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GENE THERAPY: A BOON TO MEDICAL SCIENCE

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ABSTRACT

The human genome contains ~25,000 genes that encode a wide variety of proteins called the building blocks of the cell to drive every biological process necessary for life. Although a significant advancement has been made in developing modern medicine, including chemotherapy, radiation, and surgery, results into alter the body's chemistry and create dependency over time, and offer only temporary relief by reducing disease symptoms. These issues are partly addressed by developing gene therapy. Human gene therapy is defined as the introduction of new genetic material directly into the cells of an individual to produce a therapeutic benefit. Mainly the treatment of disease is done by replacing, altering, or supplementing a gene that is absent or abnormal and whose absence or abnormality is responsible for a disease. The human genome has been an objective in the medicinal field since the recognition of the gene as the basic unit of heredity. It has gained tremendous success by understanding the capacity for gene improvement employing the correction of altered (mutated) genes or site-specific modifications that have treatment as a target. It holds considerable potential for the treatment of both hereditary genetic disorders and infectious diseases. Gene therapy has become an alternative treatment for a wide range of infectious diseases. The broad field of gene therapy promises several innovative treatments that are likely to become important in preventing diseases such as fetal abnormalities, diabetes, and cancer. Till now, Twenty gene therapy products have already been approved and over two thousand human gene therapy, clinical trials have been reported worldwide. These advances raise great hope to treat rare and inherited diseases.

KEYWORDS: gene therapy, genetic disorders, retinal, fetal.

INTRODUCTION

Currently, gene therapy is an area that exists predominantly in research, and its application is still experimental.[1] These are the products that "Initiates their effects by transcription or translation of transferred genetic material and by integrating it into the host genome.[2] Gene therapy offers the potential of a one-time cure for inherited disorders, for which current therapeutic approaches are ineffective. The approach is broad, with the potential treatment of diseases caused by recessive gene disorders, such as cystic fibrosis, hemophilia, muscular dystrophy, and sickle cell anaemia, acquired genetic diseases such as cancer, and certain viral infections, such as AIDS.[3,4] Approaches to gene therapy for infectious diseases can be divided into three broad categories: (i) gene therapies based on nucleic acid moieties, including antisense DNA and RNA; (ii) protein approaches such as trans-dominant negative proteins (TNPs) and single-chain antibodies; and (iii) immunotherapeutic approaches involving genetic vaccines.[5] Recent advances in the fields of genetics, molecular biology, clinical medicine, and human genomics have led to the development of the new stream of gene therapy. It delivers genetic material to the cell to generate a therapeutic effect by correcting an existing abnormality or providing cells with a new function. Applications of viral vectors have found an encouraging new beginning in gene therapy in recent years.[6] Direct in vivo administration of manipulated viral vehicles for gene delivery and ex vivo genetically engineered stem cells are the two principal approaches in advanced clinical gene therapy.[7] Nowadays researchers gain tremendous success in retinal and fetal gene therapy. Retinal gene therapy is Use to restore visual perception to the blind retina during the terminal stage of the degeneration and fetal gene therapy use to treat genetic abnormalities in the womb itself, Hence it has become a boon to medical science.[8]

HISTORY

Genetic-science studies initiated in the early 1850s, by Austrian monk and Gregor Mendel, in a series of experiments with green peas, described the inheritance pattern by observing the traces that were inherited as separate units, which we know today as genes.[9] In 1970, researchers discovered a series of enzymes that initiate the separation of the genes in predetermined sites along the DNA molecule and their reinsertion in a reproducible manner. These genetic advances prepared the scenario for the emergence of genetic engineering with the production of new drugs and antibodies, and as of 1980, gene therapy has been utilized by scientists.[10] clinical investigation of gene therapy began in 1990, when the first clinical study, for a rare immunodeficiency disorder, was undertaken at the US National Institutes of

Health. Since then, more than 2,500 clinical studies have been initiated for a broad range of applications, from a variety of monogenic diseases such as infectious diseases, complex neurodegenerative disorders, and cancer. The bar graph classifies clinical gene transfer studies by different diseases. (Figure. 1).[11]

