New Genomics Technologies for Gene Therapy

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I. The "Rise and Fall" of Gene Therapy

Gene therapy has been a topic of interest in the medical community for decades. Especially in recent years, there have been many strides made in the field. Despite many failures and controversies surrounding this field, the progress made has been extremely promising and could potentially save many lives. Two cases, in particular, have been considered the "rise and fall" of gene therapy, and have really shaped the industry as we know it.

The first gene therapy clinical trial took place in 1990, by Dr. W. French Anderson, on a four-year-old patient named Ashanti DeSilva. The patient had a genetic disease called adenosine deaminase (ADA) deficiency, which caused her immune system to be completely non-functional. The treatment she was on had stopped working and the gene therapy clinical trial was her only chance at living a normal life. The therapy involved using disabled retroviral vectors recombined with a good copy of the ADA gene, which was transferred into T cells that had been cultured from the patient. The therapy lasted two years and the patient received 11 transfusions into her bone marrow. Although the treatment did not cure her disease, it was effective enough to partially restore her immune system and allow her to lead a normal life (Naam 2005). This trial proved to the world that gene therapy can be safe, effective and without side effects. Many clinical trials emerged after this success.

Unfortunately, in 1999, a clinical trial at the Penn institute resulted in the death of an 18-year-old boy. Dr. James Wilson conducted a gene therapy clinical trial on patients with ornithine transcarbamoylase (OTC) deficiency (Sibbald 2001), a disease fatal to all carriers of the disease. A boy named Jesse Gelsinger agreed to partake in a clinical trial because, while he only had a partial deficiency, his clinical trial could have potentially saved the lives of thousands of children. The research team used adenoviral vectors recombined with the corrected version of the OTC gene, injecting it into his hepatic artery. Unfortunately, his immune system rejected the foreign vector and he passed away after just four days. This trial resulted in the FDA conducting lengthy investigations of 69 other gene therapy trials, shutting down many of them. The entire industry was turned upside down, and the public became afraid, no longer trusting this type of treatment.

In today's age, we have a much better understanding of the etiology of diseases. Clinical trials have shifted their focus to symptomatic and pre-symptomatic individuals, rather than individuals with advanced and irreversible disease. There has been an establishment of better vector safety protocols. Additionally, after the Gelsinger case, anti-inflammatory drugs are now administered as part of treatment, to suppress the immune system in the case of adverse effects. After many years of

adjusting protocol and making advances in gene therapy technology, faith has been restored in the potential success of gene therapy.

Finally, 27 years after the first clinical trial, the first ever gene therapy was approved by the FDA for commercial use. Kymriah (tisagenlecleucel), manufactured by Novartis Pharmaceuticals Corp, was approved by the FDA in 2017, for patients under 25 with a form of acute lymphoblastic leukemia (ALL). ("FDA Approval Brings First Therapy"). Using Car-T cell therapy, Kymriah has a positive response from 83% of patients for whom no other therapy worked. The other therapies in practice for leukemia are chemotherapy and stem cell transplantation. Chemotherapy attacks and kills all cells, cancerous or not, and has extreme side effects. Gene therapy, on the other hand, attacks only the cancerous cells and has minor side effects, proving to be extremely advantageous. With FDA approval, the treatment has been given a stamp of safety and is a step towards greater public acceptance. We have come a long way since the first human patient was treated by gene therapy in 1990, but the field is still in its experimental stages.

II. What is Car-T Cell Therapy? Risks, Benefits, Access

"Car-T" stands for Chimeric Antigen Receptor T cells. The production of T-cells is already present in our bodies. These cells assist B-cells in producing antibodies to help fight any foreign disease that attacks the healthy cells. However, the "CAR" portion of these cells in particular is what makes them so unique (Almåsbak 2016).

Car-T cells are engineered in the lab through a process called apheresis. First, patient's T-cells are harvested through their blood. T-cells are only found in the white blood cells, plasma, and platelet portion of the blood, so any unnecessary components can be infused back into the patient. Then, T-cells are brought to the lab to be modified by forcing them to grow chimeric antigen receptors, also known as CARs. The receptors allow for the cell to bind to malignant cells and signal other T-cells to join the fight. Our naturally occurring T-cells often have a hard time binding to the malignant cell or cannot properly signal other cells to help. When we are sick with the common cold, our natural T-cell function is enough to keep us healthy; however, this is not often the case when it comes to cancerous intruders. The newly formed CAR-T cells are multiplied and sent back to the patient's treatment center, where they are infused back into the bloodstream. This treatment option is ideal for patients who have relapsed from chemotherapy, or are looking for a more "natural" method to treat the cancer.

