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Genome Editing: New, Emerging, and Interesting Developments for Clinical Applications

Kaiser Jay Aziz

Former Associate Director, Center for Devices and Radiological Health, Food and Drug Administration, Rockville, USA

ABSTRACT

Principles of Genome Editing can be applied in the various areas of medical diagnosis and treatments ---from early process design/development through maintenance of the validated state during commercial manufacturing and post-marketing surveillance. Gene editing and clinical applications comprises of systematically assessing, monitoring, and reviewing manufacturing processes and subsequently monitoring measures to control output risks. Quality risk management (QRM) principles have been described in various FDA's regulatory guidances for several aspects of good manufacturing practices (GMPs) such as several stages of process validation and verification in the drug product lifecycle including critical quality attributes (CQA's) and monitoring critical process parameters (CPPs). A CPP is defined as a process parameter whose variability has an impact on a CQA and, therefore, should be monitored or controlled to ensure that manufacturing process produces end product of the desired quality. FDA's mission is to facilitate the premarket review and evaluation of new genomic products for clinical use. The FDA guidances emphasize quality management approach to design of studies by providing oversight and objective review based on risk-benefit analysis and guides the use of new medical products by providing patients organized data and appropriate labeling information in support of the new product's intended clinical use [1-3].

***Corresponding author**

Kaiser Jay Aziz, MB, BS, MS, PhD, FACB, FACS, Former Associate Director, Center for Devices and Radiological Health, Food and Drug Administration, Rockville, USA. E-Mail: kashj33@yahoo.com

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Introduction

Genome Editing

Genome editing is a type of genetic engineering in which DNA is inserted, deleted, modified or replaced in the genome of a living organism. In this process, an enzyme cuts the DNA at a specific desired location in the genome, and when this is repaired by the cell a change or 'edit' is made to the DNA sequence:

- Genome editing technique is used to precisely modify DNA within a cell
- This technique involves making cuts at specific DNA sequences with enzymes called 'engineered nucleases'
- The technique can be used to add, remove, or alter DNA in the genome
- By editing the genome the characteristics of a cell or an organism can be changed

A common approach in modern bio-research technologies is to modify the DNA sequence (genotype) of an organism (or a single cell) and observe the impact of this change on the organism (phenotype). Genome editing can be used to change the DNA in cells or organisms to identify the biological mechanism in cells in order to understand their biology or how they work. For disease treatments, genome editing has been used to modify human blood cells that are then put back into the body to treat conditions such as leukemia and AIDS. It could also potentially be used to treat other infections such as MRSA, other genetic conditions such as muscular dystrophy and hemophilia. Genome editing uses a type of specific enzyme known as 'genetic engineering nuclease' (GENs) which cut the genome in a specific place. These nucleases are made up of two types-- a nuclease type that cuts the DNA and a nuclease type that points DNA target designed to guide the nuclease to a specific sequence of DNA. After cutting the DNA in a specific place, the cell naturally repairs the cut. This repair process can be manipulated to make changes or edits to the DNA in that location of the genome (figure 1)

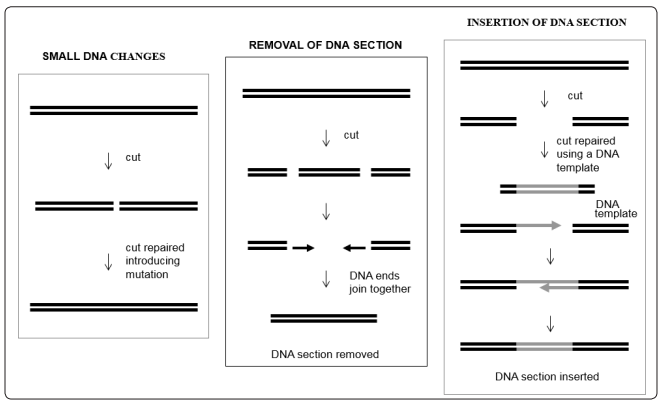

Figure 1: Illustration showing Basic Concepts of Gene Editing (Genome Editing)

Types of Engineered Nucleases for Genome Editing

Small DNA Changes

-A 'nuclease engineered' enzyme is designed to cut a specific location in the DNA. After cutting the DNA, the cell's normal

DNA repair process recognizes the damage and joins the two cut ends of DNA back together. This repair process is not always 100 per cent achievable and often a few bases are lost or added around the site of the cut when it is being repaired. This type of small change (mutation) in the DNA may affect the function of that section of DNA, which may affect the gene's function (improper function or no function at all).

Removal of DNA Section

-In order to remove a section of DNA, engineered nucleases that make cuts in either sides of the section that is desirable to remove. After cutting the DNA, the cell's normal DNA repair process recognizes the damage but may mistakenly join the wrong ends of the DNA together.

