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Navigating the Complex Testing Strategies for Viral Vector _Based Gene Therapies

Yusra A. Radeef*

*Department of Biology, College of Science, Babylon University, Iraq.

*Corresponding Email: *sci.yusra.ali@uobabylon.edu.iq*

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Abstract: Safely introducing transgenes into cells is one of greatest challenges facing the field of genetic factor analysis. Using virus-related directions has become one of the most effective means to achieve this by taking advantage of a virus's natural ability to invade and introduce their genetic material into human cells. Because of this, viral vectors are being developed to help to treat a variety of diseases. In order to assure safety and to meet regulatory requirements, thorough testing takes place through all steps of the viral vector development and manufacturing process. This article explores various viral vector testing methods, such as identify testing quality testing.

Keywords: Viral Vectors, Transgenes, Diseases, Gene Therapy.

1. INTRODUCTION

Genetic Factor Therapies

Genetic therapies are defined as a method through which healthy genetic factors are introduced into various diseased cells with the aim of producing functional genes. Therefore, this method is used to treat diseases.1

In recent years, there have been many developments in genetic engineering, including gene therapy, which involves many changes in genes with the aim of obtaining the desired gene to overcome the diseases.

Gene therapy is considered one of the best ways to overcome widespread diseases by manipulating the genes present in the tissues inside the body of the organism.

Previous scientific research has indicated that gene therapies are therapeutic methods with important effectiveness in treating diseases that have a hereditary basis and occur as a result of a defect in one gene. Among the diseases that result from a genetic defect are sickle cell deficiency and other hereditary diseases.2,3

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Principle of Genetic Therapies

It is a way to overcome diseases by obtaining the correct gene instead of the unwanted gene (Genetic changes) or Providing defective cells with many correct genes through genetic transfer, as these transferred genes work to treat the genetic defect in the patient's cells and thus treatment of diseases is achieved. The genetic diseases to be treated are inherited, meaning that the disease is transmitted from parents to children through sex cells (sperm or egg) that carry the defective gene or non-hereditary diseases that are generated in the person after his birth as a result of mutations (genetic defect). 4

A gene is defined as the genetic material present inside a living cell, and each gene performs a specific work. The work performed by the gene may be structural or functional in living cells. The results of research in recent studies have shown that the human body is composed of thirty thousand genes, while previous studies have shown that the human body is composed of a hundred thousand genes. 5, 6

All the basic information for the development of the fertilized egg up to the adult human being is carried by genes, and the characteristics may be external, such as the color of eyes, skin, hair, etc., or they may be functional characteristics, represented in the vital functions performed by living cells.7, 8

Genetic Therapies Methods

- Viral transfection.
- Liposomes.
- Ant sensitive technology.
- Biochemical alteration.
- Replacement therapy.
- CRISPR-Cas9.

Styles of Genetic Therapy:

There are two types of gene therapy depending on the type of cells targeted: The first type: the somatic type in which somatic cells are the target cells for gene therapy. This method involves genetic repairs on the cells of the body. This method does not include the sex cells present in the female, represented by the egg, and in the male, represented by the sperm and the fertilized egg.

The second type: the sexual type in which the sex cells are the target of gene therapy at the beginning of their development beforehand reaching the stage of differentiation into specific prison cell.

Other division of gene therapy:

Recent studies indicate the possibility of dividing gene therapy into different types according to the method used for the purpose of introducing the desired gene into the cells and tissues being treated. The in vivo gene therapy method involves introducing the correct gene into diseased cells and tissues for the purpose of treating them.

Ex vivo gene therapy method: This method includes genetic treatment of diseased cells outside the body of the human being, such as taking blood and marrow samples, after which these targeted cells are grown in cell farms, the desired gene is additional to the culture cells, and the genetically treated cells are returned to the body of the human being.

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In the previous two methods, reliance is placed on the type of cell to be genetically treated. The method of gene therapy outside the body is used to treat blood diseases of hereditary origin because all blood cells originate from a single stem cell from the bone marrow.

