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GENE THERAPHY : FUTURE OF GENE THERAPY AND ROLE OF GENE THERAPHY IN VARIOUS DISORDERS

[1]Mr. Prem Dilip Fendar , [2]Miss. Pinki Mulchand Rathod , [3]Mr. Dhiraj Digambar Mangam , [4]Dr. Avinash Shesharao Jiddewar

[1]Student , [2]Student , [3]Assistant Professor , [4]Principal
[1]Department of Pharmacy,
[1]Navsanjeevan Shikshan Mandals College of Pharmacy, Darwha. Dist- Yavatmal

ABSTRACT - Gene therapy, the therapeutic intervention involving the modification or replacement of defective genes to treat diseases, represents a significant advancement in medicine. As technology progresses, gene therapy is positioned to transform the treatment of various genetic disorders, including inherited conditions, cancers, cardiovascular diseases, and neurological disorders. Recent advances in gene-editing tools, such as CRISPR-Cas9, have substantially improved the precision and efficiency of gene delivery, expanding the potential applications of this approach. For monogenic disorders such as cystic fibrosis, sickle cell anemia, and muscular dystrophy, gene therapy offers the potential to address the underlying cause of the disease, potentially leading to long-term or even permanent therapeutic outcomes. Furthermore, the potential of gene therapy in treating complex diseases such as cancer and neurodegenerative disorders is becoming increasingly evident, as it can be utilized for targeted gene modifications to enhance immune responses, restore cellular function, or repair damaged tissues. Despite its promise, challenges persist, including concerns related to the safety, delivery mechanisms, and ethical implications of gene therapy. Nevertheless, ongoing research and clinical trials offer promise for the future, positioning gene therapy as a cornerstone of personalized medicine and offering potentially transformative benefits for patients suffering from a range of debilitating conditions.

Index Terms - Gene therapy, CRISPR-Cas9, Clinical trials, Transformative medicine, Cystic fibrosis, Gene delivery mechanisms, Immune response enhancement, Long-term treatment, Targeted gene modifications , Tissue repair

INTRODUCTION

Traditionally, medical treatments have primarily focused on widespread diseases and conditions requiring long-term or recurring care. This approach has left individuals with rare diseases with limited or no therapeutic options. However, the emergence of gene therapy and other cutting-edge treatments is ushering in a new era, with more ambitious goals such as disease modification and potential cures for rare conditions. Although each rare disease affects only a small number of people, there are over 6,000 such conditions, impacting 3.5–5.9% of the global population, which translates to 263–446 million individuals worldwide[1]. It's important to note that most rare diseases have a genetic basis, often involving a single gene .[2] While there isn't a universally accepted definition of rare diseases, scientific and regulatory frameworks generally use point prevalence as a threshold.[1] The European Union's regulations on orphan medicinal products define a rare disease as one affecting fewer than 50 in 100,000 people. In the United States, the Food and Drug Administration (FDA) defines it as a condition affecting less than 200,000 people (currently equivalent to about 61 in 100,000) under the Orphan Drug Act [1]. The International Society for Pharmacoeconomics and Outcomes Research (ISPOR) Rare Disease Special Interest Group calculated an average prevalence threshold of 40 in 100,000 for rare diseases [3].Consequently, the general international consensus considers a rare disease to affect fewer than 40–60 in 100,000 individuals, which is the definition used in this review. This definition, including patient numbers, is crucial for pharmaceutical companies seeking to participate in fast-track and orphan drug programs for rare disease treatments. These programs offer additional regulatory support, guidance, financial incentives, and market exclusivity. In this review, we concentrate on the current progress and future prospects of gene therapies for a subset of rare diseases – specifically, those affecting the brain and spinal cord with known genetic causes. Rare diseases are thought to impact between 3.5% and 5.9% of people worldwide [4]. The definition of a rare disease differs across regions. In the EU, a condition is deemed rare if it affects one in 2000 individuals (European Commission: ec.europa.eu/info/research-and-innovation/research-area/health-research-and-innovation/rare-diseases_en). In contrast, the United States classifies a disease as rare if it affects fewer than 200,000 people (US Food & Drug

Administration: fda.gov/patients/rare-diseases-fda). Currently, medical literature documents 6,000 to 7,000 rare diseases, with many having unknown causes. Although some rare diseases stem from infections, cancers, and autoimmune conditions, the vast majority have their roots in genetics (Orphanet: orpha.net/consor/cgi bin/Education_AboutRareDiseases.php?lng=EN).Rare genetic diseases stem from faulty genes resulting from one or more genomic mutations [5]. The malfunction of one or more Genes impairs quality of life and can lead to pre-Mature death. [4,6] Today, the therapeutic options For these diseases are limited [6]. Currently, effective treatments exist for only about 5% of rare genetic disorders [7]. However, the future holds promise as genetic therapies are anticipated to transform the management of these conditions [8,9].

