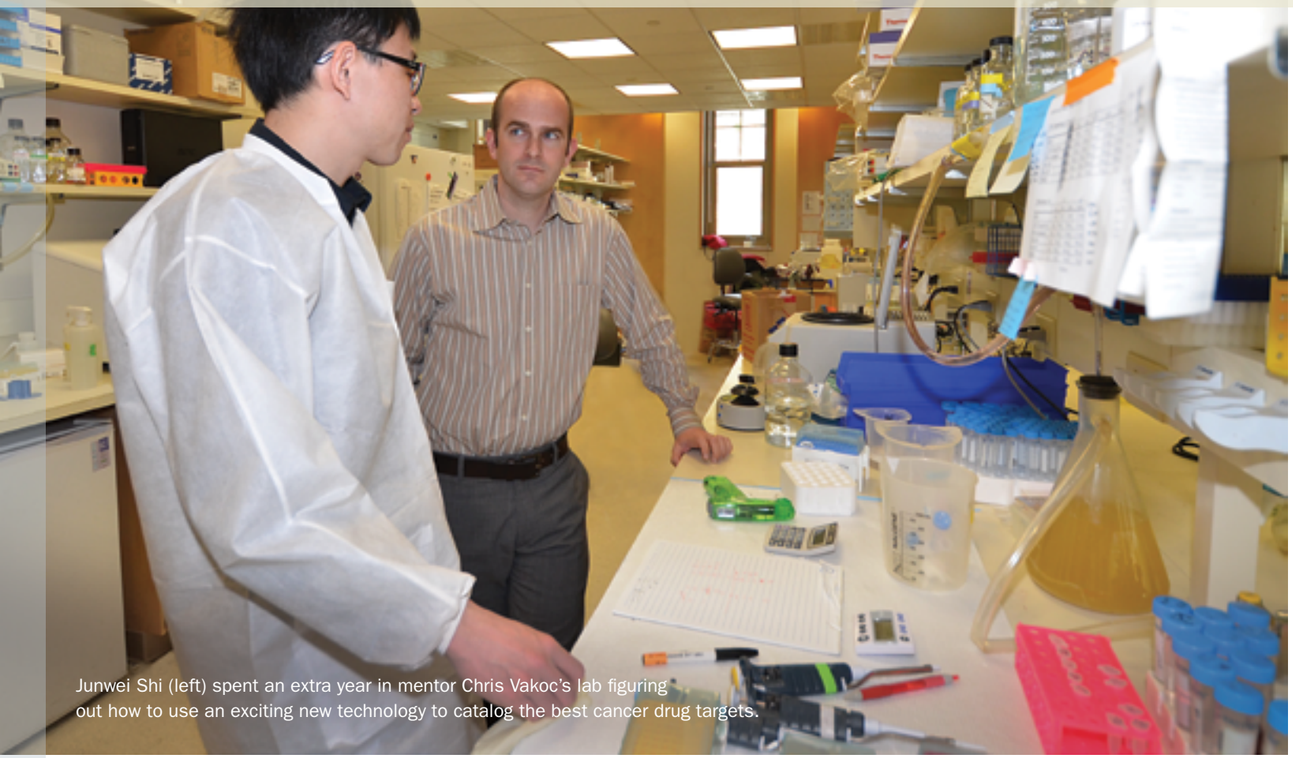
Harnessing CRISPR to target cancer



Junwei Shi (left) spent an extra year in mentor Chris Vakoc's lab figuring out how to use an exciting new technology to catalog the best cancer drug targets.

As it is embraced enthusiastically by biological researchers in labs worldwide, the powerful new gene-editing technique called CRISPR has inspired grand visions of curbing malaria, curing genetically caused human illnesses, and even bringing extinct animals like the woolly mammoth back to life.

At Cold Spring Harbor Laboratory, the lab group headed by Associate Professor Christopher Vakoc, upon learning of CRISPR a few years ago, decided to take a practical approach, and in 2015 they published a method of using the technique to find new targets for cancer drugs. In so doing, they demonstrated that "The CRISPR Craze," whatever its long-term potential, has immediately powerful implications for human health.

CRISPR refers to a method of editing the DNA letters of genomes, as one would delete and add text with a word processor. Researchers have been modifying genomes for decades, albeit painstakingly. They have never had a method so simple, precise and inexpensive.

It's based on a simple DNA-cutting mechanism that bacteria use to defend themselves against viruses and other foreign invaders. CRISPR is a two-component search-and-destroy machine consisting of an enzyme called Cas9 that cuts DNA, and a short strand of RNA that it carries, like a searchlight, to spot specific DNA sequences in a genome. Upon reaching its target along the double helix, the guide RNA positions the Cas9 enzyme over the sequence, which the enzyme then snips out.