Viral Vectors

Recent studies have shown the possibility of using viral vectors to eliminate diseases. This method involves taking the virus carrying the desired gene and transferring it into the cells to be treated, and the viruses are treated to carry the desired gene.9

Research has indicated the most important viruses used as vectors for genes used in treating diseases genetically.

1. Retrovirus: It is considered one of the viruses used to treat diseases genetically and contains ribonucleic acid. Studies have indicated the possibility of these viruses in combining the viral genome with the genetic material in the target cell. Studies have shown their limited ability to mobilize genetic material, and their ability is limited to modified genes in a specific quantity. This is used. Type of viruses in the treatment of malignant cancer diseases. 11
2. Lentivirus: This type of virus is considered more capable of transferring genes to target cells than adenovirus. Studies have indicated the ability of these viruses to treat diseases genetically because they are considered slow viral vectors that have a greater ability to transfer healthy genes to cells that divide and do not divide.12
3. Adenovirus: This type of virus is considered more efficient in transferring genes because it is frequently used as viral vectors for gene therapy. It has the ability to infect cells that are dividing or not, and it has a great ability to mobilize genetic material inside the target cells. Studies have indicated its ability to treat many cancer diseases and is also used as a method. To transport the vaccine. 13
4. Adeno-associated virus (AAV): It is considered one of the viruses used to treat diseases genetically because it is small in size, does not cause diseases,14 and has the ability to maintain the genetic expression of genes that have the ability to be modified.15

2. RELATED WORKS

Genetic factor therapy is used to treat many diseases. According to statistics from the World Health Organization, the number of people who die annually from cardiovascular diseases, immune deficiency, and genetic diseases is estimated at about 18 million people in all countries of the world, which represents 35% of the number of deaths in countries of the world. These statistics have become an incentive for scientists to pay global attention to experiments on treating various diseases by replacing the defective gene with the correct gene.

Gene editing technology was used at the Oregon Health and Science University in America, the first experiment conducted on human embryos. This technology opens different horizons in the possibility of modifying some of the genes responsible for the occurrence of diseases whose origin is hereditary in an efficient and safe manner. CRISPR technology, which represents scissors, was used to cut specific pieces of the genome to replace them with new

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pieces of DNA. Similar experiments were conducted in China, and in 2015 it was agreed at an international conference in America that the use of gene therapies on human embryos would be considered irresponsible and must be subject to safety and efficiency conditions.
"CRISPR" is not just a way to modify the DNA (which contains the total genetic characteristics of a cell) in patients: it can be used for all living organisms, bacteria, plants and animals, to help diagnose diseases, according to Mario Amendola, in charge of research at Geneton Laboratories. He adds that there are other applications that are still in the research stage, such as the possibility of "modifying animal DNA to make organs compatible" with humans, or "modifying mosquito DNA to eliminate malaria."
In 2019, eight gene therapies were available worldwide to treat patients. In 2023, 27 gene therapies (including genetically modified cell therapies) have been approved, and there are nearly two thousand treatments under development, according to figures published by Citeline, a pharmaceutical services and consulting company, in October 2023.
Be "88% of CRISPR-related therapies are in early stages of development. Therefore, we are unlikely to see another approval in the near future."
Gene therapies require very large investments. The analysis company notes that "the enormous costs of development and production, the risks of failure of clinical trials, and pressures on pricing and reimbursement processes will continue to affect these innovations."

3. MATERIALS AND METHODS

(1) Identifying the defective genetic site that needs to be compensated for, through gene transfer or gene replacement.
(2) The necessity of providing the correct gene to be given to the patient. This was available for approximately half of the number of human genes thanks to scientific progress in DNA recombinant technology. These genes are carried on vectors and cloned.
(3) At the end of the stage of obtaining the humanoid genome, it is probable toward achieve the correct gene.
(4) It must be taken into account that gene therapy does not harm the target cell as a result of the introduction of the gene to be introduced, such as the occurrence of insertional mutations through invalidating the function of the correct gene, activating the primary protein, activating the cancerous gene, or neutralizing the action of genes that suppress cancerous tumors, and thus the damage is great to the target cells and thus to the sick person.
Additional potential damage is the probability which the gene entering the cell targets cells other than the mark cells and causes a negative effect. for example the beta gene that is transmitted to the marrow cells of beta thalassemia patients, which works in white blood cells while it must effort in red blood cells.
(5) When the correct gene reaches the largest number of diseased cells, enters them then special delivery itself, it will produce the correct results and thus lead to the elimination of the disease.