These innovative approaches are expected to alleviate disease symptoms and potentially offer cures for rare genetic ailments [10,11]. Genetic therapies encompass a range of methods designed to rectify faulty genes. Among these approaches, gene therapy and gene editing stand out as two primary techniques [9,12]. (Figure 1). Gene therapy and gene editing are two distinct approaches to treating genetic disorders. Gene therapy involves introducing a functional copy of the faulty gene into cells, [5,13,14] while gene editing focuses on modifying the defective gene directly within the genome to restore its functionality. Both methods aim to address the root cause of the disease by either supplementing with a working gene or repairing the existing one, ultimately seeking to provide a cure for the condition [10,15.16]. The optimal genetic therapy should correct faulty genes without triggering oncogenes, causing unintended effects, or inducing immune or inflammatory responses [17,18]. Proto-oncogenes control cell growth and division. Chromosomal alterations can mutate these genes, transforming them into oncogenes [19]. When activated, oncogenes may stimulate unregulated cell growth, potentially resulting in cancer [20]. In clinical trials of genetic therapies, the emergence of acute T cell lymphoblastic leukemia has been documented as an adverse outcome [21]. Unintended DNA modifications occur when gene editing inadvertently alters non-target regions, resulting in off-target effects [22]. Research using animal models has demonstrated that off-target effects from CRISPR-Cas9 therapies can eliminate non-target genes, including essential or tumor suppressor genes [20]. These effects pose risks to patients, compromising the safety of these therapies and must be resolved before advancing through clinical trial stages [23]. The most frequent adverse effects of genetic therapies are immune reactions to viral vectors [24]. An immune reaction happens when the body identifies a foreign substance as harmful [25] and initiates an inflammatory response to combat it [26]. Developing viral vectors that evade immune reactions remains a significant challenge in genetic therapy research [26,27].

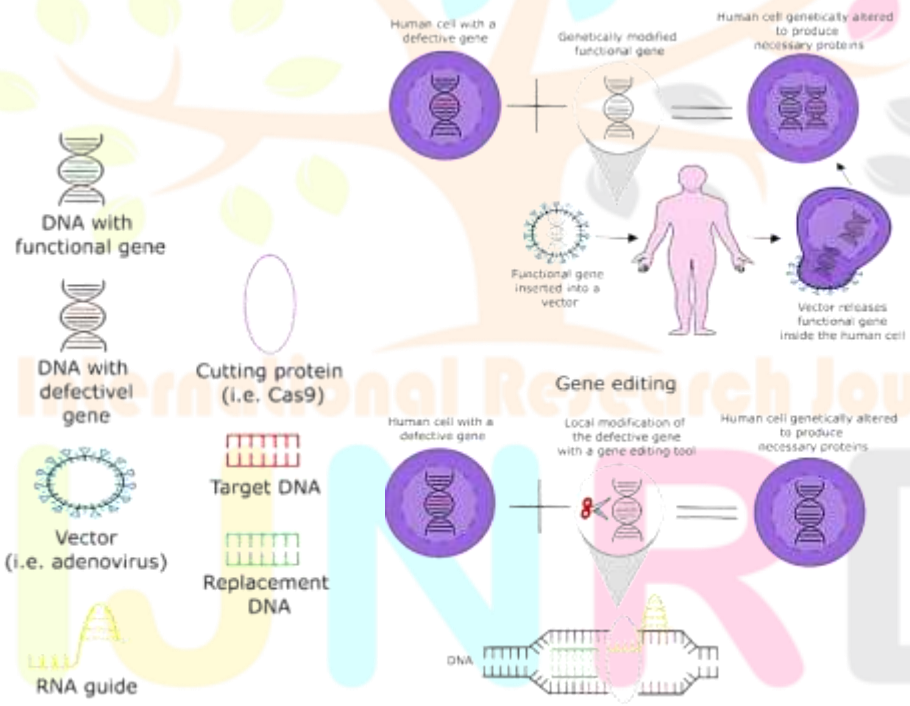

Figure 1 . Gene therapy and gene editing approaches

An optimal gene therapy should correct faulty genes without triggering oncogenes, causing unintended effects, or inducing immune or inflammatory responses [16,18]. Genes responsible for cellular growth and division are known as proto-oncogenes. [20] Chromosomal alterations can lead to mutations in these genes, transforming them into oncogenes. Once activated, oncogenes can stimulate unregulated cell proliferation, potentially resulting in cancer development [20].

A SHORT OVERVIEW OF GENE THERAPY DEVELOPMENT

In the 1970s, researchers recognized the potential of gene therapy as a promising approach for treating human genetic disorders by introducing healthy DNA to replace or complement faulty disease-causing DNA [28]. The 1980s saw the development of the idea to use viral vectors for transferring genes into mammalian cells [29]. The first approved gene therapy trial occurred in 1990, involving the viral vector-mediated transfer of the adenosine deaminase (ADA) gene to a 4-year-old patient with chromosome X-

linked severe combined immunodeficiency (SCID-X1) caused by ADA deficiency [30]. The subsequent decade witnessed numerous new trials and widespread optimism, which reached its peak with two trials that had unfortunate outcomes, leading to a temporary suspension of further gene therapy studies. The first incident involved adenovirus (Ad) vector-mediated gene therapy for ornithine transcarbamylase deficiency, where unexpected complications resulted in severe vector-associated toxicity, multi-organ failure, and the death of an 18-year-old patient [31].
In the latter instance, a gene therapy utilizing a gamma-retrovirus (γRV) vector encoding the interleukin-2 receptor gamma chain for SCID-X1 patients resulted in adverse genotoxic effects and uncontrolled T-cell clonal proliferation in six individuals. This occurred following RV integration into the host genome and subsequent activation of LIM domain only-2 (LMO2) proto-oncogenes[32]. Consequently, a moratorium on clinical trials was imposed. In subsequent years, researchers discovered and incorporated novel, safer viral vectors into new gene therapy programs, including numerous adeno-associated viral (AAV) vectors [33]. Recombinant AAVs, devoid of viral DNA and essentially functioning as non-replicable protein-based gene transfer vehicles, have gained favor in central nervous system (CNS) gene therapy. This preference is attributed to their favorable safety profile, characterized by low immunogenicity potential and pronounced neuronal tropism [34].

