CRISPR-engineered T cells in patients with refractory cancer.

E. A. Stadtmauer et al., CRISPR-engineered T cells in patients with refractory cancer. Science. ***367*** *(2020), doi:10.1126/science.aba7365.*

Reports by: Brendan Finicle[1] and Rebecca Vargas[1]
[1]Spring 2020 Journal Club, Department of Developmental and Cell Biology, UC Irvine

CRISPR-engineered T cells in patients with refractory cancer.

Scientific Report by Brendan Finicle[1] and Rebecca Vargas[1]
[1]Spring 2020 Journal Club, Department of Developmental and Cell Biology, UC Irvine

Introduction.

Shifting focus from targeting the tumor itself to targeting the host's immune system, immunotherapy has revolutionized the therapeutic landscape for cancer (*1*). The successes of immunotherapy have been dramatic – patients with fatal metastatic disease now live cancer-free due to this technology. By measure of lives saved and years added to patients' lives, immunotherapy is likely to be more successful than any other form of therapy for patients with metastatic solid tumors due to its ability to cure, not just extend survival. Despite its revolutionary impact, adoptive cell transfer therapies are limited by poor persistence due to T cell exhaustion and many tumors can acquire resistance over the course of their treatment with immunotherapy. In February of 2020, Stadtmauer *et al* publish in *Science* a critical advance to increasing the efficacy of immunotherapy in human patients by using CRISPR editing technology in T cells derived from human patients with advanced, metastatic cancer to reduce T cell exhaustion (*2*). A summary of their findings is shown in Fig. 1.

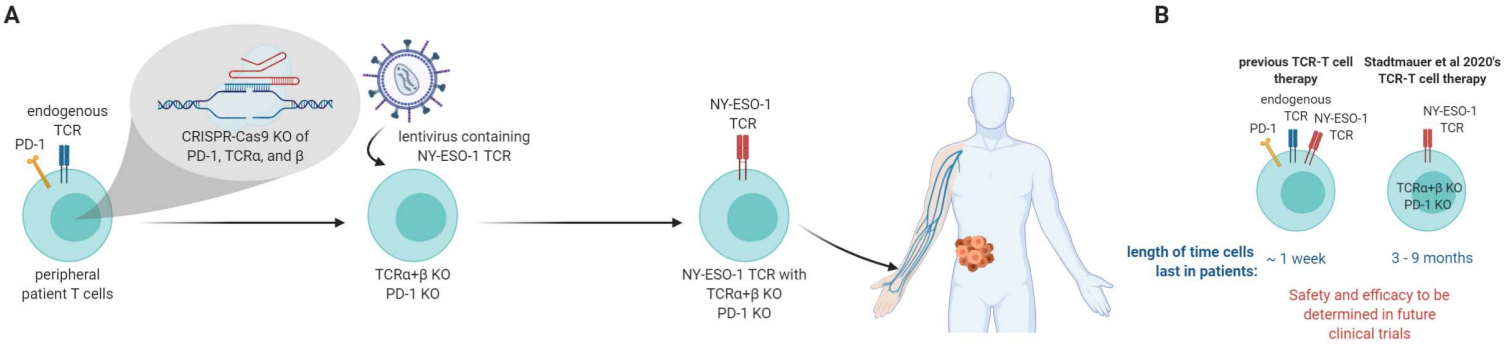


Fig. 1: Improving TCR-T cell therapy by deleting endogenous TCR and PD-1 via CRISPR-Cas9. In short, peripheral T cells were acquired from patients, and the alpha and beta subunits of the TCR and PD-1 were knocked out using CRISPR-Cas9. A transgenic TCR that targets the tumorantigen NY-ESO-1 was transduced in the T cells. Then the ex vivo engineered T cells were placed back in the patient. By knocking out PD-1 and the endogenous TCR, persistence was dramatically improved in human patients. Biorender was used to make this figure.

The current state of cancer immunotherapy.

While the anti-tumor role of the immune system has long been known, targeting the host's immune system increase the anti-tumor response is a relatively new concept. Cancer immunotherapy can be divided into two types: cell-based and checkpoint inhibitor therapies.