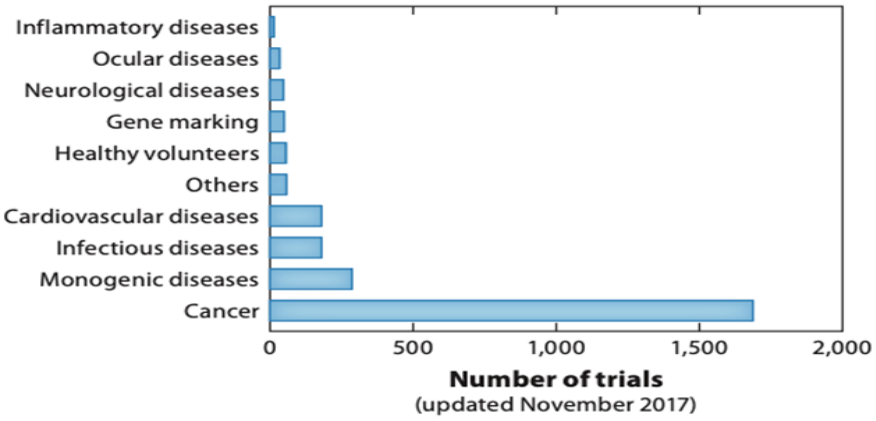


Fig 1: Number of trials of gene transfer study for different diseases.

Gene therapy continues to develop in this current decade. In 2012, the European agency approved its first gene therapy treatment. The European Medicines Agency (EMA) approved Glybera, a viral treatment for pancreatitis which is caused by the absence of a gene called lipoprotein lipase. In 2016, another European approval for a gene therapy called Strimvelis developed by GlaxoSmithKline. It is a stem cell gene therapy to treat ADA-SCID (Adenosine deaminase- severe combined immunodeficiency), patients. These cell and gene therapies are revolutionizing medicine and expected to gain tremendous success in the next few years.[12]

TYPES OF GENE THERAPY

1. Germline gene therapy

Germline therapy uses only sperm and egg cells for genetic modification so that the changes will be passed down to future generations.[13] This technique involves the incorporation of functional genes, which are integrated into their genomes. This would allow the therapy to be heritable. The therapy is highly efficient in counteracting genetic disease and hereditary disorders.[14] When germ cells or gamete are modified by the insertion of functional gene it is known as germline gene therapy. The disadvantage of this technique is that it raises alot of ethical questions because it impacts the inheritance patterns of humans. Also, high frequency of mutations is observed in this technique which can cause teratogenic consequences.[15]

2. Somatic gene therapy

The technique of somatic gene therapy involves incorporating a normal gene into the somatic cells (cells that do not produce the eggs and sperm that in turn produce the next generation) of an individual with a genetic disease, thereby permanently correcting the disorder.[16] The therapy is based on the fact that therapeutic genes are conveyed into the somatic cells.[17] This method of gene therapy includes the exclusion of some of the dysfunctional cells and introducing them with a cloned gene. The transgenic cells are then implanted into the patient's body where they perform the corrected gene function.[13] The effects of this therapy are limited to the targeted cells and are not heritable. Somatic gene therapy can be performed ex vivo and in vivo. As shown in Figure. 2, two types of somatic cell gene therapy are possible i.e. ex vivo and in vivo. In the ex vivo method, the cells with the genetic defect are Isolate from a patient and Introduce the therapeutic gene to correct gene defect. Then, the treated cells are returned to the patient via the circulation.[18] In vivo therapy differs from the ex vivo as the normal gene is introduced directly into the patient generally into the tissue requiring treatment (such as muscle, liver, or brain). Gene delivery can be carried out by viral or non- viral vectors.[19] The success of in vivo gene therapy mostly depends on the following parameters: i. The efficiency of the uptake of the remedial (therapeutic) gene by the target cells, ii. Intracellular degradation of the gene and its uptake by nucleus, iii. The expression capability of the gene. In both types, the therapeutic gene must be incorporated into a vector, usually a non-pathogenic virus, and propagated to obtain a sufficient number of new gene copies.[18,20]

