PHARMACEUTICAL VISIONS

Spring/Summer 2017 re-launch edition

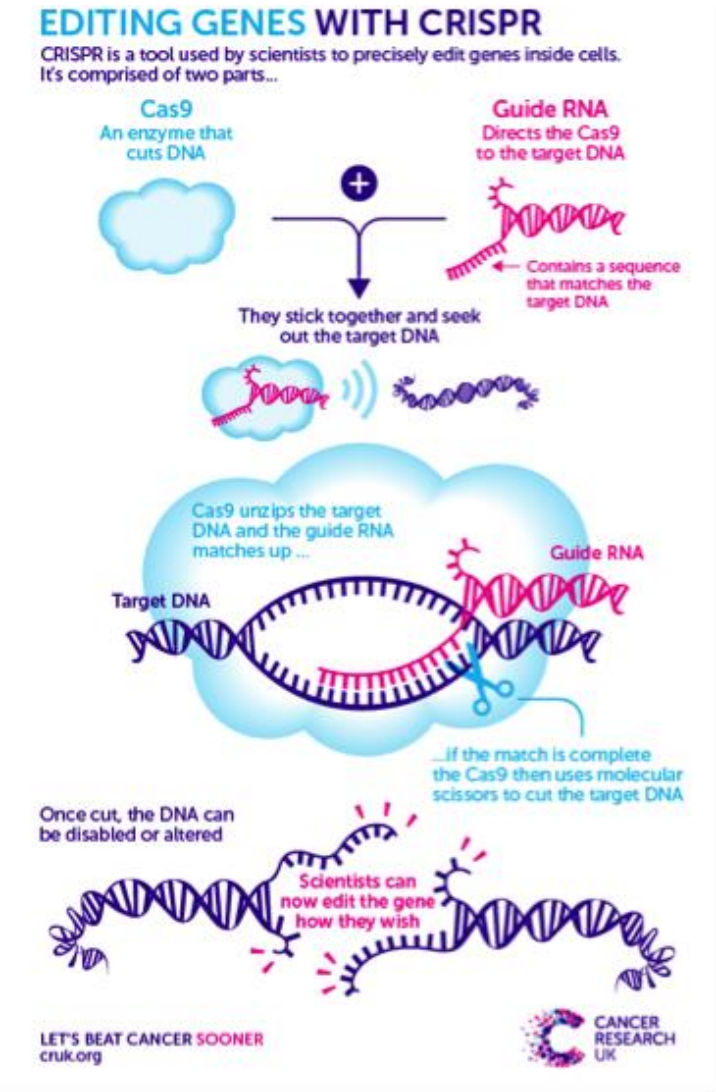
CRISPR: A New Frontier Of Cutting-edge Science

The powerful gene-editing technology CRISPR (clustered, regularly interspersed palindromic repeats) brings a whole new, and very literal, meaning to the words 'cutting-edge science'. The unprecedented ease and speed at which CRISPR and Cas (CRISPR-associated proteins) can locate and cut the genome of any species has transformed the scientific landscape and unearthed a wealth of opportunities to help feed, fuel and heal the world. *Amelia Vale explains*

The CRISPR revolution has also coincided with an explosion of readily available genomic data. After 14 years of scientific analysis, the Human Genome Project is finally bearing fruit. The sequencing of the DNA letters from our 25,000 genes has enabled scientists to unscramble the genetic mistakes behind previously untreatable diseases; making the possibility of correcting these mistakes a target within sight. The drop in the cost of sequencing the human genome – from $50 million in 2003 to just $1,000 today –

together with the arrival of CRISPR in 2013, has brought this target that much closer. Likened to word-processing software, it can cut and paste DNA letters, which could ultimately ‘fix' faulty genes forever.

CRISPR/Cas9, shortened to CRISPR, is based on an immune response that bacteria use to protect themselves against attacking viruses. When bacteria detect the invading viral DNA, they produce two types of short strands of RNA, one of which contains a sequence that exactly matches that of the invading virus. These two RNAs join forces with an enzyme called Cas9, which is a nuclease molecule that can cut DNA. The RNA molecule guides the Cas9 enzyme to the target sequence of viral DNA that it then cuts to disable the virus. Scientists have harnessed this system to cut the DNA of any organism by programming the guide RNA molecule to home in on any DNA sequence they want to change.



