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ADVANCEMENT IN RARE GENETIC DISEASE TREATMENT: A COMPREHENSIVE REVIEW OF NOVEL THERAPIES

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ABSTRACT

Rare genetic disorders collectively affect a substantial segment of the global population. Clinicians may find it challenging to distinguish between clinically identical disorders due to the lack of information available for many diseases. This causes issues with patient care and genetic counseling. In recent years, we've seen some incredible strides in how we treat rare genetic diseases, thanks to breakthroughs in genetic therapies like gene editing, gene replacement, and RNA-based treatments. Technologies such as CRISPR-Cas9, adeno-associated virus (AAV) vectors and nanotechnology have led to the development of therapies for conditions that were once thought to be untreatable, including spinal muscular atrophy, sickle cell disease, and inherited retinal disorders. Innovative delivery systems both viral and non-viral have broadened the therapeutic options available and made it possible to achieve more accurate and lasting gene corrections. Despite the promise of gene therapy, there are still some tough challenges to overcome, like immune responses, ethical issues, and the high costs involved. On future prospect, we might see a trend where gene therapy is combined with other treatment methods, such as gene editing paired with stem cell therapy, which could lead to better outcomes for patients. This review aims to provide a comprehensive overview on novel gene therapy techniques which enhance the efficiency and specificity of target gene delivery.

KEYWORDS: Rare genetic disorders, CRISPR-Cas9, Adeno-associated virus (AAV) vectors, Nanotechnology, Gene Therapy.

INTRODUCTION

The quest for effective treatments for genetic diseases has really taken center stage in today's biomedical research. This shift is largely due to the shortcomings of traditional therapies, which often only tackle symptoms instead of addressing the underlying issues. Thanks to recent breakthroughs in genetic medicine, we now have the ability to specifically modify or fix faulty genes, opening up exciting new avenues for long-lasting or even permanent cures. Various technologies have been developed in recent times which are expanding to a wide range in treating rare genetic diseases.[1]

Now, before heading towards the technologies or methods, let's study what is Rare Genetic Disorder.

Overview to Rare Genetic Disorder

Rare genetic disorders are a collection of inherited medical conditions that don't show up very often in the general population, typically affecting fewer than 1 in 2,000 people. These disorders come about due to mutations or changes in single gene or chromosome, which can throw off normal growth, development, or how the body functions.[2] Even though each rare genetic disorder might only impact a handful of individuals, together they pose a significant global health issue, affecting millions around the world; in USA they are affecting fewer than 200000, and in Europe typically affecting fewer than 1 in 2000.[3] The rarity of these conditions often results in delayed diagnoses, limited research, and a shortage of effective treatments. Patients and their families dealing with rare genetic disorders frequently encounter hurdles like misdiagnosis, a lack of clinical expertise, and steep medical expenses. However, with the progress in genomics, bioinformatics, and personalized medicine, researchers are starting to gain a clearer understanding of the molecular mechanisms behind these diseases, bringing hope for earlier detection and targeted therapies.[4]

Human genetics has undergone a revolution thanks to the quick discovery of genes linked to human disease, which has produced more precise diagnostic, prognostic, and maybe therapeutic tools. Furthermore, a better comprehension of the molecular genesis of Our understanding of how diseases spread is also changing as a result of genetic problems. Many single-gene illnesses were discovered using the classical paradigm, which is predicated on the idea that a single molecular defect is transmitted when a trait spreads throughout families.[5]

Many complicated and incurable disorders are caused by genetic diseases. The mitochondrial genome, nuclear genome, and microbial metagenome all include DNA sequences that encode the intricacy of each human and several disease risks. Applications of next-generation DNA sequencing have become important to the diagnosis of many illnesses. The burden of diseases resulting from mutations and changes in the human genetic code is enormous; known genetic disorders impact over two-thirds of miscarriages and over 5% of live births.[6]