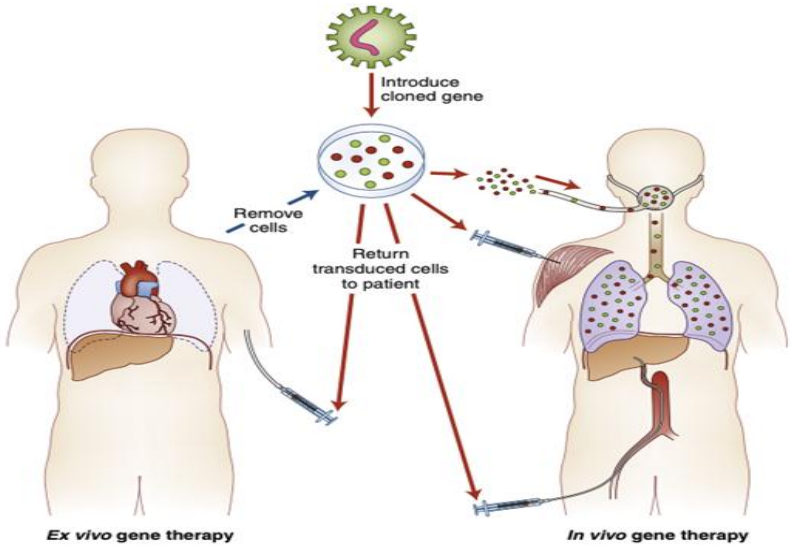


Fig. 2: Somatic gene therapy- Ex-vivo and In-vivo[21]

APPROACHES TO GENE THERAPY

1. Gene augmentation

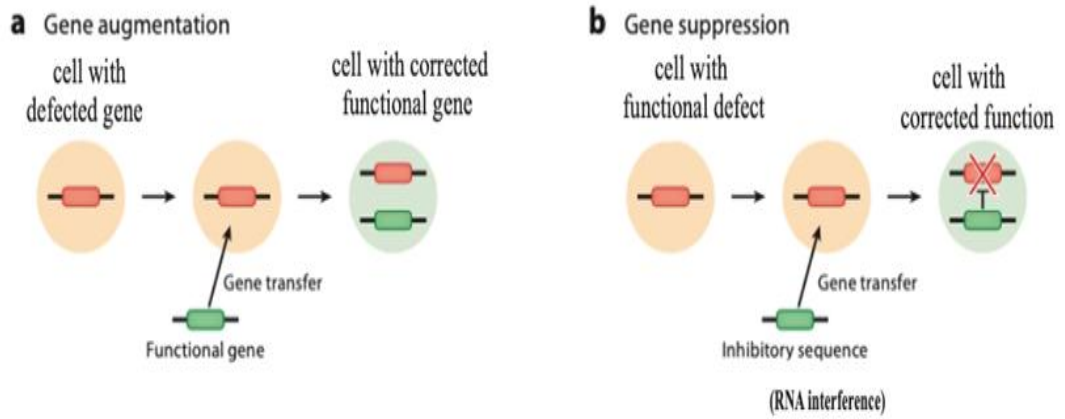
The goal of gene augmentation is to restore normal cellular function by providing a functional copy of a gene. A procedure for correcting metabolic deficiencies caused by a missing or defective gene by a healthy gene produces the necessary product without actually substituting that gene for the absent gene in the DNA.[22] gene augmentation therapy, as has been demonstrated for several visual impairments. A notable example of gene augmentation strategy is voretigene, the first gene therapy to receive U.S.FDA approval.[11]

2. Gene suppression

It involves blocking the expression of harmful genes and also restoring cellular fitness by reducing the expression of the mutated gene via RNA interference. It is mainly employed for treatment of Huntington's disease was the cellular function is lost as a result of toxic accumulation of a defective protein.[23] Gene suppression aim is to restore cellular fitness by reducing the expression of the mutated gene. To restore normal cellular protein, it may be coupled with gene augmentation to replace the lost gene function. The term "gene silencing" has also been used to describe these method.[24]

3. Gene Editing

It makes the use of a DNA repair mechanism. the gene-specific editing in mammalian cells is typically done by the induction of a DNA double-strand break at the target site and further replaced with a sequence of a functional protein.[25] Target cellular DNA is modified to correct specific mutations. CRISPER system is the current example of a gene-editing system.[26] Explained in Figure. 3.



