Gene Therapy and Gene Editing in Healthcare

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1.0 Introduction

Throughout history, humanity has grappled with various diseases, with each generation facing its own health challenges. Disease is the leading cause of death worldwide (Dattani et al., n.d.). The average life expectancy of someone with Sickle Cell Disease is 52.6 years. Cystic Fibrosis patients born between 2018-2022 are predicted to live until 56. Almost 10 million lose their lives to cancer annually. Now, humanity has the opportunity to put an end to many diseases and disorders through gene editing and gene therapy.

Being able to tamper with genes has a huge impact on the healthcare field. Numerous diseases are due to genetic mutations. For instance, Cystic Fibrosis is caused by a deletion mutation in the Cystic Fibrosis Transmembrane Conductance Regulator gene. Some mutations similar to this one can be fixed using CRISPR-Cas9, a gene editing tool used to target specific areas in the genome and modify them. An alternate option to gene editing is gene therapy. Gene therapy aims to treat disease by introducing new DNA into cells. There have been reports of success in using gene therapy to treat Adenosine deaminase deficient severe combined immunodeficiency (*Gene Therapy Offers Potential Cure to Children Born Without an Immune System*, 2021). Gene therapies are available in clinical trials which are held to high standards enforced by the Food and Drug Administration (FDA). Treatments have to pass preclinical trials before entering the four phases of a clinical trial. All clinical trials have safety and ethical concerns that must be considered. These are very important to deliberate before and throughout a clinical trial. Overall, gene editing and gene therapy have applications in treating disease. Gene editing has promise in treating Cystic Fibrosis and gene therapy has shown success for Adenosine deaminase deficient severe combined immunodeficiency. These treatments must be monitored through clinical trials. Safety and ethical considerations for each treatment should be taken into account in deciding whether the treatment is viable for patients or not.

2.0 Gene editing for Cystic Fibrosis

Gene editing tools are needed for potential treatment of fatal diseases caused by genetic mutations. Cystic Fibrosis is one of those diseases. Cystic Fibrosis (CF) is an autosomal recessive genetic disease that affects the cells that produce mucus, sweat, and digestive juices, causing these viscous substances to become sticky and thick (*Cystic Fibrosis - Symptoms and Causes*, 2021). This disease which affects 30,000 Americans (*Learn About Cystic Fibrosis*, 2022), damages the lungs, digestive tract, and other organs (*Cystic Fibrosis*, n.d.). For someone to have CF, both parents must be carriers of a mutated version of the Cystic Fibrosis Transmembrane Conductance Regulator (CFTR) gene. If both parents are carriers of the mutated gene, there is a 25% chance the offspring will have CF. A known mutation in the CFTR gene is the deletion of phenylalanine 508 (F508del) [citation]. This deletion mutation causes defective channel processing (chloride ions that pass in and out of cells aren't being regulated). As a result of this, cells that are not receiving chloride ions (mostly cells that line the entrance of the lungs and other organs) create sticky and thick mucus, blocking airways. The severity of the disease depends on other environmental and other circumstantial factors. There has been much

research on curing disorders such as CF using gene editing tools, specifically a tool called CRISPR-Cas9.