Named after the therapy, Cytokine Release Syndrome (CRS) is one of the most toxic side effects of CAR-T Cell therapy. According to the Lymphoma and Leukemia Society, "CRS is caused by the release of cytokines, chemicals that assist the T-Cells in their function." Symptoms can range from flu-like effects—nausea, chills and fever—to more severe symptoms: renal insufficiency, cardiac failure, and multiple organ failure. Other side effects include neurotoxicity, which is less common than CRS and may affect the patient's ability of speech, and B-Cell Aplasia, where Car-T Cell attacks the antigens on both healthy and cancerous B-cells in the patient's body, producing a loss in B-cell count (Sibbald 2001).

Though there are risks involved, there are also many benefits. These include high success rates, rapid recovery time, less of an invasive treatment compared to other cancer cell therapy, and short treatment time. According to Cleveland Clinic's

website, "Over 80 percent of patients who received Yescarta® in clinical trials experienced either a complete or partial response," while "Over 80 percent of children and young adults treated with Kymriah® in clinical trials had their cancer go into remission." Unlike other therapies that take an abundance of time, Car-T Cell therapy is short term, only requiring approximately two weeks of inpatient care. Aggressive therapies such as chemotherapy leave patients with occasionally long recovery times, whereas Car-T cells' recovery time is said to be more rapid. The procedure is also less invasive, summarized into seven steps: "evaluation, collecting, engineering, multiplication, conditioning therapy, infusion and recovery" (Almåsbak 2016). These are simple steps that include taking the T-Cell from the patient and genetically engineering them to attack cancer tumors.

In Kulemzin et al's article, "CAR-T Cell Therapy: Balance of Efficacy and Safety," researchers describe patients' long-term health effects improving after the treatment. Since the receptors are programmed to kill foreign intruders, they continue to do so even after the cancer is eradicated. The 2017 study showed that patients saw a boost in overall immunity because the CAR T-cells were still present in their bloodstreams (Kulemzin et al, 2016).

Many studies have surveyed groups that have undergone CAR-T cell therapy, but one story that stands out among the rest is that of Dr. Robyn Stacy-Humphries. Dr. Stacy-Humphries was diagnosed with lymphoma in 2011, after discovering inflamed lymph nodes in her neck. She decided to go through the traditional treatment of RCHOP chemotherapy. She declared remission after just three rounds of chemo, but decided to complete the next three, to make sure that all invasive cells were destroyed. Fortunately for her, she saw very little side effects after chemo, and was even able even remaining able to work after each session.

However, four years later, she received a biopsy that confirmed that the cancer in her lymph nodes had returned. This time, she chose to undergo through a combination treatment of the RCHOP chemotherapy and stem cell therapy. Unfortunately, even after successfully completing the therapy, the cancer was discovered again three months later. At this point, she decided to abandon chemotherapy and look into clinical trials on CAR-T, since she was familiar with it through her work field. Dr. Stacy-Humphries began the process of apheresis, and the newly grown CAR-T cells were infused into her.

Twenty-four hours after treatment, she felt the sensation that her tumors were melting "like ice cubes." Shortly after, she experienced hypotension as well as a low-grade fever and had to be admitted. Over the next year, Dr. Stacy-Humphries described the tumor areas as being painful and sensitive. She also had difficulty getting out of bed, flu-like aches and pains, and severe allergic reactions to insect bites. However, even though the side effects were brutal and the therapy took a toll on her mentally, physically, and financially, Dr. Stacy-Humphries says that the therapy saved her life ("Car-T Cell Therapy").

Currently, Car-T Cells are only FDA approved for patients suffering from hematologic cancers, such as lymphoma. It is not yet covered by any health insurance. However, plans for the future have been discussed by the CMS (Centers for Medical and Medicaid) and FDA. The current cost for Car-T Cell therapy is approximately $375,000-$475,000 (Hitchcock 2019).

