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THE CRISPR REVOLUTION

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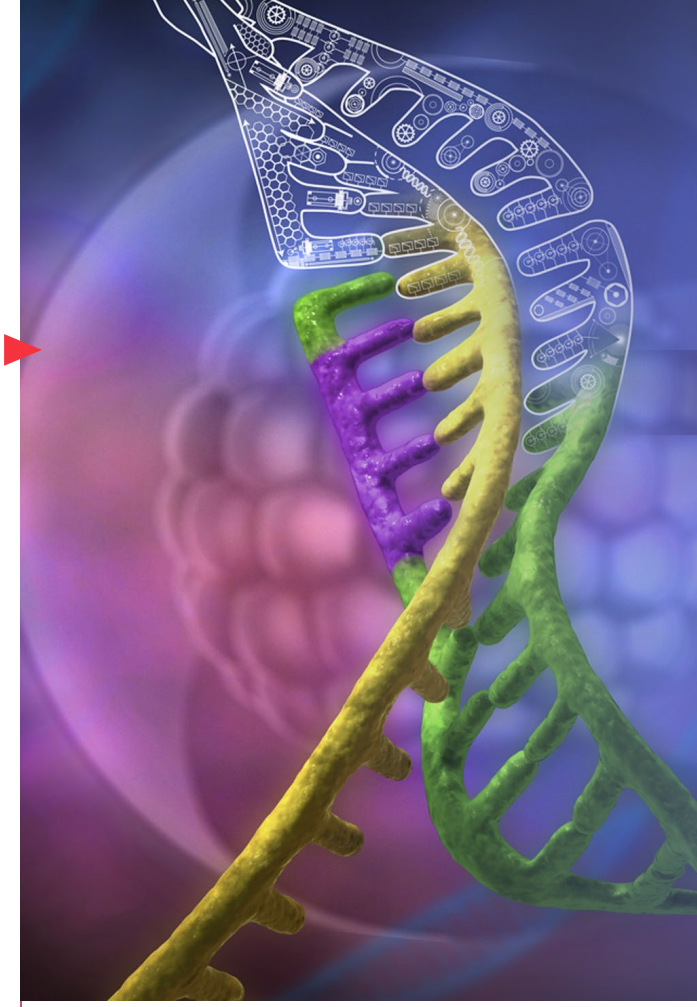

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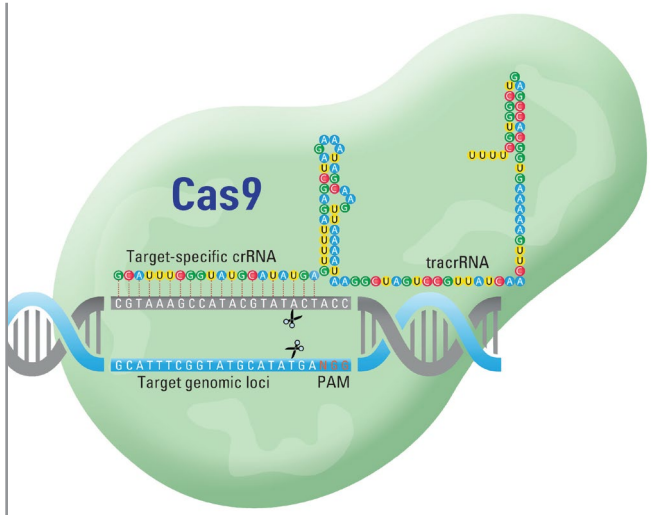
A CRISPR-Cas9 targeted double-strand break. Cleavage occurs on both strands, 3 bp upstream of the NGG proto-spacer adjacent motif (PAM) sequence on the 3' end of the target sequence.



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Part of the Cambridge Healthtech Institute Media Group, Bio-IT World provides outstanding coverage of cutting-edge trends and technologies that impact the management and analysis of life sciences data, including next-generation sequencing, drug discovery, predictive and systems biology, informatics tools, clinical trials, and personalized medicine. Through a variety of sources including, **Bio-ITWorld.com,** Weekly Update Newsletter and the Bio-IT World News Bulletins, Bio-IT World is a leading source of news and opinion on technology and strategic innovation in the life sciences, including drug discovery and development.

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Aaron Krol

> "The system is so general, so powerful, and so easy to use that it's quickly becoming a basic part of the molecular biologist's toolkit."

The CRISPR Revolution

Not too long ago, I was attending a conference in Boston where a stem cell researcher was discussing a set of genes that were very important to his cells of interest. Somewhere in the middle of his presentation, he pulled up the next slide and paused. "This slide used to talk about our problems editing these genes in our cells," he said (or words to that effect). "Of course, that was before CRISPR…"

Some scientific discoveries knock down so many barriers, so fast, that yesterday's problems suddenly look quaint. So it is with CRISPR, the gene editing technology discovered by Jennifer Doudna and Emmanuelle Charpentier in late 2012, and since adapted to dozens of organisms and untold numbers of experiments. A natural defense mechanism used by bacteria to fight off viruses, CRISPR seeks out particular, short sequences of DNA, and cuts them right out of a living cell's genome. The system is so general, so powerful, and so easy to use that it's quickly becoming a basic part of the molecular biologist's toolkit, even in labs that never dreamed they'd have the resources to go through the once-complicated process of gene editing.

In a little over two years, CRISPR has spawned huge online libraries of plasmids and RNA sequences to help get new users started; been turned into an experimental antibiotic; been modified to add, replace, and reversibly knock down DNA sequences in live cells; inspired three different companies hoping to create drugs for previously incurable diseases; and become the subject of a high-stakes patent dispute.

In short, it's been a busy time for the groundbreaking technology. To help catch you up, here are Bio-IT World's biggest CRISPR stories of the past two years.

Aaron Krol
Senior Science Writer, Bio-IT World and Clinical Informatics News

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CRISPR Knockin Mice Enable RAPID GENE EDITING

By Bio-IT World Staff | September 26, 2014

Researchers at the Broad Institute of MIT and Harvard have created a line of mice whose cells naturally express the Cas9 protein used in CRISPR gene editing. These mice can be used for virtually any gene knockdown experiment, allowing researchers to quickly and easily alter the expression of one or more regions of the genome at any stage in the mouse's life.

The Cas9 protein, when directed by a guide RNA molecule to a matching stretch of DNA, cuts that DNA directly from the chromosome, making this CRISPR method by far the most flexible tool for gene editing yet discovered. (CRISPR is named for Clustered Regularly Interspersed Short Palindromic Repeats, a kind of signature DNA sequence associated with Cas9 that, in bacteria, encodes the guide RNA.) Until now, researchers using CRISPR have created complexes that include both Cas9 and guide RNA, delivering these to cells in order to switch off or modify specific genes. However, with the new Cas9-expressing mice, only the guide RNA has to be introduced to change the native genome, an easier proposition well within the capabilities of most biology labs.

The Broad Institute team, supervised by CRISPR pioneer Feng Zhang and his colleague Phillip Sharp, also demonstrated the power of their knockin mice by inducing lung cancer-related mutations. Using an adeno-associated virus as a vector for guide RNA sequences, the team simultaneously altered the KRAS, p53 and LKB1 genes, which resulted in adenocarcinoma in the mice. The work is published in this week's Cell.

CRISPR knockin mice have already been shared with more than a dozen institutions, and are available for purchase through the Jackson Laboratory, a popular national resource for genetically modified animal models.

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Could CRISPR TRANSFORM WILD ECOSYSTEMS?

By Bio-IT World Staff | July 18, 2014

In a paper published yesterday in the open access journal eLife, George Church and colleagues discuss the potential for "gene drives" that alter the genomes of whole wild populations using CRISPR gene editing technology, and weigh the risks and public interests surrounding such initiatives. Church's lab at the Wyss Institute for Biologically Inspired Engineering at Harvard Medical School has been involved in the development of CRISPR since it first took off as a gene editing tool two years ago.

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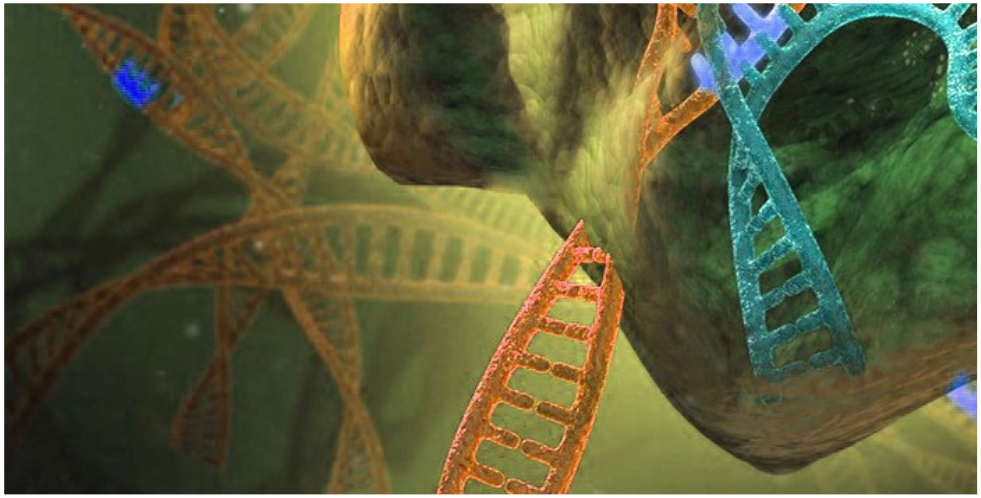
A GENE DRIVE TAKES ADVANTAGE OF MECHANISMS OF BIASED INHERITANCE, IN WHICH A GENETIC ELEMENT IS FAVORED TO BE PASSED ON TO THE NEXT GENERATION EVEN IF IT CONFERS NO ADAPTIVE ADVANTAGE, OR EVEN HARMS THE HOST ORGANISM.



Gene drives have been imagined and even attempted for over a decade, traditionally with disease-carrying species of mosquito as their targets. A gene drive takes advantage of mechanisms of biased inheritance, in which a genetic element is favored to be passed on to the next generation even if it confers no adaptive advantage, or even harms the host organism. Such genetic elements could include endonucleases, which seek out a target region of a chromosome, cut out the native sequence, and copy themselves in its place; or segregation distorters, which destroy entire homologous chromosomes during cell division, leaving only their own copies intact. Although no gene drive has yet advanced beyond the lab, a transgenic swarm of mosquitoes without a gene drive was released in Brazil earlier this year as a control measure against dengue fever.

