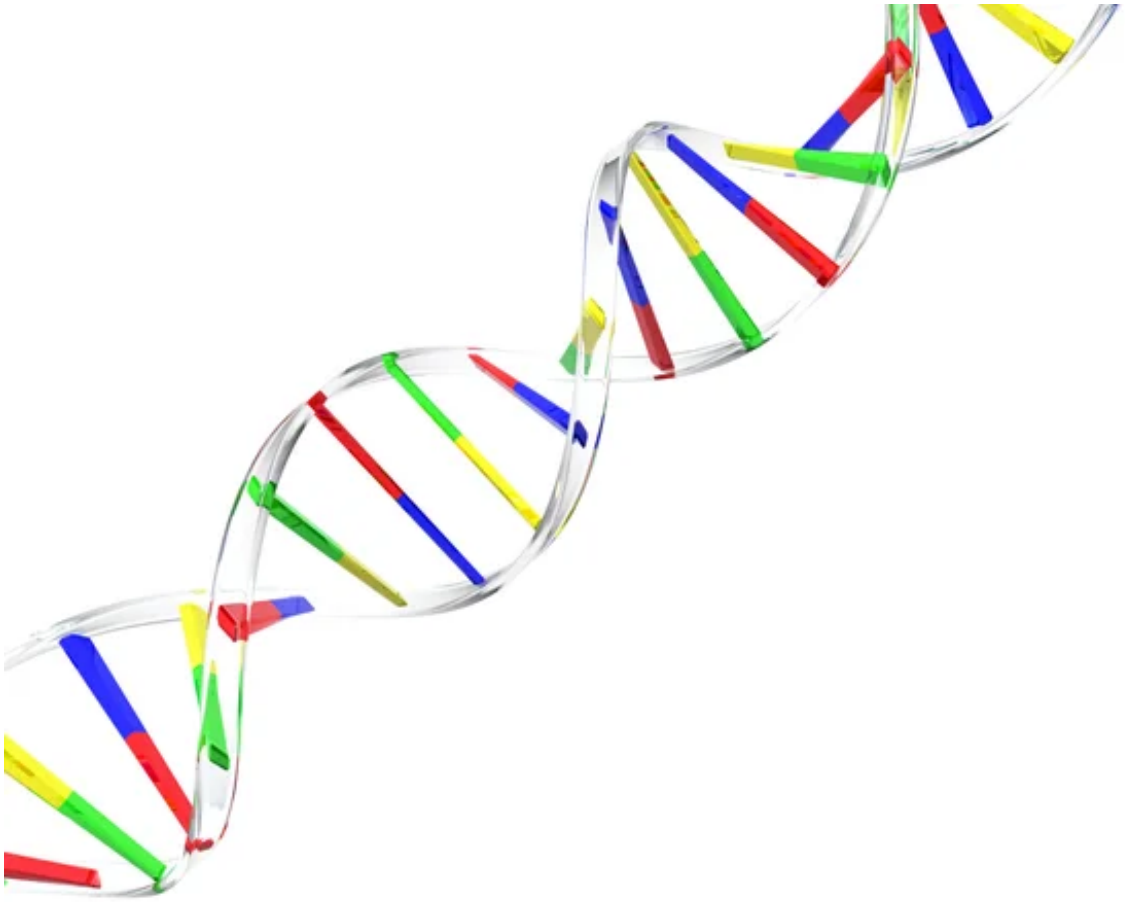
BIOTECH

# CRISPR-Edited Cells Linked to Cancer Risk in 2 Studies

The preliminary findings raise questions about one of the ways this tech edits genomes

By Sharon Begley, STAT on June 12, 2018



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Editing cells' genomes with CRISPR-Cas9 might increase the risk that the altered cells, intended to treat disease, will trigger cancer, two studies published on Monday warn—a potential game-changer for the companies developing CRISPR-based therapies.

In the studies, published in Nature Medicine, scientists found that cells whose genomes are successfully edited by CRISPR-Cas9 have the potential to seed tumors

inside a patient. That could make some CRISPR'd cells ticking time bombs, according to researchers from Sweden's Karolinska Institute and, separately, Novartis.

CRISPR has already dodged two potentially fatal bullets—a 2017 claim that it causes sky-high numbers of off-target effects was retracted in March, and a report of human immunity to Cas9 was largely shrugged off as solvable. But experts are taking the cancer-risk finding seriously.

The CEO of CRISPR Therapeutics, Sam Kulkarni, told STAT the results are "plausible." Although they likely apply to only one of the ways that CRISPR edits genomes (replacing disease-causing DNA with healthy versions) and not the other (just excising DNA), he said, "it's something we need to pay attention to, especially as CRISPR expands to more diseases. We need to do the work and make sure edited cells returned to patients don't become cancerous."

Another leading CRISPR scientist, who asked not to be named because of involvement with genome-editing companies, called the new data "pretty striking," and raised concerns that a potential fatal flaw in some uses of CRISPR had "been missed."

On the other hand, the Novartis paper has been available in preliminary form since last summer, and CRISPR experts "haven't freaked out," said Erik Sontheimer of the University of Massachusetts Medical School, whose CRISPR research centers on novel enzymes and off-target effects. "This is something that bears paying attention to, but I don't think it's a deal-breaker" for CRISPR therapies.

