

Advances in Gene Therapy: Novel Strategies for Targeted Gene Delivery and Therapeutic Applications

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Abstract- Gene therapy has emerged as a promising approach to treat genetic disorders and diseases over the last 25 years. This review explores its evolution, aims, and scope. Gene therapy corrects genetic abnormalities by introducing functional genes, potentially offering long-lasting therapeutic effects. Recent technological advancements enable researchers to target challenging organs and genetic anomalies untouched by traditional treatments. It has shown remarkable progress in treating neurodegenerative disorders and is integral to therapeutic strategies for inherited and acquired human diseases, potentially revolutionizing medicine with personalized treatments. Future research goals include identifying suitable targets, precise delivery methods, immune response management, regulatory control, and minimizing side effects for enhanced efficacy and safety. The ongoing progress and potential of gene therapy offer hope for improved patient health and quality of life.

In the context of cancer treatment, gene therapy holds the potential to deliver essential proteins and regulate gene expression, potentially replacing traditional treatments for long-term benefits. While monogenetic diseases have seen success, challenges persist for common conditions like cystic fibrosis and muscular dystrophy. Modified adenoviruses are explored as effective gene delivery vehicles to overcome these challenges. Principles of chemistry, manufacturing, and control (CMC), along with quality assurance, are crucial for gene therapy product safety and efficacy during clinical trials. Gene therapy, combined with technologies like CRISPR-Cas9, presents exciting opportunities for precision medicine, marking a milestone in personalized and targeted healthcare.

Index Terms- Gene, development, CRISPR-Cas9, barriers, cancer, DNA, RNA.

I. INTRODUCTION

Gene therapy is a promising alternative to conventional treatments for genetic disorders like cystic fibrosis and Parkinson's, aiming to modify malfunctioning genetic expression for lasting effects. Successful gene therapy relies on delivering therapeutic genes across biological barriers to the target location. However, direct introduction of bare nucleic acids has limitations due to rapid clearance and loss of expression. Therefore, researchers are investigating various delivery vehicles to encapsulate genetic material during transportation, with a focus on DNA delivery in this review.

[1] It has shown significant progress in treating neurodegenerative disorders over the past few decades. Advancements in technology, understanding of disease mechanisms, and the identification of therapeutic targets and vectors have contributed to this progress. Genetic interventions can now effectively target the root causes of these disorders, whether they have single-gene or complex origins. The sustained and potentially permanent therapeutic effects of gene therapy are particularly beneficial for treating difficult-to-reach organs like the eye, cochlea, and central nervous system. These organs are challenging to treat due to physiological barriers like the blood-cerebrospinal fluid barrier, blood-retina barrier, and blood-brain barrier. Additionally, gene therapy can manage genetic targets that traditional treatments cannot handle, allowing for gene silencing to address gain of function mutations and gene overexpression to handle loss of function mutations.

[2]

DEFINITION -

Gene therapy is a technique that modifies and develops a person's genes to treat or cure diseases or disorders. It can work through various mechanisms, such as replacing a disease-causing gene with a healthy copy of the gene. By introducing functional genes into the patient's cells, gene therapy aims to correct genetic abnormalities and restore normal cellular function. This promising approach holds the potential to revolutionize medicine by providing targeted and personalized treatments for a wide range of genetic conditions, offering hope for improved health and quality of life for patients in the future. Successful gene therapy is not limited to genetic diseases. They can spread world wide with their various applications.

T cells has received much attention as it is highly effective at erode coating B-cell leukaemia's and lymphomas that are resistant to standard therapies in cancer patients. Autologous CD8+ T cells are engineered to recognize and kill cells bearing tumour-specific antigens.

Through a CAR that combines the specificity of a monoclonal anti body with the proliferative and cytotoxic abilities of an activated.

II. HISTORICAL BACKGROUND -

Nearly five decades ago, visionary scientists proposed the idea that using exogenous DNA for genetic modification could be a promising approach to treat inherited human diseases, known as "gene therapy." This strategy was believed to offer the potential advantage of providing a lasting and potentially curative clinical benefit through a single treatment.

After almost 30 years of promise tempered by setbacks, gene therapies are rapidly becoming a critical component of the therapeutic armamentarium for a variety of inherited and acquired human diseases.