CLASSIFICATION

Genetic Disorders are classified into three main types

1. Single-gene Disorders
2. Chromosomal Disorders
3. Multifactorial Disorders

1. Single-gene Disorder

Single-gene disorders, often referred to as Mendelian disorders, are genetic conditions that arise from mutations or changes in the DNA sequence of just one gene. These mutations can throw a wrench in the normal production or function of proteins, resulting in a variety of health issues. While each single-gene disorder may be relatively uncommon on its own, collectively, they play a significant role in the inherited diseases we see around the globe.[7]

These disorders can be passed down in various ways depending on where the gene is located and the nature of the mutation, such as:

- Autosomal Dominant
- Autosomal Recessive
- X-linked Dominant
- X-linked Recessive

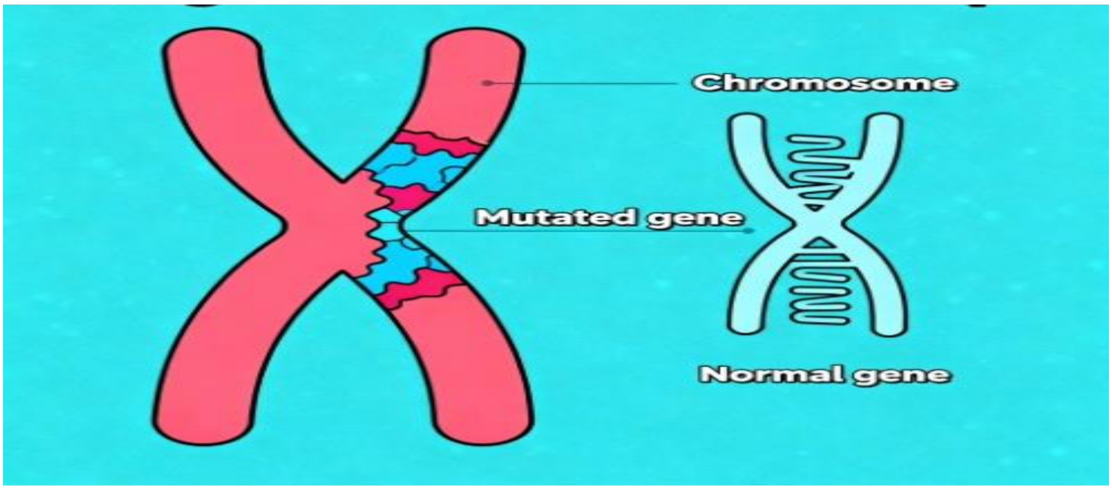

Figure 01: Single gene disorder.

2. Chromosomal Disorder

These conditions involve a change in the number or structure of chromosomes. It is further classified as Structural and Numerical Anomalies.

- **Numerical Anomalies:** When we talk about numerical anomalies, we're referring to situations where there's an extra chromosome or one is missing. A well-known case is Down syndrome, also known as Trisomy 21, where individuals have three copies of chromosome 21 instead of the typical two.
- **Structural Anomalies:**, these involve parts of a chromosome being rearranged, duplicated, or even missing altogether.[8]

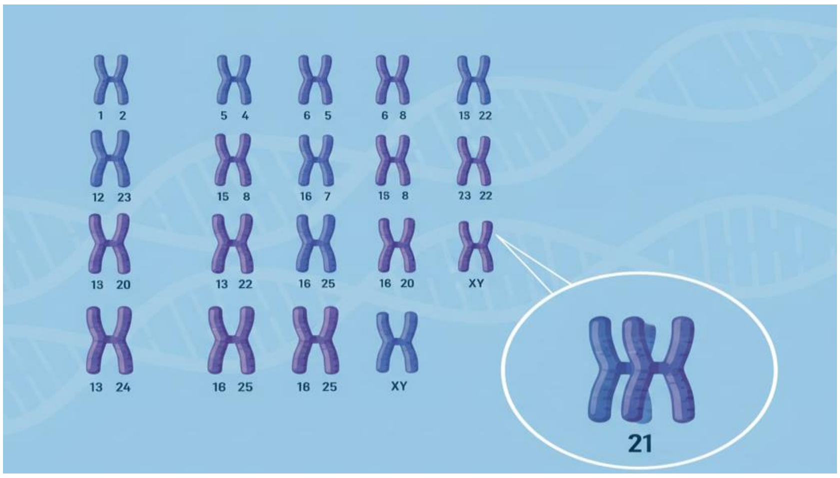

Figure 02: Chromosomal Disorder:- Trisomy 21 (Down Syndrome).

