born in the second week of November, He was about a month away from starting that pregnancy. DeWitt again tried to dissuade him, he said.

The number of those in the know was growing. Also in January, He emailed Dr. Matthew Porteus of Stanford, whom he had met at the Berkeley workshop a year before and who is trying to develop CRISPR into a treatment for blood diseases such as sickle cell (in children and adults, not embryos). In contrast to embryo editing, which alters heritable DNA and is passed on to descendants, this kind of "somatic genome editing" is much less controversial: It alters only targeted cells, making changes that go no further than the patient.

He asked if Porteus could meet the following month. One evening in February, Porteus ushered He into his Stanford office. The Chinese scientist began describing his experiments using CRISPR to edit monkey embryos, mentioning that he'd tried to start pregnancies but without success. No matter, He said: He had ethics-board approval for a clinical trial and was planning to move forward.

Porteus was blindsided and angry, but also perplexed: he knew He was in frequent communication with his Stanford colleague Hurlbut, and was shocked that those conversations apparently had no effect. "I strongly rebuked him for even considering this," Porteus recalled, adding that he told He to stop an experiment that could threaten the whole field of genome editing for disease treatment and prevention.

"He was being reckless," Porteus said. "He at least needed to speak to Chinese authorities in a formal way."

Porteus was especially concerned about the gene He intended to edit: CCR5. It makes a protein that acts as a portal by which HIV, the AIDS virus, enters cells. The medical rationale for trying to disable CCR5 was insufficient to edit it in an embryo, Porteus told He; to do so would threaten clinical use of CRISPR. Porteus also argued that He's experiment didn't come close to meeting the requirements for germline editing set out by a 2017 National Academies of Science report, such as rigorous assurance of safety and lengthy testing on lab animals. Later, He cited the report as a rationale for moving forward with the pregnancies.

"He didn't even acknowledge what he was doing might be wrong," Porteus said. "I think he was looking for someone to tell him it was a great thing to do."

Porteus thought he had dissuaded He, and didn't hear from him again until the eve of the Hong Kong summit. But if Nana and Lulu were born the second week of November, the pregnancy was just underway, or about to be, as He and Porteus sat talking.

It would later emerge, initially from an Associated Press story and later from He's summit talk, that he and his colleagues had enrolled eight couples in which the man is HIV-positive and the woman is not. After injecting a single sperm into an egg, the scientists injected their CRISPR molecules: a guide RNA that bloodhounded its way to CCR5 plus an enzyme that slashed the gene. In all, they injected 31 embryos, succeeding with 21. Analyses of cells when the embryos were 3 to 5 days old revealed a mishmash of edits. None replicated the CCR5 mutation known to protect against HIV. With the parents' permission (He said they understood the genetic niceties), He implanted 11 of them anyway.

He also spoke to Stanford's Hurlbut that February, but did not tell him explicitly that he had started a pregnancy. Hurlbut nevertheless suspected that was He's plan, and is puzzled about why He told others but not him.

"I was advising him a very gentle sort of way because I wanted to keep the conversations going," Hurlbut said. "But I told him, JK, you need to be careful. You have a bright future. You have a new baby. You could be humiliated by this" — where "this" meant an experiment that would bring the wrath of the scientific community, and possibly Chinese officials, down on him.

Like Porteus, Hurlbut wondered about targeting CCR5. Because AIDS is preventable and treatable, many scholars argue, it does not meet the threshold of "serious, unmet medical need" that would justify embryo editing. Only diseases like Huntington's, Tay-Sachs, cystic fibrosis, and perhaps familial Alzheimer's clear that bar.

He disagreed. He had been deeply moved by a visit he made to an "AIDS village" in China, with HIV-positive rates of 30 percent. In his country, he told Hurlbut, AIDS patients face enormous stigma and prejudice, struggling to find jobs, spouses, even housing. If someone with HIV wants to spare his or her child from the same misery, and if CRISPR'ing CCR5 can turn it into a form that blocks HIV infection, why is that any less justified than editing the Huntington's gene?

"He wants to help people," Hurlbut recalled of those many conversations. "He has [two] children of his own and is sensitive to the meaning of human life. He wants it to be healthy and happy. He kept stressing to me the problems that people with HIV have in China."

In addition to what Hurlbut calls "a very earnest motivation to move the science forward," something else was driving He. In more and more of his conversations, he was bringing up Edwards, the test tube baby doctor. People who spoke to He this year recall him as "grandiose," convinced he was about to accomplish something for the history books.

In describing his planned experiment to the ethics committee of the hospital that eventually approved it, He promised, "The project will stand out in the increasingly intense international competition of gene editing technologies," according to a translation by Jing-Bao Nie, a bioethicist at New Zealand's University of Otago.

"This creative research will be more significant than the IVF technique which won the 2010 Nobel Prize, and bring about the dawn of the cure for numberless genetic diseases."

ON THE STAGE IN HONG KONG: 'SOMETHING DIDN'T LOOK RIGHT'

The organizers of the Hong Kong summit knew nothing of the preparations for the PR onslaught. As they assembled their list of speakers, He wasn't on it.

