ISSN (Online): 2455-3662
EPRA International Journal of Multidisciplinary Research (IJMR) - Peer Reviewed Journal
Volume: 10| Issue: 12| December 2024|| Journal DOI: 10.36713/epra2013 || SJIF Impact Factor 2024: 8.402 || ISI Value: 1.188

RECENT DEVELOPMENT IN GENE THERAPY: VECTORS AND DELIVERY SYSTEM

Miss. Suchita S. Lathi[1], Shruti Pramod Shinde[2*], Tushar S. Sawant [3] Sudarshana R. Shelke[4], Prajakta B. Shinde[5,] Isha R. Suryavanshi[6]

[1]*Asst.Proff. (Faculty of P'ceutics) Raosaheb Patil Danve College of Pharmacy Badnapur,Jalna*
[2]*Student of Bachelor of Pharmacy, Raosaheb Patil Danve College of Pharmacy Badnapur,Jalna*
[3]*Student of Bachelor of Pharmacy, Raosaheb Patil Danve College of Pharmacy Badnapur,Jalna*
[4]*Student of Bachelor of Pharmacy, Raosaheb Patil Danve College of Pharmacy Badnapur,Jalna*
[5]*Student of Bachelor of Pharmacy, Raosaheb Patil Danve College of Pharmacy Badnapur,Jalna*
[6]*Student of Bachelor of Pharmacy, Raosaheb Patil Danve College of Pharmacy Badnapur,Jalna*
Raosaheb Patil Danve College of Pharmacy Badnapur, Jalna 431202

ABSTRACT

Gene therapy is the transfer of a genetic material to treat a disease, or at least to improve the clinical status of a patient.viruses into genetic vectors carrying the gene of interest to the target cells is the aim of gene therapy. Based on the genome's nature, these vectors are divided into RNA-based or DNA-based viral vectors .[1] Gene therapy has recently witnessed accelerated progress as a new therapeutic strategy with the potential to treat a range of inherited and acquired diseases. The process of gene therapy involves converting viruses into genetic vectors that transport the desired gene to the specific cells. These vectors are categorized as either RNA-based or DNA-based viral vectors depending on the genome's composition. Recently, there has been rapid advancement in gene therapy, which is emerging as a new method to potentially treat various inherited and acquired illnesses. Billions of dollars have been dedicated to basic and clinical research in gene medicine, with current clinical trials targeting cancer, monogenic diseases, cardiovascular diseases, and other difficult-to-treat illnesses. Gene therapy shows great potential as a molecular method to treat uncommon genetic disorders. Gene therapy works by restoring, replacing, inhibiting, and editing genes to correct the disease phenotype. Recent studies indicate that a growing percentage of gene therapy clinical trials are opting for viral vectors (64.2%) over non-viral vectors. Gene therapy of human genetic diseases essentially requires gene delivery systems. Gene therapy is a special technique that can use modified genes to treat any illness. Gene therapy shows great potential as a treatment for various conditions like genetic diseases, viral infections, and cancer. The efficacy of gene delivery systems is determined by the flexibility of targeting gene delivery systems. In recent times, several effective gene delivery systems have been successfully developed for the practical use of gene therapy. Gene therapy can permanently fix diseases by introducing normal genes to replace mutant gene deficiencies, suppressing mRNA of mutant alleles, and triggering cell death in cancer cells with transgenes that encode apoptosis-inducing proteins. Encouraging findings from clinical trials for eye disease and Parkinson's disease indicate that gene-based neurotherapeutics hold significant promise.

KEYWORDS: *Gene therapy, viral vector ,non viral vector, delivery system .*

INTRODUCTION

Beta-thalassemia Lipid-based nanoparticles AAV9 Lentivirus from Ad5 vector caused the death of an 18-year-old male in 1999. In 2000, the first successful instance of gene therapy was documented in treating X-linked severe combined immunodeficiency (SCID-X1) disorder. Regrettably, a participant in the early clinical trials was diagnosed with leukemia caused by the Moloney murine leukemia virus vector[6]. These unforeseen side effects prompted researchers to concentrate on the fundamental principles of gene therapy, with speculation already existing regarding the potential for gene therapy before the initial human coding sequence was identified. An insightful article from 1971 in the field of science highlighted numerous challenges that would be encountered in clinical gene therapy, such as developing secure viral delivery vehicles and effectively delivering genes to sufficient patient cells to fix the inherited genetic flaw. Initially investigated solely for monogenetic disease treatment, gene therapy's scope expanded quickly to include various ailments following its 1972 definition by Friedmann and Robin. The FDA authorized the first gene therapy clinical trial in 1990 to address ADA-SCID, but the intense immune reaction prompted the development of new vectors for Spinal muscular atrophy and Familial chyluria syndrome. As a result, scientific and technological progress has led to the emergence of a new era in clinical trials and facilitated the introduction of gene therapy products [7]. The State Food and Drug Administration of China gave approval in 2003 for the use of the gene therapy product Gendicine to treat head and neck squamous cell carcinoma. Gendicine is the first of its kind and utilizes a recombinant adenovirus vector containing the human p53 tumor suppressor gene. Gene therapy seeks to close the distance between the development of a disease and its treatment by addressing the root causes of the illness. The additional value of this new approach is the utilization of gene therapy to address symptoms[8]. Gene therapy therefore aims to bridge the gap

ISSN (Online): 2455-3662
EPRA International Journal of Multidisciplinary Research (IJMR) - Peer Reviewed Journal
Volume: 10| Issue: 12| December 2024|| Journal DOI: 10.36713/epra2013 || SJIF Impact Factor 2024: 8.402 || ISI Value: 1.188

between disease pathogenesis and effective therapy, aiming to correct the underlying causes of disease. The use of gene therapy to treat symptoms adds a further value to this novel alternative to classical small-chemicals pharmacology[11] .

GENE THERAPY

The process of using a gene to treat, prevent, or cure a disease or medical condition is known as gene therapy. A gene that causes disease is replaced with a healthy copy. Gene therapy is the process of modifying or correcting host genes for therapeutic purposes. It can be carried out in a number of ways, including replacing the defective gene, altering gene expression (exon skipping), supplementing with healthy genes, and, most recently, host genome editing [3]. foreign genetic material (DNA or RNA) that can be used in gene therapy to change genes that aren't working or to change the expression of genes that are [2]. Gene delivery is a therapeutic strategy that corrects genetic defects by either eliminating a harmful function or restoring native functional activity.