3.0 Gene Editing tool: CRISPR-Cas9

There are many different gene editing tools such as: Prime editing, Base editing, Zinc-finger Nucleases (ZFNs), Transcription Activator-like Effector Nucleases (TALENs), and CRISPR-Cas9. CRISPR-Cas9 particularly is a very interesting gene editing tool that swept away the gene editing field. After CRISPR-Cas9 was founded in 1987, the field of genetics experienced numerous breakthroughs. Such as the recent use of CRISPR-Cas9 in a treatment for Sickle Cell Disease and the potential for treating HIV. The ability CRISPR technology has is revolutionary–in its relatively short life it has already been used to edit genes, been the key to many theoretical propositions which are being investigated currently. CRISPR-Cas9 has also been considered for new diagnostics tools. CRISPR, Clustered Regularly Interspaced Short Palindromic Repeats, is a group of DNA sequences found in genomes of some species of bacteria. These DNA sequences are formed using fragments of bacteriophages that have infected the organism. The bacteria can then detect and eliminate similar DNA during infections, providing an immune defense system. CRISPR-Cas9 was adapted from this naturally occurring bacterial immune defense system. CRISPR-Cas systems are comprised of two main parts, a guide RNA (gRNA) and the Cas9 enzyme. The gRNA is predesigned and is short, usually around 20 bases long. The gRNA will bring the enzyme, Cas9, to the desired location where a target gene is. The gRNA is responsible for making sure the Cas9 enzyme cuts at the right area. The guide RNA binds to the wanted DNA sequence for Cas9 to find the specific location of the gene defect. The gRNA is created to have complementary bases as those in the targeted DNA sequence in the genome. This is meant to help ensure that the gRNA will only bind to the wanted DNA sequence. The Cas9 enzyme can then cut the DNA at the target site, creating a double-stranded break (DSB) to prompt repair by the natural healing processes in the cell. The break is repaired through one of two pathways: Non-Homologous End Joining (NHEJ) or Homology-Directed Repair (HDR) pathways. NHEJ repairs the break by joining the ends together. NHEJ is frequently used, it is quick but is prone to creating unwanted mutations. HDR on the other hand is more accurate but it generally takes a longer period of time to repair. HDR utilizes a template to repair the DSB. This repair pathway is essential for the CRISPR-Cas9 system to operate. Gene editing however is not the only option, as gene therapy is the oldest form of genetic manipulation.

4.0 Gene Therapy

Gene therapy is a form of gene transfer technology that results in the introduction of new DNA to cells. This includes pinpointing dysfunctional cells and replacing missing or defective DNA. Gene therapy works on the premise of using a vector to act as a carrier or delivery system for the new DNA. There are viral vectors, nonviral vectors, and engineered vectors. The main vectors used are viral but there is insight into the advantages of using nonviral vectors. This insight entails the view of nonviral vectors being safer, more versatile, and potentially reducing production costs for therapies. Viral vectors work to package exogenous DNA and carry it into target cells. The method of using viruses is popular because viruses are good at gaining entry into cells by infecting them, and they are good at carrying genetic material. Depending on what