By springtime, He and others in his lab were presenting more of their work on embryo editing, again without mentioning pregnancies, to scientific conferences. At a genome-editing meeting in Suzhou organized by Cold Spring Harbor's Asia unit, graduate student Feifei Cheng reported research using CRISPR to edit the heart-disease gene PCSK9 in human embryos. Again, it attracted little notice.

At their last planning meeting, in October in Santa Monica, Calif., the organizers of the Hong Kong summit were finalizing their list of invited speakers. They were concerned that few were from Asia, said committee member Robin Lovell-Badge of London's Francis Crick Institute. He's name came up the first evening, over pre-dinner drinks. "The context was the rumor that had been going around for a few months that He had received local ethical committee approval" to start pregnancies with genome-edited embryos, Lovell-Badge said.

When the committee began formal discussions the next day, Lovell-Badge raised the possibility of inviting He to speak in the session on embryo editing. "It was well-known that he had been conducting relevant research using mice, non-human primates and ... [human] embryos in culture," Lovell-Badge said.

The committee discussed the quality of He's research and his plans to start pregnancies. "We all thought this was far too premature," Lovell-Badge said, but "it was felt that the summit was an opportunity to bring him into line": that immersing He in discussions of ethics and safety for three days "would curtail any plans he had" for pregnancies.

He accepted the invitation immediately. Lovell-Badge received He's slides on Oct. 31. They expounded such ethical principles as "mercy for the needy," genome editing "only for disease, not for vanity," and "everyone deserves freedom from genetic disease." There was nothing about pregnancies.

The organizers, flying to Hong Kong from three continents, trickled into Le Méridien on Telegraph Bay on the weekend before the summit. By Saturday, news of the "CRISPR babies" was filtering out: A reporter at MIT's Technology Review found the listing that He had entered in China's clinical trials registry, explaining his plan to start pregnancies with CRISPR'd IVF embryos. The magazine's story on He's plans — but not the births — ran on the evening of Nov. 25 in the U.S., just after 8 a.m. Monday in Hong Kong. About two hours later, the Associated Press, which had interviewed and filmed He for months after being tipped off by He's public relations adviser in April to the pregnancy, scrambled to run its story, including news of the births. He arrived at the hotel on Monday, after an hour-plus drive from Shenzhen with a colleague.

After dashing across town for a 3:30 press conference, where they mostly begged off on commenting about He's unverified claim, several of the organizers made the return trip to the hotel — and, for some of them, dinner with He in the hotel's Nam Fong restaurant.

"That," said bioethicist and organizing committee member Alta Charo of the University of Wisconsin, "was an interesting dinner."

Over the Cantonese buffet, Charo fired questions at He, focusing on the bioethics of his experiment: how he recruited families, what he told them, why he chose CCR5. He opened his laptop and showed a spreadsheet, but it was in Chinese, leaving the scientists hardly better informed about key details. Asked why he had kept his experiment a secret, He said he had presented his work at science meetings. But he had never said he was going to establish pregnancies, and seemed not to understand why that was such a great leap beyond the embryo experiments he did talk about.

He also spoke of Edwards, the test tube baby pioneer, with starry-eyed admiration. "He seemed to think that what he had done would vault him into the scientific pantheon, too," Charo said. "He was just oblivious" to the fact that scientists and others around the world greeted his announcement with horror. But he also feared for his safety, showing his dinner companions what he called a threatening message on his phone, and worried that reporters would find him. Nervous and scared, He stormed off.

The organizers were left wondering, would He show up for his summit talk two days later?

The 500-plus people packing the standing-room-only auditorium at Hong Kong University on Wednesday wondered the same thing. Security men with earpieces crisscrossed the front of the room. Camera crews crowded the periphery. When Lovell-Badge introduced He, there were several anxious seconds before he emerged through a side door and took the stage.

"He was just oblivious."

ALTA CHARO, BIOETHICIST AND LEGAL SCHOLAR, UNIVERSITY OF WISCONSIN

He whipped through his 59 slides so quickly that it was only later, when they had a chance to scrutinize them, that scientists grasped how flawed the research was; He had introduced mutations into the girls with unknown consequences and that might not even protect against HIV.

But even amid the rush of data, audience members were growing more and more appalled at what they saw as sloppiness.

"I remember staring at the chromatogram," said Kathy Niakan, a biologist at the Francis Crick Institute, referring to a graph showing the prevalence of different DNA sequences. "I remember not seeing what he was claiming had happened. Something didn't look right."

After the presentation, Porteus and Lovell-Badge joined He on stage for an awkward Q&A. Porteus aimed to get specific answers to simple questions: How many embryos have you tried to modify? How accurate were the modifications? But He was cagey. Only after Porteus, who looked as if he wanted to be anywhere but there, circled back did He acknowledge a second, very early pregnancy.

As audience members pressed He on the ethical and regulatory scrutiny of his trial, He never acknowledged that what he did might have been wrong. "For this particular case," He said, "I feel proud."