Several diseases and disorders, such as hemophilia and other conditions, blindness from Retinitis pigmentosa, leukemia, inherited neurological disorders, cancer, heart and blood vessel diseases, Parkinson's disease, Alzheimer's disease, and cystic fibrosis, have been treated with gene therapy in human clinical trials. When the first approved gene therapy trial employing retroviral vectors expressing adenosine deaminase (ADA) to treat ADA deficiency—a disorder that results in severe combined immunodeficiency—began in the 1990s, gene therapy trials gained impetus. Long-term maintenance of ADA activity after multiple treatments was noted in one of the two patients treated in this experiment, suggesting that this medication may have beneficial and long-lasting benefits [[. Gene therapy's comparatively long-term effectiveness is one of its main advantages. Following the successful transfer of a therapeutic gene

The drawbacks of using peptides in recombinant medicine, including limited bioavailability, instability, high production costs, clearance rates, and severe toxicity, are addressed via gene therapy[2].Gene knockdown, deactivating problematic genes, inserting a new gene to treat a disease, and replacing dysfunctional genes with therapeutic genes are some of the ways that gene therapies work [24]. For hereditary retinal disorders, gene therapy can quiet a dominant gene, replace a damaged gene, improve or modify a functional gene, or change the RNA to create a functional protein. Each gene therapy vector has advantages and disadvantages that determine which gene types it is appropriate for [25] .

Vectors

A vector is a means of introducing genetic material into a cell in order to fix a gene that has been mutated or damaged. There are two categories of vectors, which are as follows:

1) Viral vector
2) Non – viral vector

1) **Viral Vector**

A viral vector is a means of introducing genetic material into a cell in order to fix a gene that has been altered or damaged. The 1980s saw the first appearance of viral vectors [9]. In 1984, the vaccinia virus was employed as a vaccine vector to shield chimpanzees from Hepatitis B. Viral vectors are used in DNA-based gene delivery systems to transfer genetic materials to the host cells [4]. Viral vectors must frequently be administered repeatedly in order to provide a beneficial therapeutic impact, but this tactic can also strengthen the immune system's defenses against the vector [12]. 0 Clinical application of viral vectors is determined by factors such as stability, toxicity, safety, ease of manufacture, and transgenic expression efficiency.31 Additionally, the different kinds of vectors are depicted by viruses with single-stranded (ss) or double-stranded (ds) genomes that have both RNA and DNA [19] . There are now 18 active clinical trials for in vivo gene treatments that use more recent viral vectors besides AAV, Ad, and HSV. Lentivirus, arenavirus, measles virus, MVA, fowlpox virus, VSV, human cytomegalovirus, retrovirus, and Sendai virus are some of these more recent viral vectors. Different indications for these viral vector-based gene treatments are developing, depending on the characteristics of certain viral vectors [20] . In the treatment of monogenic inherited diseases, blood disorders, chemotherapy-resistant cancer (acute lymphocytic leukemia, or ALL), disease modeling for chronic and neurological disorders like Parkinson's, Alzheimer's, neuropathies, and so forth, gene therapy using viral vectors has consistently produced positive results in gene transfer [3].

Types of Viral Vectors

- Adenoviral
- Lentiviral
- Retroviral
- Herpes simplex viruses

Adenoviral

Adenoviral particles lack lipids and membranes, they remain stable in solvents like ethanol and ether. Well-characterized, non-integrated, linear dsDNA viruses that are roughly 26–40 kb long, non-enveloped, and covered with icosahedral particles, adenoviruses have a diameter of about 950 Å (not including elongated fiber proteins) and a molecular weight of about 150 MDa. They swiftly infect cells and are not reproduced [13]. Human adenoviruses (AdVs) are non-enveloped viruses with a double-stranded DNA genome of 26–45 kb and an icosahedral capsid that measures 90–100 nm in diameter [23]. The majority of AdVs produce mild infections in humans, such fever or cough, but others can cause serious illnesses or multi-organ dysfunctions in immunocompromised people [23].

The majority of AdVs produce mild infections in humans, such fever or cough, but others can cause serious illnesses or multi-organ dysfunctions in immunocompromised people [23]. Even though AdV vectors are said to have potent immunogenic effects, they are nonetheless often used in lab experimental models and even in gene therapy for conditions like cancer. The Chinese FDA authorized Gendicine, a recombinant AdV vector that expresses p53, in 2004 for the treatment of head and neck cancer [21]. DNA-containing non-integrating viruses known as adenoviruses (AdVs) may infect both dividing and non-dividing cells [3]. AdVs have two main disadvantages:

ISSN (Online): 2455-3662
EPRA International Journal of Multidisciplinary Research (IJMR) - Peer Reviewed Journal
Volume: 10| Issue: 12| December 2024|| Journal DOI: 10.36713/epra2013 || SJIF Impact Factor 2024: 8.402 || ISI Value: 1.188

immunogenicity and a lack of long-term expression, which limit their usage in in vivo settings.

Lenitiviral

LVs are enclosed viruses that range in size from 80 to 120 nm. They have two copies of a 9 kb single-stranded genomic RNA, which is subsequently reverse-transcribed into double-stranded DNA in order to integrate into the host chromosome [23]. Human immunodeficiency virus type 1 (HIV-1), simian immunodeficiency virus, feline immunodeficiency virus, and equine infectious anemia virus have all been used to create various LV systems [23]. Ex vivo gene therapy applications, such as the stable integration of CAR (chimeric antigen receptor) cDNA into T cells, have been the primary usage of LV vectors. This is due to LVs' many characteristics, which include their broad tropism via VSV-G pseudotyping, huge genetic capacity (up to 8 kb), and capacity to infect both proliferating and non-dividing cells [23]. Furthermore, because LVs are protected by a cellular lipid bilayer, they are less immunogenic than AAV or AdV.Transduction of both dividing and nondividing cells, adaptability to various cell types, lack of viral proteins following transduction, delivery of complex genetic elements, and relative ease of vector manipulation and production are some of the traits that make lentiviruses attractive tools for gene therapy [14]. Lentivirals are viral systems that transfer genes to non-dividing cells but lack the ability to replicate and have tiny, retrovirus-like viral proteins [13]. The two main drawbacks of lentiviral vectors, such as insertional mutagenesis and genomic integration, restrict their in vivo uses to the same degree as those of AAV vectors [3]. Retinal genes that cannot be packaged in AAV vectors because of their small 4.7 kb packaging capacity, such as ABCA4 (associated in Stargardt's illness) and Myo7A (related in Usher's syndrome), are very effective when lentiviruses are used. Lentiviruses have an advantage over other viral vectors since they can infect both quiescent and growing cells.