Methods of Delivering the Healthy Gene to Target Cells

Recent studies have indicated that viruses are one of the important biotic trajectories in genetic therapies, as the virus is the carrier of the desired gene, and through it, the gene enters

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the target cells, settles there, expresses itself, and thus treats the target cell. The furthermost imperative problem fronting genetic therapies operations is selecting the virus that carries the desired gene. A change occurs in the cells targeted by retroviruses, as these viruses enter the genetic material of the sick person's cells and form part of his nuclear material. When viruses enter an infected cell, the viral ribonucleic acid is transmitted to the nuclear material of the cell and turns into DNA. It is part of the DNA of the infected person and develops regularly.
Recent studies have shown the possibility of transferring the correct gene to an infected cell through an inactivated virus, but this technique has some drawbacks, including that these viruses have the ability to move in totally kinds of infected cells and are not specific to entering one kind, and this leads to a lack of transfer effectiveness.

4. RESULTS AND DISCUSSION

Gene therapy is one of the important methods in treating many diseases, as recent studies have shown the possibility of using gene therapy methods in treating many diseases with impressive success. Diseases that have been treated genetically include immunodeficiency, retinitis, and leukemia. 16
Recent studies have indicated that there is a great diversity of techniques used to treat diseases genetically. These modern techniques include the treatment of malignancy, immune diseases, and diseases of the circulatory system, but these techniques have some drawbacks, such as the possibility of unanticipated results of gene therapy, and the results may be deadly.
One of the possible errors in these techniques is inserting the desired gene into the incorrect place or inserting it into the healthy gene, which may cause it to stop working. One of the disadvantages of this method is an abnormal increase in cell activity and the occurrence of cancerous diseases. It is also possible for the gene to reach reproductive cells and cause undesirable results, and these results have dangers at the community level.17
Genetic ttherapy methods are used to treat cancer diseases, which can be considered an immune disease that occurs as a result of the failure of the patient’s immune system to function due to the inability of the immune system to recognize cancerous cells. From this it becomes clear that gene therapy does not treat genetic diseases only, but rather extends to treating non-genetic diseases. In large areas of the world. The lymphocyte antigen gene is introduced from a healthy cell into cancerous cells, after which the cancer cells produce the antigen and the immune system works to distinguish it and remove it directly. Thus, this is considered a way to eradicate malignant skin cancer.

There are non-specific methods known as non-sensitive methods that use these techniques to treat cancerous diseases by preventing the function of the gene that causes the cancerous diseases and thus works to nullify the function of the protein produced by this gene, thus turning a typical cells into a tumorous cells.18
There is another, non-specific method, by inserting an ribonucleic acid strand that can combine thru messenger ribonucleic acid and stop its work by preventing it from completing the translation process and stopping it from being converted into a protein. As a result, both of the previous techniques lead toward the similar effect that is stopping the construction of tumorous and typical cell proteins.

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We conclude from what was mentioned previously that it is possible to treat diseases genetically through the use of DNA or ribonucleic acid in both ways, internally and externally. Recent studies have shown that there is additional method to treat diseases genetically through injecting a gene that activates the medication used to treat cancer cells. . Its action is specific to cancer cells, and this method is good because it only works on cancer cells and does not purpose damage to healthy cells. This gene can be used to eliminate cells adjacent to cancer cells, and this method is called release therapy.

5. CONCLUSION

The Gene Therapy Unit aims to use genetically engineered viral vectors to deliver functionally effective therapeutic genes into cells. The primary goal of the unit is to discover new gene therapies using clinical vectors that have translational applications to enhance patient health. The unit conducts theory-proving studies that include gene cloning and the design and production of clinical viral vectors.

Ethical Approval
Systematic search includes submitting to the ethics of scientific studies in the ministerial committees affiliated with the Ministry of Higher Education in Iraq.

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