IPWatchdog

CRISPR Modified CAR T-Cells Bolster Immuno Oncology Arsenal

By Sanjeev Mahanta, Ph.D., J.D. / August 27, 2018

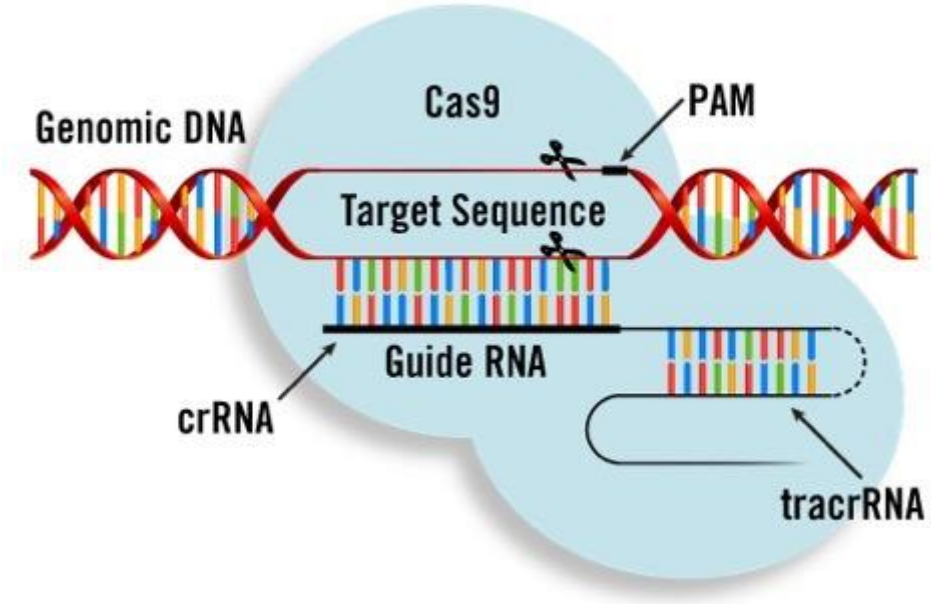
CRISPR (pronounced "crisper") and CAR T-cell therapy are genetic technologies that have generated continued excitement during the last several years. CRISPR is a genome editing tool that allows alteration of a genome. Compared to other genome editing tools, it is faster, cheaper, and more accurate. Its applications are numerous, ranging from agriculture to gene therapy. On the other hand, CAR T-cell therapy is a form of adoptive cellular immunotherapy. Adoptive cellular immunotherapy involves infusion of cells into a patient to help the patient's immune system fight disease. In CAR T-cell therapy, the infused cells are a type of immune cell known as T-cells. Significantly, these cells are derived from the very patient undergoing therapy and modified genetically prior to being transferred back into the patient to destroy cancer cells.

Both technologies are experiencing exponential growth. A recent patent landscaping study that considered patent publications since January 2007, identified 4800 unique CRISPR related patent applications spread across about 1750 INPADOC (International Patent Documentation) families. As to CAR T-cell therapy, adoptive cellular immunotherapy, generally, was named as the Clinical Cancer Advance of the Year 2018 by the American Society of Clinical Oncology. Also, in 2017, FDA approved the first two CAR T-cell therapies, Kymriah™ and Yescarta™, indicated, respectively, for a form of leukemia and a form of lymphoma.

While CAR T-cell therapy holds great promise in the fight against cancer, significant hurdles remain preventing realization of its full potential. Several innovative approaches are under investigation to overcome these hurdles. The present article provides a peek into this area of innovation by focusing on the invention described in a recently published patent application, WO/2018/115887, relating to novel CAR T-cells engineered using CRISPR. Improvements described in WO/2018/115887 are designed

to dramatically simplify CAR T-cell therapy and has the potential to significantly bring down the cost of the therapy. Currently, CAR T-cell therapy is highly expensive. According to a recent report, a single dose of Kymriah™, (which, incidentally, is sufficient), costs $475,000.

