A GENE THERAPY APPROACH TO THE DESIGN OF PERSONALIZED MEDICINE

Abstract

Gene therapy is the process of altering a cell's genetic makeup to affect how it behaves. It involves employing viral and non-viral vectors for efficient gene delivery. Ad vectors are successful because of their high levels of transduction efficiency, a wide range of tropism, and scalable production techniques. Non-viral methods offer security, significant gene transfer, and lower toxicity. Examples include sonoporation, electroporation, and gene guns. Microinjectors, needle injection, hydrodynamic gene transfer, jet injection, sonoporation, hydrostatic pressure, and mechanical massage are examples of emerging delivery techniques. The therapeutic landscape for several illnesses, including ALS, cancer, haemophilia, epilepsy, and monogenic diseases, may be drastically changed by gene therapy. Clinical trials are now being carried out for ALS individuals who have SOD1 mutations, C9orf72 hexanucleotide repeat expansions, ATXN2 trinucleotide expansions, and FUS mutations as well as sporadic illnesses with unknown genetic aetiology. The therapeutic landscape has been widened by advances in CNS targeting, gene delivery, and gene editing technologies. Treatments for human cell-based gene therapy have been developed in recent years to address a number of therapeutic indications and tissue targets. The goal of the Somatic Cell Genome Modifying Consortium is to improve in vivo genome modification for addressing human health issues in a variety of tissue types and disease states. The management of the immune system and genetic modification technologies will be the focus of future study.

Keywords: Genetic, Gene therapy, Gene Delivery, Vectors, Gene modification

Author

Kiruba Mohandoss
Assistant Professor
Department of Pharmaceutics
Sri Ramachandra Faculty of Pharmacy
Sri Ramachandra Institute of Higher Education and Research (DU)
Porur, Chennai.
mkirupa@sriramachandra.edu.in

I. INTRODUCTION

Gene therapy is a promising technique for treating genetic disorders, involving the introduction of therapeutic genes into a patient's cells to modify their function or correct genetic abnormalities. Efficient gene delivery to target tissues or cells is a critical stage in this process and is achieved using two types of vectors: viral and non-viral. Viral vectors, such as retroviruses, adenoviruses (Ads), and adeno-associated viruses (AAVs), have been widely used for in vivo gene delivery due to their ability to efficiently enter target cells and express therapeutic genes. Delivering an effective transgene ex vivo, which entails removing cells from the body and growing them elsewhere, is an alternate technique. The four main techniques utilised in gene therapy are gene silencing, gene insertion, replacement of genes, and modifying genes.

In the initial stages of gene therapy, Dr. Stanfield Rogers conducted a trial to treat two sisters afflicted with hyperargininemia, a condition caused by a deficiency of the enzyme arginase, leading to elevated arginine levels in the blood. Despite efforts, the trial could not effectively address the issue, as the viral vector derived from the Shope papilloma virus used in the treatment failed to encode for arginase production, hindering the correction of the genetic defect. Similarly, in 1980, Dr. Martin Cline attempted to perform gene therapy on two -thalassemia patients by introducing recombinant -globin genes into their bone marrow cells. However, the surgical procedure proved unsuccessful in achieving the desired genetic correction.

Vascular vector gene therapies achieved therapeutic success in the early 1990s with a trial conducted by French Anderson, Michael Blaese, and Steven Rosenburg treating Ashanthi DeSilva with adenosine deaminase deficiency severe combined immunodeficiency illness (ADA-SCID) utilising an ex vivo strategy. There are a lot of promising viral vector-based treatments for genetic disorders thanks to 40 years of virus research.

1. **In Clinical Trial Phases:** In human gene therapy studies, cytokines, antigens, suicide enzymes and tumour suppressors are the most frequently transferred gene types. These categories now account for 55.3% of trials since the introduction of pathogen-specific antigens in vaccines. Growth factors were transferred in 7.5% of trials, the majority of which focused on cardiovascular diseases. Receptor genes and deficient genes were used in 8.0% of studies. Replication inhibitors were used in 4.3% of trials, while 2.9% of trials transferred marker genes. Antisense or short interfering RNA was transferred in 1.8% of trials and oncolytic viruses in 2.1% of trials..

   Gene therapy trials account for 78.6% of all studies, the majority of those that are in phase I or I/II. Phase II studies make up 16.7% of all trials, while phase II/III trials make up 4.5%. Trials at this stage are becoming more prevalent, which shows growth towards clinical usability.

2. **India's use of Gene Therapy:** With the primary objective of advancing and raising awareness of gene and cell therapy in India, the ASGCT Indo-UK Workshop on Clinical Gene Therapy was held on October 9, 2021 that brought together researchers, scientists, medical professionals, advocacy groups, and entrepreneurs from India and the UK. Key topics of discussion included gene therapy for Leber congenital amaurosis, CAR-T cell

therapy for leukaemia and lymphomas, and haemophilia. Delegates from the Indian Council of Medical Research (ICMR) and the government body NitiAayog provided valuable insights into regulatory preparation for clinical trials and government assistance mechanisms. The workshop fostered collaboration and increased awareness, laying the groundwork for further advancements in gene therapy in India.

