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Gene editing and therapy – A new tool: CRISPR/Cas9

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One of the most important scientific discoveries of the past few years is a powerful new gene editing technology known as *CRISPR/Cas9.* Gene editing opens the door to treatments for an array of diseases, and holds tremendous potential to treat various inherited & acquired diseases.

In the past nine years, scientists have figured out how to exploit a quirk in the immune systems of bacteria to edit genes in other organisms - plants, mice, even humans. With CRISPR/Cas9, they can now make these edits quickly and cheaply, in days rather than weeks or months [1-2].

This new technology is used now to treat many inherited diseases to reduce the severity of genetic diseases, treat the same type of hearing loss in people, treat sickle-cell anemia. Thalassemia, Malaria vectors like mosquitoes.

A big concern is that while CRISPR is relatively simple and powerful, it isn't perfect. Scientists have recently learned that the approach to gene editing can inadvertently wipe out and rearrange large swaths of DNA in ways that may imperil human health.

This follows recent studies showing that CRISPR-edited cells can inadvertently trigger cancer. That's why many scientists argue that experiments in humans are premature. The risks and uncertainties around CRISPR modification are extremely high. In November 2017, a scientist in China, He Jiankui, reported that he had created the world's first human babies with CRISPR-edited genes: a pair of twin girls resistant to HIV. The announcement stunned scientists around the world. The director of the National Institutes of Health, Francis Collins, said the experiment was "profoundly disturbing and tramples on ethical norms" [3-5].

What is CRISPR /Cas-9?

In 1987, when Japanese scientists studying E. coli bacteria first came across some unusual repeating sequences in the organism's DNA. "The biological significance of these sequences," they wrote, "is unknown". Other researchers found similar clusters in the DNA of other bacteria (and archaea). They gave these sequences a name: *Clustered Regularly Interspaced Short Palindromic Repeats - or CRISPR.*

In 2007, food scientists studying the Streptococcus bacteria used to make yogurt showed that these odd clusters actually served a vital function: They're part of the bacteria's immune system [6-7].

When bacteria are under constant attack from viruses, they produce enzymes to fight off viral infections. Whenever a bacterium's enzymes manage to kill off an invading virus, other little enzymes will come along, scoop up the remains of the virus's genetic code and cut it into tiny bits. The enzymes then store those fragments in CRISPR spaces in the bacterium's own genome [8].

When a new viral infection occurs, the bacteria produce special attack enzymes, known as Cas9, that carry around those stored bits of viral genetic code like a mug shot. When these Cas9 enzymes come across a

virus, they see if the virus's RNA matches what's in the mug shot. If there's a match, the Cas9 enzyme starts chopping up the virus's DNA to neutralize the threat. It looks a little. In 2011, Jennifer Doudna of the University of California Berkeley and Emmanuelle Charpentier of Umeå University in Sweden were puzzling over how the CRISPR/Cas9 system actually worked [9-10].

The scientists soon discovered they could "fool" the Cas9 protein by feeding it artificial RNA - a fake mug shot. When they did that, the enzyme would search for anything with that same code, not just viruses, and start chopping. In a landmark 2012 paper, Doudna, Charpentier, and Martin Jinek showed they could use this CRISPR/Cas9 system to cut up any genome at any place they wanted.

While the technique had only been demonstrated on molecules in test tubes at that point, the implications were breathtaking. Further advances followed. Feng Zhang, a scientist at the Broad Institute in Boston, in *Science* in February 2013 showing that CRISPR/Cas9 could be used to edit the genomes of cultured mouse cells or human cells [11-12].

In the same issue of *Science*, Harvard's George Church and his team showed how a different CRISPR technique could be used to edit human cells.

Since then, researchers have found that CRISPR/Cas9 is amazingly versatile. Not only can scientists use CRISPR to "silence" genes by snipping them out, they can also harness repair enzymes to substitute desired genes into the "hole" left by the snippers (though this latter technique is trickier to pull off). So, for instance, scientists could tell the Cas9 enzyme to snip out a gene that causes Huntington's disease and insert a "good" gene to replace it. What makes CRISPR so revolutionary is that it's so precise:

The Cas9 enzyme mostly goes wherever you tell it to. And it's incredibly cheap and easy: In the past, it might have cost thousands of dollars and weeks or months of fiddling to alter a gene. Now it might cost just $75 and only take a few hours. And this technique has worked on every organism it's been tried on [13].

So what can we use CRISPR for?

CRISPR can help speed up genome screening, and genetics research could advance massively as a result.

1) *Edit crops to be more nutritious:*
2) *New tools to stop genetic diseases:* Scientists are now using CRISPR/Cas9 to edit the human genome and try to knock out genetic diseases like hypertrophic cardiomyopathy, Huntington's disease or cystic fibrosis, sickle-cell disease, epilepsy and on the BRCA-1 and 2 mutations linked to breast and ovarian cancers. Diabetes Scientists have even shown that CRISPR can knock HIV infections out of T cells.
3) *Powerful new antibiotics and anti-virals:* Other researchers are working on CRISPR systems that target viruses such as HIV and herpes.
4) *Gene drives that could alter entire species:* Scientists have also demonstrated that CRISPR could be used, in theory, to modify not just a single organism but an entire species. It's an unnerving concept called "gene drive."
5) *Creating "designer babies":* It's not entirely far-fetched to think we might one day be confident enough in CRISPR's safe to use it to edit the human genome - to eliminate disease, or to select for athleticism or superior intelligence.

In February 2017, a report from the National Academy of Sciences said that clinical trials could be green lit in the future "for serious conditions under stringent oversight." But it also made clear that "genome editing for enhancement should not be allowed at this time." Society still needs to grapple with all the ethical considerations at play here.

The WHO in March 2019 decided to weigh in on genome editing after Chinese biophysicist He Jian kui said in November that he had modified the genomes of two girls to make them resistant to HIV. Late last month, China's health ministry issued draft regulations to restrict the use of gene editing in people.

Last month, a group of ethicists and scientists - including some of the inventors of CRISPR - published an opinion piece in *Nature* calling for "a fixed period during which no clinical uses of germline editing whatsoever are allowed"

In an accompanying response, the leaders of the US National Academy of Sciences, the US National Academy of Medicine and the UK Royal Society opposed such a moratorium, arguing that "we must achieve broad societal consensus before making any decisions, given the global implications of heritable genome editing"

The registry recommended by the WHO committee is an attempt to bridge the gap until the world agrees on a framework to govern gene editing in people. The panel says that it should cover studies of the clinical applications of human genome editing – including both changes to the germline and techniques that alter a person's genes in ways that won't be inherited. The latter has not generally been controversial. For example, if we edited a germline, future generations wouldn't be able to opt out. Genetic changes might be difficult to undo [14-16].

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