CRISPR-Cas9: A Future in Treating Type 1 Diabetes - A Review

Alvina Shaik

Abstract:

Type 1 Diabetes is a very prevalent disease in today's population; approximately 8.4 million people have it. Symptoms of Type 1 Diabetes include taxed breathing, vomiting, weight loss, and extreme fatigue (American Diabetes Association [ADA], 2023). Currently, there is no cure for Type 1 Diabetes, even though it is a very prevalent disease. Gene editing is a new technology that could provide more permanent solutions to T1D ailments. The CRISPR-Cas9 technology has enabled researchers to use gene-editing technology to begin to develop treatments and possible cures for Type 1 Diabetes. CRISPR-Cas9 is a gene-editing mechanism that can be used to modify DNA, ultimately providing the possibility to cure or even prevent genetic diseases. It is derived from bacterial mechanisms of protection from harmful bacteriophages. When infected by a bacteriophage, the CRISPR-Cas9 bacterial immune system can create spacer sequences as a memory of infection. When matching viral DNA reinfects the cell, the CRISPR-Cas9 system will utilize the spacer sequence to create a guide RNA (gRNA) that directs Cas9 to the invading viral DNA in order to cut the DNA and prevent replication, stopping the infection. Although gene editing technology is new, researchers are testing the use of CRISPR-Cas9 technologies to treat Type 1 Diabetes. CRISPR Therapeutics and ViaCyte, Inc. announced the beginning of the first clinical trial in which genetically edited cells would be used to treat Type 1 Diabetes. Together, the companies are developing the CRISPR-edited VCTX210 stem cell therapy. When discussing genetic editing as a cure for Type 1 Diabetes, there are many safety and ethical considerations that must be taken into account: the principles of beneficence and non-maleficence, the accessibility of the treatment, the long-term effects and unintended consequences of such treatments, and patient autonomy. This paper discusses the genetic causes of Type 1 Diabetes, the process of CRISPR-Cas9 gene-editing, current steps being taken to cure Type 1 Diabetes using CRISPR-Cas9, and the safety and ethical considerations of using gene editing technology.

Introduction:

Type 1 Diabetes (T1D) is a very prevalent disease in today's population, with almost 9.5% of the world having it, according to a 2020 study (Mobasseri et al., 2020). T1D is an autoimmune disorder in which the immune system destroys pancreatic β cells, the cells that synthesize, store, and release insulin (Steck, A. & Rewers, M., 2016; Bertrams, J., 1984). Generally, people with T1D inherit genetic risk factors from their parents, but environmental factors trigger onset. According to the American Diabetes Association, the HLA-DR3 or HLA-DR4 are associated with autoimmune diseases and are found in most Caucasian people with T1D. More generally, the entire HLA class II alleles are linked to a greater predisposition to T1D In fact, HLA class II alleles account for up to 30% - 50% of genetic Type 1 Diabetes risk (Steck & Rewers, 2016; Noble, J. A., & Valdes, A. M., 2011). When HLA genes are working normally, they process and present antigens to CD4+ T lymphocytes. When mutated, however, this activity is inhibited. As a result, there is generally a loss of CD4+ T lymphocyte activation, which impacts the onset and regulation of the immune response (Cruz-Tapias et al., 2013; Crux, N. B., & Elahi, S., 2017; Jordanova, E. S., 2003; Nordquist H., Jamil R.T., 2023). However, advances in the medical field have enabled researchers to use gene-editing technology to

explore potential treatments for T1D. One of the most common gene-editing tools is CRISPR-Cas9.