The conference aimed to develop homegrown gene therapy technology with the approval of CAR-T cell therapy for academic trials at the Advanced Centre for Treatment, Research, and Education at the Cancer/Indian Institute of Technology in Bombay and the Christian Medical College in Vellore. Clinical trials for commercial gene therapy are not currently being conducted in India, but that will change in the second half of 2022.

The Department of Biotechnology (DBT) and the Wellcome Trust-DBT India Alliance have provided funding for multiple preclinical studies employing viral vectors to address diseases like haemophilia, Leber congenital amaurosis, thalassemia, and more. India is actively investing to establish itself as a competitive manufacturing hub for gene and cell therapy products. To facilitate gene therapy trials in the country, the Indian Council of Medical Research (ICMR) and DBT jointly developed the National Guidelines for Gene Therapy Product Development and Clinical Trials. Gene therapy has promise for treating inherited and hereditary illnesses because of its safety, potential for precision targeting, high gene transfer, toxicity, and affordability. There are, however, still very few therapeutic uses. The work's key objectives are to improve transfection efficiency, limit tissue damage, reduce toxicity, and improve targeting specificity. Ongoing work is needed to create new tactics and improve existing systems.

A Gene Therapy Advisory and Evaluation Committee (GTAEC) with its headquarters at the ICMR now include the Directorate General of Health Services, the Central Drugs Standard Control Organisation, the Department of Science and Technology, the Medical Council of India, and other governmental organisations. The GTAEC will also direct prospective participants and provide advice on clinical trial submissions for review. Gene therapy has promise for treating inherited and hereditary illnesses because of its safety, potential for precision targeting, high gene transfer, toxicity, and affordability. There are, however, still very few therapeutic uses. The work's key objectives are to improve transfection efficiency, limit tissue damage, reduce toxicity, and improve targeting specificity. Ongoing work is necessary to create new tactics and improve old systems.

Young professionals in medical colleges all over India were contacted by the ASGCT-sponsored symposium to spread the word about the event and the ongoing advancements in gene therapy. The organisers are requesting the ASGCT to assist as a matchmaker between members of the society and those engaged in India-based research, clinical trials, vector production facilities, and cell manufacturing. The ASGCT is also asked to promote twinning projects in order to foster collaboration and hasten progress.

3. **Industry View:** At the ASGCT Global Outreach meeting, the significance of the industry in advancing universal access to gene therapy was underscored. While this class of therapeutic medications holds great promise, there are technical and logistical challenges that necessitate further research. Issues like immunogenicity, integrational mutagenesis,

and the persistence of therapeutic effects require additional study for long-term solutions. Manufacturing cell and gene therapies remains costly and technically demanding, demanding substantial upfront investments in product development. To make gene therapy more widely available and affordable as a mainstream therapeutic option, efforts will be required to enhance production capacity and reduce costs in the future. Gene therapy has promise for treating inherited and hereditary illnesses because of its safety, potential for precision targeting, high gene transfer, toxicity, and affordability. There are, however, still very few therapeutic uses. The work's key objectives are to improve transfection efficiency, limit tissue damage, reduce toxicity, and improve targeting specificity. More work will be needed to increase manufacturing and reduce prices once gene therapy becomes a mainstream treatment option.

Late-phase clinical trials have considered involving Limited Member States (LMICs); however, their participation in early-phase trials has been limited by small cohort sizes. To ensure safety and obtain relevant findings, consistent and controlled supportive care is essential. Establishing centers of excellence capable of characterizing gene changes, providing access to affected individuals, and conducting controlled trials with cutting-edge therapeutic drugs would be advantageous to both small and large developers of cell and gene therapy products.

Both academic gene therapy researchers and industry professionals aiming to develop commercial products express concerns about the absence of a streamlined regulatory structure for swift examination and approval of clinical trials. Ethical considerations hold equal importance in the realm of gene and cell therapies. Furthermore, the industry must carefully evaluate the laws pertaining to liability and intellectual property rights to navigate these novel therapeutic approaches successfully.

Due in part to the high cost of these therapeutic agents, access to trials involving gene and cell therapy is limited in LMICs. One possible advantage of novel reimbursement structures, such as installment payments over time and rebates, has been mentioned: the industrialisation of gene therapy medications in industrialised nations. However, access to licenced gene therapies can only be made possible by compassionate and managed access programmes, which do not reach all eligible patients. As technological developments lower the cost of production, more research will be required to examine improved access alternatives in LMICs.(Cornetta et al., 2022)

II. Ad VECTORS

Ad vectors are useful in gene therapy because of their high transduction efficiency, long chromosomal persistence in the host cell, broad tropism for numerous tissue targets, and scalable production techniques. Human serotypes HAd2 and HAd5 are the source of contemporary advertising vectors. The main objectives of Ad vector development are potent innate immune responses to capsid proteins and potent adaptive immunological responses to freshly synthesised viral and transgenic products.