Retroviral

If the target cells are actively proliferating, retroviral vectors generated from the Moloney murine leukemia virus (MOMLV) can transduce a broad range of target cells and integrate into the host genome [15]. Retroviruses are diploid, single-stranded, circular-enveloped RNA viruses that belong to the Retroviridae family. They have a diameter of about 80–120 nm with a genomic size of 7–11 kb. Although retroviruses are the cause of diseases including AIDS, leukemia, and cancer, major advancements in treatment have been made possible by their use as a vector in gene therapy [13].

RVs have the capacity to permanently alter cells' genetic makeup by integrating their cargo into dividing cells. Nevertheless, RV vectors show poor in vivo efficiency, and this characteristic raises the danger of insertional mutagenesis [10]. Retroviruses are a class of viruses that may integrate into host cell chromosomes and change RNA genomes into double-stranded DNA, like the human immunodeficiency virus (HIV). The integrase enzyme can insert the virus's DNA into any part of the host genome, which is a challenge when employing retroviruses for gene therapy. The function of one of the host cell's primary genes will be compromised if the genetic material is put in the middle of it (insertional mutation).

Herpes Simplex Virus

HSV has the ability to multiply and infect a wide range of neurons and epithelial cells, including cancer cells [16]. It is possible for Adeno-associated viral vectors (AAV) to introduce a gene into the 19th chromosome. AAV comes in five varieties, each with unique characteristics. While AAV-5 can transfer a gene into glial cells and neurons, AAV-2 can only transfer a gene into neurons. The primary attribute of AAV vectors is their low immunogenicity
and cytotoxicity [17]. This class of viruses infects a particular kind of brain cell using double-stranded DNA. A frequent human disease that produces fever blisters and cold sores is type 1

Herpesvirusinfection.[10]

The human neurotropic virus known as the Herpes Simplex Virus is mostly utilized in the nervous system for gene transfer. Though it may be inactivated and result in a lytic cycle of viral replication, the wild HSV-1 virus can invade neurons and evade the host's immune response. As a result, a mutant strain of HSV-1 that cannot replicate is usually employed [1]. In order to create recombinant HSV-1 vectors, some or all immediate early (IE) genes, such as ICP4, which is necessary for viral replication, have been deleted [17].

2) **Non-Viral Vectors**

These types of delivery systems transfer genetic material to cells without the use of viruses. In order to transfer only the bare DNA, nonviral vectors usually use a plasmid-based gene delivery mechanism, often in combination with physicochemical techniques that make transfection easier. Therefore, although an innate immune response may still happen, the nonviral method may be less immunogenic and possibly safer than viral vectors.

Although the nonviral gene transfer technique is straightforward, it often has a lower effectiveness than the majority of viral vector-mediated gene transfer techniques. Furthermore, unless selection is used on ex vivo transfected cells, nonviral transfection is usually temporary and results in transient expression of the transgene [15]. In order to introduce genetic elements through cell membranes, non-viral gene delivery is purposefully mediated by physical means. Needlestick injection, ballistic DNA injection, sono-poration, photo-poration, magneto-fection, and hydro-poration are examples of the physical way of gene delivery. Using a needle to directly introduce genetic elements is known as needle injection [4]. In order to accommodate bigger genetic transfers, the synthetic vector can bind to condense the genetic information and engage electrostatically with RNA or DNA. Then, endocytosis allows non-viral chemical vectors to enter cells. Generally speaking, there are two non-viral vectors: polymers and liposomes. By forming lipoplexes, liposomes enable gene transfer in liposome-based non-viral vectors. When positively charged liposomes and negatively charged DNA come into contact, lipoplexes are naturally created.

ISSN (Online): 2455-3662
EPRA International Journal of Multidisciplinary Research (IJMR) - Peer Reviewed Journal
Volume: 10| Issue: 12| December 2024|| Journal DOI: 10.36713/epra2013 || SJIF Impact Factor 2024: 8.402 || ISI Value: 1.188

Non-viral vectors based on polymers interact with DNA to create polyplexes.[4]

Types of Non Viral Vector / Physical Method

- Cationic lipids
- Polymeric nanoparticles
- Electroporation[15]
- Sonoporation
- Gene gum
- Ormacity[1]
- Injection of naked DNA
- Photoporation
- Magnetofection
- Hydroporation

1.Electroporation

An electromagnetic pulse that makes holes in a cell membrane to let genetic elements in is known as electroporation [15]. One technique for transferring DNA from cell membranes is electroporation, which involves brief, high-voltage pulses. The membrane becomes permeable to nucleic acid due to the temporary formation of tiny pores on its surface brought on by electrical shock. Although electroporation is applicable to many cell types, its usage in clinical settings has been restricted due to high cell death rates [1].

2.Gene Gun

Another physical technique for DNA transfer is particle bombardment, also known as a "gene gun." This technique involves coating the DNA with gold particles and then putting it inside a machine that gives it the force it needs to enter the cell. However, DNA can cause a tumor if it is found in the wrong location within a genome, such as in a tumor suppressor gene. In clinical studies involving patients with X-linked severe immunodeficiency (X-SCID), this approach was attempted. Three out of twenty patients had their T cell leukemia successfully treated when HSCs were infected with a retrovirus that contained the modifying gene[1].

3.Sonoporation

This technique introduces DNA into a cell by using an ultrasonic frequency. DNA moves throughout the cell as a result of this event, which is seen as an ultrasonic cavitation in the cell membrane [1]. A sound wave called sono-poration makes holes in a cell membrane so that genetic elements can enter.