CRISPR-Cas9:

CRISPR-Cas9 is a gene-editing mechanism that can be used to protect the body from viral infections and prevent genetic diseases. It is derived from bacterial mechanisms of protection from harmful bacteriophages. Clustered regularly interspaced short palindromic repeats (CRISPR) are short DNA sequences sometimes found in prokaryotic genomes. These sequences are repeated many times throughout the genome with different “spacer” sequences between them. These spacers match DNA sequences found in bacteriophage genomes. Cas9 is an endonuclease, which is a type of enzyme that can cut DNA. If a virus infects a bacteria, the bacteria will use its Cas9 enzymes to cut the viral DNA and store pieces of it (spacers) in its genome as a memory of the infection. Bacteria then transcribe these spacers into guide RNA, which binds to Cas9 and guides it to the invading DNA sequence. Ultimately, the CRISPR-Cas9 system can bind to matching viral DNA found in the cell and cut its DNA to prevent replication, stopping the infection (Bio-Rad, n.d.; Jiang, F. et al., 2016).

In the field of gene editing, scientists utilize the CRISPR process found in bacteria to create precise genetic changes that can address genetic diseases or create new traits. In the CRISPR-Cas9 process, Cas9 proteins bind to single guide RNA (sgRNA), an engineered RNA that is a fusion of two separate, naturally occurring RNAs: the guiding region and scaffolding region. The guiding region, which is part of the CRISPR RNA (crRNA) in nature, is complementary to the target region Cas9 cuts. Scientists modify this sequence to match their own genetic targets. The scaffolding region is the trans-activating CRISPR RNA (tracrRNA) and forms a scaffold that binds in a crevice of the Cas9 protein. The protein-RNA complex then binds through the Protospacer adjacent motif (PAM). When Cas9 binds with the PAM site, it separates the DNA strands to allow for binding of the sgRNA. If the sgRNA is complementary to the DNA, the Cas9 will cut it (Bio-Rad, n.d.; Jiang, F. et al., 2016).

After the DNA sequence is cut, the cell works to repair the cut. DNA repair can occur in two ways: Non-homologous end joining (NHEJ) and homology-directed repair (HDR). In NHEJ, enzymes facilitate the rejoining of the ends of the double-stranded break. This process is error-prone: the cell may randomly insert or delete nucleotides. This haphazard process results in mutations that can disrupt gene function and expression. In HDR, enzymes can fix the break using donor template DNA created by scientists. These template DNA strands may include a desired sequence surrounded on both sides by ‘homology arms’ that match the sequence upstream and downstream of the cut. As a result of the repair process, a complementary DNA strand is created (Bio-Rad, n.d.; Jiang, F. et al., 2016).

Researchers are testing the use of CRISPR-Cas9 technologies to treat T1D. On November 16, 2021, CRISPR Therapeutics and ViaCyte, Inc. announced the beginning of the first clinical trial in which genetically edited cells would be used to treat T1D. Currently, the clinical trial is still in phase 1. Together, the companies developed the CRISPR-edited VCTX210 stem cell therapy (CRISPR Therapeutics, 2021). This therapy constructs ViaCyte’s CyT49 pluripotent human stem cell line using CRISPR Therapeutics’ CRISPR-Cas9 and RNP electroporation gene editing technology and could potentially facilitate the development of a beta-cell replacement product. The VCTX210 stem cell therapy is designed to allow patients withT1D to create insulin from a small medical implant (VC-02) containing millions of pancreatic endoderm cells produced from CRISPR genetically modified stem cells. The VC-02 allows for

the direct interaction between blood vessels and implanted cells (GenEdge, 2022). In the past, cell replacement therapies have generally faced a significant challenge: when the cells are implanted in the body, the immune system attacks them because it views the cells as foreign intruders. As a result, patients are prescribed long-term immunosuppressants, which can lead to many dangerous side effects, such as increased risk of cancer and increased risk of infection. Researchers are hopeful that the genetically edited cells will be able to evade detection and destruction by the body's immune system (Sum, V., 2022; Cohrt, K., 2021). The VCTX210 stem cell therapy sets a path to potentially expand the treatable population by removing the necessity for immunosuppression with implanted cell therapies. However, new gene-editing technologies have faced ethical and safety concerns from the scientific community.