Since the first generation of E1A-deleted Ad vectors was generated, many techniques have been developed to improve the durability, efficiency, longevity of gene transfer, and safety of these vectors. During the first generation's engineering, transgene cassettes as long

as 4.5 kb were employed to replace the E1A/E1B region. However, the first generation had two significant drawbacks: (2) It is possible for spontaneous homologous recombination between the vector and engineered E1 region from HEK293 to result in replication-competent adenovirus during genome amplification. (1) De novo expression of Ad proteins can still cause the host immune response, clearing cells that have been transduced with the vector.

In the first generation of Ad vectors, the E1A/E1B region was changed with transgene cassettes up to 4.5 kb in size. Recombinant Ad cannot multiply in host cells as a result, thus compatible cell lines like HEK293 must be used. fortunately, the first generation has two significant drawbacks: the host immune system can be activated by de novo manufacture of Ad proteins, and replication-competent adenoviruses might emerge as a result of probable spontaneously homologous recombination among the vector's original and altered E1 region.
In the second generation, more early gene regions (E2a, E2b, or E4) are deleted to make room for larger transgenic cassettes (10.5 kb). These novel vector designs include deletion of the E4 region, temperature-sensitive rAd vectors, and DNA polymerase (Pol) protein encoded by E2b.

These Ad vectors significantly lower late gene expression and the acytotoxic T-lymphocyte response when administered in vivo. However, deletion of the E2 and/or E4 genes leads to decreased titers and adverse effects on viral vector amplification. Despite these alterations, the native Ad late genes that are still present in the vector genome can still harm cells and induce host immunogenicity.

The only viral sequences present in third-generation ad vectors, also known as "gutless" or "helper-dependent" ad vectors, are ITRs and packing signals. The cargo genes in these high-capacity adenoviral vectors (HCAds) can be up to 36 kb long. HCAd production needs a second helper virus (HV) with loxP sites added to flank the packing signal. HCAds are less immunogenic, have a larger cargo capacity, and take longer to transduce. However, removing the helper adenovirus from vector preparations may improve the efficacy and security of HCAd vectors in vivo.

III. ADVANCEMENT AND LIMITATIONS

The genetic disorder known as Friedreich's ataxia is caused by a GAA hyper expansion of intron 1 of the frataxin gene. This mutation suppresses FXN, a mitochondrial enzyme required for iron metabolism and homeostasis, resulting in neurodegeneration and cardiac failure. Clinical studies have demonstrated that current therapies, which emphasise boosting frataxin expression and mitochondrial activity, are unsuccessful at halting neurodegeneration.

Recent studies on in vivo and ex vivo gene therapy methods indicate potential as a one-time therapy in FRDA animal and cell models. Through CRISPR/Cas9 gene editing of the FXN gene, it is possible to restore native frataxin expression. Another potential therapeutic strategy for autologous transplantation is ex vivo gene editing in hematopoietic stem and progenitor cells. (Gonçalves & Paiva, 2017)

Limitation

The argument over genetically altering germlines has persisted for a while in science, with bioethics playing a significant role in determining the risks and ethical ramifications.

- Chinese scientists reported the first CRISPR-Cas9 genetic editing of embryonic cells in 2015 to introduce the CCR5 gene mutation to confer HIV resistance.
- 4 out of 26 embryos were effectively changed, according to the genetic study, demonstrating the need for procedure improvement.
- The controversy over genetic editing has been reignited by these recent papers, and the Japanese Ethics Committee has confirmed the validity of the study and egg donor consent.
- The first effort for altering a healthy human embryo was allowed in the United Kingdom, but American research organisations have remained cautious, repeating their opposition to this kind of experiment while waiting for advancements in technology and ethical considerations.(Gonçalves & Paiva, 2017)

IV. NON-VIRAL METHOD

Non-viral techniques provide security, substantial gene transfer, less toxicity, and simple preparation. For targeting that is unique to a tissue or cell, they can be altered with ligands. They do, however, have drawbacks, including ineffective transfection and subpar transgenic expression. The efficiency of gene delivery, which is mostly influenced by delivery vectors, systems, or transfer processes, is crucial to the success of gene therapy.

Non-viral gene delivery limits

Rapid delivery of nucleic acid materials to the target cell population, effective cell absorption, and transport into the proper cellular compartment are necessary for effective gene therapy. The successful delivery of genes by non-viral means is hampered by several factors, including the extracellular transport of DNA, which can be quickly destroyed by some nucleases in plasma after systemic administration. A rapid removal of DNA from the circulation can result from the reticuloendothelial system's (RES) detection and absorption of DNA complexes. Since DNA can be broken down by ECM nucleases, transporting DNA in the extracellular matrix (ECM) provides additional challenges.