In the eLife paper, "Concerning RNA-guided gene drives for the alteration of wild populations," Church and his colleagues note that CRISPR will make it much easier for a far wider variety of labs to engineer future gene drives in any species they choose. CRISPR is more specific, more durable after multiple replications, and easier to target to a chosen site on the genome than any previous gene editing technique. Writing that "we hope to initiate transparent, inclusive, and well-informed discussions concerning the responsible evaluation and application of these nascent technologies," the authors consider in detail how multiple obstacles to a CRISPR-based gene drive could be addressed. They also float a number of real-world applications, including the control of disease vectors, elimination of invasive species, and agricultural pest control.

The authors further note that a successful gene drive, once released into the wild, would be difficult to control and would not respect any artificial boundaries like political borders in spreading through a species. They make certain broad suggestions for responsible conduct of gene drives, including education and engagement of the public. "[A]ll decisions involving the use of suppression drives must involve extensive deliberations including but not limited to ecologists and citizens of potentially affected communities," they write.

More specifically, the authors list certain precautions that any lab considering a gene drive could take to make the effects as precise and reversible as possible. They propose field trials with small populations that contain any desired genetic changes, but not the gene drives to favorably spread them, in order to observe the ecological impacts of the modified organisms. They also insist that after a gene drive is released, wild samples should be captured and sequenced periodically to monitor how the relevant genes are dispersing through the population, and "recommend that all laboratories seeking to build standard gene drives capable of spreading through wild populations simultaneously create reversal drives able to restore the original phenotype."

"These precautions," they add, "would allow the effects of an accidental release to be swiftly if partially counteracted."

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ADVANCES IN GENETIC ENGINEERING

at the 2014 American Society for Microbiology Meeting

By Bio-IT World Staff | May 22, 2014

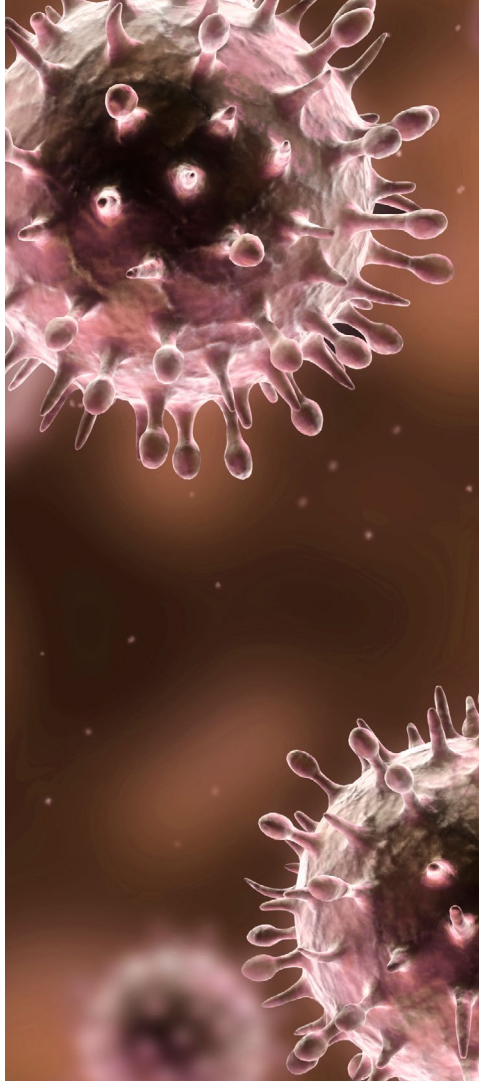
The annual meeting of the American Society for Microbiology (ASM) came to a close this Tuesday after three days of scientific sessions in Boston. Presentations at the event showed that rapid advances in the methods and reach of genetic engineering, which have been felt across the life sciences in the past year, are causing a particular stir in microbiology labs. Bacteria, with their small, nucleus-free genomes and open trade in plasmids, have traditionally been a first port of call for gene editing, and the talks at ASM provide an early look at the latest developments in the field.

The technology making the biggest impact this year has been CRISPR, which over a matter of months in late 2012 and early 2013 went from being a curiosity of the bacterial immune system to the most promising and adaptable new tool in the genetic engineer's toolkit. The CRISPR system has allowed scientists to combine "guide RNA" sequences that match a specific stretch of DNA in a target genome, with a Cas9 protein that cuts both strands of DNA at that locus. This offers an unprecedented ability to easily remove, add, or modify selected sequences of DNA. In his ASM presentation on Tuesday, Stanley Qi of the UCSF Center for Systems and Synthetic Biology said simply, "Cas9 is magical."

Qi had come to ASM to present a re-engineered version of the Cas9 protein in which the nuclease, the domain that cuts DNA, has been made inactive. This "nuclease-deficient Cas9," or dCas9, still binds to the genome at the targeted site, but instead of cleaving the DNA, it blocks transcription of the gene where it sits. Like RNA interference, or RNAi, this "CRISPRi" system can be used to silence genes one by one.

CRISPRi can also be made reversible, by combining dCas9 with an inducible promoter. In the presence of an inducer, the target genes are silenced, while removing the inducer restores partial gene transcription. Qi also suggested that dCas9 could be useful for labeling DNA, if combined with fluorescent proteins, or for modifying the genome at specific loci without carving out the native sequence.

Qi shared one application of CRISPRi, an extensive partial knockdown study of essential genes in Bacillus subtilis. By exposing B. subtilis to different combinations of CRISPRi and 35 different antibiotics, Qi's lab was able to identify genes that contribute to antibiotic resistance. CRISPRi is useful for this kind of project because it is relatively simple to achieve partial suppression of essential genes, which does not kill the bacterium outright but may increase susceptibility to certain antibiotics, and because guide RNAs can be engineered very rapidly to target dCas9 to different genes. Qi and his colleagues even extended the project to knockdown combinations, in which pairs of genes were suppressed together, a reflection of how quickly and easily CRISPRi **can be scaled up.**



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Stephanie Yaung, of George Church's lab at the Wyss Institute for Biologically Inspired Engineering, demonstrated a newly-discovered property of CRISPR that further distinguishes it from other gene editing methods like zinc finger nucleases or TALEN. All these technologies can be targeted to specific sequences in the genome, but most can only be guided to the four traditional, unmodified DNA bases. Methylated bases have proven a greater challenge to target.

To see if the same was true of CRISPR, Yaung and her colleagues tried to infect CRISPR-protected strains of E. coli with bacteriophages whose genetic payloads included methylated bases, including 5-hmC. Although the CRISPR defense systems specifically guided Cas9 to viral genes that included the methylated bases, Cas9 was still able to cut the viral genomes at the targeted sites. This suggests that modified bases will not deter Cas9, a finding with important implications for gene editing in mammalian cells, which tend to be much more heavily methylated than bacteria.

DAUNTING CHALLENGES

Gene editing may not be the only field where CRISPR eventually becomes a widespread technology. Mark Mimee, from Timothy Lu's Synthetic Biology Group at MIT, described the early stages of an intriguing effort to use CRISPR as a narrow-spectrum antibiotic. The idea makes a certain amount of intuitive sense: in nature, bacteria use CRISPR as an antiviral, and the ability to direct Cas9 to specific DNA sequences suggests it could be highly discriminating in attacking only selected pathogens.

The prospect is also highly appealing, given the scientific community's growing alarm at the problem of antibiotic resistance, and calls for new narrow-spectrum drugs. Said Mimee, "[we are] asking the question, can we make a programmable-spectrum antimicrobial?... Can we get a list of antibiotic resistance genes, of virulence genes, of genes we consider bad, and make an antimicrobial to selectively find these cells, kill these, and spare all other cells in a population?"

In initial experiments, Lu's lab has chosen antibiotic resistance genes in E. coli as targets, and built guide RNAs to direct Cas9 to these genes. Using phagemids to deliver the CRISPR system to mixed populations of E. coli, the group has found that it can drastically reduce the prevalence of only those strains carrying the chosen antibiotic resistance genes. The system used is not modified as an antibiotic; rather, the presence of the CRISPR system itself seems to cause cell death, an effect the group hypothesizes is due to native toxin-antitoxin systems in the bacterial genome that are destabilized by Cas9 activity. Mimee observed that the team was even able to kill E. coli when targeting genes located on plasmids, rather than the bacterial chromosome, and could also build CRISPR systems targeting two antibiotic resistance genes simultaneously.

The system is not a fully-functional antimicrobial – the team was never able to destroy the targeted populations entirely, and in the first in vivo experiments, on infected waxworms, an intervention with CRISPR-carrying phagemids only improved survival by 30%. Nevertheless, the notion of a "programmable-spectrum" antimicrobial is an exciting one, and one can hope that further experiments may increase the lethality of CRISPR without diminishing its specificity.

George Church was also present at ASM, to deliver one of his trademark whirlwind tours of cutting edge work among his wide circle of collaborators. On Tuesday, in a talk titled "Synthetic Ecologies," his subject was the wholesale "re-coding" of E. coli genomes, removing entire basic elements of their genetic dictionaries.

Church, along with a variety of collaborators prominently including Farren Isaacs of Yale and Marc Lajoie at Harvard, has been working since at least 2011 to strip entire codons from a bacterial genome. This work began with a project to replace every TAG codon in E. coli with the synonymous TAA codon. TAG was chosen because it is the rarest codon in the organism's genome, occurring in 314 locations, and because as a stop codon it may have been less essential to the cell than an amino acid-encoding sequence. The feat was achieved by inserting fragments that included TAG>TAA changes, 10 at a time, into 32 separate populations of E. coli. These populations were then paired up to exchange DNA, resulting in 16 populations with 20 changes each, and the process was repeated until all 314 changes coexisted in a single strain. As Church pointed out, this method of genetic engineering is so fast that the major time constraint is not how quickly new strains can be produced, but how quickly the researchers can check that their pairings have created the right genetic combinations. "We can make on the order of a billion genomes a day," said Church. "We can screen, in this case, 100,000 genomes a day, so the bottleneck is the screening."