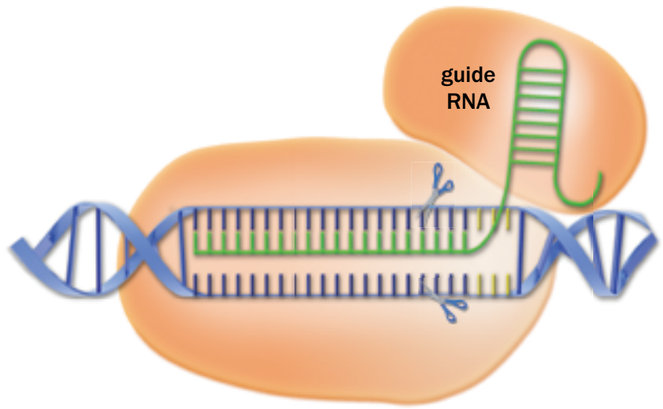
In 2012–13, scientists in California, Massachusetts and Sweden demonstrated that CRISPR can be used to cut and paste DNA in many living things, including human cells, even though Cas9 is not native to the human system.

An inspiring decision

Along with the rest of the research world, Junwei Shi, at the time a Ph.D. student in the Vakoc lab, read exciting reports about using CRISPR in human cells and started to think about adapting it for his own work. This happened to coincide with an important meeting in 2013 with his thesis committee. "They told me, 'It's time to finish your Ph.D. and move forward with your training!'" Shi recalls.

But, says Vakoc, "he actually begged them to stay." He wanted another year in Vakoc's lab to try CRISPR, even though he didn't need to do that work, as Vakoc explains. "Junwei already had published several important papers, one of which reported the discovery of a powerful leukemia drug target called BRD4 that's now the focus of a clinical trial. Even though he had completed all his requirements to graduate, he was driven to make more discoveries, even if it meant a delay in moving to the next career step—which we all found pretty inspiring."

That extra year began slowly, as Shi spent 3 months optimizing CRISPR and tackling the question of how to deliver it into human leukemia cells. Once he could use it to cut DNA anywhere in the human genome, the question, he says, became: "*where* do we want to cut along *specific* genes?" The question gets at why CRISPR has generated so much excitement: it is a reprogrammable cutting machine. The DNA scissors can be sent to any target in the genome, simply by providing the correct genomic "address" to the RNA guide strand carried by the Cas9 enzyme.



CRISPR-Cas9 is a reprogrammable DNA cutting machine that is being used to edit genomes in many organisms for research purposes. Its primary component, the Cas9 enzyme (orange), cuts genomic DNA (blue). The enzyme is directed with exquisite specificity to its target—essentially any sequence along the genome—by hitching it to a strand of "guide" RNA (green) whose sequence is complementary to that of the DNA target. Upon finding and pairing with it, Cas9 snips out the target segment. It can either be deleted or replaced with another DNA sequence (not shown here).

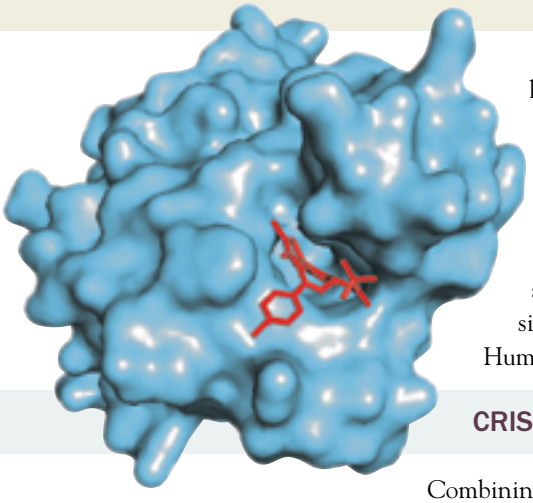
Emmanuelle Charpentier (left) and Jennifer Doudna, two of the pioneers in adapting CRISPR-Cas9 to genome editing, seen last year at a major Cold Spring Harbor meeting on CRISPR that drew over 400.

As it happened, CSHL had expertise in making the kind of short RNAs needed to program Cas9. Greg Hannon (now an adjunct professor), along with Leemor Joshua-Tor and other CSHL colleagues, made important discoveries in the early 2000s about the workings of another genome-regulating system called RNA interference, or RNAi. Hannon's team built large libraries of small RNA molecules which could be used in experiments to "knock down" expression of any of the 21,000 human genes, often in a single experiment. With CRISPR, one doesn't knock down, *i.e.*, reduce, expression of a gene as in RNAi but instead knocks out the gene or a part of it entirely, either deleting it or replacing it with a modified sequence.

But, as Shi asked: *where* to cut? He started with a gene the team knew well. He picked *BRD4*, which encodes a protein that leukemia cells cannot live without, as the team discovered in 2011. That finding led to the current clinical trial of a drug that inhibits BRD4 and has a powerful killing effect on cells of acute myeloid leukemia (AML) while not causing significant harm to healthy cells.