In recent years, there have been significant advancements in genome editing technologies, which are founded on engineered or bacterial nucleases.

In 1996, an advisory panel from the National Institutes of Health (NIH) reached the conclusion that the disappointing clinical outcomes observed in gene therapy were a result of inadequate understanding of the biology of the viral vectors, the target cells and tissues, and the diseases being treated. [3]

III. AIMS AND SCOPE -

The Journal of Drug Delivery and Therapeutics is a peer-reviewed publication catering to academic and industrial researchers in Pharmaceutical Science and Therapeutics. It encompasses Medical Sciences (medicine, surgery, ophthalmology, gynaecology and obstetrics, paediatrics, orthopaedics, microbiology, pathology and laboratory medicine, medical education, medical ethics, community medicine and public health, anatomy, physiology, pharmacology) and Pharmaceutical Sciences (Biotechnology, Biochemistry, DRA, Phytomedicine/Ayurveda, Pharmaceutics, Drug Delivery, Pharmaceutical Chemistry, Pharmaceutical Analysis). [4]

India's gene therapy research rapidly improved with government financial assistance, now ranking third among major Asian countries with gene therapy laboratories.

Gene therapy's publication growth remained stagnant despite a 6% annual rise in authors, indicating limited appeal and low research activity. [5]

The scope of gene therapy is vast and encompasses a wide range of genetic and acquired diseases. It holds promise in treating various genetic disorders, such as cystic fibrosis, muscular dystrophy, sickle cell anaemia, and inherited metabolic disorders. [6]

IV. FUTURE GOALS -

Identifying an appropriate target for gene therapy.

Getting a therapeutic transgene into the right cells (and only those cells) in the right amount.

Delivering the transgene with a vector that doesn't trigger an immune response or, in the case of certain viral vectors, revert to a pathogenic form.

Providing the appropriate regulatory elements for turning the gene on and off at the correct time.

Keeping the transgene in the target cell long enough for it to do its job.

Keeping the transgene from causing damage elsewhere (e.g., spurring development of neoplasms or autoimmune disease, which could happen if the transgene expresses a protein new to the patient's body. [8]

V. GENE THERAPY'S FUTURE IN ANOTHER 25 YEARS -

350 years have passed since the Royal Society was founded, but only 25 years have gone by since we first showed that transferring genes to patients could work. In these last 25 years, we've made a lot of progress, and gene therapy has been tested on patients in studies, bringing some benefits. Looking ahead, it's likely that we'll use gene therapy to provide important proteins that our bodies need for a long time. Imagine delivering antibodies through gene therapy to protect us from diseases or to help treat cancer and autoimmune diseases. This could be done in specific places to reduce side effects. We might also control the amount of gene therapy using small molecules that are safe for treatment. Gene therapy could even replace some traditional treatments, like using genes to regulate insulin for treating type 1 diabetes.

Inherited problems caused by single gene issues have sometimes been treated by putting modified cells into the bone marrow. In the future, we might fix these problems by directly correcting the genes, but it will need special treatments for each different issue. We could also use gene editing to treat certain eye problems caused by a single gene. But for common issues like cystic fibrosis or muscular dystrophy, getting the right genes to enough cells in places like the lungs or muscles is still tricky. Many companies might focus on adding genes for common problems like heart diseases or cancer in the long run. Using injectable methods for delivering genes is also a good choice because they can be made and given out like regular medicines. However, for treatments where cells need to be changed outside the body first, we'll still need to make modified cells locally. To do this, we need to find better ways to create and purify the viral carriers we use for gene therapy. A lot of the methods used now are just upscaled versions of methods from research labs. [9]

VI. TCR GENE-MODIFIED T CELLS FOR CANCER IMMUNOTHERAPY-

Gene transfer of cloned TCRs isolated from tumour infiltrating T cells represents another approach for T-cell-based cancer immunotherapy, especially for tumour antigens not expressed on the cell surface.

In TCR gene therapy, the patient's T cells are again engineered *ex vivo* to redirect their specificity toward a particular tumour antigen.