Safety and Ethical Considerations:

When discussing genetic editing as a cure for T1D, there are many safety and ethical considerations that must be taken into account. When treating a T1D patient with genetic editing methods, providers must apply the principles of beneficence and non-maleficence, or maximizing the benefits gained and minimizing the harm done by the treatment. When appropriately managed, T1D is usually not life-threatening; it poses some difficulties in the patient's everyday life, such as consistently managing blood sugar, weight, and diabetic medications. As a result, the provider must weigh the risk-benefit ratio carefully to determine whether administering such a new treatment to a patient is genuinely beneficial. Proponents of this therapy must also take the accessibility of the treatment into account. About 8.4 million people have T1D. CRISPR-Cas9 treatments can be costly, so policymakers and insurance companies must ensure equitable access to treatments for such a common disease so as not to worsen existing healthcare disparities (National Human Genome Research Institute, 2017). Before suggesting genetic editing as a treatment for T1D, providers should consider the long-term effects and unintended consequences of such treatments. CRISPR-Cas9 technology was first described as a bacterial immune system in 2002 and was first used on humans in October 2016; CRISPR-Cas9 is still a very new technology (Gostimskaya I., 2022). As a result, there is little information on the long-term effects of gene editing. Also, genetic editing can make errors, accidentally editing the genome in unwanted places. The unknown long-term effects and off-target effects of gene editing technologies must be considered when suggesting them as treatments for T1D (National Human Genome Research Institute, 2017). Providers must also respect the autonomy of the patient when discussing gene editing treatments for T1D with patients. CRISPR-Cas9 technologies are very new in the field of genetics and medicine, and little is known about their long-term effects on patients. Also, patients who correctly manage their T1D can live long, healthy lives, mostly uninhibited by their condition. With this information in mind, the patient may choose not to be treated with gene editing. On the other hand, the patient, knowing that gene editing has the potential to cure them of their condition, may want to be treated with gene editing. Ultimately, the provider must provide the patient with accurate information, ensure they understand the treatment, and allow them to make informed decisions about their health uncoerced (National Human Genome Research Institute, 2017).

Conclusion:

New technologies such as CRISPR-Cas9 have paved the path toward the treatment of Type 1 Diabetes, a disease that impacts a substantial number of people. However, as the medical field makes advancements, the scientific community must ensure the ethical use of

such technologies. This way, people from all backgrounds can benefit equally from humanity's successes, and the world can take steps toward becoming a healthier place.

References:

American Diabetes Association. (2023). Understanding Type 1 Diabetes. American Diabetes Association. https://diabetes.org/about-diabetes/type-1

Bertrams J. (1984). The HLA association of insulin-dependent (type I) diabetes mellitus. Behring Institute Mitteilungen, (75), 89–99.

Bio-Rad. (n.d.). CRISPR-Cas Gene Editing Teaching Resources. Bio-Rad. https://www.bio-rad.com/en-us/applications-technologies/crispr-cas-gene-editing-teaching-resources?ID=Q58I0DWDLBV5

Cohrt, K. (2021). First CRISPR Therapy for Type 1 Diabetes Set to Enter Clinical Trial. CRISPR Medicine News. https://crisprmedicinenews.com/news/first-crispr-therapy-for-type-1-diabetes-set-to-enter-clinical-trial/

CRISPR Therapeutics. (2021). CRISPR Therapeutics and ViaCyte, Inc. to Start Clinical Trial of the First Gene-Edited Cell Replacement Therapy for Treatment of Type 1 Diabetes. CRISPR Therapeutics. https://crisprtx.com/about-us/press-releases-and-presentations/crispr-therapeutics-and-viacyte-inc-to-start-clinical-trial-of-the-first-gene-edited-cell-replacement-therapy-for-treatment-of-type-1-diabetes