The Karolinska and Novartis groups tested CRISPR on different kinds of human cells —retinal cells and pluripotent stem cells, respectively. But they found essentially the same phenomenon. Standard CRISPR-Cas9 works by cutting both strands of the DNA double helix. That injury causes a cell to activate a biochemical first-aid kit orchestrated by a gene called p53, which either mends the DNA break or makes the cell self-destruct.

Whichever action p53 takes, the consequence is the same: CRISPR doesn't work, either because the genome edit is stitched up or the cell is dead. (The Novartis team

calculated that p53 reduces CRISPR efficiency in pluripotent stem cells seventeenfold.) That might explain something found over and over: CRISPR is woefully inefficient, with only a small minority of cells into which CRISPR is introduced, usually by a virus, actually having their genomes edited as intended.

"We found that cutting the genome with CRISPR-Cas9 induced the activation of … p53," said Emma Haapaniemi, the lead author of the Karolinska study. That "makes editing much more difficult."

The flip side of p53 repairing CRISPR edits, or killing cells that accept the edits, is that cells that survive with the edits do so precisely because they have a dysfunctional p53 and therefore lack this fix-it-or-kill-it mechanism.

The reason why that could be a problem is that p53 dysfunction can cause cancer. And not just occasionally. P53 mutations are responsible for nearly half of ovarian cancers; 43 percent of colorectal cancers; 38 percent of lung cancers; nearly one-third of pancreatic, stomach, and liver cancers; and one-quarter of breast cancers, among others.

The Novartis team was trying to see how it could increase the efficiency of CRISPR editing of pluripotent stem cells. Because this kind of stem cell can morph into virtually any kind of cell, it might be able to treat a variety of diseases. Neuroscientist Ajamete Kaykas of the company's Institutes for BioMedical Research in Cambridge, Mass., got CRISPR's efficiency at inserting or deleting chunks of DNA up to 80 percent. Unfortunately, when CRISPR worked, it was because p53 didn't, which raises cancer concerns.

As a result, the Novartis paper concludes that "it will be critical to ensure that [genome-edited cells] have a functional p53 before and after [genome] engineering." The Karolinska team warns that p53 and related genes "should be monitored when developing cell-based therapies utilizing CRISPR-Cas9."

The p53 finding doesn't mean CRISPR is toast. For one thing, "the two papers present preliminary results," biochemist Bernhard Schmierer of the Karolinska, co-leader of

its study, told STAT. “It is unclear if the findings translate into cells actually used in current clinical studies.”

For another, the p53 problem might be worse with Cas9 than with other DNA-cutting enzymes used in CRISPR. And, crucially, it probably affects only one avenue of genome-editing.

CRISPR edits genomes in either of two ways. It slices out a chunk of disease-causing DNA, in a process called non-homologous end joining (NHEJ), or gene disruption. That’s how CRISPR Therapeutics is going after sickle cell disease. Alternatively, CRISPR both cuts out a disease-causing stretch of DNA and replaces it with healthy nucleotides, in homology-directed repair (HDR), or gene correction. Several university labs are investigating HDR to treat Duchenne muscular dystrophy, among many other diseases.

In the normal, mature cells she and her team studied, Haapaniemi said, gene disruption “can happen even when p53 is activated.”

That’s good news for CRISPR Therapeutics’ sickle-cell and thalassemia programs as well as for Editas Medicine’s lead product, targeting a form of blindness, and others in its pipeline, all of which use NHEJ gene disruption. It also should not affect the gene-disruption approach that Intellia Therapeutics and Regeneron are taking to transthyretin amyloidosis.

CRISPR-based editing of T cells to treat cancer, as scientists at the University of Pennsylvania are studying in a clinical trial, should also not have a p53 problem. Nor should any therapy developed with CRISPR base editing, which does not make the double-stranded breaks that trigger p53. Developed by Harvard’s David Liu, base editing replaces a wrong DNA “letter” with the right one, without cutting, and is the basis for startup Beam Therapeutics.

The p53 problem, however, might affect other products that companies hope to develop via gene correction, including glycogen storage disease, cystic fibrosis, and severe combined immunodeficiency.

It's also a potential problem for stem cells. There, the Novartis team showed, p53 inactivation seems to be necessary for both NHEJ disruption and HDR correction. (Novartis' Kaykas said he could not speak to a reporter without clearance from the company's communications office.) That could be an issue for therapies using CRISPR'd stem cells: The same dysfunctional p53 that allows CRISPR to work its magic also makes cells likely to become cancerous.

Which raises an obvious question—if successfully CRISPR'd cells can seed cancers, why hasn't this been seen before, and why haven't the many CRISPR'd mice developed tumors?

Karolinska's Haapaniemi said the effect shows up in large-scale experiments like hers and Novartis' "but can be missed in small-scale studies where people only focus on editing one gene in one cell type." In speaking to other scientists, she said, "it seems that other teams have noticed the effect of p53 on editing," but have not highlighted it.

Jacob Corn of the University of California, Berkeley, said that although his lab has seen evidence of p53 activation in a few cases, they have "looked hard for growth effects after editing in [hematopoietic stem cells] and found nothing." He has "no reason to doubt the pluripotent stem cell results" in the new research, he added.

As for why no one has reported CRISPR'd mice getting cancer, Haapaniemi said, "This is a good question." One reason might be that "laboratory mice are killed early," perhaps leaving too little time for them to develop cancer.

But Corn said he and others "have all been looking for the possibility of cancer. So far, no one has seen evidence of [it] based on p53 status or induced by editing."

Nevertheless, he called the two papers "important, since they remind everyone that genome editing isn't magic."

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