The process of delivering genes via non-viral techniques is likewise constrained by intracellular obstacles. Passing through the negatively charged cell membrane is one of the most difficult procedures for effective gene transfer. However, there are physical and chemical ways to make it easier for DNA to enter the cytoplasm. Physical techniques like the gene gun, electroporation, and sonoporation enable the temporary development of pores on the cell membrane, allowing DNA to freely enter. Gene vectors like cationic lipids or cationic polymers are used to chemically package and compress DNA, generating complexes that are quickly ingested by cells by endocytosis.

If DNA escapes the endosomes before they develop, it will eventually be digested by lysosomal hydrolytic enzymes. After DNA uptake, endosomes containing DNA will change into digesting lysosomes. The endosome membrane can be damaged by the use of pH-responsive amphipathic peptides or lipid components, and cationic polymers like

polyethyleneimine (PEI) can cause the "proton sponge effect."Non-viral techniques provide security, substantial gene transfer, less toxicity, and simple preparation. For targeting that is unique to a tissue or cell, they can be altered with ligands. They do, however, have drawbacks, including ineffective transfection and subpar transgenic expression. The efficiency of gene delivery, which is mostly influenced by delivery vectors, systems, or transfer processes, is crucial to the success of gene therapy.

Another important barrier to DNA entrance into the nucleus is the nuclear envelope. Large macromolecules cannot freely enter the nucleus due to the NPC's modest diameter (9nm), which only permits the unimpeded passage of small or medium-sized molecules. Significantly better nuclear entrance may be the cause of the increased transfection efficiency in gene carriers modified with NLS. Plasmid DNA alterations, such as the addition of SV40 sequences, can also improve transfection effectiveness.

Cells in the S and G2/M phases have much higher transfection efficiency than those in the G1 phase, indicating that the cell cycle has a considerable impact on the nucleus entrance of DNA. Given that tumour cells divide more quickly than normal cells, this non-viral gene delivery property that depends on the cell cycle may have applications in the treatment of cancer.

V. EMERGING DELIVERY METHODS

The advantages of relative safety, the capacity to transmit large-sized genes, reduced toxicity, ease of preparation, etc. make non-viral techniques very promising. Non-viral techniques still have several drawbacks, such as poor transgene expression and limited transfection efficiency, which limit their practical applicability. A number of gene carriers and processes have developed as a result of the many initiatives that are being made over the past decade to improve the effectiveness of gene transfer.

1. **Microinjectors:** Nucleic acid is mechanically injected into living cells using a micropipette through a procedure called microinjection. It is a straightforward, affordable, successful, repeatable, and non-toxic approach to delivering genes. However, it may have poor transgenic expression levels and persistence and necessitates manual programming of every cell. In immunisation methods, when transgene expression is strong, microinjection has a lot of potential. Naked DNA can be made more active physically or chemically, but it has poor cellular entrance and low gene expression. (Barber, 1911)

2. **Needle Injection:** Muscle, liver, skin, brain, and tumours have all been given direct local injections of bare DNA. This method is straightforward, secure, and appealing for clinical uses such as cancer gene therapy and heart function enhancement. However, it has drawbacks like low gene expression and quick blood nuclease breakdown. To get around these problems, a high-pressure-mediated technique called hydrodynamic gene transfer has been developed.(Losordo et al., 2002)

3. **Jet Injection:** Since 1947, the needle-free medication delivery technique known as jet injection has used highly pressurised gas to create a fast, ultrafine stream that can enter target cells' DNA. This approach is ideal for a variety of tissues, including muscle, skin, fat, and breasts, thanks to its better gene transfer efficiency and flexibility in gas pressure.

Jet injection, except for local hyperemia, oedema, and minimal bleeding, has demonstrated positive outcomes in the inhibition of malignancy.(Lysakowski et al., 2003)

4. **Gene Gun:** The use of gene gun technology to introduce genes into different tissues and tumours was originally intended for plant transformation. Fired at target cells or tissues are heavy metal particles coated with plasmid DNA, facilitating penetration and DNA release. The effectiveness of gene transfer and tissue harm is influenced by variables such as particle size, speed, and dose. Although straightforward and secure, temporary transgenic expression restricts its application, particularly in rapidly dividing cells. To enhance microcarrier preparation, speed up the procedure, and lessen tissue injury, changes have been implemented. (Gao et al., 2007)