This re-coding project was first published, short of completion, in 2011, but at ASM Church described major developments over the past year. The E. coli strain with no instances of the TAG codon is now complete and thriving. "It's fully viable, and it's resistant to two viruses that we've tested," said

IN NATURE, BACTERIA USE CRISPR AS AN ANTIVIRAL, AND THE ABILITY TO DIRECT CAS9 TO SPECIFIC DNA SEQUENCES SUGGESTS IT COULD BE HIGHLY DISCRIMINATING IN ATTACKING ONLY SELECTED PATHOGENS.

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Church, indicating one advantage of a genome-wide codon change. The incompatibility with natural organisms' genomes confers viral resistance, and prevents the exchange of genetic material with naturally occurring bacteria.

More radically, the group has gone on to reintroduce TAG codons into re-coded E. coli strains, but change their meaning. With no TAG stop codons, the organism no longer relies on release factor 1 (RF1) – the protein that recognizes TAG as a stop codon – to accurately transcribe its DNA into RNA. The group therefore stripped RF1 from the bacterium, and instead introduced protein-tRNA complexes that read TAG as coding for two new amino acids that do not occur naturally. The new amino acids, p-azidophenylalanine and 2-naphthalalanine, were plugged into a gene for green fluorescent protein, which the organism successfully translated. The hope is to eventually use similar organisms to create useful proteins that could not be built using the natural repertoire of 20 amino acids.

Church reported that work on new re-coded E. coli strains is progressing steadily, most ambitiously an effort to remove every instance of 13 rare codons simultaneously from 42 essential genes, which has been partially successful.

The scale of genetic engineering challenges being tackled by ASM participants is remarkable, and shows how rapidly basic research in this field is moving. Microbiology is often viewed as a testing ground for gene editing techniques, but with increasingly sophisticated synthetic organisms, and new leads on antimicrobial discovery, the editing of bacterial genomes is also looking increasingly important in its own right.`

BROAD CLAIMS PATENT ON CRISPR TECHNOLOGY

By Bio-IT World Staff | April 15, 2014

In April 2014, the U.S. Patent and Trademark Office issued the first patent to cover CRISPR-Cas9 gene editing, awarding the Broad Institute a patent for a version of the CRISPR-Cas9 system used to modify the DNA of mammalian cells.

The technology covered by this patent was first demonstrated in a January 2013 Science paper by senior author Feng Zhang, who has previously discussed his work on CRISPR-Cas9 with Bio-IT World for a feature on the gene therapy company Editas, of which Zhang is a founding member. While CRISPR-Cas9 exists in nature as a defense mechanism against viruses shared by many species of bacteria, the elaborate system of DNA-cutting proteins and guide RNA sequences requires extensive engineering to function in eukaryotic cells, and to insert new genes where the targeted host DNA is excised. These modifications are patentable under U.S. and international law.

In announcing the patent, the Broad Institute reemphasized its commitment to sharing insights and capabilities in CRISPR gene editing with scientists around the world. "Consistent with the Broad's mission to accelerate the understanding and treatment of disease, we are committed to empowering the global research community by making this technology broadly available to scientists for research around the world," said Institute Director Eric Lander in a press statement.

CRISPR has been cited as a very promising mechanism for curing genetic disease in humans, and has already been demonstrated in live mammals including cynomolgus monkeys and mice, in addition to its use in numerous basic research studies. The underlying chemistry of the Cas9 protein continues to be explored.

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Gene Therapy's NEXT GENERATION

By Aaron Krol | January 29, 2014

If you're in the business of drug discovery, you've got to be ready for the long haul. Even small startup companies trying to deliver new treatments will have to pour tens of millions of dollars into research and development over the course of years, only to run the risk of failing in clinical trials and being left with nothing to show for their efforts. One recent study published in Nature Biotechnology suggested that only around 10% of drug indications that enter clinical trials ever reach FDA clearance.

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So it takes a special kind of chutzpah to start a company around a type of treatment that has never met with FDA approval. Yet the founders of Editas Medicine, based in Cambridge, MA, are optimistic that now is the time to enter the business of gene therapy – almost a quarter century after the first clinical trials of gene therapy in the U.S. began, without producing a single commercial product to this day. Editas is betting that recent advances in the technology of genetic engineering have leapfrogged previous generations of therapies, opening a huge market niche for a company at the cutting edge of gene editing if it's game to try its hand at treating genetic diseases.

"That's what Editas allows us to do," says Feng Zhang, one of the company's academic founders. "We can go into the native genome, the natural DNA in the cell, and then make a modification in the genome to correct deleterious mutations." And they can do it with technologies that didn't exist when their competitors entered the gene therapy arena.

The classic paradigm of gene editing has been to use an engineered virus to deliver a healthy human gene to a patient with a deleterious mutation. The virus copies its own DNA – stripped of disease-causing material, and including the healthy gene – somewhere into the patient's cellular DNA, allowing the patient's cells to produce normal proteins. More recently, zinc finger nucleases (ZFNs) have been used to edit the genome with much greater precision. This technology incorporates Cys2His2 zinc fingers, a class of protein structures that bind to a specific short sequence of DNA. Combining pairs of these zinc fingers with an enzyme that cuts DNA allows researchers to choose a specific stretch of DNA to remove from the genome, and even add a second sequence to replace it. Zinc finger nucleases have the advantages of carving out disease-causing mutations, and being targeted to areas of the genome where inserting new DNA doesn't run the risk of disrupting genes that are already there; this is the gene editing technology driving companies like Sangamo Biosciences, which currently has two gene therapies in phase 2 clinical trials.

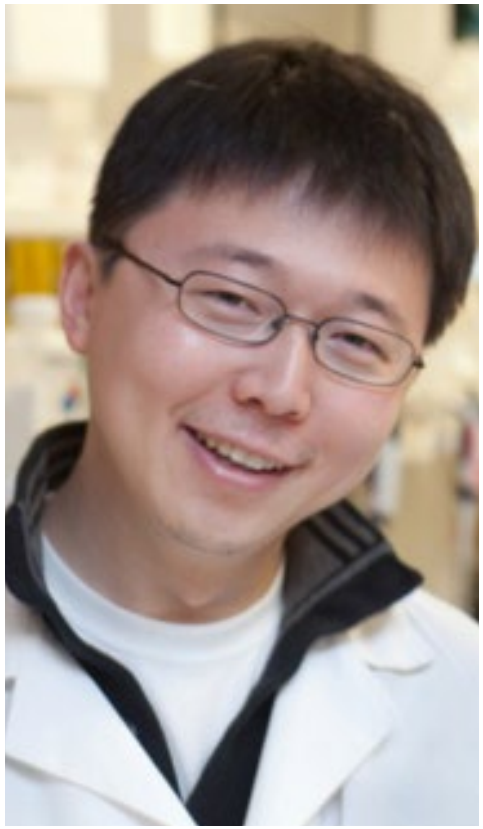
THE TECHNOLOGY

Zhang, whose academic lab is part of the Broad Institute of MIT and Harvard, has years of experience working with both ZFNs and the similar TALEN* system. But in early 2011, he read a paper that turned his attention instead to a quirky feature of certain bacterial genomes called CRISPR.

The paper, published in Nature under lead author Josiane Garneau, demonstrated how CRISPR functions as a defense mechanism against bacteriophages – the viruses that attack bacteria. CRISPR stands for "clustered, regularly interspaced short palindromic repeats," and it was first noticed as a peculiar pattern in bacterial DNA in the 1980s. A CRISPR sequence consists of a stretch of 20 to 50 non-coding base pairs that are nearly palindromic – reading the same forward and backward – followed by a "spacer" sequence of around 30 base pairs, followed by the same non-coding palindrome again, followed by a different spacer, and so on many times over.

Researchers in the field of bacterial immunology realized that the spacers were in fact short sequences taken from the DNA of bacteriophages, and that bacteria can add new spacers when infected with new viruses, gaining immunity from those viral strains. What Garneau and her colleagues showed was the mechanism that made the system work: the spacers are transcribed into short RNA sequences, which a protein called Cas9 uses to find the same sequences in invading

**TALEN stands for transcription activator-like effector nuclease. Like ZFN, TALEN combines an enzyme that cuts DNA with a protein that binds to specific DNA sequences. The same cleavage domains can be used in both systems, but TALEN's binding domain is a TAL effector, a protein that bacterial species in the Xanthomonas genus use to modify the genomes of the plants they parasitize.*



Feng Zhang, one of the founders of Editas and a professor of biomedical engineering at MIT.
Image credits: Editas Medicine

"WE CAN GO INTO THE NATIVE GENOME, THE NATURAL DNA IN THE CELL, AND THEN MAKE A MODIFICATION IN THE GENOME TO CORRECT DELETERIOUS MUTATIONS."

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viruses and cut the viral DNA at the targeted site.

"That was a pretty interesting paper, because it showed Cas9 will cut DNA," Feng Zhang told Bio-IT World. "And Cas9 uses short RNA sequences to be able to cut the DNA." Immediately, the system suggested a new method of gene editing: CRISPR-Cas9 complexes could be paired with RNA sequences that target any sites researchers were interested in cutting. The system also had some clear advantages over ZFN or TALEN. Because Cas9 does the cleaving on its own, and carves out an entire stretch of DNA instead of making a single cut, engineers wouldn't have to combine the system with a cleaving enzyme, or use paired proteins that bind to both ends of the locus they want to modify.

In the fall of 2012, a team including Jennifer Doudna and Emmanuel Charpentier went on to show that CRISPR's natural guiding system, which features two distinct types of RNA, could be replaced with a single sequence of artificially-produced guide RNA, or gRNA, without compromising its effectiveness. This opened up the possibility of rapid engineering, where only the gRNA sequence would have to be modified to target CRISPR to different areas of the genome. These gRNA fragments would be much easier to engineer than the binding agents of ZFN or TALEN, which use their own elaborate coding schemes to target DNA bases.

Finally, in January 2013, Zhang's lab published a paper in Science that hit the major benchmark for gene editing: they successfully used a CRISPR-Cas9 system to modify DNA in mammalian cells, both mouse and human. As a flourish, the group encoded multiple gRNA sequences into the same CRISPR array, and showed that Cas9 cleaved out all the relevant sites of the genome. "One advantage of CRISPR [is] you can use it to target multiple genes at the same time," says Zhang. "And that is actually something that really sets it apart from TALENs or zinc finger nuclease."

THE COMPANY

CRISPR was ready for use creating new strains of model organisms, or in crop engineering, or for any of a hundred important research applications. But from the beginning, the pioneers of CRISPR-Cas9 editing had higher ambitions for the system.