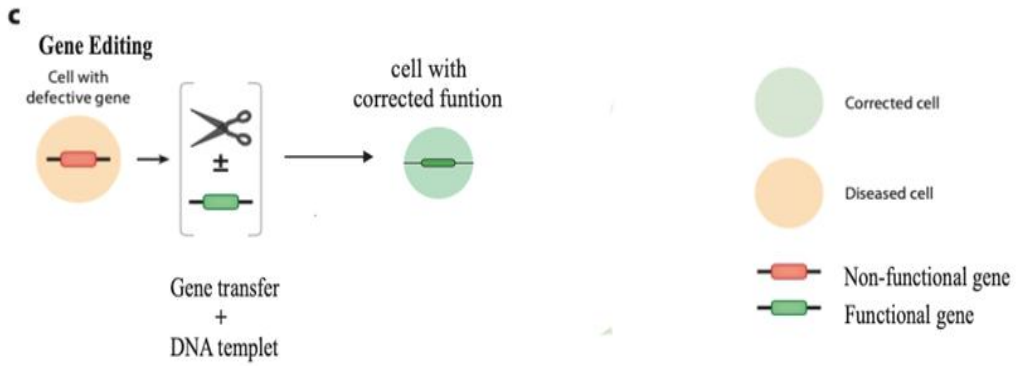


Fig 3: Various approaches of gene therapy.

DELIVERY SYSTEMS FOR GENE THERAPY

Although a systemic intravenous route can be applied to deliver the genetic material to the cells, local delivery methods are more commonly used for gene delivery. Viral vectors are one of them, this method works well for localized conditions but it cannot be used for treating systemic disorders. The most important method of gene delivery is in vivo.[27] In this method, viral, or non-viral vectors are used to deliver the therapeutic material to the defective target cells or tissue in the body. A wide variety of physical and chemical methods including needles, gene guns, electroporation, sonoporation, are being used to deliver genetic material to target cells.[28–30] However, none of them is more efficient than viral vectors in delivering therapeutic genes to the target cells due to their inherent shortcomings and operational complexity. The various methods are enlisted in Table no.2.with its therapeutic application.[31–34]

Table 1: Methods of gene delivery.

Method	Name	Entry mechanism	Therapeutic indication
Viral Methods	Adenovirus (AV)	Receptor mediated endocytosis	pancreatic neuroendocrine tumour, cancer
	Adeno-Associated Virus (AAV)	Endosomal escape and microtubule transport.	Haemophilia A and Haemophilia B, Familial hypercholesterolaemia, Retinal gene therapy.
	Herpes Simplex Virus (HSV)	Receptor binding with conformational changes.	Neuronal Disorders, Foetal gene therapy.
	Retrovirus (RV)	Membrane fusion, Internalization.	Cancers and Monogenic diseases
Non-Viral Methods	Ormasil	Silica based nanoparticles	Alzheimer's disease, Huntington disease
	Injection of naked DNA	cellular uptake	Intrinsic muscle *disorders*
Physical methods	Electroporation	Use of high-voltage short pulses	Breast cancer, Prostate cancer and Melanoma
	Gene Gun	particle bombardment	Neurodegenerative diseases

APPLICATIONS OF GENE THERAPY

1. Retinal gene therapy

Retinal degenerative diseases are major causes of blindness worldwide. These diseases are characterized by the progressive loss of several cell types of the retina, resulting in irreversible vision loss.[35] The neuroretina is a light-sensitive membrane present in the back of the eye, with specialized sensory properties to enable the capture of light by photoreceptors. Its function is to convert the light signal into electrical signals through a phototransduction process, and further integration and processing of the electric impulses into an image at the central nervous system. The light-sensing cells which form the outer retina are categorized into cones and rods based on their ability.[36] Eye possess a favourable natomical and immunological characteristics, hence it has been at the forefront of translational gene therapy for inherited retinal dystrophies (IRDs), which are progressive degenerative diseases caused by a lack of normal proteins encoded by specific genes involved in retinal cellular structures, phototransduction and the visual cycle.[37] The administration route is in fact a major determinant of the specificity and efficacy of retinal gene delivery. The two most common modes of administration have been utilized: intravitreal and subretinal injections. Represented in Figure.4.[38] Subretinal injections are more invasive and reach the Retinal pigment epithelium and photoreceptor area. Intravitreal injections are easier to administer and are considered better for targeting the inner retina, which is adjacent to the vitreous cavity.[39] The subretinal space is relatively immune-privileged thus the exposure to foreign antigens, including viral vectors used for gene therapy, is generally well tolerated and does not elicit potentially damaging immunologic reactions.[40,41]