"Junwei tested CRISPR by targeting Cas9 to snip out different parts of the *BRD4* gene in human leukemia cells," says Vakoc. This led to a big discovery. "Junwei noticed that of the many places to cut the gene, there is only one kind of sequence to cut if you want to kill the leukemia cells efficiently. You cut the DNA that encodes the pockets on the surface of the BRD4 protein where our drug, JQ1, binds."

This provided a basis on which to apply CRISPR to leukemia and cells of other cancer types, on a massive scale: program CRISPR to target binding pocket motifs—DNA



3D model of the BRD4 protein, showing how JQ1 (red), a drug now in clinical trials, blocks a key binding pocket that leukemia cells depend upon to grow and spread.

letters that encode such features on proteins of interest. A comprehensive set of such motifs is known, thanks to extensive genome annotation work performed since completion of the Human Genome Project.

CRISPR scanning

Combining this knowledge with Shi's optimization of CRISPR and methods of producing massive numbers of small RNAs, the team arrived at a powerful way to discover new drug targets, not only in leukemia, but across all cancer cell types. Vakoc and Shi call their method CRISPR scanning. It's the fruit of Shi's "extra" year—which extended to 2 years before Shi this winter accepted a tenure-track assistant professorship in the cancer biology department at the University of Pennsylvania.

In a single experiment, the team uses thousands of CRISPR "scissors" to cut out different DNA regions in a given gene. Each Cas9 enzyme is loaded with a different guide RNA. When piggy-backed to an inactive virus, each enzyme and its guide RNA infects a single cancer cell, grown in a culture dish. "It's called multiplexing, and in one experiment, you can make thousands of different cuts in the genome and track the impact on cancer cell death," Vakoc says.

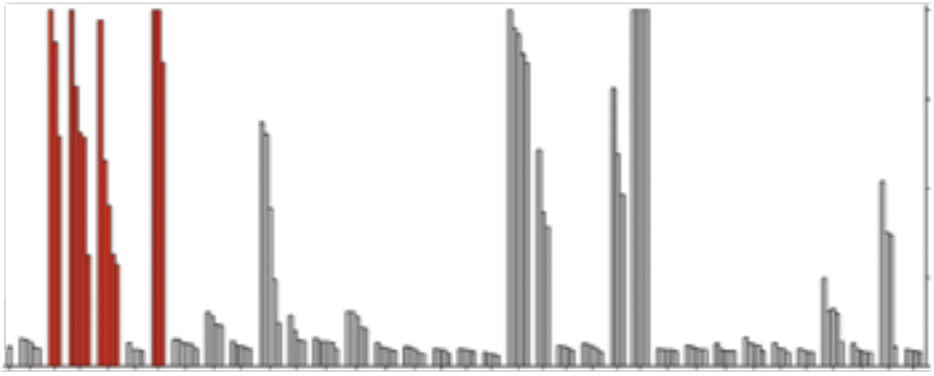
In effect, the scientists use CRISPR to mimic, in thousands of binding pockets at once, the impact of a drug blocking the pocket, just as JQ1 blocks a key pocket on BRD4. Most of these actions won't slow growth of the cancer cell. It's the few that do—pockets that, when blocked, cause cells to die—that the team is looking for. These needles in DNA haystacks are made visible on what the scientists call a Manhattan plot—a few "skyscrapers" of cell-killing activity along an otherwise flat horizon.

"This technology allows us to map every active surface of every protein that every cancer requires," says Vakoc. "There are other ways of finding proteins that cancer cells need. Our approach reveals not just the proteins but information about which surface features on them that we want drugs to hit in order to have dramatic killing effects."

The team used CRISPR to screen 192 gene-regulatory domains of interest in mouse AML cells, and found all six previously known drug targets as well as 19 previously unidentified binding pockets that the cells could not live without. These are immediate targets of interest in drug discovery—DNA addresses of binding sites for future drugs that could have powerful killing effects on cancer cells. Adding to the immediacy of the potential benefit, this screen tested only those targets that current pharmaceutical science already knows how to hit. They're "druggable targets," in the language of chemists.

Ahead of the competition, the Vakoc lab is able to use CRISPR to find these powerful targets in cancer thanks in part to the dedication of Junwei Shi. "His success," says Vakoc, "is a good example of what young researchers at the Lab can aspire to. You can come to Cold Spring Harbor and help advance a field."

Peter Tarr



Shi and Vakoc hitched numerous RNA guide molecules to Cas9 enzymes in order to make literally thousands of cuts in leukemia cancer genes. This "Manhattan plot" registers the "skyscrapers" where particular binding pockets on the cancer cells, when blocked or inactivated, have powerful killing effects on the cancer. Red peaks are previously known targets. Grey peaks are among 19 powerful new targets discovered in a single experiment.