"I thought that maybe we could start a company to use CRISPR to treat genetic diseases," Zhang says. "And so I went to my boss here at the Broad Institute, Eric Lander, and I asked him if he would be interested in working on starting a company using the CRISPR technology." Lander recommended a few venture capital firms that specialized in the life sciences and might be willing to take a risk on a game-changing technology in gene therapy – a field that was inherently risky, haunted by past failures, and certainly years away from promising any return on investment.

Three firms – Polaris Partners, Flagship Ventures, and Third Rock Ventures – stepped to the plate, providing $43 million in startup capital in November of 2013 for a company the founding team decided to name Editas. "The type of investing that we do, and the type of company we're attracted to, is really big idea science and platform technology," says Kevin Bitterman, a principal at Polaris Partners who is now serving as the Interim President of Editas. "What we really get excited about are the Editas types of technologies: really transformational, broad platforms that can lead to a number of different exciting products."

Bitterman was part of the early discussions about who belonged in the company and what kinds of disease applications it could tackle. Through Zhang and colleagues at the Broad Institute, a lot of the biggest players in the early development of CRISPR – as well as TALEN, which will also play a role in the company's research and development – were approached to gauge their interest in tackling gene therapy with these new technologies. "We all decided pretty quickly that it made more sense to band together," Bitterman told Bio-IT World, "not to form a company in this space, but to form the company in this space."

The Editas team of academic founders includes

Kevin Bitterman, Interim President of Editas.

"WHAT WE REALLY GET EXCITED ABOUT ARE THE EDITAS TYPES OF TECHNOLOGIES: TRANSFORMATIONAL, BROAD PLATFORMS THAT CAN LEAD TO A NUMBER OF DIFFERENT EXCITING PRODUCTS."

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both Zhang and Jennifer Doudna, as well as Keith Joung of Massachusetts General Hospital, and George Church and David Liu out of Harvard. "They're really the heart and soul of this company," says Bitterman. "We're building this company around their science, so they're much more than advisors… [They're engaged] in helping think about the indications, in helping us recruit the appropriate people to the company."

"Many of the scientists that we have hired to get Editas off the ground are coming out of the founders' labs," he adds. "They're the folks that have gotten their hands dirty already with the technology." Editas is investing heavily in updating its lab space in Kendall Square, and bringing a strong in-house scientific team on board. The founders' academic labs will continue doing foundational work in making CRISPR-Cas9 ready for therapy, but all the drug development will be Editas' responsibility.

The company plans to hire 25 to 30 scientific staff in 2014. Their first hire, Morgan Maeder, came from founder Keith Joung's lab, where she worked with both ZFNs and TALENs, and was first author on a study using CRISPR to activate gene expression in human cells. "She's worked on all three platforms, and has been working in genome editing for many years as well," Joung told Bio-IT World. "So when the opportunity came up to join Editas, I think it was a good fit."

THE CHALLENGE

If there's one thing everyone wants to know about a new gene therapy company, it's which diseases they think they can cure. There are hundreds if not thousands of genetic diseases that might someday be amenable to gene therapy, most of them vanishingly rare. They affect different tissues, demanding different vectors for drug delivery. They can be caused by a single well-defined mutation, or by many barely-understood ones. For some conditions, the mutated genes will just have to be carved out, while for others they will have to be replaced.

Editas is still considering where its technology can do the most good. "The first thing that we look at is the unmet need," says Bitterman. "This is potentially such a transformational technology, it's less exciting for us to go after an area where there's already therapy and we can potentially make it slightly better… We want to go after areas where there's truly no other option."

But practical considerations can make or break any company whose ultimate goal is to get treatments through clinical trials. "We have to be able to deliver Cas9 into the disease-causing tissue," Zhang points out, "so we have to think about what kinds of delivery systems are available – whether it's viral, or whether it's chemical… The second criterion is, what kind of modifications can we achieve efficiently? The most efficient process using CRISPR right now is to knock out a gene. And that means for genetic mutations that have a dominant negative effect, we can use CRISPR to remove that dominant negative gene." That implies simple Mendelian diseases might be promising first targets for Editas, while polygenic conditions might be further down the road.

Bitterman says that Editas has a prioritized list of possible disease indications, but unsurprisingly, the company isn't yet ready to name any. There's a lot of groundwork to be laid before any specific therapeutics go into development. While Editas works to set up a private lab that can tackle therapies, many of the founders are working furiously in their academic labs to make CRISPR-Cas9 specific enough for safe use in humans.

Specificity is a huge concern in gene therapy. A clinical trial in 2003 became a frightening setback for the industry when four infants being treated for severe combined immunodeficiency (SCID) developed leukemia. The virus carrying the corrective gene had inserted its genetic payload into a known proto-oncogene, causing cancerous mutations. Since then, anyone working in gene therapy has been extremely cautious about where the cellular genome is being changed.

CRISPR-Cas9 seems like a promising vehicle for

Keith Joung, one of the founders of Editas and Associate Chief of Pathology for Research at Massachusetts General Hospital.

"… IT'S IMPORTANT TO GET THESE TOOLS INTO THE HANDS OF ACADEMIC RESEARCHERS WHO WANT TO USE THEM."

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very precise changes to the genome. The gRNA sequences have traditionally been 20 bases long – a convention borrowed from the bacteria that use the CRISPR-Cas9 system naturally – which in theory is more than enough for the targeted sequence to only occur once in the human genome. For comparison, zinc fingers need to be combined in groups of three just to get to nine bases of specificity.

But studies have shown that gRNAs can sometimes lead Cas9 to stretches of DNA that differ from their 20-base sequences by as many as five bases. These off-target hits could have dangerous effects on the cellular machinery, and need to be made vanishingly rare, if not eliminated altogether, before any therapy can go ahead.

This is exactly the sort of foundational science that the founders' labs can contribute to the Editas project, while also advancing the state of the art for CRISPR-Cas9 users around the world. Just this week, founder Keith Joung published a paper in Nature Biotechnology demonstrating that the specificity of CRISPR-Cas9 can be improved by shortening the gRNA sequences.

"It is totally counterintuitive," says Joung. "I think most people, including us originally, would think you should just make [the gRNA] longer," so that more mismatches need to occur before the gRNA will bind to the wrong area of the genome. Yet his lab found that truncated gRNA sequences of 17 or 18 bases, or tru-gRNA, were actually far better at reducing the number of off-target hits, without compromising the ability to hit the real target.

"We could see, in some cases, as much as five thousand-fold improvements or more in the specificity of a truncated guide RNA versus the full-length guide RNA," says Joung. His working hypothesis is that full-length gRNA has too much binding energy, and that force is enough to overcome a few mismatches. By reducing the number of matches, "we're reducing the binding energy in the system to a point where it's just sufficient now for activity… [making] it much more sensitive to any kind of mismatch at the guide RNA-DNA interface."

Discoveries like this move the CRISPR-Cas9 system forward, but they also underscore just how young the technology is. Other pushes to improve specificity include using paired nickases: modified Cas9 proteins that only cut one strand of DNA, so that in theory two nickases need to bind to opposite strands before the DNA will be cleaved. But as Joung points out, even measuring specificity is a process full of uncertainty. Current methods, like T7 endonuclease I assays, can only look at one region of the genome at a time, so researchers have to guess where off-target cuts are likely to take place; any unexpected off-target effects will pass undetected.

"What we need next is something that is lacking for all of the gene editing platforms," says Joung, "which is a method that is unbiased, genome-wide or global in cells, that says where [these off-targets are] occurring." In the meantime, even by the current, less sensitive measurements, neither tru-gRNA nor paired nickases are enough on their own to bring off-target effects down to zero.

Nevertheless, by gene editing standards CRISPR is moving forward at lightning speed. Less than a year after proving that CRISPR-Cas9 works in human cells, Feng Zhang's lab was able to produce a library of almost 65,000 gRNA sequences targeting over 18,000 human genes, for in vitro studies of human cells. These resources also fuel future research into CRISPR, as many of the founders take an open science approach to the system's development. Zhang's library of CRISPR plasmids is freely available on Addgene, while Joung has been contributing for years to the online resource ZiFiT, a program that helps researchers identify target sites for gene editing with ZFNs, TALENs, or CRISPR; he has already incorporated tru-gRNAs into the ZiFiT software.

"These are broadly applicable technologies," says Joung, "and I think it's important to get these tools into the hands of academic researchers who want to use them." Zhang agrees. "Something like CRISPR is a foundation tool," he says. "I think making sure these foundation tools are open is important… What my lab has done is try to make the information, the technical know-how, as well as the physical reagents, as accessible to everybody as possible."

This generous philosophy may seem at odds with building a competitive company, but everyone at Editas seems to agree that their edge has to rest on talent, not on crowding out the field. "Our sense is that this is an important and broad area," says Bitterman, "and there already are multiple companies working in this area. And we think that's a good thing. There's way too many diseases that this can tackle for one company to go after."

It's certainly true that there's more than enough unmet need to go around – if any company is ever able to break through the frozen regulatory environment for gene therapy. There have been some modest signs of a coming thaw, and the Editas team is hopeful that they've come along with the best new technology at just the right time for the markets to open up.

"Gene therapy is already used in the clinic," points out Zhang – just not in the U.S. "In Europe there's a drug called Glybera, which is a gene therapy product… Especially today, we're really benefiting from a lot of the hard work that people have done in the past decade."

"It's still a very, very new field," he adds, "so there's a lot of learning that everybody has to do, and I think a lot of thoughtful discussions need to happen to figure out what is the best way forward. But I think it will definitely change."

For now, that change is still being outpaced by the transformation in gene editing technologies in the lab. But with each new addition to the arsenal of genetic engineering, a new crop of companies rises up to see whether the right tool is finally available for the job. Given the promise of gene therapy to not just treat some of the world's most debilitating and intractable diseases, but potentially cure them, we can be grateful that there are still companies like Editas willing to bet that the finish line is finally in sight.

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IP Deals Shed Light on EDITAS MEDICINE'S STRATEGY for CRISPR Gene Therapies

By Aaron Krol | December 2, 2014

Although the CRISPR-Cas9 system was only discovered in the fall of 2012, it has already attracted three companies betting that this powerful gene editing tool can be turned to drug development. Editas Medicine and Intellia Therapeutics, both of Cambridge, Mass., and their Swiss counterpart CRISPR Therapeutics, are all exploring CRISPR-based biotech drugs for indications as varied as infectious disease, cancer, immune disorders, and rare genetic diseases.