Approximately ten years after the initial gene therapy, Europe approved Glybera R© (alipogene tiparvovec) in 2012 to treat lipoprotein lipase deficiency [35]. In 2016, Strimvelis R©, an ex vivo hematopoietic stem and progenitor cell (HSPC) gene therapy, received approval for ADA-SCID treatment [36]. Three years later, Zynteglo was authorized in Europe to treat beta-thalasemia [37]. Luxturna R© (voretigene neparvovec), the pioneering gene therapy for inherited eye disorders, gained approval in the US and Europe in 2017 and 2018, respectively. Subsequently, Zolgensma R© (onasemnogene abeparvovec) was introduced. This gene therapy, which targets motor neurons in the central nervous system with axonal extensions into the peripheral nervous system, received approval for treating spinal muscular atrophy in the United States and Europe in 2019 and 2020, respectively., respectively [38]. The most recent addition is Libmeldy R©, an ex vivo gene therapy using lentivirus vector (LV)-transduced autologous CD34-positive hematopoetic stem and pluripotent cells (HSPCs), which received European approval in 2020 for treating metachromatic leukodystrophy [39].

A FDA (Cellular & Gene Therapy Guidances, July 20, 2018) and EU commission (Directive 2001/83/EC, Part IV of Annex I) define human gene therapy as a biological medicinal product containing recombinant nucleic acid administered to humans to regulate, repair, replace, add, or delete genetic sequences for disease treatment or cure. Quality treatment envelops three fundamental regions: (1) in vivo vector-mediated quality treatment, (2) ex vivo cell transduction quality treatment, and (3) genome altering [40]. While antisense oligonucleotide (ASO) treatments are not the focus of this review, they may be briefly mentioned when relevant.

HISTORY AND FUTURE

History

On September 14, 1990, a young female patient received treatment at the National Institutes of Health's Clinical Center in Bethesda, Maryland. The procedure was conducted by Dr. W. French Anderson and his team at the facility. They extracted white blood cells from the patient's body and introduced genes responsible for producing ADA into these cells. Subsequently, the modified cells were reintroduced into the girl's system. Following this treatment, a significant enhancement in the patient's immune function was observed. Concurrently, research into gene therapy applications continued for various medical conditions. Notably, individuals suffering from melanoma, a type of skin cancer, underwent gene therapy as a form of treatment.

Future

The field of gene therapy has reached a critical juncture. The recent achievements in genetic medicine have opened doors for a more extensive range of treatments and established the groundwork for cutting-edge technologies. This commentary provides an overview of current advancements and projections for the immediate future. The field of gene and cell therapy has experienced a significant revival in the last five years, with the first approved treatments emerging after decades of research (Fig. 2). These include pioneering oligonucleotide-based therapies (Spinraza , Exondys 51 , Vyondys 53), three cell therapies (Kymriah, Yescarta, Tescartus), and two in vivo gene therapies (Luxturna and Zolgensma), with more on the horizon. These treatments address various clinical conditions and tissue targets, such as neuromuscular disorders, inherited vision loss, and cancer.

While these approved therapies are transformative for affected individuals, they also demonstrate broader implications for the field and establish a foundation for developing treatments for numerous other conditions. As an example, Luxturna and Zolgensma have demonstrated effective AAV gene delivery to the human retina and central nervous system in living organisms, treating conditions such as Leber's congenital amaurosis and spinal muscular atrophy., respectively, has paved the way for AAV-based therapies targeting the liver and skeletal muscle to treat hemophilia [41] and Duchenne muscular dystrophy [42], respectively. Likewise, initial technological advancements in ex vivo lentiviral and retroviral gene transfer to T cells that led to adoptive cell immunotherapy have been extended to modify hematopoietic stem cells, enabling treatments for inherited disorders like sickle cell disease and beta thalassemia, recently approved in the European Union and currently under review in the United States [43].

Although the potential to apply these early gene therapy successes to other conditions and patient populations is promising, next-generation technologies are substantially expanding the impact of these medicines on treating human disease (Fig. 3). For example, a major challenge to wider application remains the immune response to gene delivery vectors and products of foreign transgenes. Consequently, some of the most influential work in the near future will focus on controlling the human immune system. Despite the remarkable success of many AAV-based gene therapies, up to 50% of patients are currently ineligible for treatment due to pre-existing immunity to the viral capsids [44]. Recent advancements and ongoing clinical trials have led to

technological breakthroughs to overcome this immune obstacle, such as engineering modified AAV capsids that evade pre-existing neutralizing antibodies [45,46] and methods for temporarily removing antibodies from circulation [47].