Checkpoint inhibitors: anti-PD-1 and PD-L1: Checkpoint inhibitor therapies use therapeutic antibodies to inhibit receptors on immune cells or tumor cells that turn off the immune response. PD-1 is a checkpoint receptor expressed by CD8-positive T cells and normally functions as a cell surface protein that prevents autoimmunity by turning off the immune response caused by inflammatory T cells (*3*, *4*). During a standard infection, inflammatory T cells secrete IFNγ. In response to sustained exposure to IFNγ, surrounding cells will upregulate expression of PD-L1, the ligand for PD-1, which binds to and activates PD-1 on CD8-positive T cells. PD-1 activation shuts down CD8-positive T cells and limits the killing of host cells. In the cancer context, tumor cells will upregulate PD-L1 surface expression to evade attack by CD8-positive T cells. Checkpoint inhibitor immunotherapy exposes tumor cells to the immune system by inhibiting PD-1 or PD-L1. Blocking binding of PD-1 with PD-L1 unleashes activated tumor-reactive cytotoxic T lymphocytes and produces sustained anti-tumor responses. Indeed, the efficacy of this approach is illustrated by the approval of monoclonal anti-PD-1 antibodies and an anti-PD-L1 antibody for the treatment of multiple advanced, aggressive, and metastatic cancers (*3*, *4*). In summary, checkpoint blockade therapies have represented a major advance in cancer therapy, especially in the treatment of deadly metastatic diseases like melanoma.

Cell-based immunotherapy: TCR-T and CAR-T: Instead of enhancing the anti-tumor immune response by unblocking inactivated immune cells, immune cells can also be engineered to target tumor cells via a process known as adoptive cell transfer. Recent advances in genome editing and gene transfer technology have produced TCR-T and CAR-T cell therapy.

TCR-T cell therapy involves isolating peripheral T cells from a patient and introducing physiologic T cell receptors (TCR) that recognize tumor antigens (*5*). TCRs are receptors expressed by T cells that are responsible for mediating T-cell killing by recognizing antigens on target cells. In this case, the TCRs are cloned from tumor infiltrating lymphocytes isolated from the patient. Because TCR activity depends on the major histocompatibility complex (MHC) system for antigen presentation and recognition, tumors can acquire resistance by turning off antigen presentation. To avoid MHC dependence, researchers have cloned TCRs that recognize tumor antigens widely expressed by many tumor cells. For example, NY-ESO-1 is a cell surface protein that is normally only expressed in male germ cells but is overexpressed by many tumor cells, especially sarcomas, melanomas, and myelomas (*6*). While its function remains elusive, it is a useful antigen for engineered immune cell recognition as NY-ESO-1 targeting TCRs can be cloned into T cells to facilitate targeted destruction of NY-ESO-1-expressing tumor cells.

CAR technology involves engineering T cells with chimeric antigen receptors (CARs) that link single chain antibodies recognizing tumor cell surface antigens to the intracellular portions of TCRs (*1*). CAR-T therapy has produced major success in many cancer classes and have saved patients with late stage disease previously thought to be incurable. Unfortunately, CAR-T cell therapy produces a dangerous side effect: cytokine release syndrome (CRS). When large number of T cells (or other white blood cells) are activated, they release inflammatory cytokines. The released inflammatory cytokines produce wide ranging effects on the body that can be life threatening. TCR-T cell therapy produces CRS with lower frequency than CAR-T cell therapy.

While ex vivo expanded and engineered T cells can produce amazing anti-tumor responses, this technology is currently limited by T cell exhaustion. T cell exhaustion is the reduced function of T cells due to over-stimulation and upregulated expression of inhibitory receptors. When ex vivo engineered T cells are supplied to patients (e.g., TCR-T or CAR-T cell therapy), PD-L1 expressed by tumor and host cells acts in concert with PD-1 to turn off T cell function. Additionally, in the context of TCR-T cell therapy, transgenic TCR expression competes for cell surface expression with endogenous TCR subunits. In summary, endogenous PD-1 and TCR expression in ex vivo T cells reduces T cell function and persistence, limiting the anti-tumor response.

Improving TCR-T cell therapy by deleting endogenous TCR and PD-1 via CRISPR-Cas9 technology.