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The Cas9 protein, first found in bacteria cutting up the genomes of invasive viruses, is in many ways the perfect tool for genetic engineering. The protein can be directed to almost any locus in any genome, where it cuts through both strands of DNA, and can be modified to add new genetic material to fill the gap. And unlike other systems like TALEN and zinc finger nuclease, CRISPR does not use its own elaborate molecular code to find its DNA target; it only requires a matching strand of RNA, making it easy to use and extremely flexible. (CRISPR stands for "clustered regularly interspaced short palindromic repeats," a description of the signature bacterial DNA sequences that first pointed scientists toward Cas9.)

"It's not just exciting science," says Katrine Bosley, a serial biotech executive who became the first permanent CEO of Editas Medicine this summer. "It really is the beginning of a new frontier in genomic medicine. You think about the kinds of therapies you can potentially create with genome editing, and it's a lot of things we've aspired to do for years and years."

However, there are obstacles to CRISPR's use in humans: Cas9 makes occasional off-target cuts that could be dangerous, and like most complex biomolecules, it's difficult to introduce into cells, especially inside living bodies. This week, Editas announced a series of intellectual property agreements that the company hopes will shore up its path to creating a safe and effective platform for CRISPR-based drugs. Three separate deals, with the Broad Institute and Harvard University, Massachusetts General Hospital, and Duke University, respectively, will grant Editas exclusive license to use key CRISPR technologies in pursuit of human therapies. Together, these deals cover one issued patent, and numerous provisional patent applications.

"This is a unique combination of licensing rights across these multiple institutions," says Bosley. "Each one has important, foundational science in the field of genome editing, but the combination of the three gives us a critical mass to make new therapies."

CRISPR DELIVERY

Many of the properties licensed to Editas come from the labs of the company's scientific founders: Feng Zhang of the Broad Institute, David Liu and George Church at Harvard University, and Keith Joung from Massachusetts General. (Editas' fifth founder, Jennifer Doudna, is co-discoverer of the CRISPR-Cas9 system.) The Duke University properties come from the lab of Charles Gersbach, who is not a member of Editas but is partnering with the company on specific R&D programs.

One of the licenses secured by Editas this week, from David Liu's lab at Harvard, covers a new drug delivery system, an important piece of the puzzle for turning CRISPR from a research tool into a therapeutic agent. Getting a large biomolecule like Cas9 past cell membranes and into a cell's genetic material is a serious challenge. Liu's solution, described in a Nature paper this October, uses a coat of cationic lipids to sneak the protein inside cells. Cas9, or another gene editing protein like a TAL effector, is attached to an RNA or DNA molecule with a strong negative charge, which lets the entire complex bind with the positively-charge lipid structure. A fusion of the lipid coat with the cell membrane then allows the packet of gene-editing material to pass safely through.

Importantly, Liu and colleagues have already shown that this system can work inside the bodies of living organisms, successfully engineering cells in the inner ears of mice to express a red fluorescent protein. This is significant because Editas' competitors, CRISPR Therapeutics and Intellia, have both stated that they will concentrate first on ex vivo applications, modifying cells outside the body before reinjecting them, for instance as part of an immune attack on cancer.

With the cationic lipid delivery system, however, Editas may have a shot at testing out in vivo drugs earlier in its development process, something that Bosley says is "certainly within the scope of programs we've started to work on." She adds that she expects this tool to be Editas' first delivery vehicle, for both in vivo and ex vivo programs. The active pursuit of in vivo therapies would be a landmark for CRISPR gene editing, opening up otherwise intractable hereditary diseases as possible targets for treatment.

"IT'S NOT JUST EXCITING SCIENCE. IT REALLY IS THE BEGINNING OF A NEW FRONTIER IN GENOMIC MEDICINE."

Katrine Bosley, CEO of Editas Medicine

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"SOMETHING LIKE CRISPR IS A FOUNDATION TOOL."

Feng Zhang, The Broad Institute

OPEN FOR INNOVATION

The IP licensed to Editas in this week's agreements also includes the first and only issued patent related to CRISPR, covering Feng Zhang's work modifying the system to work in mammalian cells; and Keith Joung's work with guide RNA to help minimize off-target cuts to the genome.

While the details of each agreement are different, negotiators have tried to strike a balance between Editas' desire to gain a competitive edge in drug development, and open science principles that seek to make CRISPR technology broadly available, which are important to the company's scientific founders. "Something like CRISPR is a foundation tool," Zhang told Bio-IT World in an interview this January. "I think making sure these foundation tools are open is important… What my lab has done is try to make the information, the technical know-how, as well as the physical reagents, as accessible to everybody as possible."

Editas' deal with Harvard and the Broad Institute includes a clause that opens the relevant IP to third parties who want to pursue gene targets Editas is not working on itself. Editas hopes to pursue a large and diverse selection of gene targets, both internally and through partnerships with academic labs and other companies. However, the space of diseases that could potentially be impacted by CRISPR is so large that a single company would be hard pressed to monopolize the field.

"It is important that the technology be pursued broadly," says Bosley. "If there are targets we're not pursuing, and someone has a good idea to do that, I think it's great to have this provision where they can come to us, or come to one of the institutions, and work together to find a way they can pursue it."

Editas has not yet shared the specific diseases or genes it plans to go after, except to say that its active programs include both relatively simple applications of CRISPR, like attacking viral RNA and DNA, and more complex ones, like modifying human cells inside the body to repair disease-causing mutations — including, perhaps, the mutations behind Duchenne muscular dystrophy, the focus of Charles Gersbach's research. Bosley says that the company will begin announcing targets over the course of 2015 as its priorities take shape.

"We're still at least a couple years from the clinic," she adds. "We want to advance these a little further, and have more data to share, before we start talking about specific programs."

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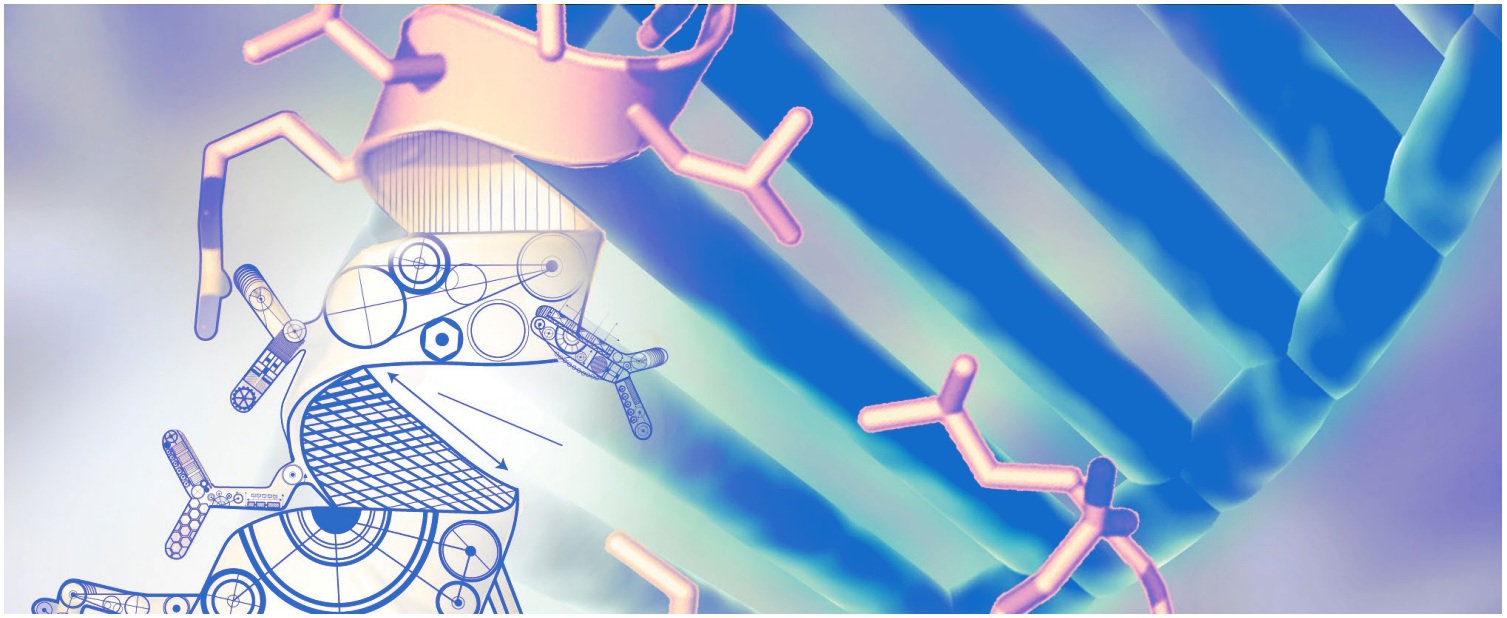

NEW CAS9 MOLECULE

Points the Way to Viral Delivery of CRISPR Systems

By Aaron Krol | April 1, 2015

Startup biotechs and major pharmaceutical companies alike are working overtime to apply the recently discovered CRISPR genome editing technology to treating or even curing human diseases. Derived from a natural defense mechanism of bacteria, the CRISPR system uses a class of proteins called Cas9 to engineer precise cuts in cellular DNA, making it possible for scientists to flexibly delete or insert genes nearly anywhere in the genome.

There are obstacles to using CRISPR in living organisms, however, and perhaps the biggest is the problem of delivering Cas9 to cells in the body. The most popular vector for gene therapies, in which new DNA sequences are inserted into the human genome to combat the effects of disease-causing mutations, has long been the adeno associated virus (AAV), which can deliver a genetic payload to a single, well-defined region of the genome without noticeable side effects. However, AAV vectors have been largely unavailable to CRISPR scientists, because these viruses can only carry payloads up to a certain size — and a standard Cas9 system, including the Cas9 molecule itself and multiple guide RNA sequences to direct Cas9 to its DNA target, comes in over the limit.

Today, a paper in Nature demonstrates a way around this limitation, successfully engineering genetic changes in mice using an AAV-delivered CRISPR system. The work comes from the lab of Feng Zhang, of the Broad Institute of MIT and Harvard, who first demonstrated that CRISPR genome editing could be performed in mammalian cells in 2013.