The industry impact

With genetic science racing ahead, the industry has also moved up a gear. This has generated a multibillion dollar biopharmaceutical sub-sector which is developing and manufacturing products made from a diverse range of living organisms. Called biologics, the sector spans numerous markets beyond medicine, including agriculture and biofuels, and is expected to grow in value from $209,779 million in 2016 to $479,752 million by 2024. [1]

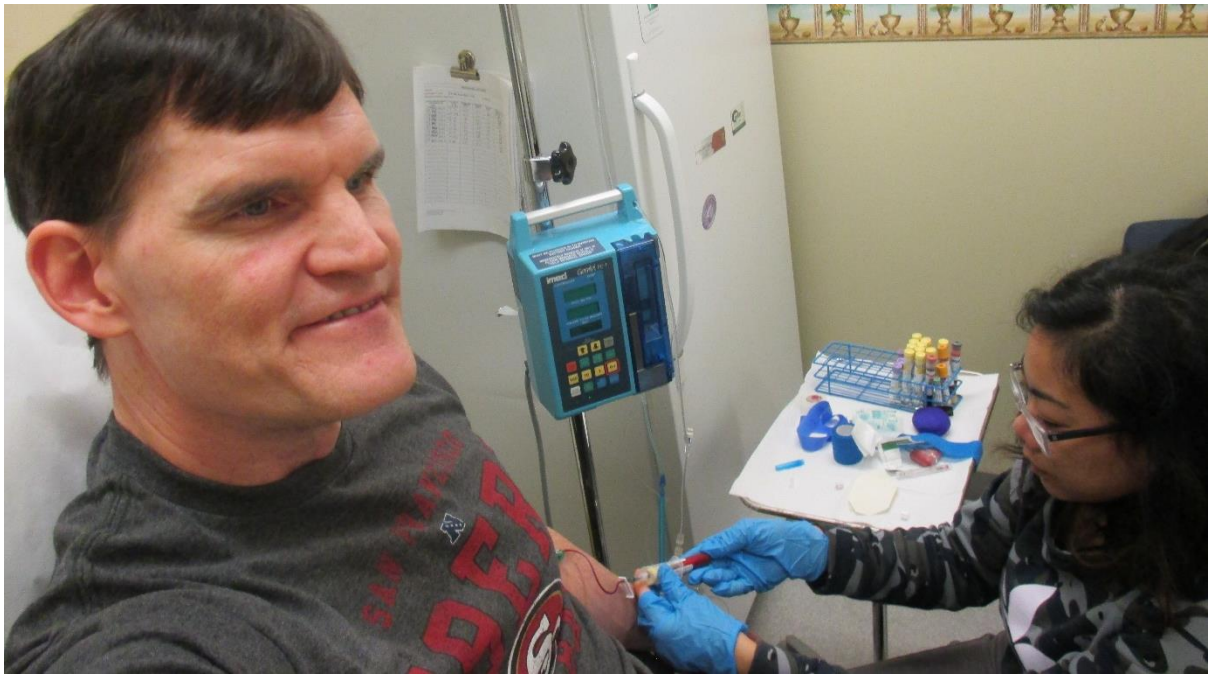
Considering that until six years ago, CRISPR was used for little more than making yogurt, the potential of this rising star is nothing short of meteoric. In 2011, two groups of scientists at opposite ends of the United States began studying the possibility of using CRISPR to cut and edit DNA. As the scope of the technology became increasingly apparent, a battle broke out for the rights to the new use of CRISPR. Finally, the case between Professor Feng Zhang's lab at the Broad Institute of Harvard and the Massachusetts Institute of Technology (MIT), and Professor Jennifer Doudna and Emmanuelle Charpentier's group at the University of California, Berkeley, was decided in a patents' court.

Earlier this year, the key CRISPR genome editing inventions were awarded to the Broad Institute. At the tender age of 36, the decision has secured Professor Zhang a place in medical history. Since 2013, the Broad Institute has awarded more than a dozen licences for commercial use of its CRISPR technology to industry giants, including GE Healthcare, Evotec AG, AstraZeneca and the agricultural firm Monsanto.

Professor Zhang has also capitalised on his achievements and launched the start-up Editas Medicine, which commanded an opening share price of $22.50 last year. Editas now holds exclusive licences to all applications of Zhang's CRISPR inventions for diseases, and the work to turn them into cutting-edge treatments is well underway. Editas already has a diverse pipeline which includes eye diseases, blood disorders, cancer, lung and liver conditions.

'Of the estimated 6,000 diseases that are caused by a genetic mutation, more than 95 per cent have no approved therapeutic option,' Professor Zhang points out. 'Where options do exist, they often only treat the symptoms of disease or modify the course of disease, but not the root cause. By turning the gene on or off or manipulating it in some other way, we are addressing the underlying genetic defect,' he explains.

CRISPR is very much a work in progress according to Professor Zhang. Despite having a wife and daughter, he often returns to his lab after dinner and works into the early hours. 'There is definitely room for improvement. Firstly, we can make the RNA guide more accurate by getting the RNA to be better matched, so fewer off-targets. And secondly, we can improve the cleavage part. In fact, we have already found four other cleavage molecules which are more versatile and specific than Cas9,' he says. [2]



ED WALSH RECEIVING HIV GENE EDITED CELLS

The efforts with HIV

CRISPR is far more effective than the cumbersome genome editing tools Professor Zhang first used. At the time, however, they were seen as a major breakthrough. In 2010, HIV patients were the first to receive gene-editing treatments, in an attempt to block off the receptor that the virus uses to enter immune system T-cells. Although some trial participants have seen modest improvements [3], one patient believes the treatment actually put his life at risk.