4.Hydroporation

The hydrodynamic capillary effect that controls cell permeability is known hydro-poration.[4].

5.Magnetofection

The process of concentrating nucleic acid particles into target cells by complexing magnetic particles with DNA and an external magnetic field. To expose the DNA-containing substance to only one cell layer, magnetic particles are complexed with DNA and a magnet is positioned beneath the cellular tissue culture container. The therapeutic gene binds to the magnetic nanoparticles in this approach, which is predicated on the idea of targeted medication delivery. The complex deposition and transfection rate are additionally accelerated by the electromagnetic field gradient created by the ground beneath the cell culture media [1].

6.Ormasil

Another non-viral technique is the use of Ormasil, which is silica or modified organic silicate. Silica is a wonderful choice for gene delivery because of how easy it is to work with. Because of its low toxicity, silica is most frequently used in gene therapy in combination with amino silicones and nanoparticles. Delivery in the presence of serum, however, decreases the effectiveness of this technique because of the limiting factor of the reaction between serum proteins.[1]

7. Photoporation

This process involves using a ray pulse to make holes in a cell membrane so that genetic materials can enter.

ISSN (Online): 2455-3662
EPRA International Journal of Multidisciplinary Research (IJMR) - Peer Reviewed Journal
Volume: 10| Issue: 12| December 2024|| Journal DOI: 10.36713/epra2013 || SJIF Impact Factor 2024: 8.402 || ISI Value: 1.188

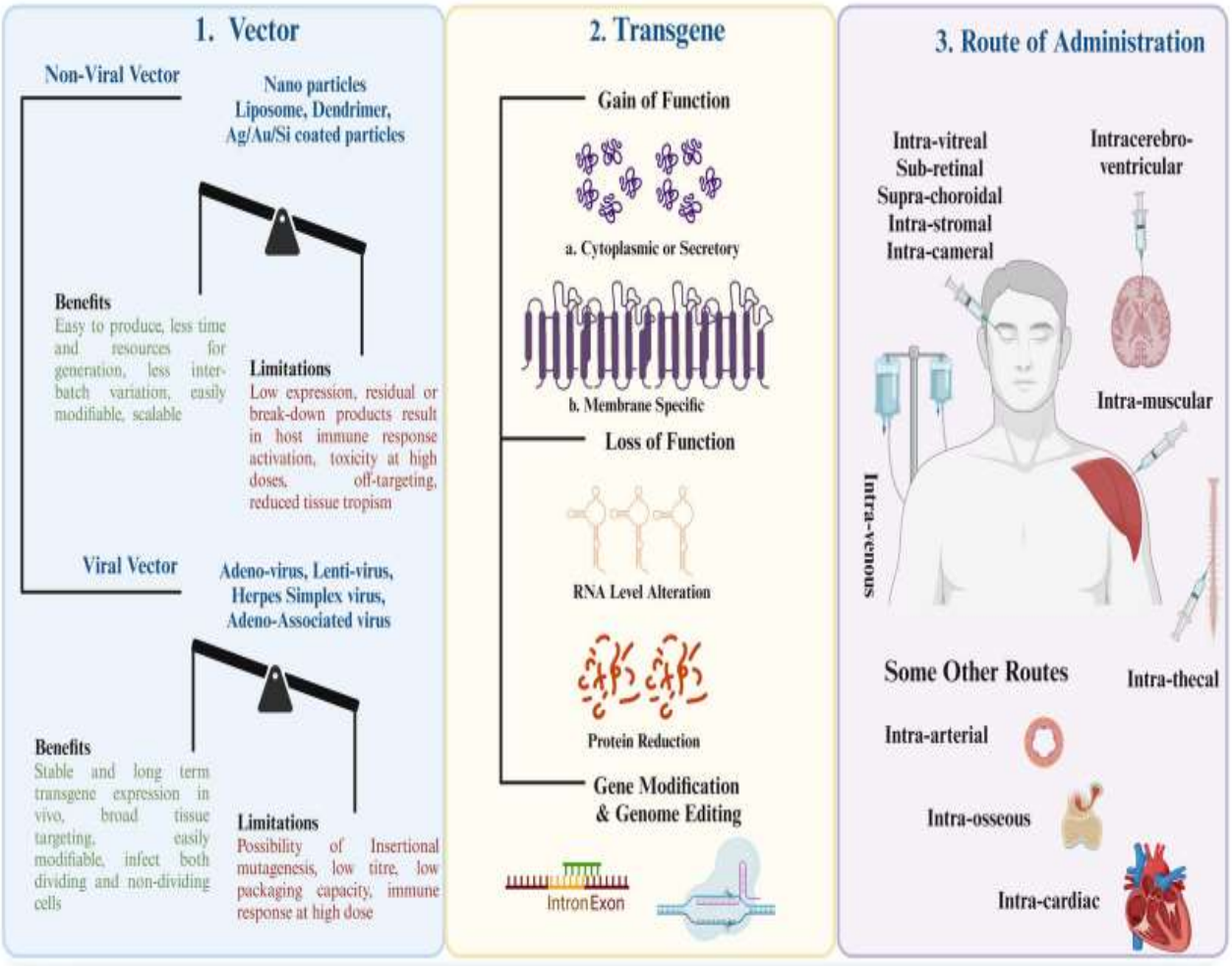


Fig no 1 : Development of Vector System [3]

Gene Delivery System

Virus-Mediated gene delivery systems make use of the virus's capacity to introduce its DNA into host cells [4]. Ex vivo modified cells, viral vectors, and non-viral vectors are among the gene delivery methods available for gene transfer and treatment. Direct injection of naked DNA, lipofection, and gene gum are the most widely utilized non-viral delivery methods for gene transfer in vivo. These techniques' low transduction efficiency is its main flaw. Viral vectors have been effectively used to deliver directly into the in vitro and in vivo environments due to their high delivery efficiency. Targeting uncommon or incurable illnesses that frequently lack traditional treatments is made possible by advancements in gene delivery. Actually, over 70% of uncommon illnesses are are monogenic, and only 8% of these illnesses can be cured with a medication that has FDA approval [10]. Ex vivo modified cells, viral vectors, and non-viral vectors are among the gene delivery methods available for gene transfer and treatment. The gene gun, lipofection, and direct injection of naked DNA are the most widely utilized non-viral delivery methods for gene transfer in vivo. These techniques' low transduction efficiency is its main flaw. Both in vitro and in vivo, genes have been effectively delivered into the pituitary gland using viral vectors, which have a high delivery efficiency [11].