THE FALLOUT: PRAISE, THEN CONDEMNATION

At first, He got the plaudits he expected. In China, the initial reaction was laudatory, emphasizing that this scientific first belonged to the Middle Kingdom. The government-backed People's Daily treated He's experiment as a "milestone accomplishment China has achieved," Otago's Nie wrote.

That attitude quickly flipped. The People's Daily story was almost immediately pulled down. More than 100 Chinese scientists issued an open letter condemning He's experiment. By Thursday, Chinese officials said they had halted the research and were investigating. Scientists called for independent verification of He's (still unproved) claims.

The reverberations quickly traveled more than 8,000 miles to the leafy campus of Rice in Houston. Deem, the AP had reported, was deeply involved in He's experiment, including being present when parents gave consent to have their embryos CRISPR'd. Deem is also a co-author on a paper He tried to publish that described additional CRISPR experiments in early embryos, but without establishing a pregnancy.

That Monday morning, Deem was accompanied into his students' office area by Yousif Shamoo, Rice's vice provost for research, according to someone with direct knowledge of the events. They told his graduate students and postdocs to turn over their files and research records as part of a university investigation into Deem's role in the project.

Although people at Rice and former students of Deem knew he had some research collaborations in China (Deem and He had co-authored at least three papers since 2016), his graduate students and fellows were shocked when they read about Deem's involvement in He's experiment. For one thing, in his lab at Rice Deem does not even do "wet biology," meaning experiments with living cells or biochemicals, let alone clinical research. The school said it had no knowledge of the work.

In a statement last week, attorneys for Deem said, "Michael does not do human research, and he did not do human research on this project." They did not respond to questions about what AP reported.

Scientists and others who have spoken to or emailed He, who has been on leave from his university since February, say he remains upbeat. He expresses confidence he will be vindicated both by the official investigations and by history.

History's verdict remains to be written. But no one believes CRISPR science has seen its last bombshell, and not only because of the second pregnancy He said is underway. Even those who condemn his experiment doubt it will be more than a speed bump on the road to editing of embryos to prevent severe inherited diseases. "We have to acknowledge there is interest in using [CRISPR] clinically," Doudna said. To those calling for a moratorium or an outright ban on such research, she has one response: "It's too late."

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UP NEXT

First CRISPR clinical trial backed by U.S. companies launches

By Sharon Begley

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BIOTECH

First CRISPR clinical trial backed by U.S. companies launches

By Sharon Begley @SXBEGLE | AUGUST 31, 2018

The first clinical trial of CRISPR-Cas9 sponsored by U.S. companies has launched, testing the genome-editing technique in patients with the blood disorder beta thalassemia, according to an announcement posted Friday on the U.S. clinical trials website.

The Phase 1/2 clinical trial, co-sponsored by Vertex Pharmaceuticals and using an experimental treatment from CRISPR Therapeutics, will be conducted at a single hospital in Regensburg, Germany, and aims to recruit up to 12 adults with the inherited disease. Although it was only a matter of time before the start of the first company-sponsored CRISPR clinical trial, Editas Medicine's experimental treatment for a rare form of blindness was widely expected to be the first in the clinic.

The gene-editing therapy that will be tested, called CTX001, is intended to treat both beta thalassemia and sickle cell disease.

Beta thalassemia is caused by a mutation in the HBB gene that reduces the amount of the oxygen-carrying blood protein hemoglobin a patient is able to produce.

Rather than correcting the disease-causing mutation in gene directly, the therapy targets a region of DNA that acts like a brake on production of a form of hemoglobin that the body usually stops making after the first months of life, called fetal hemoglobin.

In the rare cases when a genetic variant keeps production turned on well into childhood and even adulthood, even people with β-thalassemia (or sickle cell) have enough healthy hemoglobin to avoid the worst symptoms of those sometimes-fatal diseases.

The CRISPR therapy being tested is called ex vivo, meaning that blood cells are taken from a patient and altered in the lab (much like CAR-T cancer therapy) and then returned to the patient. In this case, CRISPR molecules are introduced to the blood cells via electroporation, and the genome editor alters the fetal hemoglobin "brake." If all goes well, the CRISPR'd cells will then produce red blood cells that contain fetal hemoglobin.

"This is one important step of many toward bringing the promise of this new technology to patients with serious diseases like sickle cell and beta thalassemia, and we are thrilled to be at the forefront of what we believe may be a fundamental change in the treatment of disease," Vertex spokesperson Heather Nichols said.

In May, CRISPR Therapeutics and Vertex announced that the Food and Drug Administration had placed a "clinical hold" on the companies' application to test CTX001 in sickle cell in a U.S. study, but that the planned Phase 1/2 trial in Europe in adults with β-thalassemia was on track. That is the trial that is now recruiting patients.

A spokesperson for CRISPR Therapeutics did not immediately respond to a request for comment.

A handful of CRISPR studies are underway at research centers in China. A trial led by researchers at the University of Pennsylvania is testing the use of the gene-editing technology in some cancers.