These tumour-specific engineered T cells are then reinfused back to the patient, where they recognize tumour antigen in the context of HLAs in the tumour microenvironment. Various clinical trials have employed genetically modified TCRs to treat a wide variety of cancers (synovial cell sarcoma, neuroblastoma, melanoma, and colorectal cancer) with long-term tumour regression. [10]

CRISPR-Cas9
CRISPR-Cas9 is a groundbreaking gene-editing technology that has revolutionized the field of genetics by enabling scientists to make targeted modifications in the DNA sequences of living organisms. The name "CRISPR" stands for "Clustered Regularly Interspaced Short Palindromic Repeats," a distinctive pattern of DNA sequences initially discovered in bacteria and other microorganisms. Complementing CRISPR is "Cas9," which refers to the CRISPR-associated protein 9, functioning as molecular scissors during the gene-editing process. [11]

Originally identified as part of the bacterial immune system, CRISPR-Cas9 serves as a defence mechanism against invading viruses and foreign genetic material. However, researchers have ingeniously harnessed this system and adapted it into a powerful tool for precise and efficient gene editing in various organisms, including plants, animals, and even humans. This transformative technology opens up new possibilities for treating genetic diseases, advancing agricultural practices, and conducting fundamental research in genetics and biology. [12]

The CRISPR/Cas system originally belongs to the prokaryotic acquired immune system, playing a crucial role in defending bacteria against invading phages. When a bacterium is first infected by a phage, it incorporates small fragments of the phage's DNA between short palindromic repeats into a specific region of its own genome known as the CRISPR locus. This process effectively "memorizes" the genetic information of the invading phage. [13]

VII. GENE THERAPY IN CANCER TREATMENT –

In recent decades, remarkable technological and scientific advancements have paved the way for gene therapy to emerge as a promising approach in treating various diseases. While monogenetic diseases have well-defined biological and clinical trial endpoints, it is in the field of cancer that gene therapy has shown particularly exciting potential. Cancer, being one of the most devastating and prevalent diseases worldwide, has become a major focus for gene therapy research and development.

The ability to target specific genetic mutations and cellular pathways associated with cancer has revolutionized the field, offering new avenues for treatment that were previously unimaginable. Promising clinical data from early trials have provided encouraging results, further fueling the momentum behind gene therapy as a potentially transformative cancer treatment. As research continues to advance, gene therapy holds great promise in the fight against cancer, offering hope for improved outcomes and better quality of life for patients facing this formidable disease. [20]

The field of cancer gene therapy holds immense promise and offers a plethora of exciting potential treatments. [21]

Gene therapies possess all the profiles required for advances in cancer therapy, including a novel therapeutic agent with a unique mode of action, multiple mechanisms of cell death, and the potential for synergy with conventional management, as they target the disruption of normal cell proliferation and apoptosis process that underlies cancer development. [22] Recent progress in gene therapy has been fuelled by the identification of bone marrow stem cell populations and the successful transduction of other long-lived hematopoietic cells, making this approach feasible for testing in clinical trials. Several recent reviews have succinctly summarized the advancements in gene therapy, highlighting the significant developments in the field. [23]

VIII. CHALLENGES OF GENE THERAPY IN CANCER TREATMENT -

Despite being fully aware of the challenges faced by investigators, Cancer Gene Therapy (CGT) has adapted its peer review process to accommodate these difficulties. [24]

Despite the encouraging outcomes from initial clinical trials, the delivery of the HF10 virus for breast cancer patients has been restricted to local-regional administration. However, the assessment of systemic effects from this therapy in breast cancer patients is still pending and has not been conducted yet. [25]

Breast cancer cells stimulated the secretion of chemokine CCL5 from mesenchymal stem cells (MSCs), and this chemokine acted in a paracrine manner to enhance cancer cell motility, invasion, and metastasis. [26]

Cancer has a considerably lengthy latency period, meaning that the clinical symptoms or disease diagnosis are far less frequent compared to the incidence observed during autopsy [12-28]. This extended timeframe offers ample opportunities for modifying genetic damage through gene replacement or the introduction of genes with tumour-suppressing capabilities. [27]

IX. ADENOVIRUSES AS GENE-DELIVERY VEHICLES -

For gene therapy to work well, we need effective and safe ways to deliver therapeutic genes into the body's cells. It's a bit like delivering a special kind of medicine. These genes are much bigger than regular drugs and need help to reach the right cells. We use carriers called vectors to package and guide the genes to the right place. But there are challenges in this process, like making sure the genes stick to cells, get inside them, travel to the centre (nucleus), and actually start working.