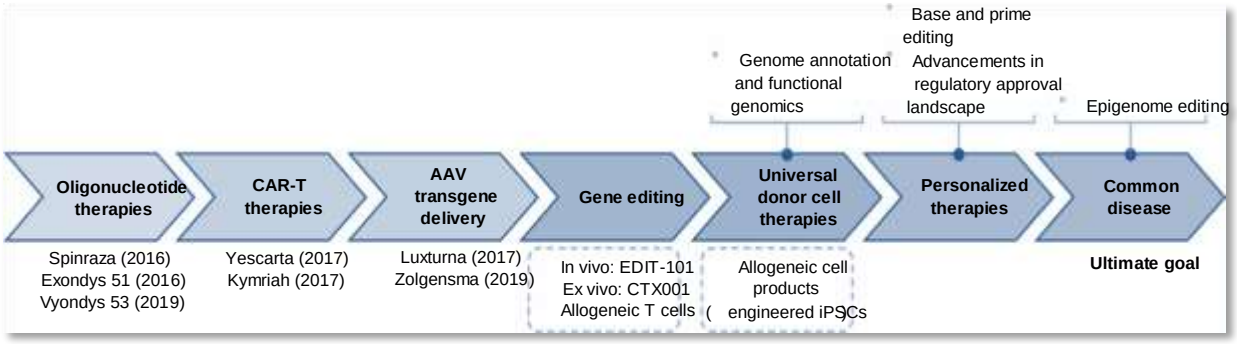

figure.2:- timeline depicting milestones (in colored arrows) towards gene therapies for common disease.- below each milestone, the text displays approved treatments along with their approval years, as well as potential therapies under investigation (indicated by dashed boxes). to achieve future milestones (listed as bullet points), additional research is necessary to explore alternative therapeutic strategies and address fundamental scientific questions.

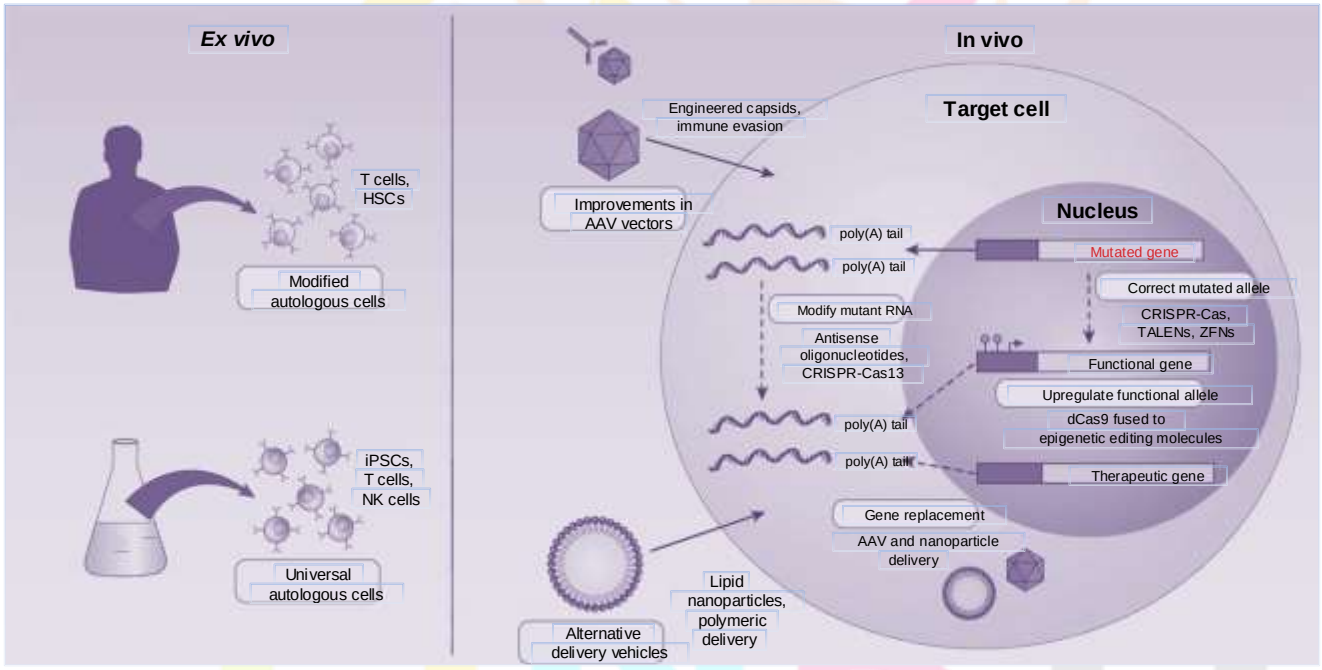

figure. 3 :- schematic of ex vivo and in vivo strategies (shown in blue boxes) for treating genetic diseases - _in ex vivo methods (depicted on the left side), cells from the patient can be extracted and genetically altered to produce a therapeutic outcome, while cells from donors can be manufactured and made available as ready-to-use products. in contrast, in vivo approaches necessitate the targeting of particular cells to either enhance the expression of a therapeutic gene or rectify pathological processes, enabling functional gene expression (indicated by dotted arrows).

Moving beyond initial gene therapies that focused on transgene delivery, gene editing technologies are ushering in a novel treatment approach based on precise alterations of human genomic sequences. Although gene editing therapies first entered clinical trials in 2010 as a method to prevent HIV infection in T cells [48], it wasn't until the past year that the first demonstration of disease-modifying efficacy was shown in clinical trials using CRISPR-based gene editing for sickle cell disease and beta-thalassemia (CTX001) [49]. This groundbreaking success, coupled with the promising safety profile observed so far in human trials involving gene-edited T cells and HSCs [49,50] , has paved the way for highly anticipated outcomes from ongoing and upcoming clinical trials of in vivo genome editing. These include a current trial of AAV-based gene editing in the retina (EDIT-101) [51] , and a planned trial for non-viral nanoparticle-based delivery of CRISPR to the liver (NTLA-2001) [49,52].