Insertion of DNA Section

A DNA repair system may be hijacked by inserting a section of DNA into a genome via the process of genome editing. In a normal cell division, the entire DNA is copied so that the two resulting daughter cells can receive a complete copy of the genome. If there is a break in one copy of the DNA, the cell repairs the break by using the other copy as a template. This process ensures that both copies of the DNA match again and this process is known as "homology-directed repair". In some situations, after the DNA has been cut, a modified piece of DNA similar in sequence to the site of cut, may be introduced. In this situation, the cell may use the modified piece of DNA as the template to repair the break, filling the break with a copy of the new DNA. As above described situation may be useful to insert a new section of DNA, or replace an existing section of DNA with an altered version (i.e., this approach can be useful to correct a point mutation within a gene).

Genome Editing Systems

There are different types of 'engineered nucleases' used in genome editing. They all contain a nuclease function to cut the DNA and a DNA-targeting point by recognizing the DNA sequence they need to cut (Figure 2)

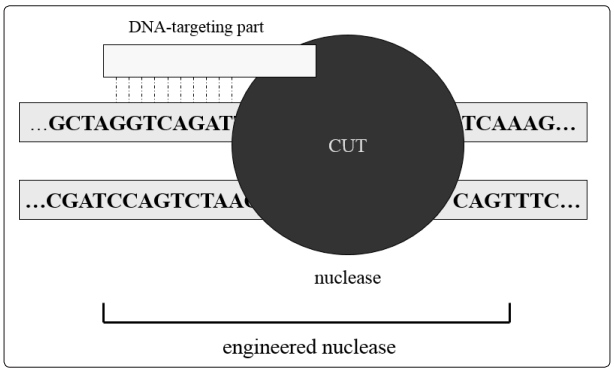

Figure 2: Illustration showing the basic structure and function of engineered nucleases used for genome editing.

The main difference being:

- RNA-based containing a short sequence of RNA that binds to the target DNA to be cut
- Protein-based containing a protein that recognizes and binds to the target DNA to be cut

CRISPR-Cas9 (Clustered Regularly Interspaced Short Palindromic Repeats)

This is most common technology system considered to be efficient for genome editing. CRISPR technology is based on DNA targeting which consists of an RNA molecule designed to bind to specific DNA bases through complementary base-pairing. Cas9 stands for CRISP- associated protein 9, and is the nuclease part that cuts the DNA. The CRISPR-Cas9 system was originally discovered in bacteria that uses the technology to destroy the invading viruses (Figure 3)

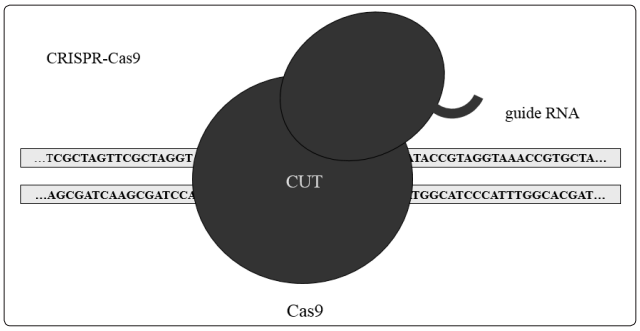

Figure 3: Illustration showing the components of CRISPR-Cas9 in Stem cells to diagnose/treat/cure disease

ZFNs (Zinc Finger Nucleases)

The DNA binding part of ZFNs is made of zinc-finger proteins, which each bind to about three DNA bases, however, different combinations of zinc-finger proteins bind to different sequences of DNA. The nuclease part of ZFNs is normally a FokI nuclease, which cuts the DNA. Two Fokl molecules come together to make a cut in the DNA, so that a pair of ZFNs are made, one binding to each strand (Figure 5)

TALENS (Transcription Activator-Like Effector Nucleases)

The DNA binding region of TALENs is made of transcription activator unit. There are four different TALE regions- one for each DNA base, in such a way that engineered nuclease can bind to specific DNA sequences. The nuclease part of TALENs must be together to make cut in the DNA (Figure 4)

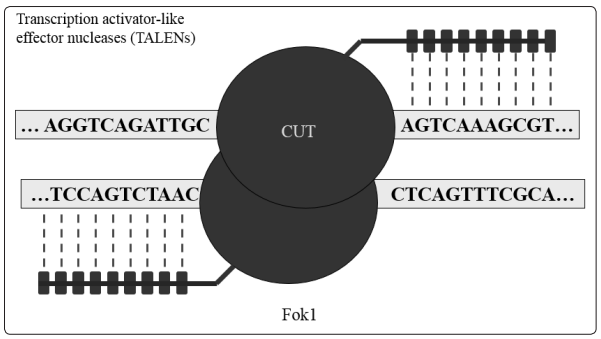

Figure 4: Illustration showing the components of Transcription activator-like effector nucleases (TALENs)