Some viruses that have been changed in labs work really well as carriers. These modified viruses have been used a lot, especially one type called murine retroviruses, but they have limits. They're great for studies outside the body (ex vivo), where cells are changed in a dish. But they're not so good at working inside the body because they need cells to divide to infect them. That's a problem because many cells in our body don't divide all the time.

Other carriers, like human adenoviruses, show promise for delivering genes in the body (in vivo). They can deliver genes into different types of cells that divide and even those that don't. This makes them more versatile for gene therapy in the body.

Scientists initially thought about using human adenoviruses as vehicles to treat cystic fibrosis because they have a liking for cells in the lungs. Adenoviruses come in over 40 types and can cause various mild illnesses like diarrhoea or a sore throat, but they don't usually lead to serious problems or cancer. These viruses don't have an outer layer and carry a 36-kb genetic code that has both early genes (which make regulatory proteins) and late genes (which make structural proteins).

Using adenovirus as a delivery vehicle is a good idea because it can make a lot of clean modified virus. This virus can infect specialized cells that don't divide. To make it safe, scientists tried changing the virus to not cause infections anymore. They first thought about completely replacing the part of the virus that makes structural proteins with the new gene. But that turned out to be really hard. So, they tried a different method. They deleted the genes in the virus that make it active and cause other viral genes to work (Fig. 1). Without these controlling genes, the virus can't replicate inside the body, and its active genes stay quiet. These early-generation adenoviral vehicles can spread in lab settings and carry new genes to different types of cells.

Scientists have tested the use of modified adenoviruses for treating cystic fibrosis, a genetic condition that affects lung function. In cystic fibrosis, a faulty gene leads to lung problems and repeated infections. They tried putting adenoviruses with the correct gene into the lungs of rodents. This led to a lot of the new gene being produced in most lung cells.

They then did safety tests in animals like monkeys before starting small tests in cystic fibrosis patients. They found that concerns about viruses causing problems by changing, reproducing, or spreading weren't really happening. There was some inflammation at the site of gene transfer, but this depended on the dose and species.

However, there were some issues. The effect of the new gene wasn't long-lasting, and giving more doses of the virus didn't really help. Also, the adenoviruses didn't transfer the new genes into lung cells as well as they had hoped. These limitations made researchers less excited about using these modified adenoviruses as a treatment for cystic fibrosis. The main problem with the first-generation adenoviral vectors, which are used for genetic treatment of chronic diseases, is that they can only express the therapeutic gene temporarily. This is a limitation because the protein produced by the gene becomes undetectable after a few weeks, and trying to express the gene again using the same vector is not effective. This happens partly because the body's immune system responds to the vector and the cells it infects, similar to how it responds to any viral infection.

When the therapeutic gene is introduced using the adenoviral vector, the body's immune system reacts by producing immune cells that can destroy the cells infected by the vector. This leads to a loss of the gene's expression and causes inflammation. Additionally, the immune system generates antibodies against the vector, which can neutralize the vector's effects if it's used again.

To address these immune-related issues, scientists have modified the vectors to reduce the production of viral-related substances that trigger the immune response. Another strategy involves using medications that suppress the immune system's activity to prevent immune cells from attacking the vector-infected cells. By combining an improved vector with methods to temporarily calm down the immune response, it might be possible to overcome these problems caused by the body's reactions.

In the context of using adenoviral vectors for cystic fibrosis, there's a disagreement arising from initial clinical trials about how well these vectors are able to transfer genes into the airways. In these trials, when a small amount of the viral vector is introduced into the lung through a bronchoscope (a medical device used to see and treat airway conditions), gene transfer into airway cells is observed, but only at a low dose of the vector. Similar results were seen in animal studies. Another approach was to deliver the vector specifically to the cells lining the nasal passages.

The idea behind targeting the nasal lining was that these cells could be a good model for the cells that line the airways within the lungs, and they are also easier to access for experimental purposes. However, three separate groups of researchers discovered that gene transfer using adenoviral vectors is highly inefficient in the untreated nasal lining of people with cystic fibrosis.