In vivo and ex vivo are the two methods for delivering the desired gene. The genetic material is delivered directly to the patient's body using the in vivo method, either as a naked gene or encapsulated in a particle. The ex vivo method, on the other hand, entails removing the target cells from the patient or a healthy donor, genetically altering them, sometimes growing them, and then giving them to the patient. As a result, the patient and the transmission vector are not in direct contact.

ISSN (Online): 2455-3662
EPRA International Journal of Multidisciplinary Research (IJMR) - Peer Reviewed Journal
Volume: 10| Issue: 12| December 2024|| Journal DOI: 10.36713/epra2013 || SJIF Impact Factor 2024: 8.402 || ISI Value: 1.188

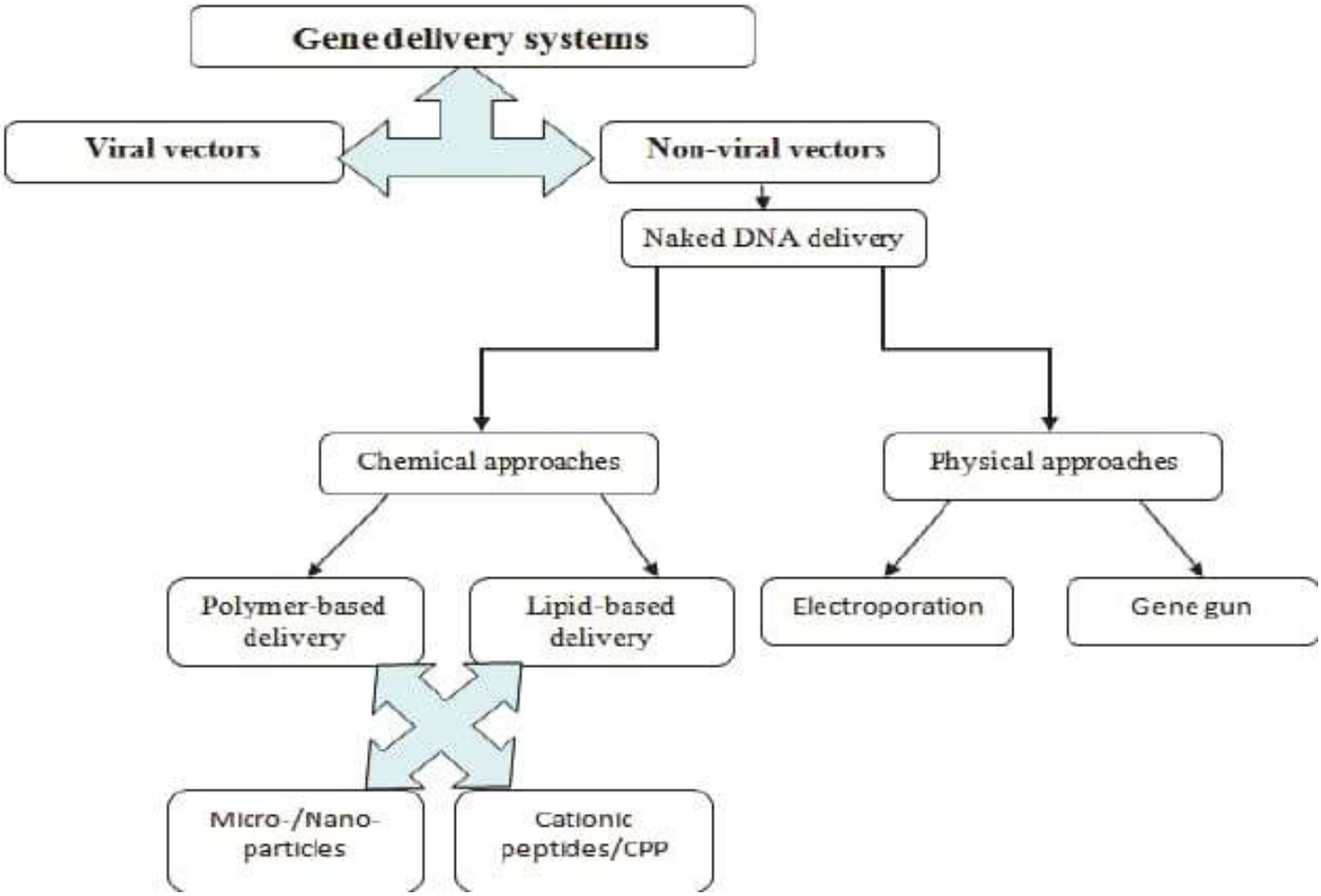


Fig no 2 . Gene Delivery System[18]

Gene Editing Technology

A sequence-specific DNA binding module is used in genome editing technology to insert, remove, or modify DNA at specific loci in a living cell's genome. One of the most important methods for gene engineering is genome editing, which began with meganucleases, zincfinger nucleases (ZFNs), and transcription activator-like effector nucleases (TALENs). Importantly, Drs. Jennifer Doudna and Emmanuelle Charpentier's 2012 discovery of clustered regularly interspaced short palindromic repeats (CRISPR)-Cas9 (CRISPR associated protein 9) as a programmable nuclease greatly increased the potential for genome editing [23].

In prokaryotes, clustered regularly interspaced short palindromic repeats (CRISPR) were initially identified as an effector of the adaptive immune system. CRISPR is made up of a collection of little DNA sequences that are present in prokaryotic genomes and were obtained through prior bacteriophage infections. Prokaryotes can use it as a defense mechanism to prevent reinfection by similar bacteriophages. The use of gene editing for human diseases was made possible by the subsequent development of this technology into a tool for modifying genes in eukaryotic cells.[30]

Apllications

- Gene Therapy: Treating genetic diseases by editing genes in vivo.
- Cancer Treatment: Editing genes to selectively kill cancer cells.
- Gene Editing for Rare Diseases: Treating rare genetic disorders.
- Regenerative Medicine: Using gene editing to generate healthy cells for transplantation.
- Infectious Disease Treatment: Editing genes to confer resistance to infectious diseases .

