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CRISPR Therapeutics: New Emerging Developments and Clinical Applications - IVT BLOG

By **Kaiser Jay Aziz** **Sep 24, 2021 7:00 am EDT**

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ABSTRACT

CRISPR gene editing is a genetic engineering technique applied in clinical applications in which the genomes of living organisms may be modified. It is based on the principles of the CRISPR-Cas9 antiviral defense system. It is based on delivering the Cas9 nuclease complexed with a synthetic guide RNA into a living organism cell and that organisms' genome can be "cut" and –"paste" at a desired location, allowing existing genes to be modified for desired outcome (i.e., CRISPR for Precision Medicine). CRISPR gene editing harnesses the natural defense mechanisms of some bacteria to cut human DNA strands. Then the DNA strand either heals itself or injects a new piece of DNA to mend the gap. Studies have been reported in Lung Cancer diagnosis and treatments. CRISPR-based engineering techniques have been developed for T Cells and Stem cells applications (i.e., Gene Corrections in Hematopoietic Stem Cells for the Treatment of Blood and Immune System Diseases). Even though earlier CRISPR methodologies were used for performing simple DNA edits, recent applications include the ability to delete genes or insert genes and edit regulatory regions in a wide range of cell types. The role of CRISPR in human therapeutics is currently focused on utilizing CRISPR techniques to perform either in vivo editing of human cells–everything from the head, eye all the way to neurons and liver cells--or performing ex vivo therapies. The FDA's new genomic CRISPR technology based products approval process begins with review and evaluation of preclinical studies in order to establish and characterize the proposed product's safety profile. New genomic products must be shown to be safe and effective for the FDA approval process. The sponsor of the new genomic product must show that the product is safe and effective in human subjects.[1]

INTRODUCTION

Pharmacogenomics (PGx), the development of how one's genes may affect an individual's response to new treatments, has evolved in the translational stage of various specialties in medicine.[2,3,4] New developments regarding Clustered Regularly Interspaced Short Palindromic Repeat (CRISPR) technology and its CRISPR Associated proteins (Cas9, Cas12a, Cas13) essentially offer a programmable, sequence-specific means to target the DNA or RNA sequence of your choice; however, Cas13 is a unique enzyme in that it targets RNA. The use of multiple Cas nucleases (i.e., Cas12a, Cas13a and Cas14a) offers opportunity for a large range of sequences, thus providing diagnostic information on a broader range of diseases. CRISPR technology comprises of two components, a nuclease, e.g., Cas9, which acts like a pair of scissors and is responsible for cleavage of double stranded DNA, and a single guide RNA, which forms a complex with the nuclease and guides it to the target site. This technology can be designed to make a break at a specific target sequence in the DNA within a living cell,

allowing an analyst to modify almost any locus in the genome of the organism.5 Cas9 is the most commonly used nuclease in CRISPR gene editing to target specific sequences of genetic code and edit DNA at precise locations, which may make it possible to correct the DNA mutations underlying diseases like sickle cell disease, Huntington's disease and cystic fibrosis. However, while CRISPR/Cas9 gene editing offers a challenging opportunity in genome editing, and it can function in various cell types and species, but challenges remain due to low target site specificity, sensitivity and standardized performance characteristics. These developments offer opportunities for medical applications and products for clinicians utilizing genetic editing in a clinical setting. This technology consists of standard base-pairing of the CRISPR guide RNA bound by the Cas protein to the targeted DNA or RNA, which then can be predictably cleaved or bound in the case of catalytically inactive Cas exonuclease. This technology opens implementation and utilization of genetic editing in clinical applications. These types of applications provide several design advantages over other similar systems, such as zinc finger nucleases (ZFNs). In contrast to CRISPR/Cas technology, ZFNs must be engineered specifically for each target, whereas CRISPR/Cas requires only the guide RNA target specific design. The CRISPR/Cas technology presents remarkable medical opportunities inclusive of genome editing. Current clinical studies involve CRISPR modifications of T-cells in immuno-oncology patients by removing certain genes targeted by checkpoint inhibitors.5,6

DISCUSSIONS

There are clinical studies in patient harvested T-cells modified by CRISPR/Cas for HIV treatments by targeting either the latent genomic virus or by removing genes encoding viral cell surface receptors to prevent additional T-cell infections. There are examples where engineered Chimeric Antigen Receptor (CAR) T-cell based therapies are available. There are other examples that consist of extracted cells modified and infused in proximal time frames. There are other projects based on mini-chromosomes (covalently closed circular DNA, or cccDNA) responsible for chronic hepatitis B infections targeted by CRISPR/Cas for interhepatic HBV clearance. Other examples are based on studies involving edited disease-causing genes for conditions such as Fanconi anemia/cystic fibrosis in cell lines or human organoids raising the prospect for future CRISPR/Cas editing to remove treatment concerns or carrier status by modifying the germline of patients and their offspring.5,6

Technical Issues and Concerns: There are three major technical CRISPR/Cas issues:

- Incidence of off-target effects leading to potential second site cleavage events and potential undesirable alterations.
- Concerns over repair of any CRISPR/Cas cleavage. The technology is satisfactory in removing targets and repairing the excised region.
- Delivering any ribonucleic acid protein complex in vivo management challenges.