3. Multifactorial Disorder

Multifactorial diseases are intricate conditions that emerge from the interplay of various genes (known as polygenic inheritance) and environmental influences. Unlike disorders caused by a single gene mutation, these multifactorial diseases develop through the interaction of genetic tendencies and factors like lifestyle choices or environmental exposures, which can include things like diet, stress, pollution, and infections.[9]

Figure 03: Multifactorial Disorder:- Gene-Environment Interaction in Disease.

Effect on Families and Individual

The effects of rare genetic disorders reach far beyond just the individuals who are diagnosed; they ripple through their families and communities as well. Those living with these conditions often encounter a host of difficulties, such as being misdiagnosed, having limited treatment options, and struggling to find access to specialized care [10]. It's important to recognize that conducting clinical trials can be quite challenging, especially when patient populations are small. The rarity of certain conditions often hinders research efforts and makes it tough to develop effective medications.[2]

To effectively address the difficulties they present, it is imperative to comprehend uncommon genetic illnesses, including their diagnosis, categorization, prevalence, and impact. There is promise for better diagnostic tools as genetic research continues to progress and treatment choices that might improve the lives of people afflicted by these illnesses. The advancement of programs that assist patients and their families in managing uncommon genetic disorders will depend heavily on greater understanding and cooperation among medical professionals, researchers, and advocacy groups.[4]

GENE THERAPY

Advancement in Gene Therapy

Gene therapy is an innovative medical approach designed to treat, prevent, or even potentially cure diseases by making direct changes to a person's genetic material. This process usually involves introducing, removing, or editing specific genes within an individual's cells to fix or compensate for genes that are abnormal or not functioning properly.[11] Thanks to developments in molecular biology, genetics, and biotechnology, gene therapy has undergone substantial change since its inception in the late 20th century. Its progress is still influenced by ethical and regulatory frameworks, which guarantee that treatments are accessible, safe, and effective.[12]

Techniques and Tools used in Gene Therapy

Table 01: Technology's and Tools that are use in gene therapy.

Technology/Tool	Primary Action	Examples
CRISPR/Cas9	Gene correction/editing	Sickle Cell Disease[13]
Viral Vector-Based Therapy	Gene replacement/delivery	Retinal gene therapy[14]
Nanoparticles	Nonviral gene delivery	mRNA delivery, repeat dosing[15]

CRISPR/CAS9 SYSTEM: GENOME EDITING TECHNOLOGIES

Overview

Researchers have long hoped to use genetic alterations to treat illnesses or possibly develop the ability to accurately and permanently fix any part of an organism's DNA.[16] In recent years, the rapid advancements in genome editing tools have opened up exciting new possibilities in the medical field. Gene editing has emerged as a powerful technique for treating various disorders, making significant strides and greatly enhancing our ability to correct and even creatively modify the genomes of eukaryotic cells.[17,18]

Genome editing technologies have truly transformed the landscape of genetic research and therapeutic applications, enabling us to make precise changes to DNA sequences. Among these innovative tools, CRISPR/Cas9 (Clustered Regularly Interspaced Short Palindromic Repeats" (CRISPR) and CRISPR-associated protein 9 (Cas9)) stands out as a game-changer, thanks to its simplicity, efficiency, and versatility. This system, which originates from a bacterial immune response, employs a guide RNA (gRNA) to steer the Cas9 endonuclease to specific spots in the genome, where it creates double-strand breaks (DSBs) [19]. After the double-strand breaks (DSBs) occur, the cell's repair mechanisms kick in, allowing for the introduction of specific genetic changes through methods like non-homologous end joining (NHEJ) or homology-directed repair (HDR).[20]