Contemporary gene editing techniques employ nuclease-based systems to sever DNA strands and activate DNA repair mechanisms to introduce desired genetic modifications. While these methods are just beginning clinical trials, numerous advanced editing technologies are emerging to enhance precision, accuracy, efficiency, and applicability across various disease categories [53,54]. For instance, the development of base editing and prime editing has enabled precise genomic alterations without

DNA breaks or reliance on endogenous DNA repair pathways [53]. RNA-targeted editing technologies allow for temporary and reversible gene expression modifications without permanent genomic changes, potentially improving efficiency and safety [54]. Additionally, epigenome editing technologies offer advantages such as adjustability, reversibility, and the potential for lasting effects after transient editor activity, which can be inherited through cell division [55].

Concurrently, the range of DNA-targeting systems continues to grow, particularly with the rapidly increasing diversity of CRISPR-Cas systems derived from engineered variants, various bacterial species, and distinct classes of CRISPR targeting mechanisms [54]. The swift advancement of these editing technologies is poised to revolutionize our approach to gene therapies and significantly expand the scope of treatable human diseases.

Functional genomics and our understanding of human genome regulation represent another area of innovation that will profoundly impact the gene therapy field in the near future. For example, the function of approximately 6,000 out of 20,000 human genes remains unknown [56]. In addition to enabling gene editing therapies, CRISPR technologies are facilitating the functional analysis of these gene sequences [57]. Furthermore, while scientific studies and therapeutic interventions have traditionally focused on genes, 98% of our genome consists of non-coding DNA containing epigenetic regulators responsible for over 90% of susceptibility to common diseases[58].

Notably, the first demonstration of therapeutic efficacy using a CRISPR gene editing approach (CTX001) involves editing a distal gene regulatory element to alter gene expression, rather than modifying the underlying genetic mutation [59]. Although initiatives such as the NIH ENCODE Consortium have mapped more than two million gene regulatory elements across hundreds of human cell types and tissue samples, the function of only few sites is known [60].

Elucidating this genomic "dark matter" will unveil new areas of disease biology and classes of therapeutic targets, enabling novel approaches to combat human diseases through gene therapy, gene editing, and other modalities.

PRINCIPLE

Through the process of homologous recombination, a faulty gene could be replaced with a functional one.

- Alternatively, the defective gene might be corrected via targeted reverse mutation, restoring its normal functionality.
- Additionally, it's possible to modify the expression level of a specific gene, adjusting how much it is activated or suppressed.

APPROACHES

1. Hereditary adjustment
- Substitution treatment
- Remedial quality treatment

2. Quality exchange
- Physical
- Chemical
- Organic

3. Quality exchange into particular cell lines
- Physical quality treatment
- Germ line quality treatment

4. Genetic counseling approach (Quality addition) Other shapes of hereditary building incorporate quality focusing on and quieting of particular qualities utilizing designed nucleases such as zinc finger nucleases, designed I-CreI homing endonucleases, or nucleases produced from TAL effectors. This approach is right now being utilized in a few human clinical trials [61].

VECTORS IN GENE THERAPY

Some of the diverse sorts of infections utilized as quality treatment vectors

Retroviruses

It is group of viruses capable of producing double-stranded DNA versions of their RNA genomes. These genomic copies can be incorporated into the chromosomes of cells they infect. The Human immunodeficiency virus (HIV) exemplifies this class of viruses.

For instance, one challenge in retroviral gene therapy is the unpredictable nature of the integrase enzyme. This enzyme can insert the virus's genetic material at any random location within the host's genome. If this insertion occurs within an existing gene of the host cell, it can disrupt that gene's function (insertional mutagenesis). Should this disruption affect a gene controlling cell division, it may lead to uncontrolled cellular proliferation, potentially resulting in cancer. Recent efforts to address this issue have begun to explore the use of zinc finger nucleases [62].

Adenoviruses

It is a group of viruses characterized by their double-stranded DNA genomes. These pathogens are known to infect humans, causing ailments that affect the respiratory system, intestines, and eyes. One notable example of an adenovirus is the pathogen responsible for the common cold.

Adeno-associated viruses
It is a group of diminutive, single-stranded DNA viruses capable of integrating their genetic material into a specific location on chromosome 19.

Cis and trans-acting elements
Replication-defective vectors invariably include a "transfer construct," which contains the gene intended for transduction, also known as the "transgene." This construct also incorporates essential sequences for the viral genome's general operation, such as the packaging sequence, replication repeats, and, when necessary, elements for initiating reverse transcription. These components are referred to as cis-acting elements because they must be located on the same DNA segment as the viral genome and the gene of interest to function properly [63].

Herpes simplex viruses
It is belong to a category of double-stranded DNA viruses that specifically target neurons. One common human pathogen in this group is Herpes simplex virus type 1, which is responsible for causing cold sores [64].

GENOME EDITING IN GENE THERAPY

Genetic modification techniques allow for the alteration, removal, or addition of nucleotides, as well as the adjustment of gene expression and epigenetic modifications [65]. In single-gene disorders affecting the central nervous system, a mutation that causes either loss of function or gain of toxic function can be addressed by specifically targeting and editing the mutation to restore normal cellular behavior. The current toolkit for genetic editing includes ZFNs, TALENs, and CRISPR/Cas systems [66,67].