Crux, N. B., & Elahi, S. (2017). Human Leukocyte Antigen (HLA) and Immune Regulation: How Do Classical and Non-Classical HLA Alleles Modulate Immune Response to Human Immunodeficiency Virus and Hepatitis C Virus Infections?. Frontiers in immunology, 8, 832. https://doi.org/10.3389/fimmu.2017.00832

Cruz-Tapias, P., Castiblanco, J., Anaya, J.M. Major histocompatibility complex: Antigen processing and presentation. In: Anaya JM, Shoenfeld Y, Rojas-Villarraga A, et al., editors. Autoimmunity: From Bench to Bedside [Internet]. Bogota (Colombia): El Rosario University Press; 2013 Jul 18. Chapter 10. Available from: https://www.ncbi.nlm.nih.gov/books/NBK459467/

GenEdge (2022). CRISPR Therapeutics, Sweet Spot: ViaCyte Dose First Patient with Cell Therapy for Type 1 Diabetes. Gen Genetic Engineering and Biotechnology News. https://www.genengnews.com/gen-edge/sweet-spot-crispr-therapeutics-viacyte-dose-first-patient-with-cell-therapy-for-type-1-diabetes/#:~:text=type%201%20diabetes.-,VCTX210%20is%20designed%20to%20engineer%20ViaCyte's%20CyT49%20pluripotent%20human%20stem,the%20need%20for%20chronic%20immunosuppressants.

Gostimskaya I. (2022). CRISPR-Cas9: A History of Its Discovery and Ethical Considerations of Its Use in Genome Editing. Biochemistry. Biokhimiia, 87(8), 777–788. https://doi.org/10.1134/S0006297922080090

Jiang, F., Taylor, D. W., Chen, J. S., Kornfeld, J. E., Zhou, K., Thompson, A. J., Nogales, E., & Doudna, J. A. (2016). Structures of a CRISPR-Cas9 R-loop complex primed for DNA cleavage. Science (New York, N.Y.), 351(6275), 867–871. https://doi.org/10.1126/science.aad8282

Jordanova, E. S., Philippo, K., Giphart, M. J., Schuuring, E., & Kluin, P. M. (2003). Mutations in the HLA class II genes leading to loss of expression of HLA-DR and HLA-DQ in diffuse large B-cell lymphoma. Immunogenetics, 55(4), 203–209. https://doi.org/10.1007/s00251-003-0563-z

Mobasseri, M., Shirmohammadi, M., Amiri, T., Vahed, N., Hosseini Fard, H., & Ghojazadeh, M. (2020). Prevalence and incidence of type 1 diabetes in the world: a systematic review and meta-analysis. Health promotion perspectives, 10(2), 98–115. https://doi.org/10.34172/hpp.2020.18

National Human Genome Research Institute. (2017). What are the Ethical Concerns of Genome Editing?. National Human Genome Research Institute, National Institute of Health. https://www.genome.gov/about-genomics/policy-issues/Genome-Editing/ethical-concerns

Noble, J. A., & Valdes, A. M. (2011). Genetics of the HLA region in the prediction of type 1 diabetes. Current diabetes reports, 11(6), 533–542. https://doi.org/10.1007/s11892-011-0223-x

Nordquist H, Jamil RT. Biochemistry, HLA Antigens. [Updated 2023 Apr 24]. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2023 Jan-. Available from: https://www.ncbi.nlm.nih.gov/books/NBK546662/

Steck, A. K., & Rewers, M. J. (2011). Genetics of type 1 diabetes. Clinical chemistry, 57(2), 176–185. https://doi.org/10.1373/clinchem.2010.148221

Sum, V. (2022). B.C. researchers launching clinical trial for first genetically engineered stem cell-based therapy for type 1 diabetes. The University of British Columbia. https://news.ubc.ca/2022/05/12/b-c-researchers-launching-clinical-trial-for-first-genetically-engineered-stem-cell-based-therapy-for-type-1-diabetes/