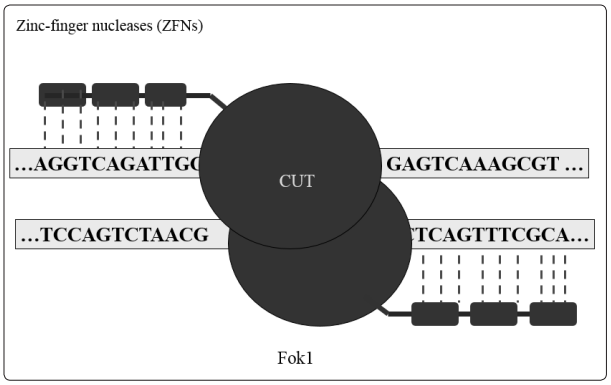

Figure 5: Illustration showing the components of zinc-finger nucleases (ZFNs)

Medical Applications

Gene Therapy

The ideal situation would be to replace the defective gene with a normal allele at its natural location. Gene targeting through ZFNs or TALEN based approaches can also be used to modify defective genes at their endogenous chromosomal locations. Examples are treatment of x-linked severe combined immunodeficiency (X-SCID) by Ex vivo gene correction with DNA carrying the interleukin-2 receptor common gamma chain (IL-2RY) and the correction of Xeroderma pigmentosum mutations in vitro using TALEN insertional mutagenesis by the retroviral vector genome induced leukemia in some patients. A problem that can be avoided by application of ZFNs or TALEN type of technologies. Gene editing is used to generate modified custom immune cells. Examples are modification of T-cells to inactivate glucocorticoid receptors resulting in immune cells being fully functional but resistant to the effects of commonly used corticosteroids T-cells expressing chimeric antigen receptors have been reported using TALEN technology. These T-cells can be engineered to be resistant to anti-cancer drugs and to invoke immune responses against targets of interest. The first clinical use of TALEN-based genome editing was in the treatment of CD19+ acute lymphoblastic leukemia. In this study, modified donor T-cells were engineered to attack the leukemia cells to be resistant to Alemtuzumab, and to evade detection by the host immune system.

Eradicating Diseases

CRISPR-Cas9 technology is currently being researched in eradicating diseases such as yellow fever, dengue, zika, West Nile, Schistosomiasis, Leishmaniasis and Lyme disease. There are currently non-clinical vaccines (animal studies) that are being researched. The CRISPR-Cas9 techniques are currently being developed to modulate the population of bacterial species by targeting clinical genotypes epidemiological isolates. These studies are being proposed to enable the beneficial bacterial species over the harmful ones by eliminating pathogens, which is advantageous over broad spectrum antibiotics. Therapeutic studies targeting human viruses such as HIV, herpes, and hepatitis B virus are being developed. These studies are being proposed to target the virus or the host to disrupt genes encoding the virus cell surface receptor proteins. Extensive research is being conducted on CRISPR-Cas9 in correcting genetic mutations which cause genetic diseases such as Down syndrome, spina bifida, anencephaly, Turner and Klinefelter syndromes using targeted gene therapy. Studies are being conducted by using CRISPR technology to target specific genes in cancer cells via genome editing to block cell proliferation and tumorigenicity of cancer cells.

Developmental Aspects of Pandemic Situations

Multiple disease outbreak models have been developed to evaluate impact of safety and effectiveness. Estimating the scope of a disease outbreak must address consideration of factors such as population susceptibility, effective/ineffective dose, incubation period, modes of transmission, duration of illness, mortality rate, effectiveness of treatment interventions and population migration. Environmental systems comprise of the release of epidemiological agents before the onset of symptoms in exposed subjects. Such systems have been known for the detection of epidemiological agents (i.e., bacteria, virus etc) in indoor and outdoor settings. Testing technological products are being developed and used for these types of agents' identification based on real-time polymerase chain reaction (PCR). Available testing technologies are based on detecting an environmental biological agent (i.e., virus particles). An ideal testing situation is dependent on accuracy, sensitivity, and specificity of the testing methodology dependent on efficiency of the sample collection system comprising of location and placement of the monitors, and the concentration of the organism in the air sampled by the collector. Rapid and accurate diagnosis of potential viral agents is crucial for identifying and lessening the impact of an infectious threat. A major effort is to enhance diagnostic capacity is the Laboratory Response Network (LRN), established by the CDC (Center for Disease Control). Monitoring systems using PCR and antibody-conjugated-technology have greater sensitivity and specificity than other methods for biological agents' detection.

