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Can we really cure Genetic Diseases

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Abstract : Advances in biochemistry, molecular biology and biotechnology have paved a new way to understand the genetic basis of inherited diseases. It always remained a dream of researchers to replace the defective genes with good ones and cure the genetic disorders. Gene defects result in failure to synthesize a functional protein or in the synthesis of a dysfunctional protein. Gene therapy is defined as the insertion of functional genes into target cells to replace or supplement defective genes so as to achieve the therapeutic goals. The newly introduced genes will encode proteins and correct the deficiencies that occur in genetic diseases. Apart from inherited genetic disorders with single nucleotide polymorphism (SNP),the major thrust area of gene therapy are a number of acquired diseases such as malignancies, immunological disorders , including AIDS, cardiovascular neurological and infective diseases in many of which even short-term expression of the introduced gene could be therapeutic. Safe methods have been devised to do this, using several viral and non-viral vectors. Two main approaches emerged: in-vivo gene therapy and ex-vivo gene therapy. The important vectors employed in ex-vivo modification are Viruses (Retrovirus, Adenovirus, Adeno-associated virus, Herpes Simplex Virus), Human Artificial Chromosome (HAC), Bone Marrow Cells etc. The major concern with viral gene delivery is immunogenicity. The residual viral elements can be immunogenic, cytopathic, and/or recombinogenic. Non-viral vectors are far less efficient than viral vectors in terms of transfection efficiency, but they have advantages due to their low immunogenicity and their large capacity for therapeutic DNA. Gene transfer protocols have been approved for human use in inherited diseases, cancers and acquired disorders. In 1990, the first successful clinical trial of gene therapy was initiated for adenosine deaminase deficiency. Since then, the number of clinical protocols initiated worldwide has increased exponentially. Although preliminary results of these trials are somewhat disappointing, but human gene therapy dreams of treating diseases by replacing or supplementing the product of defective or introducing novel therapeutic genes. Unlike conventional treatments, most of which provide symptomatic alleviation, gene therapy eliminates genetic defects that cause a variety of diseases including genetic disorders, cancers and cardiovascular diseases, that is, gene therapy promises “cure” in a sense.
Key Words : Gene therapy, viral and non-viral vectors, in-vivo and ex-vivo gene therapy, Human artificial chromosome(HAC), Immunogenicity, Transfection efficiency.

Introduction:

A **gene** is the molecular unit of heredity of a living organism. It is used extensively by the scientific community as a name given to some stretches of deoxyribonucleic acids (DNA) and ribonucleic acids (RNA)

that code for a polypeptide or for an RNA chain that has a function in the organism. Living beings depend on genes, as they specify all proteins and functional RNA chains. Genes hold the information to build and maintain an organism's cells and pass genetic traits to offspring.

A modern working definition of a gene is "*a locatable region of genomic sequence, corresponding to a unit of inheritance, which is associated with regulatory regions, transcribed regions, and/or other functional sequence regions* ".[1] [2]

Gene therapy could be a way to fix a genetic problem at its source. By adding a corrected copy of a defective gene, gene therapy promises to help diseased tissues and organs work properly. This approach is different from traditional drug-based approaches, which may treat symptoms but not the underlying genetic problems. Gene therapy replaces a faulty gene or adds a new gene in an attempt to cure disease or improve our body's ability to fight disease. Gene therapy holds promise for treating a wide range of diseases, including cancer, cystic fibrosis, heart disease, diabetes, hemophilia and AIDS.

Most commonly, gene therapy uses a vector, typically a virus, to deliver a gene to the cells where it's needed. Once it is inside, the cell's gene-reading machinery uses the information in the gene to build RNA and protein molecules. The proteins (or RNA) can then carry out their job in the cells.

But gene therapy is not a molecular bandage that will automatically fix any genetic problem. While many disorders or medical conditions can potentially be treated using gene therapy, others are not suitable for this approach.