Almost all scientists using CRISPR today choose to use a Cas9 molecule derived from the bacterium Streptococcus pyogenes. "The reason the S. pyogenes has been used as the standard is because it

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was the only Cas9 that was very reliable, in terms of efficiency, and also had a broad targeting range," explains Le Cong, co-lead author of the new paper and a postdoctoral researcher at the Broad Institute. (His fellow lead author, Fei Ann Ran, presented some results from this paper at the Molecular Medicine Tri Conference this February.)

The efficiency refers to how reliably the Cas9 molecule cuts both strands of DNA at a targeted site, while the targeting range refers to how many potential regions of the genome the molecule can interact with. All Cas9 molecules need to make cuts next to specific PAM (protospacer-associated motif) sequences, but the S. pyogenes Cas9 uses the PAM sequence NGG, which is so common across the genome that it can in effect be targeted almost anywhere.

However, S. pyogenes Cas9 (or spCas9) was not always the default choice. Cong, who received his PhD under Zhang and was a lead author of the original paper demonstrating CRISPR genome editing in mammalian cells, recalls that this early work used Cas9 derived from Streptococcus thermophilus instead. Cong and his colleagues reasoned that another Cas9 molecule from another well-characterized species might be suitable to use with an AAV vector. "We wanted to find a new Cas9 that is of equal efficiency and targeting range [to spCas9], but smaller," he says.

To find candidate molecules, the lab partnered with Eugene Koonin of the National Center for Biotechnology Information, who studies the evolution of Cas9 molecules across species of bacteria. Koonin, in collaboration with the Zhang lab, had built phylogenic trees tracing the relationships between distantly related Cas9 molecules. From a set of over 600 molecules, the lab ultimately chose six new ones for detailed screening.

"The rationale was, if we could only sample a few Cas9s, we wanted to sample representative ones across the evolutionary space," says Cong. After initial in vitro tests of these molecules in human kidney cells, the authors found that one of them, derived from Staphylococcus aureus, was comparably efficient to spCas9 This new saCas9 was a promising candidate for in vivo experiments: at just over 1,000 amino acids in length, it is short enough to include in an AAV cartridge, and its PAM sequence, NNGRRT, is fairly well represented across the genome.

"WE EXPECT THE DIVERSITY OF THE CAS9 SYSTEM TO HAVE EVEN MORE VERSATILE AND POTENTIALLY BETTER CAS9 SYSTEMS."

Le Cong, Broad Institute

To test the system in animals, the lab engineered AAV vectors with saCas9 systems to remove two different genes from two different groups of mice. One experiment targeted the APOB gene, which encodes a protein involved in lipid transport, while the other targeted PCSK9, a gene with major therapeutic implications because people who do not express this gene have markedly low levels of LDL cholesterol and lowered risks for cardiovascular disease. (Both of these genes are especially active in the liver, important because AAV is relatively easy to deliver to liver cells.)

Both experiments were highly successful: in the PCSK9 knockout mice, over 40% of liver cells showed deletions of the targeted gene within one week of a single treatment. Even more significantly, in both populations of mice, the researchers were able to detect phenotypic changes as a result of their treatments.

"To achieve phenotypic changes, and demonstrate a physiological change in the animal, you have to be able to hit many cells," says Cong. "It requires a very efficient and scalable system." The AAV-delivered saCas9 system appears to meet this high bar: in the APOB knockout mice, telltale accumulations of lipids could be found in the liver, while the PCSK9 knockout mice had blood cholesterol levels 40% lower than wildtype mice and almost nonexistent blood levels of the Pcsk9 protein.

One surprising result of these experiments was that the DNA of the affected mouse cells appeared to contain very few off-target cuts, where Cas9 sheared the genome in places other than the targeted region, even weeks after treatment. "We didn't observe any off-target cleavage above the level of detection of our assay," notes Cong, who says this result was unexpected. Earlier in vitro experiments had suggested that saCas9, like spCas9, would cause low but detectable numbers of off-target cuts, which could be a serious concern in human therapies, potentially leading to difficult-to-predict side effects.

Cong speculates that the AAV vector, which by design delivers only a limited payload of Cas9, may have contributed to the apparent absence of off-target effects. However, he cautions that more research is needed before anyone can rule out off-targets with this method. "We think in vivo specificity is a very complicated issue," he says. "Longer-term study is necessary to really assess the long-term toxicity, or off-target effects of the system." Importantly, cells that have received AAV payloads will continue to produce Cas9 throughout their lifespans, meaning there is a potential for off-target cuts to accumulate over time.

SaCas9 is a promising tool for therapies based on CRISPR — so much so that Editas Medicine, a therapeutics company to which Zhang serves as a scientific founder and adviser, is already experimenting with this new molecule. However, Cong notes that this paper only scratches the surface of Cas9 diversity in the natural world.

"This is a first step, but there are so many other Cas9s out there in the metagenomic space, we expect the diversity of the Cas9 system to have even more versatile and potentially better Cas9 systems," he says. "And we can also rationally design a Cas9 system, now that we have knowledge of multiple Cas9 systems instead of just one. Metagenome mining and rational design are really important now to engineer and improve Cas9."

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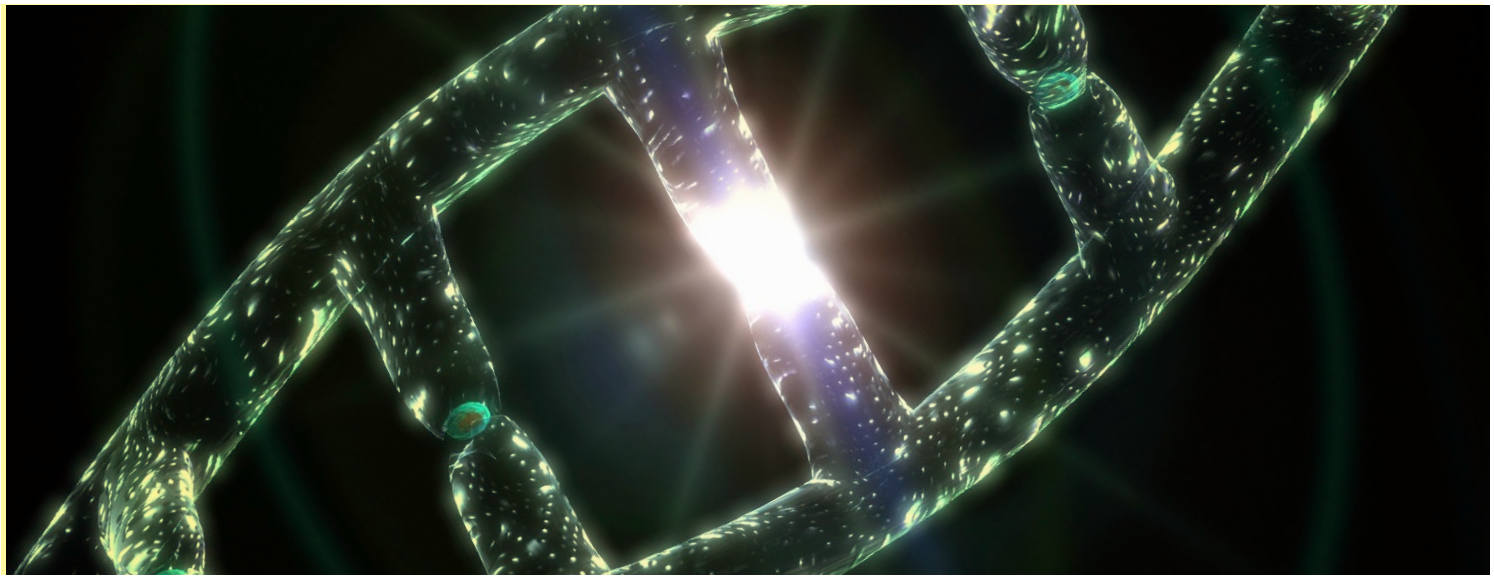

NOVARTIS DEAL

Signals Big Pharma's Entrance in CRISPR-Based Therapies

By Aaron Krol | January 7, 2015

There's an episode of the sitcom 30 Rock where Steve Martin, playing a genius entrepreneur, whispers his pitch for a new business venture to a potential investor: "Wind power. Bandwidth. Chinese market." The investor's jaw drops with a gasp.

To the biotech community, today's deal between pharma giant Novartis and recent startups Caribou Biosciences and Intellia Therapeutics might feel much the same: "CRISPR. Stem cells. CAR-T therapy." Check off your disruptive biotech bingo cards.

To recap briefly, CRISPR is a rapidly emerging technique that lets scientists edit the genomes of living cells, much more flexibly and easily than ever before. In fact, CRISPR is such a leap forward in gene engineering that startups are all but tripping over each other to be the first to try it out as a drug — despite the fact that no gene therapy has ever been approved for sale in this country.

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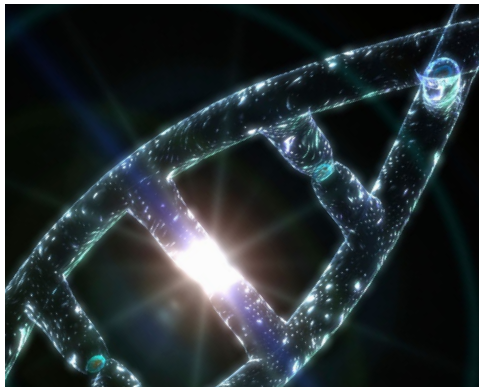
CAR-T therapy, meanwhile, is a method of fighting cancer in which a patient's T-cells are extracted from the body, trained to attack tumor cells, grown by the billions and then administered as a drug. CAR-T treatments are still in early clinical trials, but for certain blood cancers they have been outlandishly effective in these studies, all the way up to complete responses in outright majorities of patients. While all the usual caveats about early trials apply, few cancer programs have ever managed to look so promising at such a late stage of development.

Stem cells, of course, are this close to being the cure for everything, and have been for twenty years.

Under the terms of today's agreement, Novartis will have exclusive rights to use Intellia's CRISPR platforms to develop CAR-T therapies; Novartis and Intellia will form a joint development plan for a CRISPR-engineered stem cell program, which could lead to drug candidates for one or both of the partners; and Novartis will also gain a non-exclusive license to all of Caribou's intellectual property. Meanwhile, Intellia will continue to pursue its own drug pipeline, internally or with other partners, with new funding from Novartis and licenses to undisclosed Novartis patents.