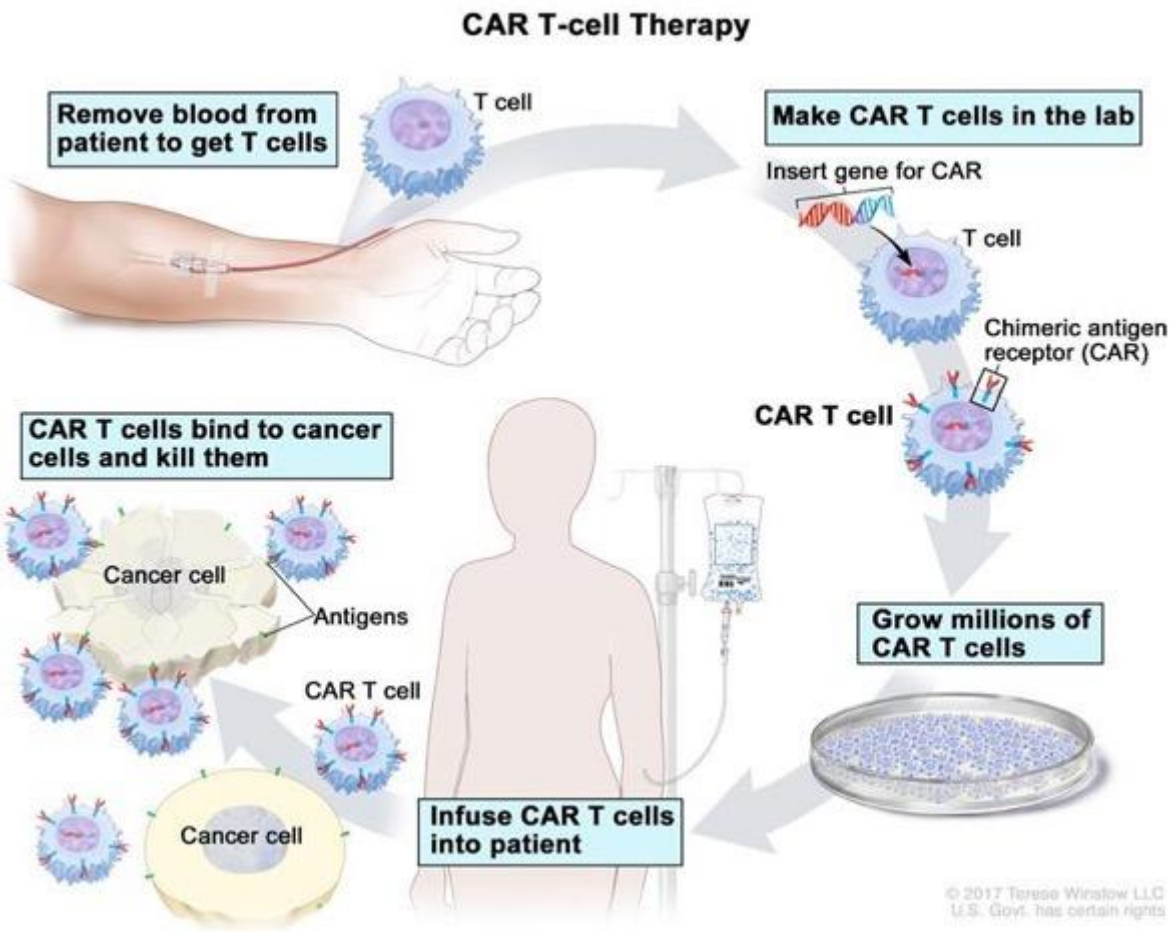
To appreciate the power of CRISPR and its use for improvements to CAR T-cells, a basic understanding of both technologies is helpful. By CRISPR is meant CRISPR-Cas9, which is short for clustered regularly interspaced short palindromic repeats and CRISPR-associated protein 9. Cas9 is an enzyme that cuts foreign DNA. The CRISPR-Cas9 system was adapted from a naturally occurring genome editing system in bacteria. In this system, CRISPR is a specialized region of DNA having two distinct features: (i) repeats of nucleotides (the building blocks of DNA) distributed across the region and (ii) spacers, which are pieces of DNA interspersed among these repeats. Bacteria capture snippets of DNA from invading viruses and use them as the spacers. This allows the bacteria to "remember" viruses it was previously attacked by (or closely related ones) to help fight off future attacks by those viruses. In a repeat attack by a virus, a portion of the CRISPR is used as a template to produce a so-called CRISPR RNA ("crRNA") having a sequence complementary to both a repeat and a spacer DNA. Then, Cas9, through binding to the crRNA and another RNA called tracrRNA (trans-activating crRNA) is guided to a target site on the virus genome that is complementary to a 20-nucleotide stretch of the crRNA. Once at the site, Cas9 cuts both strands of the DNA double helix (see drawing below), thereby inactivating the virus.



