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Editorial

A race to bring CRISPR to the clinic

It has now been two years since we first wrote about the therapeutic potential of CRISPR-Cas9 in our May 2015 Editorial "CRISPR–Cas9 Based Therapeutics: Not So Fast".

At the time, we shared serious concerns resonating within the community that the potential for undesirable off-target mutations was significant, and that the technology was not ready for introduction into humans as a gene modification strategy. Since then, the field has moved at lightning speed. Researchers have made great strides toward eliminating off-target effects and improving specificity. This has been achieved in part by modifying the Cas9 enzyme, using shorter guide RNAs, and by reducing the amount of Cas9 expression within the cell, for example. Furthermore, the ability to detect and monitor any potential off-target mutations has been greatly improved, with extremely sensitive detection methods such as CIRCLE-seq. This technique combines PCR amplification of regions containing Cas9 cleavage sites with next-generation sequencing to provide highly accurate detection of CRISPR-Cas9 mutation sites.

Along with a substantial increase in targeting specificity, the repertoire of practical applications for CRISPR-Cas systems has also expanded—within both the basic and clinical realms. For example, in the April 28th 2017 issue of *Science*, researchers report the development of SHERLOCK—a nucleic acid detection method combining the specificity offered by CRISPR-Cas (in this case Cas13a) with isothermal amplification to detect attomolar levels of target RNA or DNA. The authors were able to use SHERLOCK to detect very low levels of Zika virus (ZIKV) and to distinguish ZIKV from dengue virus, which can be a challenge with current diagnostic tools, given the genetic relatedness of these two flaviviruses. To further demonstrate utility, they also applied the technique to detect antibiotic resistance genes within pathogenic bacteria, and to identify mutations in cell-free tumor DNA. One can imagine a range of potential diagnostic and research applications for this technology in the future.

In addition to this exciting new tool, the CRISPR-Cas system has been used in countless recent basic research applications including screens to identify genes essential for tumorigenesis, identification of host factors relevant for pathogen survival, and for the creation of disease-relevant preclinical models. Cellular, organoid, and animal models have recently been created using CRISPR for a wide range of diseases including cancer, atherosclerosis, and neurological diseases. CRISPR has also been used to *treat* disease in mammals, within a mouse model of Duchenne muscular dystrophy (DMD) where the mutated dystrophin exon was removed. Researchers delivered the Cas9 gene modifying system using an AAV-9 vector—systemically in neonatal mice, and both systemically and locally in adult mice. Dystrophin expression was induced in differentiated muscle cells and cardiomyocytes as well as muscle cell precursors. Partial recovery and enhancement of muscle function was observed, providing *in vivo* proof-of-principle that this method may someday be applied to treat DMD and other human genetic diseases.

There are also multiple examples where CRISPR-Cas9 has been used to eliminate viruses such as HIV from infected cells, including several *in vivo* mouse models mimicking various aspects of disease—including latent infection. One can imagine a future where CRISPR-Cas9 will be an important contributor to efforts aimed at HIV cure. The next step for CRISPR-based HIV therapy will be to test the system in non-human primates, and to take measures to prevent viral escape, before introducing this antiviral approach into humans.

However, perhaps the most exciting new development in the CRISPR field is that the method has now been cleared for a small number of clinical trials in humans, which will use the approach to alter genes expressed within human somatic cells. The first person to receive CRISPR-based therapy (in late 2016) was a man with an aggressive form of lung cancer who had not responded to other treatments. Chinese researchers removed immune cells from the patient, and used CRISPR-Cas9 to knock-out the gene encoding PD-1 before expanding the cells and infusing them back into the patient. As with other successful immunotherapies that have targeted this checkpoint inhibitor, the hope is that by removing PD-1, the cells can then be kick-started to fight the cancerous cells in the patient's body. This trial is expected to expand and include more patients, and a second clinical trial in China looking at using this application for the treatment of a range of cancers began in April 2017. Results have not yet been announced.

A similar immunotherapy trial has also been given the green light in the US, at the University of Pennsylvania. This clinical trial will differ somewhat from the Chinese trials, in that the researchers plan to use CRISPR to target both PD-1 and the endogenous T-cell receptor within engineered CAR-T cells, which have been designed to specifically attack melanoma cells.

The results for these pilot CRISPR-Cas9 trials are, of course, eagerly anticipated by the entire biomedical research community. Has Cas9 specificity been improved enough that we can safely assume off-target effects will not be harmful to the patient, even though in this case only somatic cells will be altered? How will this approach compare to conventional checkpoint inhibitor strategies? Initial results are expected at some point this year, and could pave the way for other somatic cell therapies. We may still be some way off from CRISPR-based germline therapies, but positive endpoints and demonstrable safety outcomes from these trials would be a thrilling step forward.

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