5. **Electroporation:** Negatively-charged DNA can enter cells using the electroporation technique, which uses high-voltage electrical currents to briefly produce tiny nanometric pores on the cell membrane. *In -vitro* and *in-vivo* gene delivery were the first applications for it in 1982 and 1991, respectively. Nearly all examined tissues, including muscle, liver, lung, skin, and various tumours, are amenable to in vivo electroporation. Sakai M. et al. recently reported a localised gene transfer by electroporation.They discovered that the electroporated lobe's hepatocytes were the only ones to efficiently express the transgene. This discovery made it possible to provide DNA to a specific tissue via blood circulation and electroporation for localised gene delivery. Interleukin (IL)-12 was electroporated into the tumours of patients with metastatic melanoma in the first documented human experiment of gene transfer. The outcomes demonstrated that electroporation-mediated gene delivery is a reliable, titrable, safe method. Electrical current strength, the amount of time between discharges, DNA concentration, and DNA type are all variables that affect how effectively genes are transferred. The effectiveness of transfection can also be impacted by the recipient animals' age and the effectiveness of the injected plasmid DNA in the tissue.(Taniyama & Morishita, 2006)

6. **Sonoporation:** Ultrasonic waves are used in sonoporation, also known as ultrasound-facilitated gene transfer, to cause cell membrane permeabilization and enable intracellular gene entrance. Air-filled microbubble contrast agents can increase the effectiveness of gene transfer. Sonoporation is a passive diffusion process as opposed to electroporation, which is propelled by electric force. It provides security, non-intrusion, and the capacity to transfer genes without undergoing surgery. Local gene transfection can be accomplished by either altering contrast agents with site-specific ligands or co-administering DNA and contrast agents via blood circulation. Although sonoporation can improve the rupture of blood-brain barriers, it still has a significant flaw due to its limited efficiency. (Tomizawa, 2013)

7. **Hydrodynamic Gene Transfer:** High hydrostatic pressure is used in hydrodynamic gene transfer to transport genes to interior organs; the liver exhibits the highest levels of gene expression. DNA, RNA, proteins, or synthetic substances can be delivered safely, easily, and effectively into the tissues of tiny animals with this technique. The efficiency of delivery relies on the kind of organ, injection amount, rate, and concentration of the useful ingredient. Large injection volumes, however, present a problem for clinical use because people cannot tolerate quick injections of 8% body weight. Modifications have

been used to lessen liver damage in large animal pigs, such as catheter-based approaches. (Yoshino et al., 2006)

8. **Mechanical Massage:** To boost gene expression in the liver of mice, Liu F. et al.(Liu & Huang, 2002) reported adopting a mechanical massage technique for delivering genes. This technique damages the membranes of liver cells, allowing plasmid DNA to enter. According to the research, mechanical massage can improve survival by reducing endotoxin-induced deadly fulminant liver failure. No receptor engagement was seen during the process, which involved pressure-mediated effects.

Due to its safety, possibility for precise targeting, high gene transfer, toxicity, and affordability, gene therapy holds promise for the treatment of inherited and hereditary disorders. However, there are still only a few therapeutic applications. The main goals of the work are to increase transfection effectiveness, decrease toxicity, increase targeting specificity, and lessen tissue damage. To develop new strategies and enhance existing systems, ongoing work is required.

VI. GENE THERAPY TREATMENT

1. **ALS:** Due to the limited treatment targets and difficulty in reaching the brain and spinal cord, amyotrophic lateral sclerosis (ALS) poses special difficulties for gene-therapy-based techniques. But recent developments in CNS targeting, gene transport and gene editing methods have expanded the therapeutic window. Patients with SOD1 mutations, C9orf72 hexanucleotide repeat expansions, ATXN2 trinucleotide expansions, and FUS mutations, as well as those with sporadic disease without a known genetic basis, are undergoing clinical studies. The treatment environment for CNS illnesses like SMA may be permanently changed through gene therapy. It can enter the brain's safest areas through minimally invasive techniques, including the cerebral cortex and spinal cord. Truly effective treatments for ALS are anticipated to develop as delivery techniques, vector specificity, and cargo efficacy advance.(Amado & Davidson, 2021)

2. **Cancer:** Numerous cancer types, including gastrointestinal, lung, gynaecological, skin, and urological cancers, are the focus of the majority of gene therapy trials. Among the methods are gene-directed enzyme prodrugs, immunotherapy, oncolytic virotherapy, and tumour suppressor gene insertion. The p53 gene is the most frequently transmitted tumour suppressor gene, and some trials combine it with chemotherapy or radiation therapy. In oncolytic virotherapy, viruses are used to target and multiply in cancer cells, killing them through lysis. Gene-directed enzyme prodrug therapy, which focuses on the expression of the genes that encode the enzymes that transform prodrugs into lethal drugs, improves the effectiveness of a number of chemotherapies. (Ginn et al., 2013)

3. **Haemophilia**: Gene therapy offers the potential to treat individuals with haemophilia by establishing continuous endogenous factor VIII or factor IX (FIX) expression after the transfer of a functional gene to replace the haemophilia patient's defective gene. Because a small rise in blood factor levels in severely affected patients is associated with a significant reduction in the bleeding phenotype, haemophilias are particularly well-suited for gene therapy. In severe haemophilia B, a stable dose-dependent rise in FIX levels was shown by the St. Jude/UCL phase 1/2 experiment in 2011. AAV gene treatments are likely

to change the way haemophilia A and B are treated. The development of curative therapies will benefit from the availability of strong evidence for transgenic FVIII and FIX expression throughout the long term at therapeutic levels. (Nathwani, 2022)