Simon Levin, 2017, CRSPR in the classroom

For genome editing, Cas9 can be directed to cut any region of a genome by simply changing the nucleotide sequence of crRNA to make it complementary to a target DNA in the genome. In practice, crRNA and tracrRNA are fused to create a single so called "guide RNA." Once the DNA is cut, the cell's natural repair mechanisms are triggered to correct the damage. This process is prone to error, resulting in accidental insertion or deletion of nucleotides that can disrupt a gene and destroy its function. Alternatively, the break can be fixed with a desired sequence of nucleotides used as a filler. The latter process allows precise insertion of exogenous DNA of choice to modify a genome.

CAR T-cell therapy, as already noted, is a form of adoptive cellular immunotherapy. It involves collecting T cells from a patient, growing the cells in the laboratory to increase their numbers, inserting an artificial gene into the cells, and transferring the cells back to the patient to help fight disease (see the diagram below). T cells are a type of immune cell which, like many other immune cells, circulate in the body to detect foreign organisms such as bacteria and viruses, and even cancer cells, which can appear different from normal cells.



CAR T-cell therapy, NCI Dictionary of Cancer Terms

For detecting foreign cells, T cells use molecules known as T cell receptors (TCRs), which act as feelers to scan for molecules present only on the surface of infected cells. Upon detecting such a cell, the TCR signals the T cell to respond. One T cell subset, the cytotoxic T cells, respond by destroying the foreign cell. Another T cell subset, the so-called helper T cells, respond by producing cytokines, which are molecules that have effect on other cells. Some of these cytokines help the cytotoxic T cells perform their cell-destroying function.

The artificial gene inserted into the T cells must be able to detect the unique protein on the cancer cell surface. This gene, the chimeric antigen receptor (CAR), is assembled by fusing two components: a detection domain, made from recognition elements of an antibody that binds the unique tumor associated protein on the cancer cell surface, and a signaling domain. At the most basic level, the signaling domain is made of a

membrane spanning region and the intracellular part of the T cell receptor associated protein CD3-ζ. Once transferred into the patient, the CAR T-cell can recognize a cancer cell through the detection domain leading to destruction of the cancer cell.

Turning now to WO/2018/115887, this patent application describes the engineering of a so-called universal or off-the-shelf CAR T-cell. One embodiment of such CAR T-cell is shown below in Fig. 3 of the application. In this drawing, the cell labeled Cytotoxic T cell is the CAR T-cell and the one labeled Malignant B cell is the cancer cell. CD20 is the cancer associated protein targeted by the CAR on the CAR T-cell.