Unlike Steve Martin's venture, this one does have a natural synergy to it. CAR-T already relies on genetically manipulating T-cells, programming them to produce antigen receptors that bind to unique cancer cell proteins. CRISPR is an obvious choice of tools to handle this step. And the specific type of stem cells covered in the agreement — hematopoietic stem cells (HSCs), the precursors to blood cells — could be genetically altered to treat hereditary blood disorders, including sickle cell disease and all forms of thalassemia. The premise would be to grow patient-derived HSCs, alter them with CRISPR to correct the disease-causing mutations, and transplant them to provide a renewable source of healthy blood cells. Novartis has not actually specified that such a therapy is in the works, but it seems more than likely, especially as researchers

NOVARTIS WAS ONE OF TWO MAJOR INVESTORS WHEN INTELLIA WAS FOUNDED, AND IT'S NOW ABUNDANTLY CLEAR WHY THE BIG PHARMA COMPANY WAS EAGER TO GET IN ON THE GROUND FLOOR WITH THIS LATE ENTRANT INTO THE CRISPR THERAPY SPACE.



at Johns Hopkins have already demonstrated that CRISPR can be used for highly specific editing of adult stem cells.

Novartis is already a leader in CAR-T therapy, with multiple programs in the area, including one drug that has won breakthrough status from the FDA and is now in Phase II trials for acute lymphoblastic leukemia. Caribou Biosciences and Intellia Therapeutics are both CRISPR-based startups founded in large part on the research of Jennifer Doudna, one of CRISPR's two co-discoverers: Caribou works on basic science applications, while Intellia focuses on human therapies. In fact, Novartis was one of two major investors when Intellia was founded less than two months ago, and it's now abundantly clear why the big pharma company was eager to get in on the ground floor with this late entrant into the CRISPR therapy space.

Exactly what intellectual property Novartis is accessing through Intellia is an open question. The only issued patent around CRISPR — currently being challenged by Doudna and others — belongs to the Broad Institute, and is being licensed to a competing company, Editas Medicine. But with a line to one of the first scientists to propose CRISPR as a gene editing technique, Intellia is in a good position to retain some key rights when all the patent disputes have shaken out. (Intellia licenses its IP from Caribou, which gets its own rights directly from Doudna's lab at UC Berkeley.)

Regardless, the major takeaway today is that CRISPR therapies are now big business. Editas, the first company to enter the field, has seemed to prefer to strike out on its own (while not actually ruling out pharma partnerships). It's even snatched up its own vector for in vivo therapies, a sign of serious clinical ambitions. CRISPR Therapeutics, the European counterpart to Intellia and Editas, has made it known that it would love to work with outside partners, but is yet to announce any collaborations.

The linking of Novartis and Intellia, however, brings the resources of one of the world's largest pharmaceutical companies to bear on CRISPR for the first time. Novartis has a massive R&D budget, a lead to maintain in CAR-T, and a reputation for investing wisely in emerging technologies. There's every reason to think it regards CRISPR as more than just a curiosity, and will be willing to spend heavily to bring the technique to the clinic.

While financial details have not been disclosed, Intellia has also stated that funding from Novartis will prop up its own R&D efforts for the five-year term of the partnership. The fledgling company now looks to be a very serious contributor to the push for CRISPR-based therapies — and that, we can hope, is good news for patients.

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JUNO THERAPEUTICS PARTNERS WITH EDITAS MEDICINE

on CRISPR-Engineered Cancer Immunotherapies

By Aaron Krol | May 27, 2015

Juno Therapeutics, the immunotherapy company whose $265 million IPO last year has helped make it one of the world's flushest biotechs, has announced a deal with Editas Medicine to co-develop cancer therapies using gene editing technology. The agreement includes a $25 million upfront payment to Editas and an additional $22 million research investment, and could balloon to as much as $700 million in milestone payments if Editas delivers on three as-yet-unspecified therapeutic programs.

The sums involved here are remarkable not just because of their size, but because Juno itself is a young company, launched barely 18 months ago, whose first therapies are not expected to reach the market until at least 2017. It's a testament to how hyper-charged the biotech funding environment has become that an "established" player like Juno is taking on a role more typical of big pharma, providing major seed money to the next generation of promising biotechs in collaborations to boost its pipeline. The Editas partnership is the second major expenditure Juno has announced just this month, after acquiring Stage Cell Therapeutics in another deal whose final cost could total in the hundreds of millions of dollars.

"IT'S EASY TO IMAGINE CRISPR BECOMING THE BACKBONE OF THE SCALED-UP CAR-T AND TCR PRODUCTION PROCESSES THAT WILL BE NEEDED TO GIVE THESE THERAPIES A BROADER REACH."

Juno has amassed the funding for these aggressive ventures on the back of its chimeric antigen receptor T-cell (CAR-T) and T-cell receptor (TCR) therapies, two emerging technologies in which a patient's immune cells are extracted from the body, engineered to recognize tumor cells, and readministered as cancer-fighting agents. Juno therapies in early clinical trials for leukemia and lymphoma have shown promising results, placing the company near the head of a large pack of CAR-T hopefuls.

While oncologists (and investors) have high hopes for these programs, they are not without risk. The FDA is only beginning to evaluate CAR-T treatments, whose powerful efficacy comes with a side effect, a massive immune response sometimes called a "cytokine storm," that could only be acceptable in a disease as dire as cancer. Two cancer patients in Juno-sponsored trials have died from complications of these cytokine storms — but many more have gone into complete remission, often in cases where their diseases would previously have been considered all but incurable.

Editas and Juno make a good match. The specialty of the Editas team is CRISPR, an extraordinary gene editing technique that allows scientists to make highly precise changes to the genomes of living cells, adding and removing DNA almost at will. This technique has excellent synergy with the kind of T-cell engineering performed at Juno — the older gene editing methods currently supporting CAR-T are more labor intensive, less flexible, and less precise. It's easy to imagine CRISPR becoming the backbone of the scaled-up CAR-T and TCR production processes that will be needed to give these therapies a broader reach. The combination of these technologies is also a great shortcut to the clinic for Editas: the enormous challenge of delivering CRISPR systems to the right cells in the body is not a problem in CAR-T, where T-cells are extracted and reprogrammed in the lab.

Of course, with great promise comes plenty of competition, and Juno is not the first CAR-T player to link up with a CRISPR partner. That distinction belongs to Novartis, the big pharma company notable for its embrace of young biotechnologies, which forged a partnership with Editas competitor Intellia Therapeutics earlier this year. Novartis, thanks to a breakthrough status designation by the FDA, should be first to market with a CAR-T treatment if the agency ultimately approves its drug CTL019 for one or more types of blood cancer; Juno is close behind in its regulatory timeline.

A further wrinkle to this story: Editas and Intellia are embroiled in a patent dispute, which at heart concerns the right to administer CRISPR systems in mammalian cells. A very broad patent on this technology is currently held by the Broad Institute of MIT and Harvard and licensed to Editas, based on work performed in the MIT lab of Feng Zhang, an Editas scientific founder. That patent, and other related intellectual property, is being challenged by (among others) Jennifer Doudna, the co-discoverer of CRISPR gene editing who dropped out of Editas and is now a founding member of Intellia.

Better get used to these entanglements: as the venture capital money continues to flow into biotech, the stakes will keep getting higher for companies whose huge valuations are riding on equally huge clinical results. Juno and Novartis only recently settled their own intellectual property fight, with a royalty deal that gives Juno a small stake in Novartis' programs.

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CRISPR in the GERMLINE

By Bio-IT World Staff | March 20, 2015

There is a renewed urgency to public conversations about the ethics of genome editing, thanks to the emergence of CRISPR, a gene engineering technology so effective and easy to use that scientists are racing to keep up with its potential applications.

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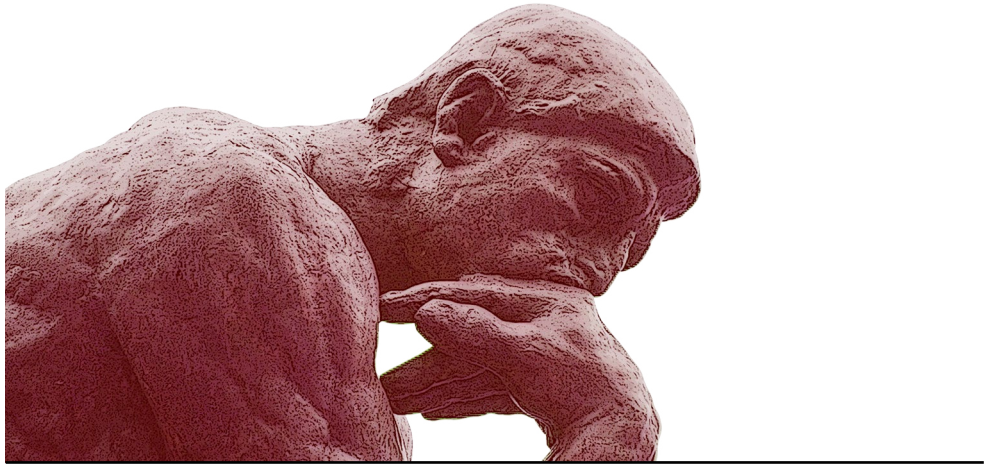
This is a profound change from past techniques for making precise edits to the genome, such as zinc finger nucleases or TALENs, which were so finicky and complex that only a few expert labs were following up on genome editing as a practical tool in the life sciences and medicine.

Yesterday, two significant publications on this issue appeared in the journal Science. The first, a policy statement, advocates for a global, voluntary moratorium on using CRISPR in human germline experiments. Unlike edits to somatic cells, which could be used to cure genetic diseases in single individuals, edits to germline cells could be passed from parent to child, making any changes induced by CRISPR permanent additions to a species' genetic variation. The authors of the policy article note that there is not yet a consensus on how specific CRISPR edits are — the technology is known to make "off-target" cuts to the genome, cutting DNA at sites similar, but not identical, to those deliberately targeted, raising the possibility of unintended effects. Notably, the lead author of this article is Jennifer Doudna, who along with Emanuelle Charpentier made the original discovery that CRISPR could provide a programmable method for editing genomes. The New York Times has additional coverage.