4. **Epilepsy:** Gene therapy can cure complex forms of epilepsy with many vulnerable mutations and environmental effects, making it a possible alternative to conventional pharmacological treatments for the condition. Potential anti-epileptogenic, anticonvulsant, or disease-modifying effects have been demonstrated in preclinical animals. Choosing the ideal therapeutic target, meanwhile, is still difficult. It is anticipated that new strategies like optogenetics, chemogenetics, and genome-editing technologies will advance gene therapy. To assure effectiveness and prevent side effects, numerous solutions must first be tested in clinical settings for safety and tolerability.(L. Zhang & Wang, 2021)

5. **Monogenic Diseases:** By introducing functional genes into proliferating stem cells, gene therapy seeks to permanently cure monogenic disorders. 161 trials are focusing on hereditary monogenic illnesses, with cystic fibrosis, severe CCID syndromes, and chronic granulomatous disease accounting for 22.4% of the total. In treating certain disorders, gene therapy has demonstrated long-lasting and clinically significant therapeutic advantages. (Ginn et al., 2013)

VII. IMPACT OF GENE THERAPY

1. **Fomivirsen:** Isis Pharmaceuticals and Novartis Ophthalmics have come up with the antisense oligonucleotide (ASO) Vitravene, also known as Fomivirsen, to treat CMV retinitis in HIV-positive individuals. The FDA has approved its marketing as the first gene-silencing antisense therapy. CMV retinitis, which is linked to reduced CD4 counts and absolute CD4 lymphocyte levels in peripheral blood, affects 30% of HIV-positive patients. On days 1 and 15 of treatment, 330 mg intravitreal injections are indicated, followed by 330 mg every four weeks. The chemical has a half-life clearance in the human vitreous body of about 55 hours. Clinical studies show that Fomivirsen is effective in treating the signs and symptoms of CMV retinitis. The commercialization of these medications was stopped in Europe and the USA in 2002 and 2006, respectively, due to the development of highly active antiretroviral therapy (HAART), which significantly decreased the prevalence of CMV retinitis.

2. **Gendicine:** Gendicine (rAd-p53), a gene therapy drug, was developed by Shenzhen SiBiono GeneTech to treat head and neck squamous cell carcinoma (HNSCC). The recombinant adenovirus replaces the human serotype 5 adenovirus's E1 region with human wild-type Tp53, enhancing Tp53's anticancer properties by inducing apoptotic pathways, obstructing DNA repair, and seizing survival pathways. The drug is produced in HEK293 cells by co-transfection of an Ad5 genome recombinant plasmid and the Tp53 expression cassette shuttle vector. The most typical adverse effect of gendicine is a self-limiting fever that lasts for two to six hours. The drug has been examined in 16 human clinical studies for more severe types and stages of head and neck cancer, malignant glioma, ovarian cancer, and hepatocellular carcinoma. Treatment with Gendicine has resulted in a stronger overall response and higher survival rates when compared to control groups. Combining chemotherapy and radiation led to higher survival rates for nasopharyngeal cancer. (W. W. Zhang et al., 2018)

3. **Pegaptanib:** Pegaptanib, a 28-mer RNA oligonucleotide, has been approved by the FDA for the treatment of advanced instances of ovarian cancer, hepatocellular carcinoma, malignant glioma, and head and neck cancer. It is the first therapeutic RNA-structured aptamer that has been given FDA market approval. Due to the fact that it prevents VEGF165 from attaching to the heparin-binding site, pegaptanib is an effective treatment for AMD. In two clinical trials involving 1186 participants, pegaptanib shown effectiveness in reducing visual acuity from baseline without dosage response. However, because aflibercept, ranibizumab, and bevacizumab produced improved visual outcomes, Pegaptanib sales decreased. Despite having a small market share, pegaptanib is still an effective alternative for treating AMD. (Solomon et al., 2014)

4. **Oncorine:** The FDA has approved pegaptanib, a 28-mer RNA oligonucleotide, for the treatment of advanced ovarian, hepatocellular carcinoma, malignant glioma, and head and neck cancer. It is a newly developed clinical RNA-structured aptamer that has been given the go-ahead to be used commercially. Pegaptanib dramatically lowers visual acuity in clinical trials without a dose-response.