In Stadtmauer *et al*, the authors attempt to increase TCR-T cell persistence in human patients with advanced refractory myeloma and metastatic sarcoma. Expression of the checkpoint receptor PD-1 is a critical limitation to adoptive T cell therapies because it causes T cell exhaustion. Additionally, while supplementation of exogenous transgenic TCRs to T cells provides tumor antigenicity, the transgenic TCR competes for binding with endogenous TCR subunits. Thus, Stadtmauer *et al* hypothesized that deleting endogenous TCR and PD-1 ex vivo in peripheral T cells obtained from patients with refractory cancer would improve persistence and the anti-tumor function of engineered T cells. They also introduced a synthetic, cancer-specific TCR transgene (NY-ESO-1) to facilitate recognition of tumor cells by the engineered T cells.

Generation of patient T cells expressing NY-ESO-1 and lacking PD-1 and TCR expression. Because PD-1 and endogenous TCR are hypothesized to contribute to T cell exhaustion, Stadtmauer et al isolated peripheral T cells from patients and deleted PD-1 and endogenous TCR used CRISPR genome editing technology. As a pilot phase I clinical trial, peripheral T cells were isolated from three patients, one with metastatic sarcoma and two with advanced refractory myeloma. The TCR consists of two subunits: the α (TRAC) and the β (TRBC). After electroporating patient T cells with recombinant protein dCas9 along with sgRNAs targeting PD-1 (PDCD1), TCRα (TRAC), and TCRβ (TRBC), cells were expanded and disruption of target gene expression analyzed. Disrupted expression of TRAC, TRBC, and PDCD1 was observed in between 20-60% of cells. NY-ESO-1 TCR was also supplied by lentiviral transduction. One concern with using CRISPR-Cas9 technology to edit the genome of cells used in human patients is the occurrence of off-target effects. The fidelity of the CRISPR-Cas9 gene editing used in Stadtmauer *et al* was found to be high, with only few off-target mutations

found in the population of T cells. In addition to off-target effects, the double-strand breaks caused by CRISPR-Cas9 can result in chromosomal translocations and rearrangements. While translocations were observed in the engineered cells, they were at the limit of detection of their PCR-based assays occurring at much less than 1% of the population. Additionally, when infused into human patients, the percent of engineered T cells with chromosomal translocations dramatically was reduced, suggesting negative selection in vivo. Functionality of T cells expressing NY-ESO-1 and lacking TRAC, TRBC, and PDCD1 (termed NYCE cells) was compared to T cells expressing NY-ESO-1 or lacking TRAC, TRBC, and PDCD1 alone by *in vitro* co-culturing with tumor cells expressing the antigen for NY-ESO-1. Interestingly, the NYCE cells that express the NY-ESO-1 TCR but lack PD-1 and endogenous TCR expression had higher anti-tumor cell activity than NY-ESO-1 TCR expressing cells with intact PD-1 and endogenous TCR. The increased anti-tumor activity was attributed to lack of endogenous TCR, which is thought to compete for expression with the transgenic NY-ESO-1 TCR. However, it would be interesting to see whether PD-1 knockout alone also increases T cell potency.

T cells expressing NY-ESO-1 and lacking PD-1 and endogenous TCR have sustained in vivo expansion and persistence in human patients. When the engineered cells were injected into human patients, rapid expansion and long-term persistence of cells was observed with no adverse side effects (including cytokine release syndrome). The engineered T cells remained stable in each patient's circulation for 3 to 9 months. Comparing these results to previous clinical trials using T cells with intact expression of PD-1 and endogenous TCR, this is a significant increase in persistence. Prior trials showed that T cells expressing PD-1 and TCR only lasted in the patients' circulation for a mean half-life of approximately one week. Importantly, tumor biopsies showed that the engineered T cells reached the tumor at similar levels to those in the circulation. Engineered T cells represented 5-10% of the circulating peripheral blood mononuclear cells. However, TRBC-edited cells showed up at the lowest frequency likely because knocking out this gene occurred at low frequency and may not have been favorable to T cell expansion.

Clinical responses: Because this was just a small phase I human pilot study, efficacy of the engineered T cells cannot be determined. The goal was to show that knocking out endogenous TCR and PD-1 enhanced T cell persistence. However, of the three patients within the study, the best outcome was stable disease in two of the patients. One patient showed regression of a large abdominal mass that was sustained for 4 months, but other metastases progressed. Interestingly, tumor biopsies showed tumor evolution following treatment. There was a reduction in expression of target antigens NY-ESO-1 and/or LAGE-1, the antigens for the synthetic TCR NY-ESO-1 TCR. This is consistent with an on-target effect of the infused T cells, and suggests that additional tumor antigens may be required to see full tumor response.

Summary of findings.