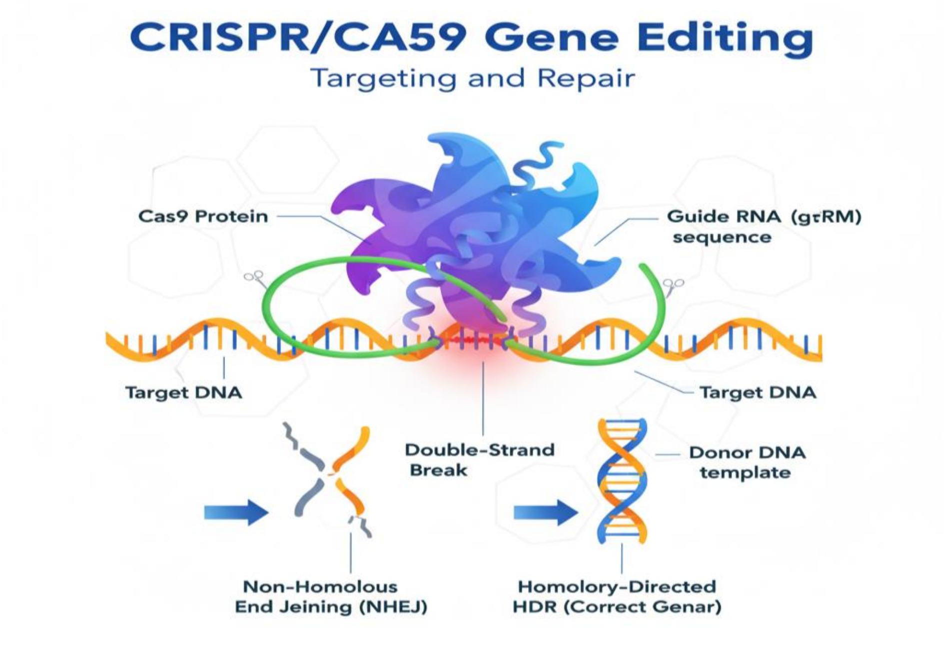

Figure 04: CRISPR/Cas9 Gene Editing.

This technology has found its way into a variety of fields. In the medical world, it's making strides in treating genetic disorders like beta- Thalassemia and Alzheimer's.

Table 02: Few mutations causing genetic diseases.

Genetic Diseases	Alterations/Changes that can be fixed using Crispr/Cas9	Target Gene	Reference
β- Thalassemia	IVS2-654 (C > T)	HBB	[21]
Huntington	p.(G1n302) and p.(Tyr539Cys)	RNF216	[22]
Alzheimer’s	H214N, R220P	Presenilin 1	[23]
Limb girdle muscular dystrophies type 2B and 2D	c.5713C>T; p.R1905X and missense c.229C>T; p.R77C	Dysferlin and alpha sarcoglycan	[24]

There are currently clinical trials using genome editing technologies, with CRISPR/Cas9 leading the way in therapeutic applications. Hematological illnesses are the focus of the first trials, in which patients are reinfused modified cells to fix genetic abnormalities.[25] Early findings have shown encouraging results, with some patients exhibiting notable clinical improvements. For instance, a research involving beta-thalassemia patients revealed that hematopoietic stem cells modified with CRISPR resulted in a sustained production of healthy red blood cells.[26] However, there is still debate regarding the long-term safety and effectiveness of these therapies, which calls for close observation and more research to assess any possible off-target effects and unforeseen repercussions of genome editing.

Advantages

1. CRISPR/Cas9 is a groundbreaking tool that allows scientists to precisely target and modify specific DNA sequences. This means we can edit genes with incredible accuracy, which is incredibly helpful for fixing genetic mutations.[27]
2. This tool is quicker and more effective than many earlier gene-editing methods, enabling swift changes to several genes at once.[28]
3. CRISPR/Cas9 is an incredibly versatile tool that works across a wide range of organisms, from plants and animals to humans. This makes it a fantastic asset for basic research, disease modeling, and developing new therapies.[29]
4. Cost Effectiveness.