When scientists looked into how adenovirus enters cells, they found a potential explanation for why there's a difference in how efficiently genes are transferred in nasal versus lung cells. Adenovirus requires certain "receptors" on the surface of cells to enter them. One of these receptors is missing in the nasal lining, but it is present in the cells of the lower airways. This could account for the lower effectiveness of gene transfer in nasal cells compared to cells deeper in the lungs.[28]

X. GUIDING PRINCIPLES FOR THE CHEMISTRY, MANUFACTURING AND CONTROL AND QUALITY ASSURANCE OF GENE THERAPY PRODUCTS-

When a new gene therapy product (GTP) is being developed for clinical trials, it's important to provide regulatory authorities with detailed information about how it's made and tested. This includes the entire process from development to manufacturing and testing. One key aspect is ensuring that the production takes place in facilities that meet high-quality standards known as good manufacturing practice (GMP) and good laboratory practice (GLP).

Manufacturers need to focus on choosing a production facility that meets these standards. They also need to submit information about the chemistry, manufacturing, and control (CMC) processes to the regulatory authorities when applying for permission to conduct clinical trials with the new gene therapy product.

To make it simpler, think of it as preparing a comprehensive report that explains how the gene therapy product is created, how it's tested to ensure its quality, and the specific facilities and procedures used during production. This report is crucial for getting regulatory approval to proceed with clinical trials. The main emphasis is on maintaining high-quality manufacturing and testing environments, which is essential for the success and safety of the gene therapy product.

Vector component:

Vector Sequence: This means you should explain the molecule's structure and provide a detailed analysis of its genetic code. Also, describe the parts that control how the gene works.

Viral Vector (if used): If the gene therapy uses a virus to carry the gene, you need to provide information about the virus. This includes its physical and chemical traits, as well as the parts that help it function. You should also talk about a helper molecule used and the covering of the virus.

Bacterial Vector (if used): If the gene therapy uses bacteria to carry the gene, you need to explain the properties of these bacteria. This includes their physical and chemical characteristics, how they grow, any special genetic markers they have, and the parts that control how they work.

In simpler terms, you're basically sharing detailed information about the building blocks of your gene therapy. If you use a virus to transport the gene, you need to describe the virus and its components. If you use bacteria, you need to explain the properties of those bacteria. This information helps regulators understand how your gene therapy works and ensures its safety for clinical trials.

Cellular components:

Whether you're using cells from the same person (autologous) or from someone else (allogeneic) for your gene therapy, you need to provide information about certain things:

Cell Source:

This means you need to explain where you're getting the cells from. Are they from the person who will receive the treatment (their own cells) or from a different person?

Methods for Cell Handling:

You should describe how you're preparing these cells. This includes how you're getting the cells ready to be used in the treatment. This involves things like getting the cells moving, making them more active, growing more of them, collecting them, and making sure you're not losing too many cells in the process.

Genetic Changes (if any):

If you're making changes to the genes in these cells, you need to say what technology you're using to do that. This might involve editing the genes to fix a problem or make them work differently.

In simpler terms, if you're using cells from the same person or someone else, you need to explain where these cells are coming from and how you're treating them to be ready for the treatment. If you're changing their genes, you also need to say how you're doing that. This information helps regulators understand how you're handling the cells and making sure everything is safe and effective for the treatment.

Other components:

Reagents:

Reagents are like ingredients used in the making of the gene therapy product (GTP). They can affect how well the GTP works, but they're not actually present in the final product that's given to patients. You need to give details about how much of each reagent you use, where you get them from, what quality they are, and at which step of the manufacturing process you use them. Imagine making a cake- the flour, sugar, and eggs are like reagents. They're important for making the cake taste good, but you don't find these individual ingredients in the finished cake. So, when you're talking about reagents, you're explaining the things you're using to make the gene therapy work properly.