Initially Ed Walsh, a writer from San Francisco, was keen to help advance HIV research. Having been recently diagnosed, Ed had a robust immune system, so he thought he had nothing to lose. But the trial was far longer and harder than he had anticipated with complications and side-effects at every stage. 'I know that my T-cell level went from 1200 [cells/mm3] to about 200 during the study, so I was on the verge

of developing AIDS, and since then it has not gotten over the 600s. My platelet level is still on the low side as well. It's been a really scary time,' Ed reflects.

With CRISPR now entering the fray, HIV might just have its work cut out for it – literally. In early May researchers at the Lewis Katz School of Medicine at Temple University proved that they can engineer CRISPR to excise HIV DNA from T-cells and eliminate infection in mice cells.[4] By shutting down HIV's replication and infiltrating its hidden reservoirs, which have been resistant to all other treatments, their achievement at this stage is unprecedented.

It is exactly the kind of success that is making CRISPR a magnet for innovators from other industries as well. Two brothers, Paul and Michael Dabrowski, who had previously worked for the Space Exploration Technologies Corporation, decided to turn their hands to engineering genes instead of rockets. Since launching their start-up, Synthego, last August, they have been offering synthetic RNA guides, instead of the plasmid and in-vitro transcription classics. Synthego's designs are winning custom from around the world due to their efficiency and consistency, meaning the Dabrowski brothers, despite having no genetics background, are mastering the CRISPR marketplace.

CRISPR and immunotherapy

Advancing innovations and commercialisation aside, the true measure of CRISPR's success will be how well it actually works. The first trials to use CRISPR on patients in the US will soon be underway at the University of Pennsylvania. Led by Dr Carl June and Dr Edward Stadtmauer, they hope to advance on the group's ground-breaking chimeric antigen receptor (CAR) immunotherapy studies.[5] Their genetically engineered immune cells, which 'search and destroy' cancer cells, have been surprisingly effective in treating blood cancers. However, translating this success into solid cancer tumours has been far more problematic. But CRISPR is now being brought in to advance the immunotherapy approach by treating 18 patients who either have melanoma, sarcoma or multiple myeloma.

Each patient's T-cells will be withdrawn, undergo three CRISPR edits and then be re-infused into their bone marrow. The first edit will insert a gene for the CAR protein, the second edit will remove a natural T-cell protein that could disrupt this process and thirdly, a gene for the programmed cell death protein 1 (PD-1), which normally puts the

breaks on an immune response, will be edited out. It is hoped that this triple whammy will activate the most powerful immune response humanly possible and eradicate the cancer.

However, this is not the first CRISPR gene-editing human trial: the Chinese have beaten the US to it. Last October, scientists at Sichuan University edited the PD-1 genes from the T-cells of a patient with metastatic non-small cell lung cancer. [6] They hope that this single cut will be sufficient but before the results are even released, a second team of Chinese researchers is already enrolling patients to treat five other late-stage cancers with CRISPR. [7]

Issues using human embryos

The US proposals have been slowed down by the lengthy regulatory and ethical discussions that come with the territory; whereas the rapid advance in China has raised serious concerns about the scientific and ethical consequences of CRISPR. In 2015, a group of Chinese scientists were the first to edit human embryos. An attempt to replace the β-globin gene responsible for the blood disease beta-thalassemia resulted in such poor uptake and unintended gene mutations that the researchers had to go back to the drawing board. 'Taken together, our work highlights the pressing need to further improve the fidelity and specificity of the CRISPR/Cas9 platform, a prerequisite for any clinical applications,' they admitted. [8]

Any changes made to the DNA of human embryos would affect all other cells in the body, including sperm and egg cells (known as germline cells), meaning that they would be passed on to future generations. If such unintended gene mutations were to happen in a human treatment, the consequences would be disastrous. Scientists could accidentally create new diseases, the likes of which we would be totally unfamiliar with. 'CRISPR is an enormously important path of research, particularly for diseases where adding a single gene isn't going to work,' says Professor Don Kohn, a pioneer of gene-editing at the University of California, Los Angeles. 'But I see editing embryos as a line that shouldn't be crossed.'

Last year a panel of international experts was drawn up to discuss how these new technologies should be governed in future. The consensus was that human trials for genetically engineering germline cells could be permitted but only for treating serious diseases, under strict oversight.(9) 'We agreed that we should keep an open mind about

this research,' says Professor Robin Lovell-Badge, a panel member and head of Stem Cell Biology and Developmental Genetics at the Francis Crick Institute in London. 'If treatments are going to happen, we want it to be somewhere where there are proper regulations and where society supports it,' he stresses.

But the best scientific intentions will not prevent what Dr David King – founder director of Human Genetics Alert, a watchdog group based in London – describes as consumer eugenics. 'Gene-editing will be used to enhance humans in sport, the army and then the public at large. It will be divisive and create greater inequality between those who can afford to pay for it and those who can't. It will be a disaster for our society,' he argues.

Ultimately, CRISPR could change the code of life for good. Therefore, it is vital everyone joins this debate and ensures that any future decisions and policies are for good: the good of the human race.

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