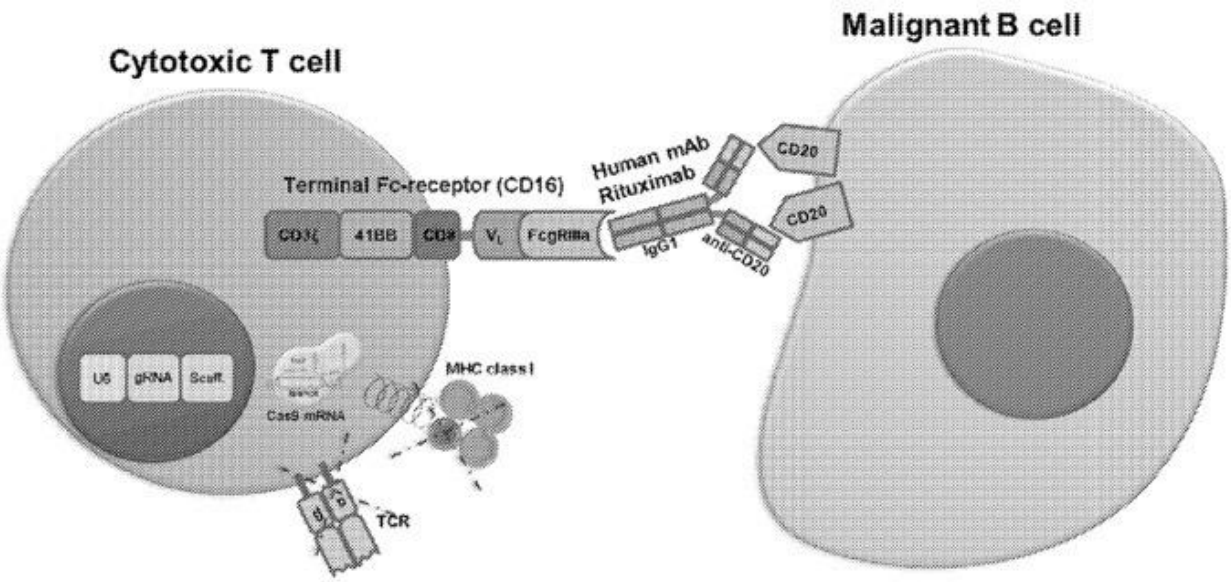


Fig. 17 of WO/2018/115887 filed 21 Dec 2017

The inventors' goal was to produce CAR T-cells for treating a patient using T-cells taken from any individual, not just the patient undergoing therapy. Currently, only T cells taken from the very patient seeking CAR T-cell therapy may be used to generate CAR T-cells lest the cells should be rejected by the patient's immune system. In other words, today's CAR T-cell therapy is personalized, one therapy for one patient. This makes the therapy both time consuming and expensive.

The engineered cells have another important feature. They can be used to treat cancers bearing different cancer associated targeting proteins, thereby dramatically simplifying the therapy. Currently, for each cancer associated targeting protein, a unique chimeric

gene – one having a detection domain that specifically recognizes that targeting protein – must be constructed.

In another improvement, the inventors used T cells isolated from umbilical cord blood. Umbilical cord blood T cells provide several advantages including the fact that the cells are not biased toward any particular immunological function, have extensive capacity to multiply, and are potent, all of which are attributes that can make a CAR T-cell more effective.

To make the CAR T-cells resistant to attack by the patient's immune system, the inventors altered the genomes of the cord blood T cells using CRISPR such that the cells lost their ability to produce MHC class I proteins (shown by a cross sign in Fig. 3). MHC class I proteins vary from individual to individual and are involved in the rejection of graft (the transferred tissue/cells) by the host immune system when there is a mismatch in MHC class I between the donor and the host.

Using CRISPR, the inventors altered the T cell genome further to also disrupt the expression of TCR (also shown by a cross sign in Fig. 3) such that the cells are rendered incapable of attacking the recipient's tissue in what is known as graft vs host disease.

The CAR T-cells were additionally modified to make them capable of targeting a cancer cell irrespective of the specific cancer associated protein displayed on its surface. To accomplish this, instead of using a detection domain derived from portions of an antibody that specifically binds to the cancer associated protein, the inventors used a portion of a protein called Fc gamma receptor (FcgIIIa in Fig. 3) which can bind to a shared region possessed by all antibodies (of a certain class e.g., IgG_1 in Fig. 3). Different antibodies used for targeting different cancer associated proteins can all thus be recognized by the engineered CAR T-cell through the FcgIIIa portion, eliminating the need to have a separate CAR T-cell, bearing a unique detection domain, for each cancer associated protein.

To conclude, CAR T-cell therapy is being refined constantly. Much like other cancer treatments, CAR T-cell therapy too has side effects, some of which are quite serious

and can lead even to death. These include the so-called cytokine release syndrome (CRS), in which there is a large and rapid release of cytokines into the blood, which can lead to high fever, troubled breathing, and dramatic drop in blood pressure. Not surprisingly, improvements that would allow control of CRS are of great interest. Another area of improvement is the simultaneous targeting of multiple cancer associated proteins to reduce chances of relapse (see Ruella, M. et al. J Clin Invest. 2016;126(10):3814-3826). With respect to universal or off-the-shelf CAR T-cells, one version of such cells has already been tested in a clinical trial and positive results obtained (Qasim, W. et al., *Sci. Transl. Med*. 2017, 9:74, eaaj2013). In this version of universal CAR T-cells, a genome editing tool other than CRISPR was used and the expression of TCR and a molecule other than MHC class I was knocked out. There is no doubt that many more improved CAR T-cell therapies will be tested in the coming months and years, some of which would likely not have been developed without CRISPR.

The Author

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