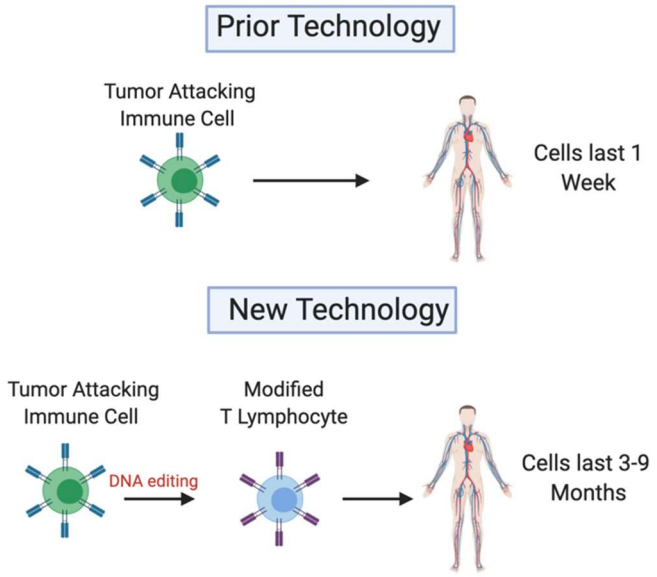
In summary, Statdmauer et al showed that knocking out the checkpoint receptor PD-1 and endogenous TCR subunits increased T cell persistence of engineered T cells, suggesting this might be a viable approach for prolonging response to adoptive cell transfer immunotherapies. Because T cell exhaustion limits persistence of cell-based immunotherapies, this represents a major advance in the field. Additionally, this approach represents another significant application of CRISPR-Cas9 technology. Future clinical trials with larger cohorts of patients are required to evaluate efficacy and safety.

References

1. *P. Sharma, S. Hu-Lieskovan, J. A. Wargo, A. Ribas, Primary, adaptive, and acquired resistance to cancer immunotherapy. Cell.* ***168****, 707–723 (2017).*
2. *E. A. Stadtmauer et al., CRISPR-engineered T cells in patients with refractory cancer. Science.* ***367*** *(2020), doi:10.1126/science.aba7365.*
3. *T. Okazaki, S. Chikuma, Y. Iwai, S. Fagarasan, T. Honjo, A rheostat for immune responses: the unique properties of PD-1 and their advantages for clinical application. Nat. Immunol.* ***14****, 1212–1218 (2013).*
4. *W. Zou, J. D. Wolchok, L. Chen, PD-L1 (B7-H1) and PD-1 pathway blockade for cancer therapy: Mechanisms, response biomarkers, and combinations. Sci. Transl. Med.* ***8****, 328rv4 (2016).*
5. *C. Yee, G. Lizee, A. J. Schueneman, Endogenous T-Cell Therapy: Clinical Experience. Cancer J.* ***21****, 492–500 (2015).*
6. *S. Gnjatic et al., (Elsevier, 2006), vol. 95 of Advances in cancer research, pp. 1–30.*

Study finds CRISPR-Cas9 editing of the human genome can fight cancer.

Lay Audience Report by Rebecca Vargas[1] and Brendan Finicle[1]
[1]Spring 2020 Journal Club, Department of Developmental and Cell Biology, UC Irvine



The genome of humans is composed of hereditary information that is necessary to build and maintain our bodies. In recent years, the possibility to change our DNA to produce a more desirable trait has become possible. This is known as gene editing, where DNA is modified within a living organism. Over the years, gene editing has become an increasingly controversial and exploited tool for the use of promoting desirable genetic changes. A gene editing tool, CRISPR-Cas9, is focused around altering DNA through extreme precision. This genetic tool uses a protein that cuts our genome and a guide molecule that tells the protein where to cut. The guide molecule can be designed to cut almost anywhere in the genome, giving scientists the ability to edit almost any gene. Editing can change the function of a gene, increasing activity or even deleting the gene. Recently, CRISPR-Cas9 technology received global interest for its use in treating human disease when it was used to induce HIV resistance by targeting and deleting a white blood cell protein necessary for HIV infection. The idea of targeting multiple genes in the genome by adding multiple guide sequences has become an attractive strategy to treat numerous diseases such as cancer. One potential application to cancer therapy is immunotherapy. Cancer immunotherapy involves rewiring our own immune system to recognize and attack tumor cells. Often times, this type of therapy involves activating an immune cell known as a T cell. In recent years, the rewiring of T cells to attack and kill tumor cells has lead to major increases in patient survival, even changing cancers previously thought to be untreatable into a curable disease.