Utilizing ZFNs and TALENs requires extensive molecular biology expertise, which has restricted their widespread use and application. In corast, CRISPR/Cas systems rely on RNA-guided nucleases and DNA-binding properties that can be easily modified using a brief RNA sequence [68,69]. These systems are categorized into two primary classes based on their nuclease effectors [70,71]: Class 1 systems (types I, III, and IV) employ a large complex of multiple effector proteins, while class 2 systems (types II, V, and VI) utilize a single Cas protein to recognize and cleave foreign nucleic acids. Due to their simpler structure, class 2 systems are more commonly used, with type II and type V CRISPR/Cas ribonucleoprotein complexes identifying specific DNA sequences through RNA-DNA base pairing and inducing double-strand breaks. The host cell responds to these breaks through homology-directed repair, where donor DNA serves as a template for repair, enabling gene editing and correction based on a healthy template DNA [72,73].

Recently, CRISPR/Cas-based genome editing has surpassed ZFNs and TALENs in popularity due to its easier engineering for recognizing unique sequences. While ZFNs and TALENs rely on protein-DNA interactions for DNA-binding specificity, CRISPR/Cas systems use sgRNAs, which are simpler and more cost-effective to design [65].

Although advancements have been made in pre-clinical research [74], only a limited number of in vivo genome editing techniques using ZFNs in the central nervous system have progressed to clinical trials. However, the opportunity to expand into CNS disorders is evident, and pharmaceutical companies should be highly motivated to advance more in vivo genome editing strategies to clinical stages for brain and spinal cord diseases. To achieve this, it is crucial to address the identified challenges and safety concerns, particularly those related to the immunogenicity triggered by genome editing tools[75], as well as improve the characterization and regulation of both on-target and off-target modifications [76].

USES OF GENE THERAPY

Cancer
Research and clinical applications related to gene therapy have primarily focused on the field of cancer. By late 2009, approximately two-thirds of gene therapy studies were dedicated to cancer research [77]. One approach involves using oncolytic viruses to introduce genes into cancer cells, leading to their destruction. Another method aims to induce oncolysis by delivering the p53 tumor suppressor gene. Gendicine, the first approved anticancer drug based on this gene therapy principle, exemplifies this approach. An additional strategy, known as suicide gene therapy, attempts to treat tumors by introducing genes that code for enzymes capable of converting prodrugs into locally active chemotherapeutic agents.

Gene therapy research and its clinical applications have primarily focused on cancer treatment. Approximately two-thirds of all gene therapy studies are cancer-related. One approach involves using oncolytic viruses to introduce genes into cancer cells, leading to their destruction. Another method involves administering the p53 tumor suppressor gene to induce oncolysis. Gendicine, the first approved anti-cancer drug based on gene therapy principles, exemplifies this approach. An additional strategy in cancer treatment is suicide gene therapy, which involves delivering genes that code for enzymes capable of converting prodrugs into localized, active chemotherapeutic agents [78].

Single Gene Disorder

Gene therapy plays a crucial role in treating various single gene disorders. These include alpha-1-antitrypsin deficiency, cystic fibrosis, muscular dystrophies, lysosomal storage disease, chronic granulomatous disease, Huntington's disease, junctional

epidermolysis bullosa, haemophilia, and ornithine transcarbamylase deficiency. These conditions represent a range of genetic disorders where therapeutic interventions targeting specific genes can be particularly effective.

Immunodeficiency

Despite years of efforts to enhance gene therapy, the first significant breakthrough since initial trials occurred in the early 1990s. Following a setback where two patients treated for X-linked severe combined immunodeficiency (X-SCID) using retroviral vectors succumbed to leukemia, clinical trials nonetheless demonstrated the substantial therapeutic advantages of gene therapy in managing both X-SCID and SCID resulting from adenosine deaminase (ADA) deficiency. Secondary immunodeficiency conditions such as Human Immunodeficiency Virus (HIV), along with primary immunodeficiency infections, have also emerged as potential candidates for gene therapy. To provide specific protection against HIV infection, transgenes can be introduced into hematopoietic stem cells or T-cells. These transgenes create an environment unfavorable for HIV-1 replication or impair HIV-1 protein function [79].

Eye Diseases

Following the initial setback in SCID, confidence in gene therapy was restored through advancements in treating Leber's congenital amaurosis. The eye's small size allows for efficient transfection of numerous ocular cells. Gene therapy shows promise for various ophthalmologic conditions, including Leber's hereditary optic neuropathy, glaucoma, macular degeneration, and red-green color blindness. An ongoing phase I study is evaluating the effects of antiangiogenic cytokine Pigment Epithelium-derived Factor (PEDF) in treating age-related macular degeneration. Research has demonstrated significant improvement in creating trichromatic color vision in adult red-green color-blind monkeys through subretinal injections of adeno-associated virus containing an L-opsin gene [80].

Cardiac Diseases

The treatment of heart diseases poses challenges due to their complex genetic origins. Researchers are conducting experiments to develop techniques for delivering genes that encode various growth factors, such as Fibroblast Growth Factors (FGF) and Vascular Endothelial Growth Factors (VEGF), with the aim of promoting blood vessel formation. Although the results did not show significant improvements in stress-induced myocardial perfusion, the observed enhancement in regional wall motion suggested a positive anti-ischemic effect. This outcome has motivated further investigation in this area of study.