Eligility for this therapy is very strict. It is limited to patients with "aggressive, refractory non-Hodgkin lymphoma" and "patients with relapsed or refractory acute

lymphoblastic leukemia up to age 25" ("CMS Proposes Coverage"). Even with these requirements, there are only a few cancer institutes that are offering this therapy.

III. What Is CRISPR Therapy? Risks, Benefits, Access, Ethics

Another helpful gene editing process is called CRISPR. "CRISPR" stands for "clusters of regularly interspaced short palindromic repeats." The technology helps scientists to easily extract and modify gene sequences and their functions, allowing them to correct mutated genes that were once deemed incurable. Here's how it works: doctors extract certain cells that can be reversed back into stem cells (Ran, F. 2013). From there, the cells are sent to a lab, where they can be modified with CRISPR. CRISPR is coded with the viral DNA, where it is used to scout and identify viral cells, then bind with an RNA enzyme called CAS9. CAS9 cuts both viral DNA and its complement strand, where CRISPR will insert the new modified genetic sequencing. The new sequencing will then be reintroduced to the patient via infusion (Cai, L. 2016).

Like any clinical treatment, there are risk factors involved. In no particular order, risks can include the following: the immune system may see the newly introduced viruses as intruders and attack them, targeting the wrong cells; viruses can affect more than one type of cells, leading altered viruses to alter other cells; altered target genes may revert back into their original functioning, causing the return of the virus; and finally, the treatment may simply create other new problems. Indeed, once physicians alter and replace the genetic sequence, it is possible to cause other problems to arise, such as insertions and deletions in the genome, causing possible tumors to form.

As there may be several risk factors in CRISPR, there are also some benefits that come with it. CRISPR has been effective in the treatment of disease that was once considered as incurable, giving not only the patient a fighting chance, but also their families. So far, gene therapy like CRISPR has been able to cure diseases like Huntington's disease, Turner syndrome, and many more incurable diseases. This type of gene therapy is a form of a precision treatment that takes the patient's unique genome and modifies their genes specifically for their treatment; it reduces the trial and waiting period that many other treatment plans require.

Currently, gene editing is a costly form of treatment. On average, a typical gene editing session costs between anything from 400,000 to one million dollars, reinforcing the belief that gene therapy is only available to wealthy people and not supported by health insurance companies. However, as more and more companies adopt gene therapy as a form of FDA approved treatment, health insurers may in the near future start to provide reimbursement programs for many patients, like those at the John Hopkins Children's Hospital (Brown 2016). Though gene therapy seems like these are only for the wealthy elite, with time, companies like Synthego will give clinics a faster way to receive genomes and translate its viral DNA into a corrected DNA. This is partly because 23andMe and other gene sequencers have begun testing and understanding genes, information which, over time, would become accessible to the world, and eventually affordable to anyone wishing to edit their genes (Dabrowski 2017).

As CRISPR-CAS9 continues to make strides in the medical gene therapy field, many wonder how far is too far for gene editing. Should scientists use these techniques of gene editing primarily for health-related issues? Or is it acceptable to

use it to edit physical traits? China's scientist He Jiankui of Shenzhen, China has successfully edited twin genomes with qualities that few people possess, like HIV resistance. He has received backlash from the medical world, since many scientists worry about gene editing causing general mutation or the mutation of disease, which can become more difficult to treat. In an interview for the Associated Press, Professor Kiran Musunuru M.D. of the University of Pennsylvania states, "We have to balance the potential benefits with the potential risk for the people involved in cases where the risks are higher than the benefits…[in that case, taking the risk] is not ethical" ("CRISPR Babies In China"). Musunuru calls for scientists to reconsider the possibility of editing embryos. Still, He Jiankui feels that it's only a matter of time before people will be requesting these edits for their own pregnancies (Jiankui 2018).

IV. Is Gene Therapy Worth It?

Any new technology that has many unanswered questions is concerning for both scientists and the general public. Without extensive research, it is difficult to predict whether a new advancement will do more harm than good. For most cases, people are often willing to take the risk if it means improving or even saving their lives. However, as outsiders that are critiquing these technologies, we raise many ethical and health concerns. Ultimately, the decision to edit a genome or make our cells tougher is a personal decision. Still, we must remember that the reason we are this advanced in the scientific field is because we took risks that benefited society more than they harmed it.