The first gene therapy vector found to target the retina was derived from adenovirus (Ad).In recent years, a plethora of viral and non-viral vectors have been evaluated for their transduction efficacy in the cells most affected by retinal degeneration, namely, RPE and photoreceptor cells.[42] Recombinant virus vectors that are replication-deficient, including adenovirus, lentivirus, and adeno-associated virus (AAV), have been widely used as vehicles to deliver the normal gene into diseased retinas and supplement the functional protein in targeted cells.[43] RP is the most common IRD, occurring approximately in 1 out of 3500 births. It is characterized by progressive loss of photoreceptor function and structural integrity in patients who complain of night blindness and constriction of the visual field.[44] Intraocular administration of the normal PDE6B- phosphodiesterase (*PDE*) gene in a mouse model preserved retinal functions and prevented photoreceptor degeneration(37). X-linked

retinoschisis (XLRS) is the most prevalent juvenile vitreoretinal degeneration in males and is caused by mutations in an extracellular adhesion protein, retinoschisin (RS1), normally expressed in the retina by photoreceptor, bipolar, and ganglion cells.[45] On December 2017, the U.S. F. D.A. approved new gene therapy, voretigene (Luxturna). Luxturna is the first gene therapy approved that can be directly administered into the eye, targeting diseases caused by mutations in the gene RPE65. Mutations in this gene can produce Leber's congenital amaurosis or retinitis pigmentosa, both rare but potentially blinding diseases. The RPE65 gene provides genetic instructions for making an enzyme that converts light to an electrical signal in the retina; mutations in the gene cause reduced enzyme activity, resulting in impaired vision. Luxturna uses a naturally occurring adeno-associated virus, modified using recombinant DNA techniques, to deliver a healthy RPE65 gene to the retinal cells via a subretinal injection to restore vision.[46]

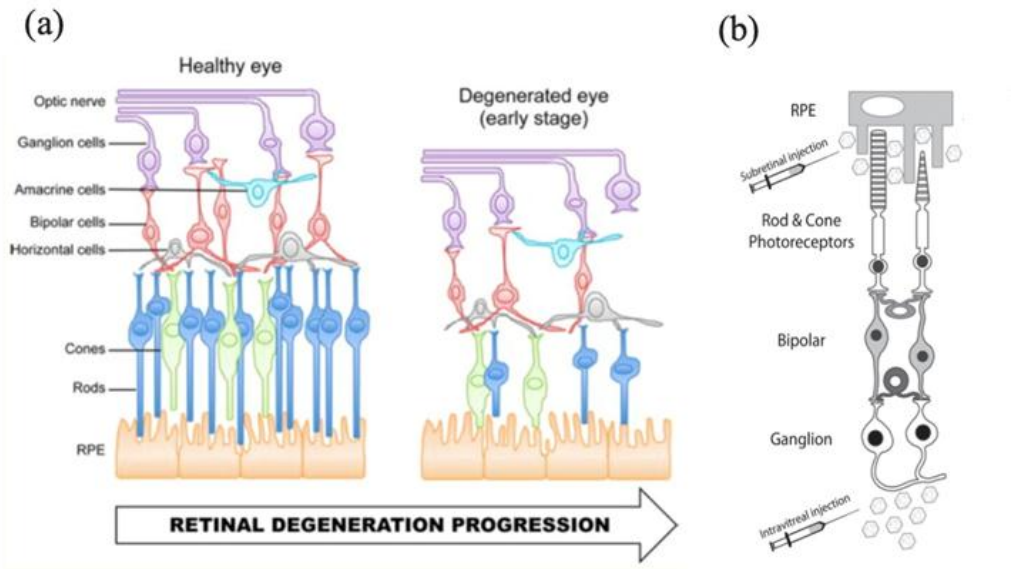


Fig. 4 a) Retinal Degeneration, b) Schematic representation of retinal delivery routes.