Meanwhile, the second publication offers a dramatic illustration of CRISPR's power in germline experiments. Two biologists from UC San Diego, Valentino Gantz and Ethan Bier, successfully used CRISPR to insert a mutation in a population of mosquitoes that caused them to produce less pigment, making the mosquitoes a pale yellow. This in itself is not remarkable: CRISPR has been used to modify traits in numerous species, including mice and cynomolgus monkeys. The twist in Gantz and Bier's experiment is that they caused their edited mosquitoes' genomes to actually produce the Cas9 protein, which makes cuts in the CRISPR system, as well as the guide RNAs that direct that protein to cut sites in the genome. As a result, an edit made to one copy of a chromosome in a cell naturally spreads to the other copy.

UNLIKE EDITS TO SOMATIC CELLS, WHICH COULD BE USED TO CURE GENETIC DISEASES IN SINGLE INDIVIDUALS, EDITS TO GERMLINE CELLS COULD BE PASSED FROM PARENT TO CHILD, MAKING ANY CHANGES INDUCED BY CRISPR PERMANENT ADDITIONS TO A SPECIES' GENETIC VARIATION.



When an edit like this is made to the germline, the result is a "gene drive," where a mutation is more likely to be passed from parent to child than chance alone would predict. In this case, when the edited mosquitoes bred with one another, 97% of their children were yellow — compared to the 25% that would be expected without the gene drive.

A Science news piece on this paper quotes George Church, a renowned genome biologist, as calling the study "a step too far." Church has himself published on the possibility of using CRISPR in gene drives, as covered in Bio-IT World, but has also demanded safeguards to make the process controlled and reversible. Practical uses of gene drives could include disease control, by making insects that carry common infections like malaria resistant to the disease-causing parasites. More drastically, gene drives have also been suggested as a way to deliberately drive a species to extinction, for example by only allowing the male sex chromosome to be passed on from father to child, gradually eliminating females from the species.

Research on CRISPR will continue to expand in scope and ambition, as labs that have never previously attempted genome editing latch onto the technique as a simple tool to explore the field. If this research is to be constrained, voluntary moratoriums among scientists are likely the fastest route to implementing certain controls while the ethics of genome editing are publicly discussed.

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OUTPOURING of COMMENTARY on CRISPR Germline Editing

By Bio-IT World Staff | June 10, 2015

Nature has been closely following the ongoing discussion on CRISPR-Cas9, and what this remarkably easy and powerful gene editing technique means for our conceptions of what is practical and ethical in altering the human genome. Particularly contentious has been the prospect of editing DNA in the human germline, which could provide complete cures for deadly genetic diseases — but could also introduce changes to genome, for better or worse, that would be passed from generation to generation. Even some of the original inventors of CRISPR-Cas9 editing have called for moratoriums on research in the germline, possibly along the lines of the Asilomar Conference of 1975 that led to voluntary restrictions on the use of recombinant DNA technology.

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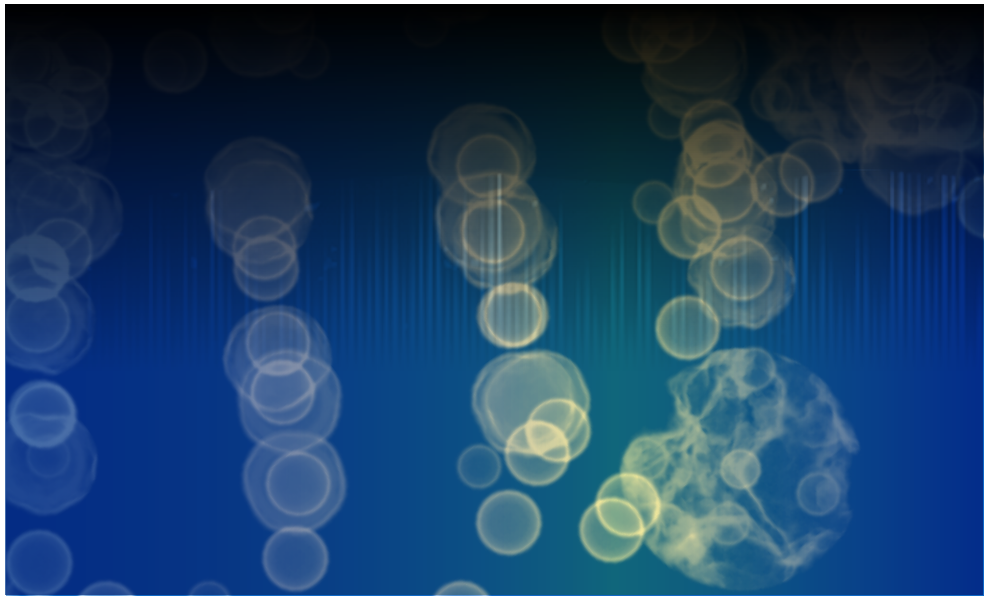

"AS IS OFTEN THE CASE, A TECHNICAL BREAKTHROUGH IS FORCING US TO CONFRONT A COMPLICATED QUESTION FAST."

- Katrine Bosley, CEO of Editas Medicine

Among Nature's coverage of this controversy has been a feature article published last month in Nature Biotechnology, weighing CRISPR's possibilities. Bio-IT World would like to extend a tip of the hat to Cormac Sheridan (@Cormac_Sheridan), who pointed out on Twitter that this article includes, in a supplementary document, complete Q&A's with over 20 experts discussing the promise of CRISPR therapeutics, the perils of editing the human germline, and what if any international norms or institutions could regulate its use. (The full article is subscription-only, but the supplementary document is free to view.) Among those interviewed are major CRISPR pioneers like Jennifer Doudna, Emmanuelle Charpentier, and Feng Zhang, and scientists with an outsize presence in biotech like J. Craig Venter.

The entire set of responses is worth perusing, but we'll reproduce just a few choice snippets here:

"The potential benefits are enormous. We are talking about cures for diseases, in which the cure itself is passed down through generations. Sobering stuff." - Jacob Corn, scientific director of the Innovative Genomics Initiative

"[T]he unanticipated effects of targeted genetic modification are a major source of concern. We still have a great deal to learn about gene regulation and networks. No one would have worried about off target effects in non-coding RNAs a few years ago." - Martin Pera, chair of stem cell sciences at the University of Melbourne

"Human germ-line engineering isn't a new concept, but we haven't had to think deeply about its management or regulation until now, because it was pretty theoretical until now. As is often the case, a technical breakthrough is forcing us to confront a complicated question fast." - Katrine Bosley, CEO of Editas Medicine

"[T]he GMO debate (in agriculture) has been incredibly badly handled, but there is a risk that we haven't learned from this and will make the same mistakes, potentially delaying or foreclosing on what could be an immensely powerful means to prevent human suffering." - Tony Perry, head of the Laboratory of Mammalian Molecular Embryology at the University of Bath

"Many parents (most likely mothers who carry an X-linked mutation) with a fatal genetic mutation who have lost a child before would take a risk to correct the genetic defect in germ cells or embryos. Is it moral to illegalize their desperate desire?" - Jin-Soo Kim, director of the Center for Genome Engineering at Seoul National University

"Asilomar has become for biology what Woodstock has become for youth culture — a mythology that's grown but that obscures how muddy the event itself was at the time." - Jonathan Moreno, professor of medical ethics at the University of Pennsylvania Perelman School of Medicine

"[H]uman germ-line engineering is inevitable and there will be basically no effective way to regulate or control the use of gene editing technology in human reproduction… One only needs to look at the proliferation of stem cell therapy clinics around the world largely in the absence of clear cut clinical data." - J. Craig Venter, chairman and CEO of Human Longevity, Inc.

For more on this issue, you can listen to Jennifer Doudna's recent appearance on Radiolab — joined also by science writer Carl Zimmer.

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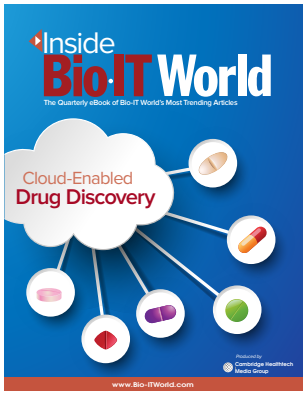

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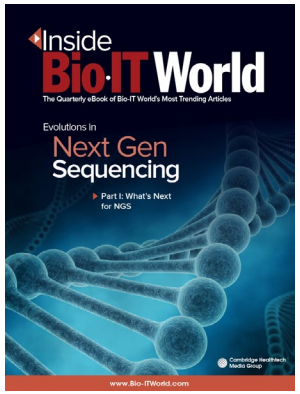
Part II: Genomics in the Clinic



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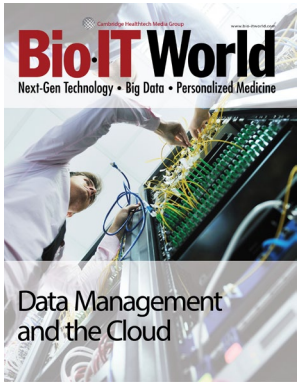
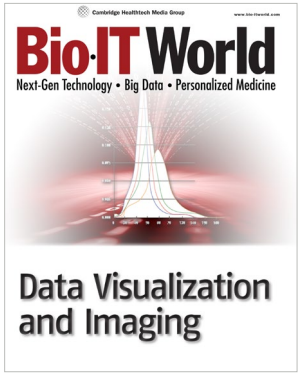


Data Management in the Cloud

Clinical Genomics & Diagnostics



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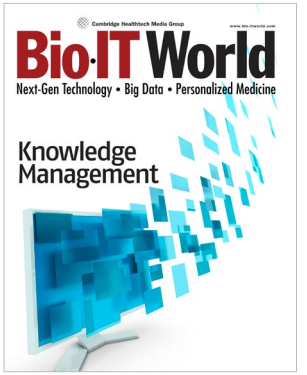
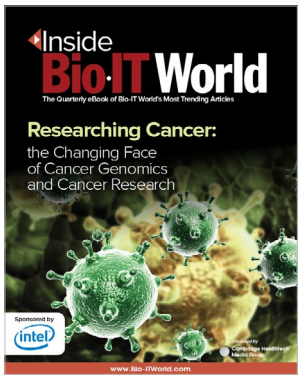
NGS Updates

Open Science

The New Clinical Trial



Knowledge Management



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