Works Cited

Almåsbak, H., Aarvak, T., & Vemuri, M. C. (2016). CAR T Cell Therapy: A Game Changer in Cancer Treatment. *Journal of immunology research*, *2016*, 5474602. doi:10.1155/2016/5474602

Associated Press. (2018). *CRISPR Babies In China* [Video]. Youtube. https://www.youtube.com/watch?v=qUiNG1iW4Ww

Association of Community Cancer Centers. (2018, July 11). *CAR T-Cell Therapy Gave Me Back My Life: Robyn Stacy-Humphries' Cancer Odyssey.* https://www.accc-cancer.org/home/learn/immunotherapy/resource-detail/car-t-cell-thera py-gave-me-back-my-life

Cai, L., Fisher, A. L., Huang, H., & Xie, Z. (2016). *CRISPR-mediated genome editing and human diseases.* CRISPR-mediated Genome Editing and Human Diseases, 3(4), 244-251. Retrieved April 11, 2019.

Cleveland Clinic. (2019, May 14). *CAR T-Cell Therapy: Risks / Benefits.* https://my.clevelandclinic.org/health/treatments/17726-car-t-cell-therapy/risks--benefits

Center for Medicare and Medicaid Studies. (2019, February 15). *CMS proposes Coverage with Evidence Development for Chimeric Antigen Receptor (CAR) T-cell Therapy*. https://www.cms.gov/newsroom/press-releases/Cms-proposes-coverage-evidence-development-chimeric-antigen-receptor-car-t-cell-therapy

Dana Farber Cancer Institute. (n.d.). *Frequently Asked Questions About CAR T-Cell Therapy.* https://www.dana-farber.org/cellular-therapies-program/car-t-cell-therapy/faq-about-car-t-cell-therapy/

Federal Drug Administration Commissioner. (2017, August 30). *FDA approval brings first gene therapy to the United States.* https://www.fda.gov/news-events/press-announcements/fda-approval-brings-first-gene-therapy-united-states

Hitchcock, S. (2019, January 09). *Does the Cost Outweigh the Benefit for CAR T-Cell Therapy?* Targeted Oncology. https://www.targetedonc.com/publications/targeted-therapy-news/2019/jan-2019/does-the-cost-outweigh-the-benefit-for-car-tcell-therapy

Johns Hopkins Medicine (2019, March 27). *CAR T-Cell Therapy | FAQ with Dr. Patrick Brown* [Video]. Youtube. https://www.youtube.com/watch v=RpD69Z-Xco4&t=425s

Kulemzin, S., Kuznetsova, V., Mamonkin, M., Taranin, A., & Gorchakov, A. (2016, August 22). CAR T-Cell Therapy: Balance of Efficacy and Safety. *Molekuliarnaia Biologiia*, *51*(2), 274-287. https://www.ncbi.nlm.nih.gov/pubmed/28537234.

Leukemia and Lymphoma Society. (2019). *Chimeric Antigen Receptor (CAR) T-Cell Therapy.* https://www.lls.org/treatment/types-of-treatment/immunotherapy/chimeric-antigen-receptor-car-t-cell-therapy#FDA%20Approved%20Treatments

Naam, R. (2005, July 3). *More Than Human.* New York Times. https://www.nytimes.com/2005/07/03/books/chapters/more-than-human.html

National Human Genome Research Institute. (n.d.) *Results From First Human Gene Therapy Clinical Trial.* https://www.genome.gov/10000521/1995-release-first-human-gene-therapy-results

Ran, F., Hsu, P. D., Wright, J., Agarwala, V., & Scott, D. A. (2013). Genome Engineering Using the CRISPR-Cas9 System. *Nature Protocols 8*(11), 2281-2308. https://www.nature.com/articles/nprot.2013.143

Sibbald, B. (2001, May 29). Death but one unintended consequence of gene-therapy trial. *Canadian Medical Association Journal, 164*(11), 1612. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC81135/

TechCrunch. (2017, May 16). *Synthego's Paul Dabrowski gets Sci Fi on us* [Video]. Youtube. https://www.youtube.com/watch?v=PHUOyy3wGUA