Gene Editing Technologies

1. CRISPR/Cas9: A popular, precise, and efficient gene editing tool.
2. TALENs: Another precise gene editing tool using transcription activator-like effector nucleases.
3. ZFNs: Zinc finger nucleases for precise gene editing.
4. Base Editing: A novel approach for precise, irreversible conversion of one DNA base to another.
5. Prime Editing: A versatile, precise gene editing tool combining CRISPR/Cas9 and reverse transcription.

Benefits

1. Precision: Gene editing enables precise modifications to the genome.
2. Efficiency: Gene editing can be more efficient than traditional genetic engineering methods.
3. Flexibility: Gene editing can be applied to various cell types, organisms, and systems.

ISSN (Online): 2455-3662
EPRA International Journal of Multidisciplinary Research (IJMR) - Peer Reviewed Journal
Volume: 10| Issue: 12| December 2024|| Journal DOI: 10.36713/epra2013 || SJIF Impact Factor 2024: 8.402 || ISI Value: 1.188

4. Therapeutic Potential: Gene editing holds promise for treating genetic diseases and improving human health.

APPLICATION

Cystic Fibrosis

- CF is a condition that causes the lungs to gradually deteriorate. Inflammation, blockage, deformity, and respiratory tract infection are some of its symptoms. There are certain benefits to directly delivering the cystic fibrosis trans membrane regulator (CFTR) gene to the target tissue, which is the cells of the respiratory tract epithelium. The host's lung's immunological and physical barriers, however, make it difficult for the gene to successfully enter the respiratory system. Research on CF has been spurred by advancements in tissue engineering, gene transfer techniques, and animal models [28].

- Adeno-associated virus AAV2 was the focus of the University of California. AAVs may be produced in vast quantities with relative ease and are safe for human use. AAV vector-delivered genes frequently exhibit long-term gene expression but are not permanently incorporated into the cell genome. The molecular modification of the virus, which made it specifically designed to enter the pig's airway cells, was a crucial component of the AAV study. In addition to their utility in CF gene therapy, these evolving vectors can be employed as a multifunctional gene transfer tool. Five variants of the AAV2 virus have been developed that are 240 times more successful than the AAV2 itself at infecting pigs' airway cells [29].

Hemoglobin-Linked Diseases

- Haemoglobin Thalassaemia is a collection of hereditary disorders that alter hemoglobin function, and about 270 million people worldwide are considered carriers. As of right now, scientists have discovered more than 200 β globin (HBB) gene variants linked to illnesses like sickle cell disease (SCD) and β-thalassaemia. SCD is an intriguing candidate for gene therapy since the most prevalent genotype of the disease is G6V, a single amino acid alteration in the HBB gene from glutamic acid to valine[10].

AIDS

- Gene therapy for AIDS included methods to stop the virus from replicating, like delivering dominant negative viral proteins to cells infected with HIV[9].

Parkinson's Disease (PD)

- This neurological condition, which affects over 4 million individuals globally, is typified by the death of dopaminergic neurons in the substantia nigra and striatum. Because the blood-brain barrier (BBB) stops medications from diffusing into the central nervous system (CNS), neurological disorders like Parkinson's disease (PD) are among the most difficult to treat with traditional therapies. Due in part to the finding that AAV9 could traverse the blood-brain barrier, significant progress has been made in overcoming this obstacle through the use of vector-mediated gene delivery. However, PD involves numerous genes, unlike the disorders described above, making gene therapy treatment more difficult [10].
- It has been established that gene therapy is successful in treating Parkinson's disease (PD). For instance, one of the suggested approaches raises the brain's concentration of GABA, a neurotransmitter whose deficiency results in Parkinson's disease. 45 volunteers with severe Parkinson's disease participated in the trial, where tubes were inserted into the brain regions related to movement. Half of the subjects received an innocuous saline solution (as the control group) while the other half received injections of viruses containing the gene that boosts the production of GABA. The movement capacity of individuals who received gene therapy improved by 23% after 6 months, which was twice as much as that of the control group [1].

Alzheimer'Disease

- The term Alzheimer's disease refers to Among the most prevalent illnesses of the nervous system are mental ones, and Alzheimer's disease (AD) is the leading cause of dementia globally for which there are now no viable treatments. The widespread buildup of faulty tau in the brain is the cause of AD and certain frontotemporal dementias (FTDs), which are referred to as tauopathies. New methods for the study of AD and other neurological illnesses have been made possible by recent advancements in gene therapy-based methodologies, particularly recombinant AAVs (rAAVs)[27].

Breast Cancer

- The most prevalent cancer in women, breast cancer, has been treated with gene therapy. Molecular chemotherapy, proapoptotic gene therapy, anti-angiogenesis gene therapy, neutralization of the mutation, immunopotentiation (improving the immune response by accelerating and extending its development and extending its duration), and genetic modulation of resistance-sensitivity are some of these. The evaluation of gene therapy's effectiveness, toxicity, and immunity in breast cancer clinical trials has started.
- Gene therapy combined with radiation or chemotherapy has shown encouraging outcomes. As a result of advancements in vector design and novel gene therapy techniques, gene therapy is now widely used to treat breast cancer. The majority of clinical trials concentrate on TSG P53. Injecting the p53 adenoviral vector intramuscularly is the recommended technique.

CHALLENGES AND LIMITATION

Technical Limitations

1. Delivery: Efficient delivery of genetic material to target cells.
2. Vector design: Optimizing vectors for efficient gene transfer.
3. Gene expression: Regulating gene expression levels.

ISSN (Online): 2455-3662
EPRA International Journal of Multidisciplinary Research (IJMR) - Peer Reviewed Journal
Volume: 10| Issue: 12| December 2024|| Journal DOI: 10.36713/epra2013 || SJIF Impact Factor 2024: 8.402 || ISI Value: 1.188

4. Targeting: Specificity and accuracy in targeting cells.
5. Duration: Maintaining long-term gene expression.

Safety Concerns

1. Toxicity: Vector-induced toxicity.
2. Immune response: Immune reactions to vectors.
3. Insertional mutagenesis: Unintended genetic changes.