the goal is, different viruses may be selected to be used as a carriers, considering their characteristics. For example, transduction efficiency refers to the ability of the virus to deliver the therapeutic gene efficiently. This may be a consideration when picking a virus. Another example is immunogenicity which is the immune response evoked by a virus. Ideally, the virus would have a lower immunogenicity so as to not disrupt the cell or cause any unwanted behaviour when trying to deliver the treatment. Contrary to a viral vector, a nonviral vector does not utilize a virus. Non-viral methods include naked DNA, which refers to the direct administration of DNA into cells. Though viral vectors have a higher success rate, they have some drawbacks including potential immunogenicity and cytotoxicity. In the case of Ashanthi de Silva, who was 4 years old and suffering from Severe Combined Immunodeficiency (SCID) when she received a novel gene therapy, a virus was used to deliver the genes encoding the enzyme Adenosine Deaminase (ADA) to replace her non-functional copy. Though gene therapy was established long before de Silva came along, this was a huge milestone. Gene therapy has an extensive history, from the initial concepts in the late 1960s and early 1970s to 1990 when the first gene therapy success story emerged. The concepts in the 1960s/1970s were rough ideas and dreams. It wasn't until 1980 when David Williams and David Nathan published a paper showing one could use a virus to insert genes into stem cells (McSwine, 2023). Gene therapy has come a long way since then but is mainly limited to clinical trials. This is a means for further testing and gathering more information on current test runs of various gene therapies. The earliest test that could be considered an early version of clinical trials, was performed in the 1970s in, West Germany. Three German sisters were afflicted with an extremely rare disease, Hyperargininemia, which affects an enzyme called arginase that controls the build-up of arginine in bodily fluids. They were treated with a rabbit virus shown to induce the creation of arginase in rabbits, thus decreasing the levels of arginine. The research and proof of concept included interviewing people previously infected with the rabbit virus. They had deficient levels of arginine in their blood. Even a person who last had contact with the virus 20 years before the interview, still had lower-than-usual levels of arginine. Which showed that the rabbit virus lowered arginine levels below normal and lasted for years. This was hope for the girls but unfortunately, the virus was of no use to them. This attempt, however, was the first step towards modern-day clinical trials. One hereditary disorder is ADA-SCID. Adenosine deaminase deficient severe combined immunodeficiency, or ADA-SCID is an autosomal recessive disorder which is caused by a mutation in the ADA gene. This gene is supposed to encode the protein adenosine deaminase (ADA). The absence or lowered levels of ADA results in the toxic build-up of deoxyadenosine. ADA-SCID is presented as a weakened immune system and can have effects on the central nervous system. In 2016 UCLA (University of California Los Angeles) made advancements in gene therapy for ADA-SCID. The gene therapy approach was extracting blood stem cells from the patient's body, and introducing the necessary genes into the cells ex vivo. The new cells are expected to produce the ADA enzyme. Skip to 2018, Orchard Therapeutics licensed the treatments, hoping to make them commercially available. They did not gain FDA (Food and Drug Administration) approval. UCLA took back responsibilities and continued to make progress before being halted to a stop in 2022 when the FDA requested changes to the newly arranged clinical trials. They are on track to proceed with clinical trials involving three to six patients each year.

5.0 CAR-T Clinical Trials

Delving further into the world of clinical trials, a current gene editing clinical trial hopes to help Lymphoma cancer patients. Cancer is a widespread disease that occurs when cells change and grow uncontrollably. A type of cancer, Lymphoma, happens in the lymphatic system, which is a system of vessels and glands dedicated to fighting infection. There are two main types of lymphomas, Hodgkin and non-Hodgkin. CAR-T immunotherapies focus on genetically engineering T cells to recognize and attack cancer cells. T-cells are lymphocytes, a type of white blood cell. Most of the CAR-T immunotherapies are autologous, editing and duplicating cells from an individual and then inserting those cells back in the patient. Although the treatment is one dose, it is expensive and time-consuming. Some patients die of their disease while waiting for the therapy and sometimes the therapeutic cells are not produced as they should. In an effort to resolve these issues, researchers have been interested in using CRISPR as an alternate form of treatment. CRISPR-based research focuses on making allogeneic CAR-T cells, cells from a fit, healthy donor. The purpose of editing these cells is to make them attack cancer cells while not being targeted by the patient's immune system. This approach reduces cost and wait time, as well as provides high-quality, capable cells. CRISPR Therapeutics is monitoring two kinds of allogeneic CAR-T cells that have been modified using CRISPR, in phase 1 clinical trials. CRISPR Therapeutics showed preliminary positive results for patients with lymphomas. As well as a dose-dependent effect on the activity of their disease, meaning the effect the doses have on the disease depends on how many doses the patient receives. The FDA has given the Regenerative Medicine Advanced Therapy (RMAT) designation to the treatment to speed up the approval of this therapy, which addresses a medical need that is currently not met. In November 2022, data was presented by CRISPR Therapeutics showing ⅔ of the patients who had a response to the treatment, 3/11 remained in complete remission after two years, and 2/11 remained in complete remission after one year following the treatment. CAR-T Immunotherapy as a gene-editing treatment for lymphoma cancer is promising and shows great hope. However, all gene editing treatments have safety and ethical concerns.

