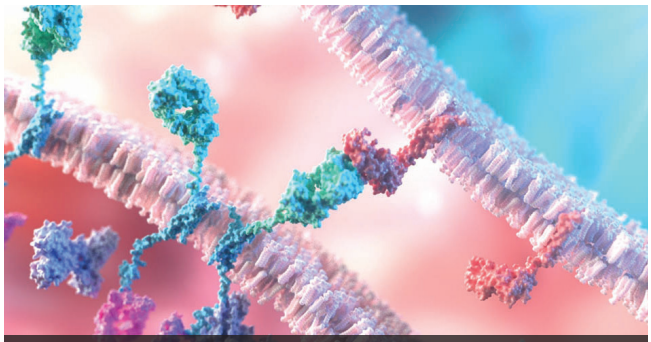
CRISPR expands CAR T cell possibilities

As interest in immunotherapy grows, so does the need for BETTER TOOLS TO ENGINEER CAR T CELLS. Could advances in CRISPR technology be the solution?



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T cells engineered to express chimeric antigen receptors (CAR T cells; bottom left) can bind to tumour cells (top right) and attack blood cancers. Engineering CAR T cells using CRISPR is now possible using a new technique and high-quality ssDNA.

In the last decade, a new immunotherapy tool has entered the clinic. T cells engineered to express chimeric antigen receptors, known as CAR T cells, have been shown to help patients with blood cancer. A pivotal study[1] in 2011 used second-generation CAR T cells to achieve sustained T cell activation and remission for the majority of patients tested.

A year later came another major breakthrough, as two groups described[2,3] a novel gene-editing tool called CRISPR-Cas9 and demonstrated its use in eukaryotic cells. These two reports helped start a new era in gene editing.

Although gene editing had the potential to improve cell engineering, it would be several years before CAR T cells and CRISPR crossed paths.

Finding the path

From the beginning, CAR T cells showed great cancer-killing potential, but generating these cells was cumbersome and complicated.

Initially, T cell engineering depended on lentiviral or retroviral vectors to deliver DNA fragments into a cell for homologous recombination. Viral vectors allow for stable integration of DNA fragments and long-term expression. However, the clinical-grade reagents needed to obtain these vectors are costly, and the vectors can only carry a limited amount of DNA which has the potential to randomly integrate into the genome.

To advance CAR T cell therapy, researchers needed to find a more efficient way to engineer long CAR sequences.

CRISPR drives the CAR

From the outset, CRISPR looked like an ideal way to engineer a T cell. It's a simple process, with minimal off-target effects, and works on a wide range of cell types. But there's one area where CRISPR has struggled.

CRISPR-Cas9 is effective at generating small mutations by creating targeted double-stranded DNA (dsDNA) breaks that are then repaired by the cell's non-homologous end-joining pathway. However, when it comes to inserting exogenous DNA using homology-directed repair mechanisms, CRISPR editing can be woefully inefficient. Yet, inserting DNA is crucial to engineering CAR T cells.

"Easi-CRISPR is a much better tool when it comes to homology directed repair," says Channabasavaiah Gurumurthy, a gene-engineer at the University of Nebraska Medical Center in Omaha, who co-invented Easi-CRISPR in 2017.

Gurumurthy and his collaborators discovered that, when it comes to using CRISPR to insert DNA into a cell, long single-stranded DNA (ssDNA) is a more effective template than double-stranded. With Easi-CRISPR, long ssDNA is injected along with a preassembled complex containing Cas9 and guide RNAs, resulting in higher rates of on-target editing and lower rates of off-target editing[4]. Alongside previous reports showing chemically synthesized single guide RNAs (sgRNA) with 2′-*O*-methyl and phosphorothioate end modifications enhanced intracellular stability and editing efficiency in primary cells[5], it was starting to look as if CRISPR-Cas9 could be an effective tool for generating both deletions and insertions within T cells.

Everything came together last year when Alexander Marson, Gurumurthy, and colleagues used Easi-CRISPR to reprogram the structure and function of human T cells without the need for viral vectors[6]. This study demonstrated that CRISPR editing of T cells using ssDNA as a homology-directed repair template was more accurate and effective for large gene insertion, with less off-target integration, than dsDNA templates.

Easing into the future

While promising, there was one important consideration. "Long ssDNA sequences are difficult to produce in the lab, especially at the high concentrations necessary for gene editing experiments," says Theodore Roth, a member of Marson's lab and first author of the study[6].

Several companies and academic developers are trying to solve this problem, working on methods to effectively generate large amounts of long ssDNA. GenScript, a global biotechnology company based in Piscataway, NJ, known for its leading DNA synthesis technologies, recently began providing ssDNAs several thousand nucleotides long, in quantities up to 100 micrograms — ideal for T cell reprogramming using CRISPR. GenScript is also one of the few companies that provides total CRISPR solutions, including HPLC-purified, chemically synthesized sgRNAs with end modifications to enhance CRISPR editing.

Gene editing is changing the way researchers approach cell engineering. Methods like Easi-CRISPR, along with improvements in DNA and RNA synthesis, are poised to further enhance CAR T cell engineering efforts, ultimately improving cancer therapy and human health.

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3. Cong, L., *et al. Science* **339**, 819–823 (2013).
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