2. Fetal gene therapy

In addition to the clinical advantages of performing gene therapy prior to birth, numerous aspects of the fetus make it a more suitable gene therapy recipient than the adult, It has the ability to integrate into the genome of the host cell.[47] The fetal gene therapy can also be termed as prenatal or in utero therapy.[48] In utero gene therapy offers several advantages in the treatment of genetic disorders such as a large amount of somatic stem cells readily available for gene transfer in the fetus and The permanent replacement of a gene into a somatic stem cell would ensure that daughter cells would also carry the gene, obviating the

need of repeated therapy during the lifetime of the affected individual.[49] Not only are the hematopoietic stem cells present in greater quantities in the fetus, but, at the appropriate gestational age, stem cells of epithelial origin are present in organs such as the intestine and the lung. Stem cells in developing organs express high levels of integrins, cell surface proteins that promote the attachment and uptake of the viral vectors. Therefore, the availability of target cells and the efficiency of gene transfer into them are improved in the fetus.[50] Another advantage of in utero gene therapy is that it greatly reduced the inflammatory response of the fetus. Because the fetus is an immune-privileged site, the immune reactions against the vector and the transgene protein are diminished.

Mainly two types of administration routes can be employed in fetal gene transfer i.e. through the amniotic fluid and via direct systemic delivery. because, Both the amniotic fluid and the fetal circulation are readily available for fetus.[51,52] Systemic delivery through the fetal circulation Injection into the umbilical vein can be used for delivery to the fetal circulation. Needle embryo-fetoscopy in combination with ultrasound guidance is used.[53] Amniotic fluid (AF) is a complex mixture of high and low molecular weight components which may have adverse effects on the transfection efficiency of the various vector systems.[54] A major limitation of intra-amniotic delivery is its dilution by the relatively large volume of the AF. In addition, without specific organ targeting a large portion of the vector may transfect the fetal skin and the amniotic membranes. Since the AF is swallowed by the fetus, reabsorbed by the gastrointestinal tract, and then excreted through the kidneys.[55] Following in utero gene transfer, multiple tissues of the developing fetus were transduced and transgene expression persisted long-term, suggesting that this approach may one day be a viable therapeutic option for diseases affecting any of the major organ systems.[56]

CURRENTLY APPROVED THERAPIES

Currently approved gene therapies are enlisted in Table no.2 with there therapeutic indication and target site.

Table 2: Currently approved gene therapies with their therapeutic indications.

Name (Brand Name)	Developer	Therapeutic Indication	Target Site	Approval
Alipogene Tiparvovec (Glybera)	Uniqure	Lipoprotein Lipase Deficiency	Liver And Pancreas	2012
Tisagenlecleucel (Kymriah)	Novartis	β-Cell Acute Lymphoblastic Leukaemia	B-Cells And T-Cells	2017
Axicabtagene Ciloleucel (Yescarta)	Kite Pharma	Non-Hodgkin Lymphoma	T- cells	2017
Patisiran (Onpattro)	Alnylam Pharmaceuticals	Familial Amyloid Polyneuropathy (FAP)	Autonomic Nervous System	2018
Zolgensma (Onasemnogene)	Novartis	Spinal Muscle Atrophy	Skeletal Muscle	2019

CONCLUSION

Gene therapy is a rapidly growing field, and it seems that scientists have only scratched the surface of its potential. The more that is discovered about gene therapy is how to optimize gene delivery vectors, and delivering a wide- scale of therapeutics. Significant progress toward the development of genetic therapies against several infectious diseases has recently been made, Fetal therapy is one of them. In the future, there is the promise of applying these techniques in several fields of Medicine and pharmaceutics. The future of gene therapy now moves towards developing safer and more efficient vectors, combining multiple existing strategies such as viral vectors with genetic engineering technologies, and personalizing the characteristics of gene therapy for the treatment of life-threatening disorders.

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