Disadvantages

1. One big worry is the possibility of unintended edits happening at DNA sites that aren't the intended target. This could lead to harmful mutations or interfere with crucial genes.[30]
2. Getting the Cas9 protein and guide RNA complex into the right cells and tissues effectively and efficiently is still a challenge, which limits its usefulness in therapy.[31]
3. When Cas9 introduces double-strand breaks, it can lead to cellular stress or even apoptosis, which might end up favoring unhealthy cells. This raises some serious safety concerns for its use in clinical settings.[32]

VIRAL VECTOR-BASED THERAPIES

Overview

A notable development in gene therapy is the use of viral vector-based medicines, which offer a way to introduce therapeutic genes into target cells. Adenoviral, lentiviral, and adeno-associated viral (AAV) vectors are the three main kinds of viral vectors that are frequently utilized. Derived from adenoviruses, adenoviral vectors are renowned for their capacity to infect a variety of cell types and their high transduction efficiency. They are appropriate for applications needing transitory gene expression, nevertheless, because they usually have a brief half-life in the target cells.[33] Lentiviral vectors, which are a type of retrovirus, have the unique ability to integrate into the host's genome. Enabling the therapeutic gene to express itself steadily and permanently. This characteristic is very beneficial for the treatment of genetic illnesses.[34] AAV vectors are less immunogenic than adenoviral vectors and are distinguished by their capacity to transduce both dividing and non-dividing cells. Because of these characteristics, AAV vectors are a viable option for numerous uses, such as neurological and ophthalmic conditions.[35] Optimizing the therapeutic potential of viral vectors requires careful engineering and design. Viral genomes can now be altered to improve their safety, effectiveness, and specificity thanks to developments in molecular biology techniques. For example, researchers can add tissue specific promoters to stimulate gene expression specifically in targeted areas while limiting off target effects.[36] Additionally, incorporating self-limiting systems can guarantee a safer therapeutic outcome by lowering the danger of insertional mutagenesis and unexpected repercussions of gene integration.[37]

Adenoviral vectors are well-known for their ability to strongly activate the immune system, making them valuable in vaccines and cancer treatments. On the other hand, AAVs are favored for in vivo gene therapy, especially when targeting non-dividing cells, thanks to their safety profile and the ability to provide long-lasting gene expression.[38] Lentiviral vectors have the unique capability to integrate into the genome, which makes them particularly effective for achieving long-term gene expression, especially in dividing cells and in ex vivo therapies like CAR-T cell immunotherapy. Viral vectors have received approval for treating genetic disorders, cancers, and chronic conditions, and they continue to play a crucial role in the ever-evolving field of gene therapy.[39]