The practical application of nucleotide target detection via cleavage methodology uses an active Cas exonuclease. In this approach CRISPR/Cas is used to specifically target the reference sequence or wild-type sequence of KRAS while leaving the versions commonly mutated in somatic cancers intact. This approach depletes unwanted sequences prior to polymer chain reaction (PCR) amplification. This approach prevents amplification of the targeted sequences and thereby enhances the amplification of rare somatic variants that may be contributing to driving a cancer growth.
2,6

CLINICAL APPLICATIONS

Gene editing studies have been reported where stem cells that had been extracted from patient's blood, edited using CRISPR-Cas9, and infused back to the patient in order to cure sickle-cell disease. The disease is caused by a mutation in a single nucleic acid replacement in a person's DNA, which causes a defective hemoglobin protein. A normal hemoglobin protein shows round and smooth red blood cells able to move easily through blood vessels and carry oxygen from lungs to the rest of the body versus twisted or curled hemoglobin proteins forming long fibers in the form of twisted shapes in the red blood cells of sickle-cell patients, which causes them to clump together and crumple into the shape of a sickle which causes oxygen not to get to the body tissues and organs, causing severe pain and devastating situations. Therapeutically, clinical cases have shown that stem cells extracted from sickle cell patient's blood, edited using CRISPR technology, activates a gene that produces a type of blood cell that appears normally in fetal stage of life (i.e., healthy fetal hemoglobin in fetal cells) thus showing that CRISPR modification works in producing normal blood cells in these patients with no sickle-cell pain attacks. Clinical pathology tests showed that bone marrow cells were producing normal fetal hemoglobin indicating that CRISPR gene edits were effective in humans. These types of clinical edits using CRISPR-based technology presents challenging opportunities for other disease mutations and treatments such as cancer, congenital blindness and bone marrow abnormalities. CRISPR technology can be useful for tumor diagnosis and treatments based on DNA sequences associated with different types of cancers and precision medical treatments. For instance, a gene known as P53 specifies the genetic codes for a protein that suppresses the growth of cancerous

tumors, which assists the body's response to damaged DNA and prevents cancerous cells from dividing. There are numerous ongoing clinical trials for various CRISPR-Cas9 clinical uses These clinical trials include potential treatments for acute myeloid leukemia, coronary heart disease and Alzheimer's disease.3,6

Gene therapy technologies involve modifying the genome of stem cells in human bodies. The CRISPR-based technology is applied when a gene is cut open and two ends of DNA are involved. The cutting and trimming mechanism provides repair of the broken gene and the gene in turn tries to recover the lost information by seeking an intact copy. Typically, a cut-open gene tries to recover the lost locus from the other copy of the gene in the cell. However, a predetermined genetic change can be incorporated into a genome. For instance, the sequence ATGGGCCCG in a gene can be changed to ACCGCCGGG or any suitable desired sequence. Thus, a mutant Tay-Sachs disease or cystic fibrosis gene can be corrected to the wild-type version.