The surface of T cells contains a protein called the T cell receptor (TCR) that recognizes proteins and other molecules and tells the killer T cells to attack cells that contain “attack signal” molecules. Beyond fighting off foreign bacteria and viruses, these T cells also patrol the human body for cancer cells. However, many cancer cells are adept at turning off the immune response by shutting down T cells. In recent years, scientists and clinicians have overcome this through taking T cells from patients with cancer and engineering them to express a TCR that tells them to attack the patient’s own tumor cells. While there have been many clinical successes using this synthetic TCR, its limitations have now come to light.

Numerous research groups have now demonstrated thatCRISPR-Cas9 gene editing technology can be used to delete the gene responsible for T cell inactivation caused by tumor cells, leading to increased tumor killing by T cells. With these promising results, researchers at the University of Pennsylvania designed the first in

human, pilot phase 1 human clinical trial to test the safety of using CRISPR-Cas9 genome editing for a cancer immunotherapy treatment. Stadtmauer and colleagues targeted naturally expressed proteins with the goal of increasing the anti-tumor activity of T cells while simultaneously increasing the length of time they would survive in human patients, thereby increasing efficacy and safety of the therapy. During the clinical trial, cells were removed from a cancer patient, engineered and transferred back to the patient. After gene editing by CRISPR-Cas9, the genetically engineered T cells were infused back into the same three patients who had advanced myeloma or metastatic sarcoma.

After confirming that CRISPR-editing worked in T cells obtained from patients, researchers found that the edited T cells killed tumor cells on a dish with high efficiency. With these exciting results, researchers infused the edited T cells back into human patients with advanced cancers. Because this was a pilot phase I clinical trial, only three patients were evaluated. However, patients infused with the CRISPR-Cas9 engineered T cells had no negative effects with no cases of the deadly response, which can be common in immunotherapies. While these preliminary findings are exciting, future studies with a larger cohort of patients will be necessary to empirically determine safety.

Genome editing isn’t perfect: editing genomes can be inefficient, leading to a lack of modification, or even cause unintended editing of off-target genes. Therefore, researchers evaluated efficiency of genome editing and potential mistakes. While most modifications were on target, there were some off target modifications that were identified that showed no negative impact on T cells. Additionally, cutting DNA by genome editing can produce errors and these errors could have deleterious effects on the T cells and potentially in human patients. This effect is called genotoxicity. Therefore, it's important to also assess genotoxicity when editing multiple genes. While authors reported toxicity, these errors declined in frequency over passage in human patients, suggesting that these rearrangements would be selected against in the T cell population over many generations of cellular expansion.

A previous limitation of using synthetic TCR T cells is T cell exhaustion in cancer patients. T cell exhaustion is the loss of functional T cells. Previous therapies using engineered T cells that were not CRISPR-edited only lasted in human patients for a little over a week. In this recent discovery, researchers predicted that by CRISPR-Cas9 editing proteins involved in T cell exhaustion, the edited T cells would have long-term persistence. The researchers showed that infusing cancer patients with CRISPR-edited, engineered T cells produced long-term persistence. These T cells stayed in patients for 3-9 months, much longer than the week provided by previous technology. This was an exciting result because it suggests that genome editing could help with cell exhaustion in cancer patients. However, currently available technology limited the researchers to only being able to analyze modified cells from one patient who had high engraftment. Tracking patients after treatment revealed that the best response was stabilizing the disease. Another response demonstrated a decrease in tumor mass that was sustained for a period of four months with other lesions progressing.

In conclusion, this study highlights the safety of CRISPR-Cas9 editing of the human genome at a clinical scale. This study was done on three patients and therefore studies on more patients would be required to fully assess the safety of this novel idea. A limitation of this study is also the types of cancers the authors chose. It would be interesting to address if this therapeutic therapy works on a broad spectrum of cancer types. This exciting work presents a new approach towards cancer treatment. While the field of gene editing is hot and scientists are ready for the next novel cancer treatment, this treatment option has many more obstacles to go through- such as expanded, large-scale human trials. While more work is to be done, Stadtmauer and colleagues have made a remarkable advancement in the race to develop a therapeutic.