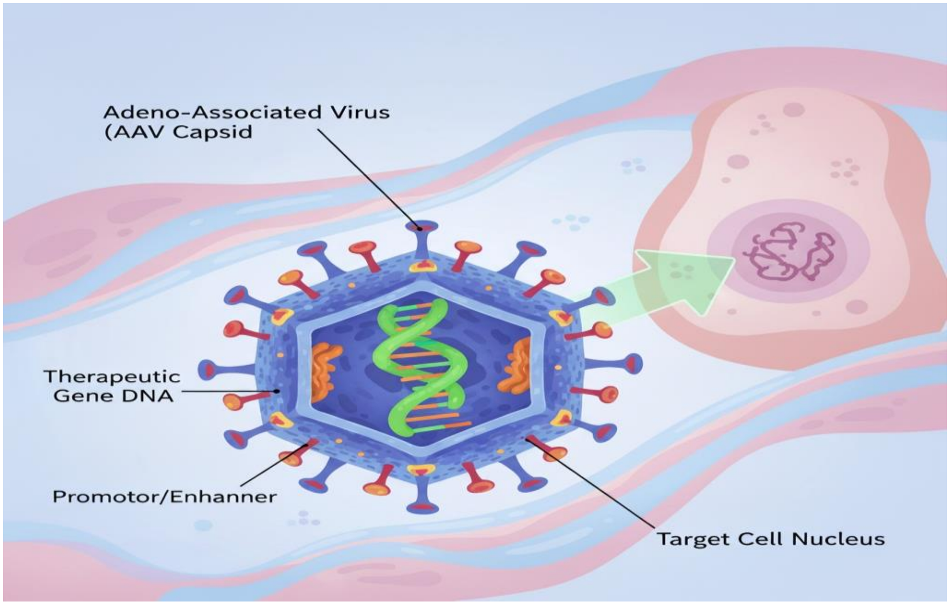

Figure 05: Viral Vector-Based Gene Therapy.

When it comes to developing viral vector-based therapies, safety and effectiveness are absolutely crucial. The risk of immune responses targeting the viral vectors can diminish their effectiveness and lead to unwanted side effects. This makes it essential to conduct thorough preclinical evaluations.[40] For example, using immunosuppressive treatments might be necessary to boost the effectiveness of adenoviral and lentiviral vectors. Additionally, there's a concern about insertional mutagenesis, especially with integrating vectors like lentiviruses, which could potentially lead to cancer. That's why it's so important to keep a close eye on patient outcomes during clinical trials to ensure the long-term safety of these therapies.[41]

Advantages

1. High gene transfer efficiency in both in vitro and in vivo.[42]
2. These methods can deliver genes for long-lasting or stable expression, particularly with lentiviruses that integrate right into the host's genome.[42]
3. Some vectors, like AAV, are known for their low immunogenicity and excellent safety profile.[43]
4. These techniques have already made their mark in FDA-approved gene therapies for genetic disorders and cancers.[43]

Disadvantages

1. Some viral vectors can trigger immune responses and inflammation, which may limit their effectiveness and safety especially when it comes to adenoviruses.[44]
2. The limited packaging capacity means there's a restriction on the size of the gene that can be delivered.[44]
3. There's also a risk of insertional mutagenesis with integrating vectors like retroviruses and lentiviruses, which could potentially lead to oncogenesis.[45]
4. Transgene expression can be temporary when using non-integrating vectors, which means you might need to administer them multiple times.[45]

NANOBOTS / NANOPARTICLES

Overview

Nanoparticles are emerging as an exciting tools for tackling genetic diseases by making it easier to deliver therapeutic genes or gene-editing tools right to the target cells. These tiny particles help shield the genetic material from breaking down in the body, boost how well cells take them in, and enable precise delivery to specific tissues or cells. They can be crafted from a variety of materials, like lipids, polymers, and inorganic substances, each offering unique benefits such as low toxicity, biodegradability, and the flexibility to be customized for controlled release and targeting specific cells.[46] When it comes to gene therapy, nanoparticles have a clear edge over viral vectors. They sidestep issues like immune reactions and insertional mutagenesis, plus they can be produced in large quantities without much hassle. Thanks to their unique physicochemical properties, nanoparticles can easily cross biological barriers and effectively deliver various types of nucleic acids, including DNA, RNA, and genome editing tools like CRISPR/Cas9. Ongoing advancements in nanoparticle design are enhancing the precision, pharmacokinetics, and safety of gene therapies, making them an essential component in the quest for personalized medicine to tackle both genetic and acquired diseases.[47]

Types of Nanoparticles used in Treatment of Genetic Disorders

Table 03: Types of Nanoparticles used in Treatment of Genetic Disorders.