Biological Limitations

1. Cell heterogeneity: Variability in cell populations.
2. Gene regulation: Complexity of gene regulation.
3. Epigenetics: Influence of epigenetic factors.
4. Gene-environment interactions: Environmental impact on gene expression.
5. Evolutionary adaptations: Potential for adaptive response

Economic Limitations

1. High costs: Development, production, and administration expenses.
2. Limited accessibility: Restrictive pricing and availability.
3. Reimbursement: Uncertainty surrounding insurance coverage.
4. Pharmaceutical industry interests: Balancing profit and public benefit.
5. Funding: Limited government and private funding.

Clinical Limitations

1. Patient selection: Identifying suitable candidates.
2. Dosing and administration: Optimizing treatment regimens.
3. Monitoring and follow-up: Ensuring patient safety.
4. Combination therapies: Integrating gene therapy with other treatments.
5. Long-term follow-up: Monitoring patients over extended periods.

CONCLUSION

Gene remedy has advanced significantly in recent times because to considerable advancements in vector development and delivery systems, which have bettered safety and efficacy. Viral gene delivery styles are made up of contagions. New viral and non-viral vectors have surfaced, and enhanced delivery ways that have been altered to be replication-deficient have made it possible to transfer genes to the target cells for expression. The maturity of gene remedy exploration has been on contagious conditions, cancer, and monogenic diseases. Though they only regard for 0.6 of all gene remedy clinical studies, autoimmune and seditious diseases are among the underrepresented conditions that gene remedy may be suitable to cure. Recent advancements in gene remedy, such the creation of CRISPR- intermediated genome engineering andnon-viral gene delivery, have significantly increased the liability of chancing gene curatives. Combining CRISPR technology with both viral andnon-viral vectors, for case, may enhance remedial sweats, according to some exploration. Over the once ten times, there have been several advancements in clinical gene remedy. One can list a number of noteworthy achievements, including the development of drugs for conditions including diabetes, Alzheimer's, Parkinson's, cystic fibrosis, and several types of cancer. To put it another way, gene remedy encompasses a variety of gene transfer ways and can be used to treat a broad diapason of ails.

SUMMARY

An overview of current developments in gene remedy vectors and delivery systems is handed in this review paper. Then, we go over the present and unborn operations of gene remedy in the treatment of several ails. also, look at the elaboration of both viral andnon-viral gene delivery vectors. In addition to pressing the difficulties and constraints related to gene delivery, we also look at the function of gene editing technologies in gene remedy and suggest possible directions for unborn study in this field. A promising system for treating contagious infections, cancer, and inheritable diseases is gene remedy. still, an effective and secure vector distribution medium is pivotal to its success. Recent advancements in viral and non-viral vectors, similar as lentivirus, retrovirus, adeno- associated contagion, and cationic lipids, are stressed in this study.

FUTURE SCOPE

Three decades after the first gene therapy study in the 1990s, the science of cell and gene therapy has regained pace. There are currently over 1000 active cell and gene therapy trials for a variety of treatments, including neurological, immunological, ophthalmology, and cancer conditions. These demonstrate the backing of numerous network partners, including international patient care organizations, pharmaceutical companies, and regulatory bodies. With assistance from numerous government agencies, regulatory authorities, etc., the growth curve of clinical trials for gene and cell therapy in India is gradually increasing.

Scientists have been expanding and refining the uses of CRISPR/Cas9 to accomplish several kinds of genome editing. CRISPR/Cas9 can cause gene disruption, deletions, knockins, or targeted editing by taking use of the appropriate DNA repair pathway[30].

REFFERENCES

1. *Yazdani, A., Alirezaie, Z., Motamedi, M. J., & Amani, J. (2018). Gene Therapy: A New Approach in Modern Medicine. International Journal of Medical Reviews, 5(3), 106-117. doi: 10.29252/IJMR-050304 .*
2. *Wang, F., Qin, Z., Lu, H., He, S., Luo, J., Jin, C., & Song, X. (2019). Clinical translation of gene medicine. The journal of gene medicine, 21(7), e3108. https://doi.org/10.1002/jgm.3108 3.*
3. *Ruchita Selot And Arkasubhra Ghosh* Grow Research Laboratory, Narayana Nethralaya Foundation, Bengaluru 560099, India . 5 January 2024. Doi: 10.1007/S12038-024-00423-0.*
4. *Sung, Y. K., & Kim, S. W. (2019). Recent advances in the development of gene delivery systems. Biomaterials research, 23, 8. https://doi.org/10.1186/s40824-019-0156-z*
5. *Casey A. Maguire & Servio H. Ramirez & Steven F. Merkel & Miguel Sena-Esteves & Xandra O. Breakefield Department of Neurology, Massachusetts General Hospital,*

ISSN (Online): 2455-3662
EPRA International Journal of Multidisciplinary Research (IJMR) - Peer Reviewed Journal
Volume: 10| Issue: 12| December 2024|| Journal DOI: 10.36713/epra2013 || SJIF Impact Factor 2024: 8.402 || ISI Value: 1.188

and Neuroscience Program, Harvard Medical School, Molecular Neurogenetics Unit, 13th Street, Building 149, Charlestown, MA02129, USA . 27 August 2014.DOI: 10.1089/hum.2012.088