5. **Rexin-G:** Rexin-G is a retroviral vehicle with a cytocidal cyclin G1 design and the first injectable gene therapy vector approved by the FDA for metastatic pancreatic cancer. The drug has a hybrid LTR promoter and a gene for neomycin resistance. It is associated with neovasculature and promotes apoptosis and cell death in cancer cells. Phase I/II trials in metastatic pancreatic cancer that was gemcitabine-resistant showed good tolerability, safety, and an acceptable survival rate. Rexin-G has shown promise in treating metastatic solid tumours that are resistant to conventional chemotherapy, as seen by its continuous clinical progress.(Chawla et al., 2010)

6. **Neovasculgen:** The Human Stem Cell Institute of Russia created Neovasculgen (PI-VEGF165), a plasmid DNA producing VEGF 165, in 2010 to treat atherosclerotic peripheral artery disease (PAD). The drug was launched in Russia in 2012 and was given the EUVED classification by the Russian Ministry of Health. The angiogenic effector neovascular initiates angiogenesis, endothelial migration, and enhanced endothelial renewal. A phase 2b/3 multicenter clinical trial found that intramuscular injection of Neovasculgen improved the ankle-brachial index, pain-free walking distance, and blood flow velocity. The FDA has not yet assessed or validated the medicine because of the treatment's minimal disease penetrance.(Chawla et al., 2010)

7. **Zolgensma:** In order to treat young infants with bi-allelic SMN1 gene mutations, AveXis, a pharmaceutical company owned by Novartis, has developed a patented gene therapy procedure known as Zolgensma. The drug is a non-replicating recombinant AAV9 modified to contain a functional copy of the human SMN1 gene, which is expressed in SMA patients' motor neurons via the CMV enhancer/chicken-actin-hybrid promoter (CB). The unique AAV9 capsid makes intravenous CNS administration possible. Preliminary results from clinical trials on zolgensma show that it is effective in treating infantile-onset SMA patients with bi-allelic SMN1 gene mutations. However, it is currently unclear whether these insights have any practical application. The drug is the most expensive form of gene therapy currently on the market but is also the most cost-effective, costing more than $2.125 million for a single injection. (Dabbous et al., 2019)

VIII. HUMAN CELL-BASED GENE THERAPY

1. **ADA:** Adenosine deaminase (ADA) insufficiency, a hereditary disease that causes severe combination immune deficiency (SCID), affects 15% of the population. Gene therapy, enzyme replacement therapy, and hematopoietic stem cell transplantation (HSCT) are now employed as treatments. The gene therapy drug Strimvelis has been approved by the European Commission for the treatment of ADA-SCID. To support cellular and immunological immunity, autologous CD34+ cells must express the human ADA cDNA sequence. The treatment has shown positive results with increased T cell subtypes and immunoglobin synthesis. (Cicalese et al., 2018)

2. **Hematopoietic Stem-Cell Transplantation:** Allogeneic hematopoietic stem-cell transplantation (HSCT) continues to be the main treatment for high-risk haematological malignancies. However, haploidentical HSCT could be unsuccessful due to graft versus host disease (GVHD). To address this issue, MolMed genetically changes partially-matched donor T cells so that they express HSV-TK as an inducible suicide gene in order to generate the Zalmoxis product, which incorporates genetically altered allogeneic T cells. When given to HSCT patients, the genetically altered donor T cells can boost immunity and prevent infections. However, donor cells occasionally mistakenly assault host cells, leading to GVHD. Zalmoxis can boost immune system reactivation and reduce infection risk after HSCT. When matched donors are not available, it may provide HSCT patients with a chance for curative benefits that will help with post-transplant GvHD control, graft versus leukaemia improvement, recurrence reduction, and other factors. (Aiuti et al., 2017)

3. **Lymphoblastic Leukaemia:** In 2012, Novartis Pharmaceuticals began investigating chimeric antigen receptor T-cell (CAR) therapies to treat acute lymphoblastic leukaemia (ALL). Young children with relapsed B-cell ALL were treated with Kymriah (tisagenlecleucel), the first CAR T-cell-based gene therapy to be granted a licence. There are four generations of CAR T-cells, each with a special mix of cytokine-producing and anti-cancer abilities. Clinical success depends on the CAR T-cells' enduring durability and ability to generate target-specific memory cells, which stop cancer relapse. Using lentiviral delivery, autologous T cell suspension was genetically altered to produce Kymriah, a CAR containing a murine single-chain antibody fragment that is specific for CD19. The dosage should be infused and depend on body weight after lymphodepleting chemotherapy. According to a phase II trial, 81% of patients with recurrent B-cell ALL experienced overall remission, with remission rates of 90% after six months of treatment and 76% after a year. (Terwilliger & Abdul-Hay, 2017)

4. **Non-Hodgkin Lymphoma:** For patients with advanced non-Hodgkin lymphoma who have tried several ineffective systemic treatments, Yeskarta is a CAR T-cell therapy. The FDA granted approval for it in 2017. As part of the therapy, CD19-directed ex vivo modulated autologous T cells are transfected with gamma-retroviral vectors. In a phase II trial, it had an objective response rate of 82%, a complete response rate of 54%, and an overall survival rate of 52%. Although it is not meant for the treatment of primary CNS lymphoma.(Neelapu et al., 2017)