6.0 Bioethics

There are numerous considerations for using gene editing to treat lymphoma cancer. One of the biggest issues that is prevalent not only in CAR-T immunotherapy but for most gene editing, is the risk of off-target effects. Off-target effects (OTEs) refer to side effects arising in an untargeted site within the genome. OTEs lead to unwanted mutations which can have several outcomes, including an impact on suppressed or active genes. There have been numerous reports of OTEs relating to CRISPR gene editing and this should be considered for CRISPR-edited T-cells. Another safety concern is translocation, which is the movement of genes to incorrect places. This can cause the cell to behave in unexpected ways such as unregulated gene expression, disruption in normal cell activity, and onset of diseases like cancer. The alteration of the T-cells can have adverse effects. The edited cells may not function as wanted and can have negative ramifications for the patient’s overall health. The new cells could trigger the patient's immune system unintentionally which could also have negative repercussions for the patient. These repercussions could include, autoimmune reactions and tissue damage. The field of gene editing and therapy is under a microscope. Researchers, scientists, and even the media are investigating the numerous bioethical challenges that the field of genetics is facing. There are four main principles of bioethics that each and every clinical

trial should abide by: beneficence, nonmaleficence, autonomy, and justice, originally proposed by Beauchamp and Childress. The first principle, beneficence, refers to one's responsibility to make sure the clinical trial benefits the patients. Additionally, the treatment should make an effort to give the patients as many advantages as possible. Weighing the benefit-to-risk ratio is a key component of any clinical trial. A benefit-to-risk ratio is the ratio of what benefits the patient will procure versus the risks they may be subject to. The trial may not be worthwhile for a patient if the risks outweigh the benefits, and considerable deliberation is required before the patient considers moving forward. It is equally important that the trial only contains patients relevant to the cause. Meaning, that all patients in the trial must be afflicted with the disease being treated or in some way relate to the overall goal of the trial. This ensures the results are true to the efficacy of the treatment. Nonmaleficence means to do no harm to the patient. This includes ensuring that the patient's suffering, ideally, zero, is kept at a minimum. Autonomy refers to the idea that every individual has worth and has the power to make rational decisions for themselves. This includes ensuring all of the patients gave consent after understanding and receiving all of the information about the trial. Trials may struggle to implement this since it could deter patients from taking part, but it is vital. All consent should not only be informed but voluntary. No patients should be forced into the trial. It is also very important that all clients are protected through the entire process, and no confidentiality agreements put in place to conceal the identity of the patient are breached. Sometimes, trials will take the additional step of keeping the patient's identity concealed from the research team. The last principle is justice, which is defined in this context as equal treatment and a fair distribution of healthcare resources. It may be hard to adhere to all of these principles, but it is crucial that these criteria are taken into consideration.

7.0 Conclusion

In conclusion, the applications gene editing and gene therapy have are enormous. They have the ability to treat and potentially cure some diseases that have plagued humans for centuries. Much research and analysis into genetic modification has been done, as well as clinical trials testing gene editing treatments. One gene editing tool, CRISPR-Cas9, has particularly been a huge help in the search for treatment options and cures for many life-threatening diseases. Cystic Fibrosis and cancer are two of many diseases that CRISPR can potentially eradicate. The wide scope of things CRISPR technology can accomplish makes it a dependable tool. Gene therapy also has a place in the healthcare industry, being one of the oldest genetic modification tools to exist. Gene therapy has proven to be useful in treating disorders such as ADA-SCID. Its complex use of vectors to perform a task makes gene therapy unique and a strong asset for gene manipulation. Though these technologies are amazing in what they do, it is important to take into account safety and ethics. Testing is crucial to make sure a treatment is safe. Even if it seems as though everything is fine, multiple tests and trials must be conducted to establish safety before proceeding with the trial. Throughout a clinical trial, it is crucial to consider bioethics. The four principles of bioethics can serve as a guide to establish that the treatment does not cross serious ethical boundaries. Investigating any potential safety or ethical concerns is vital for the ultimate benefit of the patients, as they are the ones in need.

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