VACCINOLOGY DEVELOPMENTS

- **TRADITIONAL:** Traditionally, vaccines are designed to stimulate an individual's immune system. The basic principle of traditional vaccines consists of a substance that resembles a virus, or any other pathogen delivered to an individual's body. The substance, deactivated, triggers antibodies production to protect or guard against any infection of the substance (i.e., virus or any other fragment of the virus or genetically related materials to the virus). This process is intended to keep the person's immune system checked and produce antibodies for a longer period to protect from any infection of the virus. Vaccines are produced by a variety of methods to stimulate the human immune system. The body responds by making antibodies for fighting the virus (i.e., polio, measles, mumps, rubella and chicken pox). Another traditional approach is to inject a subunit of the virus such as one of the proteins from the virus structure, allowing the human body's immune system to produce a response to encounter the actual virus (i.e., the vaccine against the hepatitis B virus). The protein fragment of the virus is considered safer to inject into a patient; however, long-term immunity is not guaranteed. Some pharmaceutical firms have pursued this approach in the year 2020 for a COVID vaccine by designing mechanisms to introduce into human cells the spiked protein similar to the coronavirus surface. There are new types of vaccines available in comparison to traditional deactivated components of the targeted virus. The new types of vaccines are composed of injections into humans a snippet of nucleic acid driven by making of proteins, acting as a guide for enzymes involved in replication and repair process. 5-8
- **NUCLEIC ACID BASED TECHNOLOGIES**: Genetically Engineered Technology: The new vaccines are based on a gene or piece of genetic coding that will guide human cells to produce components of the virus which in turn stimulate the patient's immune system. This approach was utilized in one of the earliest COVID vaccine developments, which lead into a genetically reengineered safe virus also known as adenovirus. (Adenovirus infections most commonly cause illness of the respiratory system; however, depending on the infecting serotype, they may also cause various other illnesses). Apart from respiratory involvement, illnesses and presentation of adenovirus include gastroenteritis, conjunctivitis, cystitis and rash illness. Symptoms of respiratory illness caused by adenovirus infection range from the common cold syndrome to pneumonia and bronchitis. Patients with compromised immune systems are especially susceptible to severe complications of adenovirus infection. Vaccines developed by nucleic acid technologies using adenovirus as the delivery mechanism carry a gene which codes for identifying spike proteins. The idea is that the reengineered adenovirus would make its way into human cells, where it would cause the cells to make lots of these spike proteins which in turn would stimulate the patient's immune system to make lots of antibodies. As a result of this type of mechanism, the patient's immune system would be primed to respond rapidly to any future coronavirus infection. It is essential to establish that any rapid COVID test should be confirmed by an equally sensitive and more specific test. In this regard, the confirmation procedure chosen should provide the most accurate and unequivocal results possible.
- DNA or RNA based Technology: This technology is based on introducing genetic material into a human cell that causes it to produce the components of a virus that can stimulate the immune system. The developers of DNA vaccines have utilized a method called electroporation, which delivers electrical shock pulses to the patient at the site of the injection, which opens pores in the cell membranes and facilitates the entry of DNA. The DNA vaccine delivery uses CRISPR-Cas9 protein, a guide RNA, and a nuclear localization signal that helps the DNA CRISPR-Cas9 complex enter the nucleus. The DNA vaccine complex directs the cells to make coronavirus spike proteins and thus stimulates the immune system to fight the real coronavirus. The RNA vaccine serving as a messenger RNA (mRNA) carries genetic information from DNA to the manufacturing region of the cell, where it directs protein synthesis and in COVID situations, the mRNA instructs cells to make part of the spike protein that is on the surface of the coronavirus. The RNA vaccine has an advantage over a DNA vaccine because it does not need to get into the nucleus of the cell. It works in the outer region of the cell-cytoplasm which is where the proteins are synthesized. The basic concept of RNA vaccine is based on RNA sequences that would cause human cells to make versions of the coronavirus's spike protein. CRISPR therapeutic developments consisted of

design of vaccines for the delivery mechanisms into the cell. There are developments consisting of lipid nanoparticles in tiny synthetic capsules able to carry molecules into a human cell. These developments facilitated use of genetic based vaccines providing blood tests results indicating generation of neutralizing antibodies to fight the coronavirus.[5-8]

CONCLUSION

The goal of vaccine design and development is to manufacture and consistently produce a vaccine that is safe and effective. The vaccine discovery starts with design input in terms of identification of etiologic agent, immunogenicity, adjuvant, non-clinical studies, clinical trials, and vaccine licensure (FDA approvals).[3,5,6] . The administering regimens are studied in clinical research laboratory and study designs are tested in suitable bench testing and biological models such that the vaccine candidate produces a prophylactic immune response that is safe and effective. New CRISPR based genomic drug products' applications are reviewed primarily for safety and efficacy with regard to their claims for intended clinical use. The FDA's review is conducted under IND, NDA and BLA regulations.[3,4,6] Emphasis is placed on standardized approach to evidence-based review and evaluation. These standardized reviews apply to all aspects of clinical trials, from protocol design, monitoring, auditing, to recording and reporting of clinical data presented in new product applications to the FDA. The FDA's new genomic drug approval process begins reviewing initial stages of design concepts, discovery and development of prototypes involving preclinical and clinical studies of the CRISPR therapeutic product. The FDA review emphasizes the Quality System approach to genomic product's safety and effectiveness by ensuring that essential data and appropriate labeling are presented in support of clinical use of the product.

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REFERENCES

1. Aziz, K.J. Global Harmonization and Quality System Applications for Clinical Diagnostics. Clin Lab Med, 2001: 39:76-78
2. Aziz, K.J. Clinical Trial Design: Integrating Biomarkers into Drug Development. Clin Lab and Analytical Sciences. 2007:30:10-19
3. Aziz, K.J. The FDA's new drug approval process. Drug Development & Delivery. 2018:3:50-56
4. Aziz, K.J. Risk Management: FDA's Quality Risk Management Approach to New Drug Applications. Drug Development & Delivery.2019:19:1-4
5. Aziz, K.J. Genome Editing: New, Emerging and Interesting Developments for Clinical Applications. Journal of Biotechnology & Bioinformatics Research 2021:3(1):2-4. https://doi.org/10.47363/JBBR/2021(3)127
6. Aziz, K.J. Gene Therapy: Development, Design of Studies, and Approval Process. Journal of Biotechnology & Bioinformatics Research. 2021:3(3):1-4. https://doi.org/10.47363/JBBR/2021(3)134
7. Morrow, W.J.W, et.al. Vaccinology: Principles and Practice. 2012:403-441. https://doi.org/10.1002/9781118345313
8. Milligan, G.N. and Barrett, A.D.T, Vaccinology: An Essential Guide. 2015:1-32. https://doi.org/10.1002/9781118638033