Central Nervous System (CNS) Disorders

Unlike cardiac disease, gene therapy has shown promising outcomes for neurological disorders, particularly in treating Alzheimer's and Parkinson's diseases. Currently, numerous trials exploring gene therapy for Parkinson's disease are underway, with many in phase 1 or 2, evaluating its potential, safety, and tolerability for in-vivo studies. Various approaches are being employed, such as delivering genes to putamen cell bodies for neurturin or introducing genes into the subthalamic nucleus for glutamic acid decarboxylase. In the case of Alzheimer's disease, researchers have attempted gene therapy by delivering nerve growth factor into the central nervous system [81].

NEURODEGENERATIVE DISORDERS

Spinal Muscular Atrophy (SMA) :- Spinal muscular atrophy (SMA) is defined by the deterioration of alpha motor neurons in the spinal cord, leading to muscle wasting. This condition hinders a patient's ability to perform basic functions such as walking, speaking, and breathing [82]. SMA affects roughly 10 out of every 100,000 newborns and is considered the most prevalent monogenic disorder causing infant mortality [83,84]. The disease stems from a loss-of-function mutation in the SMN1 gene, which produces a protein crucial for alpha motor neuron survival. The exact role of this protein remains not fully understood [85].

The human genome contains a similar gene, SMN2, present in multiple copies and differing from SMN1 by only a few nucleotides, notably a variant in exon 7. This SMN2 variant results in the exclusion of exon 7, producing an unstable protein. It is believed that SMN2 generates 90% truncated proteins (lacking exon 7) and 10% normal but still unstable SMN proteins. These proteins partially compensate for SMN1 loss, with the number of SMN2 copies in the genome thought to influence disease severity [82,84].

SMA is categorized into different clinical phenotypes based on symptom onset age, achieved motor functions, and SMN2 gene count. Type 0 (1 SMN2 copy) begins in utero, requires mechanical ventilation at birth, and typically results in survival under 6 months. Type 1 (Werdnig Hoffman disease) (1-3 SMN2 copies) starts before 6 months, necessitates posture, respiratory, and feeding support, with life expectancy below 2 years. Type 2 (Dubowith disease) (2-4 SMN2 copies) onset is between 7-18 months, characterized by inability to walk, need for respiratory and feeding assistance, and potential survival into adulthood. Type 3 (Kugelberg-Welander disease) (3-4 SMN2 copies) begins at 18 months, initially allowing standing and walking, though these abilities may be lost, with normal life expectancy. Type 4 (4 SMN2 copies) has adult onset, mild symptoms, and normal life expectancy [86,87].

SMA type 1 (SMA1), the most common form accounting for about 60% of cases, presents symptoms including hypotonia, motor delays, and breathing difficulties. The primary cause of death is respiratory failure due to muscle weakness [88].

Multiple System Atrophy (MSA) :- Multiple System Atrophy (MSA) is an uncommon degenerative neurological condition characterized by a combination of Parkinsonian symptoms, cerebellar ataxia, and autonomic dysfunction. This disorder affects the

striatonigral, olivopontocerebellar, and autonomic systems, with an estimated occurrence of about 2 per 100,000 individuals [89]. The primary pathological indicator is the presence of glial cytoplasmic inclusions containing insoluble protein filaments within oligodendrocytes. Consequently, MSA is classified as an α-synucleinopathy, alongside Parkinson's disease and Lewy body dementia. While the exact cause of MSA remains largely unknown, recent findings suggest potential involvement of mutations in several genes, including SNCA, COQ2, MAPT, GBA1, LRRK2, and C9orf72 [90]. At present, there is no specific treatment targeting MSA itself; therapeutic approaches focus on managing symptoms. Patients with MSA may experience relief from Parkinsonian symptoms through l-dopa treatment, while physiotherapy remains the most effective option for addressing ataxia [91].

Amyotrophic Lateral Sclerosis (ALS) :- Amyotrophic Lateral Sclerosis (ALS) encompasses a set of uncommon neurological disorders primarily affecting neurons that control voluntary muscle movement. This progressive condition has a global prevalence of approximately 5.4 per 100,000 individuals [92]. ALS is marked by the gradual deterioration of motor neurons in the brain and spinal cord, leading to a progressive loss of strength and the ability to speak, eat, move, and breathe. The majority of ALS patients succumb to respiratory failure, typically within 3-5 years of symptom onset. Genetic factors account for 5-10% of all ALS cases, with mutations in over a dozen genes identified. In European populations, roughly 34% of familial cases are attributed to defects in the C9orf72 gene (likely gain-of-function mutations [93]. An additional 15-30% of familial cases stem from gain-of-function mutations in the gene encoding copper-zinc superoxide dismutase 1 (SOD1) [93]. The FDA has approved two medications for ALS treatment: riluzole, an anti-excitotoxic glutamate antagonist, and edaravone, a free radical/reactive oxygen species scavenger believed to reduce oxidative damage. While these drugs do not effectively reverse disease progression, riluzole offers modest survival benefits, and edaravone may slow ALS advancement in its early stages [94,95].

Genetic Epilepsy Syndromes

Epilepsy, a prevalent neurological disorder, is defined by a persistent susceptibility to seizures. While its overall lifetime prevalence is roughly 800 per 100,000 individuals [96], it encompasses a diverse array of syndromes. Some of these syndromes meet the criteria for rare diseases as defined in this review (i.e., <40–60 per 100,000). Examples include Dravet syndrome (severe myoclonic epilepsy in infancy; 2.5 per 100,000), Lennox-Gastaut syndrome (15 per 100,000), West syndrome (infantile spasms; 8 per 100,000), and Angelman syndrome (5–8 per 100,000) [97,98]. These genetic epilepsies are typically identified within the initial months or years of life, with developmental regression or seizures as the primary symptomatic indicators. Given that these syndromes often resist conventional drug treatments, gene therapy may offer a potential alternative approach [99].