Stem Cell Therapies

Stem Cell is considered as an unspecialized or an undifferentiated cell that gives rise to differentiated cells of multiple different lineages. For example, all the blood cells originate from a common progenitor hematopoietic stem cell that becomes committed to differentiate along particular cell-line lineages (i.e., erythroid, megakaryocytic, granulocytic, monocytic, and lymphocytic). Recent developments have shown a polymorphism of DNA in sickle cell patients. Sickle-cell disease is an example of a recessive genetic disease, which means that both copies of an individual patient's HBB gene carries the mutation for that patient. It has been reported if only one copy has the alteration, the other nonmutated gene can produce enough normal hemoglobin to cover the negative effects of the mutated hemoglobin. Sickle-cell disease is an example of simple substitution mutation (i.e., an erroneous swapping of one letter of DNA for another). Other genetic diseases, for example, Huntington's disease is due to a mutation of the HTT gene in which the same three letters of DNA get repeated several times. Nucleotide deletions have been reported in the most common type of cystic fibrosis [1-2].

Global researchers are developing applications of CRISPR-Cas9 to study the DNA of certain organisms as well as the genetic materials in human cells (i.e., sicle-cell disease comprising of a single DNA mutation interfering with red blood cells ability to carry oxygen through the body).

Clinical trials for stem cell therapies

Human pluripotent stem cells (PSCs) are leading candidates for cell-based therapies due to their capacity for unlimited renewals and pluripotent differentiation. These developments have recently been reported for the treatment of macular degeneration, type 1 diabetes mellitus, heart failure, Parkinson's disease, spinal cord injuries. The pluripotent stem cells are making their way into clinical trials (Phases 1,2, and 3). These therapeutic developments appear to be useful beyond those that could be corrected by replacing cells in their own lineage (i.e., blood transfusion, bone marrow transplantation) [3-4].

Pharmacological Considerations

Detailed knowledge of the pathophysiology of the disease and the pharmacology of the drug actions and reactions facilitates the design of clinical studies to determine the essential data needed for the US FDA's approval process. For rare diseases pre-clinical (non-clinical) pharmacodynamic studies can be important if there exists adequate animal models as precursors for the design of clinical studies. Such studies may also include dosing and or route of administration and efficacy. For rare disease studies (deficiency diseases – requiring typically enzyme or hormone replacement) and well characterized short- and long term consequences of the deficiency, and clear understanding of pharmacokinetics and pharmacodynamics of the proposed drug compound are helpful for designing FDA's premarket approval studies. FDA regulatory requirements for licensing 'substitution products' (notably recombinant products) may be unique in comparison

to other drug compounds, provided the symptoms related to the deficiency are clearly understood and that pharmacokinetics and pharmacodynamics of the proposed drug product are well documented for clinical studies [6-7].

Conclusion

Worldwide researchers devised a powerful gene-editing tool called CRISPR-Cas9 for clinical applications. This gene editing technology has revolutionized molecular genetics and gene therapy for cutting a gene at a specific spot, allowing scientists to operate on flaws that are the root cause of many diseases such as cancer, tumor-markers, diabetes, Parkinsons, Alzheimer. The US FDA's mission is to review and evaluate gene editing tools utilizing CRISPR–Cas9 technologies in new drugs IND and NDA applications. A central theme over the past few years has been use of quality system standards to evidence-based review and evaluation. The FDA emphasizes the Quality Risk Management approach to design of studies by providing oversight and objective review of risk-benefit analysis that guides the use of new technology by providing patients organized digital data in the appropriate labeling of the product.

Acknowledgement

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References

1. Rice FH, Aziz KJ (1986) Role of adroxazine and leucogenenol in the formation of blood cells. Clin Physiol Biochem 4: 337-349.
2. Aziz KJ (1996) Clinical Molecular Biology: Concepts and Applications. Advances in Clinical Chemistry 32: 39-72.
3. Mukherjee S (2016) The Gene: An Intimate History. Scribner-Simon & Schuster.
4. Doudna J, Sternberg S (2017) A Crack in Creation-Gene Editing. Mariner Books-Houghton Mifflin Harcourt.
5. Aziz KJ (1997) Drug Monitoring: Modern Approaches to Quality Assurance. CRC Handbook of Analytical Therapeutic Drug Monitoring 19: 335-349.
6. Aziz KJ (2018) The FDA's New Drug Approval Process. Drug Development & Delivery 3: 50-56.
7. Aziz KJ (2018) FDA's Quality Risk Management Approach to New Drug Applications. Br. J Pharm Med Res 3: 1147-1152.