Nanocarrier	Particle size	Chemical Composition
Liposomes	20 – 200 nm	Phospholipids, Cholesterol
Niosomes	50 - 300 nm	Nonionic surfactants, cholesterol, charging agents
Micelles	2 – 300 nm	Amphiphilic molecules
Dendrimers	5 – 50 nm	Branched polymers
Polymeric Nanoparticles	50 – 300 nm	Different types of polymers such as polylactic acid, polysaccharides, poly(cyano)acrylates, poly(lactide-co-glycolide)
Gold Nanoparticles	5 – 200 nm	Chloroauric acid, sodium citrate

Nanotechnology has truly transformed the way we deliver drugs, offering fresh solutions to many of the challenges faced by traditional methods. Researchers have extensively explored nanoscale carriers like liposomes, dendrimers, and solid lipid nanoparticles for their remarkable ability to improve drug solubility, stability, and bioavailability.[48] The special characteristics of nanoparticles, such as their impressive surface area-to-volume ratio and their ability to cross biological barriers, make them ideal for targeted drug delivery. For example, we can modify their surfaces to attach ligands that specifically bind to certain receptors on target cells. This not only boosts uptake but also helps minimize unwanted side effects.[49]

Nanocarriers play a crucial role in shielding nucleic acids from being broken down by enzymes. Among these, lipid nanoparticles (LNPs) have emerged as the cutting-edge choice for mRNA vaccines and gene therapies, offering controlled release and reduced immunogenicity. Additionally, cationic polymers and dendrimers are also utilized for RNA derivatives.[50]

Advantages

1. Protect the genetic material from breaking down and boost its stability within the body.[51]
2. Promote effective cellular uptake and ensure smooth gene delivery across biological barriers.[52]
3. Provide flexibility in transporting different types of nucleic acids (like DNA, RNA, and CRISPR components).[53]
4. Enable controlled release profiles and can be designed for stimulus-responsive delivery.[53]
5. Can be produced easily in large quantities, are scalable, and are often biodegradable.[53]

Disadvantages

1. They can potentially cause toxicity and inflammatory reactions, which often depend on their composition and size.[54]
2. In some cases, their ability to deliver genes (transfection efficiency) isn't as high as that of certain viral vectors.[54]
3. There are also hurdles to overcome for clinical use, such as issues with stability, variations in size (polydispersity), and ensuring consistent results (reproducibility).[55]
4. Some types can build up in tissues or organs, which raises concerns about long-term safety.[55]

FINDING / CONCLUSION

Gene therapy is truly a game-changer in how we tackle genetic disorders. It harnesses the latest breakthroughs in molecular biology and genetic engineering to get to the root of these issues. We're seeing a notable shift towards precision medicine, where treatments are customized to fit individual genetic makeups, which not only boosts their effectiveness but also reduces side effects. Recent milestones, like the approval of gene therapies for conditions such as spinal muscular atrophy and certain inherited forms of blindness, highlight just how transformative this approach can be in changing the course of diseases. Plus, ongoing clinical trials are diving into cutting-edge delivery methods, including viral vectors and CRISPR technologies, which could enhance the accuracy and success of gene modifications.[56] That said, there are still hurdles to overcome, like ethical dilemmas, long-term safety concerns, and the hefty price tags that come with these therapies. The future of gene therapy depends on tackling these challenges, broadening access, and fine-tuning techniques to apply them to a wider range of genetic disorders. As this field progresses, collaboration among geneticists, healthcare providers, and regulatory agencies will be crucial to unlock the full potential of gene therapy. In the end, the ongoing advancements in this area not only offer hope for curing current genetic disorders but also for preventing them from developing in the first place through innovative treatment strategies.[57]

FUTURE ADVANCEMENT

The outlook on future advancements in treating genetic diseases is incredibly bright, thanks to the rapid progress in gene editing, gene therapy, and precision medicine. Experts anticipate that by 2036, genetic therapies will likely become the go-to treatment for many rare and inherited conditions, offering potential cures rather than just managing symptoms.[58] Key breakthroughs include the enhanced use of precise gene-editing tools like CRISPR-Cas9, which enable targeted correction or replacement of faulty genes with greater accuracy and safety. Delivery methods are also improving, with advancements in both viral and non-viral vectors, including nanoparticles, which boost targeting precision and reduce immune responses. Additionally, progress in stem cell therapies and autologous cell transplants, paired with gene modification, promises long-lasting and possibly lifelong therapeutic benefits.[59]

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