ADVANTAGES

- Scientists could potentially use gene therapy to 'silence' a gene in individuals with HIV who have not yet progressed to AIDS, potentially sparing them from the disease's symptoms and suffering.

- Gene therapy shows promise in eradicating and preventing genetic disorders like cystic fibrosis, and may offer a cure for conditions such as heart disease, AIDS, and cancer.

- Critics of gene therapy would likely opt for this treatment if it represented the final hope for themselves or their loved ones - a situation many gene therapy patients find themselves in [100].

DISADVANTAGES

- Transient effectiveness of gene therapy.

- Immunological reactions - Virally-introduced genes may elicit an immune response targeting the virus. Complications with viral carriers (potential for viral vectors to regain pathogenicity after entering the patient's body).

- Complex genetic disorders - The genetic material may fail to reach the intended cell or the correct location within the cell's DNA [101].

CHALLENGES

The potential applications of genome editing research are undeniably promising and may offer superior long-term prospects compared to retroviral vector-based gene therapy. Designer nucleases for gene addition surpass traditional retroviral vector methods by enabling targeted integration, a feature currently unattainable with retroviral vectors. Nevertheless, despite the potential for increased safety, genome editing still faces challenges.

A primary safety concern is the identification of off-target effects. To tackle this issue, considerable research has been conducted, resulting in the creation of techniques such as Digenome-seq [102] and CIRCLE-seq [103,104]. These techniques rely on adapter ligation to CRISPR-generated ends. Digenome-seq utilizes in vitro Cas9-digested whole-genome fragments to profile genome-wide Cas9 off-target effects in human cells. CIRCLE-seq creates a library of circularized genomic DNA with minimal free ends, followed by CRISPR/Cas9 RNP complex treatment, adapter ligation, and high-throughput sequencing. While these approaches show great promise, they face limitations such as NGS read length constraints. Other methods like BLISS [105] involve cell fixation, raising doubts about the accuracy of introducing DSBs as part of the screening process. Lastly, there is DISCOVER-SEQ [106].

This method relies on attracting specific DNA repair proteins, raising questions about its ability to identify all DSBs. The quantity of the engineering agent can significantly impact the same cell type, as evidenced by differences observed between engineered cord blood CD34+ using lentiviral vectors with low MOI [107] versus high MOI [108] Beyond the existing limitations in current techniques, another concern is that some off-targets may be harmless, while others could have serious implications depending on the cellular context or indication. The field recognizes this issue and is addressing it by modifying the CRISPR payload at both protein and gRNA levels, while optimizing the ideal exposure window of target cells to functional complex RNP [109].

From a regulatory standpoint, the challenges are substantial for several reasons: (a) Even a single genetic disorder caused by knockout of one gene or sequence may involve multiple mutations, some unrelated, in different patients. For instance, the consequences of disrupting the erythroid-specific enhancer within BCL11A's second intron at a population level are unpredictable. (b) Depending on the indication, even well-characterized gene therapy agents can yield unexpected results. The recent occurrence of tumor generation following lentiviral-mediated gene addition in CGD is concerning[110]. The authors reported T cell lymphoblastic lymphoma and myeloid leukemia development in 2.94% and 5.88% of tested mice, respectively, with oligoclonal composition and rare dominant clones harboring vector insertions near oncogenes. (c) Genetic engineering of HSCs presents additional obstacles as CD34+ cells are challenging to analyze karyotypically, with most cells in G0 phase. This complicates the detection of large chromosomal rearrangements resulting from designer nuclease action in patients' genomes, necessitating the development of alternative assays. Approaches introducing chromosomal deletions rather than indels will likely face difficulties transitioning to clinical trials. (d) Finally, gene therapy products are often termed "living drugs" and exhibit vastly different pharmacokinetics compared to traditional small molecules, creating challenges for regulatory agencies in assessing such products. Consequently, the progression from laboratory to clinic, and for industry to obtain marketing authorization, will necessitate collaboration among various disciplines, including researchers, physicians, industrial stakeholders, regulatory agencies, and policymakers.

CONCLUSION

Revolutionary medical and biotechnological approaches, gene therapy and gene editing, offer tremendous potential for addressing genetic disorders, improving human health, and disease prevention. Gene therapy aims to correct or compensate for defective genes by introducing, modifying, or repairing genes within an individual's cells, providing hope for previously untreatable conditions. Gene editing, particularly utilizing techniques like CRISPR-Cas9, enables precise DNA-level modifications, allowing for targeted alterations to rectify mutations or enhance specific characteristics.

Despite their transformative potential, these technologies raise concerns regarding ethics, safety, and regulation. Careful consideration must be given to issues such as unintended genetic alterations, long-term consequences, and possible misuse, especially in areas like germline editing (modifying human embryos). As research progresses, it will be essential to develop robust regulatory frameworks, ethical guidelines, and maintain ongoing scientific scrutiny to ensure responsible implementation of gene therapy and editing techniques. In summary, while gene therapy and editing show promise for significant medical advancements, their full integration into clinical practice necessitates continuous evaluation of ethical, social, and scientific implications. This approach will help maximize potential benefits while minimizing associated risks.

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