6. *Ginn, S. L., Alexander, I. E., Edelstein, M. L., Abedi, M. R., & Wixon, J. (2013). Gene therapy clinical trials worldwide to 2012 - an update. The journal of gene medicine, 15(2), 65–77. https://doi.org/10.1002/jgm.2698*
7. *Dunbar, C. E., High, K. A., Joung, J. K., Kohn, D. B., Ozawa, K., & Sadelain, M. (2018). Gene therapy comes of age. Science (New York, N.Y.), 359(6372), eaan4672. https://doi.org/10.1126/science.aan4672*
8. *Wilson J. M. (2005). Gendicine: the first commercial gene therapy product. Human gene therapy, 16(9), 1014–1015. https://doi.org/10.1089/hum.2005.16.1014*
9. *Collins, M., & Thrasher, A. (2015). Gene therapy: progress and predictions. Proceedings of the Royal Society B: Biological Sciences, 282(1821). https://doi.org/10.1098/rspb.2014.3003 (Original work published December 22, 2015)*
10. *Beitelshees, M., Li, Y., & Pfeifer, B. A. (2016). Enhancing vaccine effectiveness with delivery technology. Current opinion in biotechnology, 42, 24–29. https://doi.org/10.1016/j.copbio.2016.02 .*
11. *Castro, M. G., Davis, J. R., Xiong, W., & Lowenstein, P. R. (1999). Recent developments in gene therapy: applications for the treatment of pituitary tumours. Bailliere's best practice & research. Clinical endocrinology & metabolism, 13(3), 431–449. https://doi.org/10.1053/beem.1999.0035*
12. *Kui Wang, Forrest M. Kievitb, Miqin Zhanga, Department of Materials Science and Engineering, University of Washington, Seattle, WA 98195, USA 19 october 2016. http://dx.doi.org/10.1016/j.phrs.2016.10.016*
13. *Cevher, E., Demir, A., & Sefik, E. (2012). Gene Delivery Systems: Recent Progress in Viral and Non-Viral Therapy. InTech. doi: 10.5772/53392 http://dx.doi.org/10.5772/53392.*
14. *Kivelä, A. M., Huusko, J., & Ylä-Herttuala, S. (2015). Prospect and progress of gene therapy in treating atherosclerosis. Expert opinion on biological therapy, 15(12), 1699–1712. https://doi.org/10.1517/14712598.2015.1084282*
15. *Chuah, M. K., Nair, N., & VandenDriessche, T. (2012). Recent progress in gene therapy for hemophilia. Human gene therapy, 23(6), 557–565. https://doi.org/10.1089/hum.2012.088*
16. *Rama, A. R., Prados, J., Melguizo, C., Ortiz, R., Alvarez, P. J., Rodríguez-Serrano, F., Hita, F., Ramos, J. L., Burgos, M., & Aranega, A. (2010). E phage gene transfection enhances sensitivity of lung and colon cancer cells to chemotherapeutic agents. International journal of oncology, 37(6), 1503–1514. https://doi.org/10.3892/ijo_00000803*
17. *sato, N., Shimamura, M., & Morishita, R. (2005). Recent progress in cerebrovascular gene therapy. Currentneurovascular research, 2(3), 235–247. https://doi.org/10.2174/1567202054368353*
18. *sato, N., Shimamura, M., & Morishita, R. (2005). Recent progress in cerebrovascular gene therapy. Current neurovascular research, 2(3), 235–247. https://doi.org/10.2174/1567202054368353*
19. *Bolhassani A, Rafati S. Non-Viral Delivery Systems in Gene Therapy and Vaccine Development [Internet]. Non-Viral Gene Therapy. InTech; 2011. Available from: http://dx.doi.org/10.5772/18021*
20. *Ghosh S, Brown AM, Jenkins C, Campbell K. Viral Vector Systems for Gene Therapy: A Comprehensive Literature Review of Progress and Biosafety Challenges. Appl Biosaf. 2020 Mar 1;25(1):7-18. doi: 10.1177/1535676019899502. Epub 2020 Mar 1. PMID: 36033383; PMCID: PMC9134621.*
21. *Zhao Z, Anselmo AC, Mitragotri S. Viral vector-based gene therapies in the clinic. Bioeng Transl Med. 2021 Oct 20;7(1):e10258. doi: 10.1002/btm2.10258. PMID: 35079633; PMCID: PMC8780015.*
22. *Cring, M.R., Sheffield, V.C. Gene therapy and gene correction: targets, progress, and challenges for treating human diseases. Gene Ther **29**, 3–12 (2022). https://doi.org/10.1038/s41434-020-00197-8*
23. *Takeoka Y, Yurube T, Nishida K. Gene Therapy Approach for Intervertebral Disc Degeneration: An Update. Neurospine. 2020 Mar;17(1):3-14. doi: 10.14245/ns.2040042.021. Epub 2020 Mar 31. PMID: 32252149; PMCID: PMC7136116.*
24. *Taha EA, Lee J, Hotta A. Delivery of CRISPR-Cas tools for in vivo genome editing therapy: Trends and challenges. J Control Release. 2022 Feb;342:345-361. doi: 10.1016/j.jconrel.2022.01.013. Epub 2022 Jan 10. PMID: 35026352.*
25. *Belete TM. The Current Status of Gene Therapy for the Treatment of Cancer. Biologics. 2021 Mar 18;15:67-77. doi: 10.2147/BTT.S302095. PMID: 33776419; PMCID: PMC7987258.*
26. *Prado DA, Acosta-Acero M, Maldonado RS. Gene therapy beyond luxturna: a new horizon of the treatment for inherited retinal disease. Curr Opin Ophthalmol. 2020 May;31(3):147-154. doi: 10.1097/ICU.0000000000000660. PMID: 32175942*
27. *Kitawi, R., Ledger, S., Kelleher, A. D., & Ahlenstiel, C. L. (2024). Advances in HIV Gene Therapy. International Journal of Molecular Sciences, 25(5), 2771. https://doi.org/10.3390/ijms2505277*
28. *Gombash SE, Foust KD. Systemic Gene Therapy for Targeting the CNS. Methods Mol Biol. 2016;1382:231-7. doi: 10.1007/978-1-4939-3271-9_16. PMID: 26611590.*
29. *Oakland M, Sinn PL, McCray PB Jr. Advances in cell and gene-based therapies for cystic fibrosis lung disease. Mol Ther. 2012 Jun;20(6):1108-15. doi: 10.1038/mt.2012.32. Epub 2012 Feb 28. PMID: 22371844; PMCID: PMC3369283.*
30. *Cooney AL, Abou Alaiwa MH, Shah VS, Bouzek DC, Stroik MR, Powers LS, Gansemer ND, Meyerholz DK, Welsh MJ, Stoltz DA, Sinn PL, McCray PB Jr. Lentiviral-mediated phenotypic correction of cystic fibrosis pigs. JCI Insight. 2016 Sep 8;1(14):e88730. doi: 10.1172/jci.insight.88730. PMID: 27656681; PMCID: PMC5027966.*
31. *Yip BH. Recent Advances in CRISPR/Cas9 Delivery Strategies. Biomolecules. 2020 May 30;10(6):839. doi: 10.3390/biom10060839. PMID: 32486234; PMCID: PMC7356196.*