5. **Osteoarthritis:** Phase III studies for Invossa, a cell-mediated gene therapy approach for treating knee osteoarthritis, have been completed in the USA and Korea. It has a 3:1 ratio

of allogenic chondrocytes that are not converted and those that have been retrovirally transduced and upregulated for TGF-1. Invossa considerably reduces knee OA, discomfort, sports activities, and quality of life, according to studies. Post-marketing surveillance is now being used to assess the drug's efficacy and safety. (Shahryari et al., 2019)

IX. FUTURE DIRECTION OF GENE THERAPY

The introduction of the first approved therapies in the past five years, gene and cell therapy has experienced a great resurgence after decades of effort. Cancer, genetic blindness, and neuromuscular disease are only a few of the therapeutic indications and tissue targets that these medicines target. The success of Luxturna and Zolgensma, using in vivo AAV gene transfer to treat Leber's congenital amaurosis and spinal muscular atrophy, respectively, has spurred the development of AAV-based therapies for delivering genes to the liver and skeletal muscle to address haemophilia and Duchenne muscular dystrophy. Moreover, this method has been extended to modify hematopoietic stem cells, enabling therapies for inherited disorders like beta-thalassemia and sickle cell disease. These treatments have recently gained approval in the European Union and are now being reviewed in the United States. These advances in gene therapy are akin to the early technology development in ex vivo lentiviral and retroviral gene transfer to T cells, which ultimately led to the development of adoptive cell immune therapy.

Due to upcoming technologies, these drugs are significantly more successful at managing human diseases. Wider adoption continues to be significantly hampered by the immune response to alien transgenes and their products. The control of the immune system will be the subject of some of the most important research in the near future. For instance, up to 50% of patients are currently unable to get treatment because to immunity against viral capsids. Recent technological advancements have made it possible to go through this immunological barrier, and clinical trials are ongoing. As examples, approaches for temporarily eliminating antibodies from circulation and the development of modified AAV capsids that circumvent pre-existing neutralising antibodies are shown. Immunosuppression protocols might also provide a technique to avoid pre-existing immunity and stop adaptive immunity to the vector, allowing for re-dosing in the not-so-distant future if needed.

Besides the initial generation of gene therapy, which mostly concentrated on the delivery of transgenes, genetic modification technologies are offering a whole new modality for treatments based on precise modifications in human genome sequences. Only recently have clinical research using CRISPR-based modification of genes for sickle cell disorder and beta-thalassemia demonstrated the first instance of condition-modifying efficacy. In 2010, the first gene-editing drugs went through clinical trials as a means of protecting T cells from HIV infection. With a positive safety record for gene-edited T cells and HSCs in human trials, this novel achievement has prepared the path for the results of ongoing and planned clinical investigations of in vivo genome editing, which are highly anticipated.

The Somatic Cell Genome Modifying Consortium aims to propel in vivo genome editing for addressing human health challenges in diverse tissues and disease contexts. Focusing on delivery, safety, and system modeling, the group has secured $190 million in funding from the National Institutes of Health for a six-year duration. Undoubtedly, the

Consortium's efforts will greatly accelerate the development of gene editing therapies over the next decade and beyond.

Functional genomics and our comprehension of human genome regulation are rapidly evolving fields that will significantly influence gene therapy. For example, out of the 20,000 human genes, approximately 6,000 have functions that remain undiscovered, but CRISPR technologies offer the potential to unravel the functionality of these gene sequences. Despite 98% of our genome being non-coding DNA and epigenetic regulators contributing to more than 90% of susceptibility to common diseases, scientific research and treatment strategies have traditionally concentrated mainly on genes. Embracing advancements in functional genomics will open new avenues for understanding and harnessing the full potential of gene therapies.(Bulaklak & Gersbach, 2020)

X. CONCLUSION

The ability to safely and quickly advance potential therapies for patient benefit is being outpaced by the rapid technological advancement in gene and cell therapy. Present regulatory frameworks lack provisions for curative medicines targeting mutations specific to individual patients or limited groups. A feasible solution is to devise a universal formula capable of benefiting a broader patient population. Universal cell treatments, created through gene editing to produce immunologically stealth allogeneic donor cells evading detection by the host immune system, hold promise for regenerative therapy and adoptive cell immunotherapy. Clinical trials exploring these treatments are currently underway, and their outcomes will significantly impact the future advancement of gene and cell therapies. This approach, however, ignores the potential and active research being done to develop revolutionary innovations like base altering and prime modifying, as well as in vivo genetic mutation correction. A potential example and source of inspiration for these efforts is the effective therapy of a patient with human Battendis disease using an oligonucleotide-based medication that targets a specific genetic mutation. As we go towards personalized therapies and must adjust to the unique challenges presented by these cutting-edge technologies, regulatory sciences will soon experience one of the biggest upheavals in the discipline of cell and gene therapy.

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