Excipients:

Excipients are ingredients that are actually part of the final gene therapy product that patients receive. These ingredients might be added to help the treatment work better or stay stable. You need to provide information about how much of each excipient you're using, where you're getting them, and what specific qualities they have. Continuing with the cake analogy, imagine adding icing on top of the cake. The icing is like an excipient - it's part of the final cake that people will actually eat. So, when you're talking about excipients, you're explaining the extra things you put into the gene therapy itself to make sure it's effective and safe for patients.[29]

XI. TRANSLATION OF GENE THERAPY PRODUCTS TO NEW DRUG-

Preclinical trial requirements of gene therapy products Choosing Animal Models:

When testing a new gene therapy product before human trials, it's best to use animals that are similar in either their genes or the way they show the disease. This helps us better understand how the treatment might work.

Administration Method:

When giving the gene therapy, it's important to pick a way that targets the right place and is safe. It's better if it's not too invasive and doesn't trigger a strong immune response.

Finding the Right Dose:

To figure out how much of the gene therapy to use, it's smart to start with a small amount and slowly increase it. This way, we can see what works best without causing harm.

Checking for Effects and Safety:

We need to check if the gene therapy might cause problems in places we didn't expect or if it's harmful. We do this by looking at different factors.

Testing for Children:

If we plan to use the gene therapy on kids, we have to be extra careful. We might need to adjust the dose based on their age and make sure it's safe for them.

Checking Environmental Impact:

We should make sure the gene therapy doesn't harm the environment. We do a test to see if it shows up in the fluids of the test animals.

Extra Tests for Safety:

Depending on what's in the gene therapy, we might need to do more tests. For example, if there's something that could cause cancer, we need to check if it's safe for a long time.

In simple words, before trying a new gene therapy on people, we do tests on animals that are similar to humans with the disease. We give the treatment in a safe way and slowly increase the amount. We carefully watch for any bad effects and make sure it's okay for kids too. We also check if it's okay for the environment and do extra tests if needed. All of this helps ensure the treatment is safe and effective.

XII. CLINICAL TRIAL REQUIREMENTS OF GENE THERAPY PRODUCTS-

Planning Gene Therapy Trials:

Use animals similar to humans for testing. For patient selection, describe disease details and gather medical info. Check for existing antibodies that might react to treatment. Don't include healthy volunteers or placebo groups due to risks.

Study Design:

Group patients based on disease severity and genetics. Blinding might not be possible in some cases.

Dose and Administration:

Determine doses from preclinical studies or similar trials. Hospitalize participants before and after treatment. Monitor for immune reactions and consider temporary suppression.

Endpoints and Assessments:

Monitor safety and immunological reactions. Measure bioactivity and gene transfer. Assess functional improvements and patient experiences.

Follow-Up:

Follow patients for at least 5 years after treatment. Re-administer treatment after 1-2 years if no negative reactions. [30]

XIII. CONCLUSION-

Gene therapy is a promising approach to treat genetic disorders by modifying genes and restoring normal cellular function. Advances in technology and understanding of disease mechanisms have enabled gene therapy's application to a wide range of conditions, including challenging-to-reach organs. Future goals include precise targeting, immune response management, and safe gene expression control, holding significant potential for personalized medicine and improved patient outcomes.

In the next 25 years, gene therapy holds immense potential for delivering essential proteins, treating single-gene issues, and addressing common diseases like heart diseases and cancer. CRISPR-Cas9 technology revolutionizes genetics, enabling precise gene editing. FDA-approved gene therapy drugs and recent biotechnological advances showcase its growing significance in medicine. Advantages include the potential for permanent cures, fewer side effects, and its broad applicability across medical specialties, heralding a promising future for personalized and effective treatments.

Gene therapy in cancer treatment shows promising potential by targeting specific genetic mutations and pathways, offering hope for improved outcomes. However, challenges include delivery issues, immune responses, and temporary gene expression limitations. Modified adenoviruses hold promise for delivering genes, but efficiency varies in different cells. Overcoming these challenges requires ongoing research and innovative strategies for successful cancer gene therapy.

Guiding principles for gene therapy product development emphasize the importance of rigorous CMC processes, quality assurance, and regulatory compliance. Detailed information on vector components, cellular sources, genetic changes, and other components ensures safety and efficacy. Preclinical trials require careful consideration of animal models, administration methods, dosing, safety, and environmental impact. Clinical trials planning involves patient selection, study design, dosing, monitoring, and long-term follow-up to ensure successful translation of gene therapy products into effective treatments.

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