Gene therapy (use of genes as medicines) is basically to correct defective genes responsible for genetic disorder by one of the following approaches:-[3],[4]

- A normal gene could be inserted into a nonspecific location within the genome to replace the Nonfunctional gene (most common).
- An abnormal gene could be swapped for a normal gene homologous recombination.
- An abnormal gene could be repaired through selective reverse mutation.
- Regulation (degree to which a gene is turned on or off) of a particular gene could be altered.

Majority of the gene therapy trials are being conducted in United States and Europe, with only a modest number in other countries including Australia. Scope of this approach is broad with potential in treatment of diseases caused by single gene recessive disorders (like cystic fibrosis, hemophilia, muscular dystrophy, sickle cell anemia etc), acquired genetic diseases such as cancer and certain viral infections like AIDS.[5],[6]

Gene therapy: Historical perspectives.

Since the earliest days of plant and animal domestication, about 10,000 years ago, humans have understood that characteristics traits of parents could be transmitted to their offspring. The first to speculate about how this process worked were ancient Greek scholars, and some of their theories remained in favor for several centuries. The scientific study of genetics began in 1850s, when Austrian monk, Gregor John Mendel, in a series of experiments with green peas, described the pattern of inheritance, observing that traits were inherited as separate units, we know as genes. Mendel's work formed the foundation for later scientific achievements that heralded the era of modern genetics. But little was known about the physical nature of genes until 1950s, when American biochemist James Watson and British biophysicist Francis Crick developed their revolutionary model of double stranded DNA helix. James Watson was quoted as saying "we used to think that our fate was in our stars, but now we know, in large measures, our fate is in our genes". Another key breakthrough came in the early 1970s, when researchers discovered a series of enzymes that made it possible to snip apart genes at predetermined site along a molecule of DNA and glue them back together in a reproducible manner. Those genetic advances set the stage for the emergence of genetic engineering, which has produced new drugs and antibodies and enabled scientists to contemplate gene therapy. A few years after the isolation of genes from DNA, gene therapy was discovered in 1980s.[7]

Gene therapy was first conceptualized in 1972, with the authors urging caution before commencing gene therapy studies in humans. The first FDA-approved gene therapy experiment in the United States occurred in 1990, when Ashanti De Silva was treated for ADA-SCID.[8] By January 2014, about 2,000 clinical trials had

been conducted or had been approved using a number of techniques for gene therapy.[9] Although early clinical failures led many to dismiss gene therapy as over-hyped, clinical successes since 2006 have bolstered new optimism in the promise of gene therapy. These include successful treatment of patients with the retinal disease Leber's congenital amaurosis,[10],[11],[12],[13] X-linked SCID,[14] ADA-SCID,[15],[16] adrenoleukody strophy,[17] chronic lymphocytic leukemia (CLL),[18] acute lymphocytic leukemia (ALL),[19] multiple myeloma,[20] haemophilia[16] and Parkinson's disease.[21] These clinical successes have led to a renewed interest in gene therapy, with several articles in scientific and popular publications calling for continued investment in the field[22],[23] and between 2013 and April 2014, US companies invested over $600 million in gene therapy.[24]

In 2012, Glybera became the first gene therapy treatment to be approved for clinical use in either Europe or the United States after its endorsement by the European Commission.[25],[26]

Approaches for gene therapy:

The process of gene therapy remains complex and many techniques need further developments. The challenge of developing successful gene therapy for any specific condition is considerable. The condition in question must be well understood, the undying faulty gene must be identified and a working copy of the gene involved must be available. Specific cells in the body requiring treatment must be identified and are accessible. A means of efficiently delivering working copies of the gene to the cells must be available. Moreover diseases and their strict genetic link need to be understood thoroughly. There are two approaches to achieve gene therapy.

1. Somatic cell gene therapy:

The non-reproductive (non-sex) cells of an organism are referred to as somatic cells. These are the cells of an organism other than sperm or egg cells e.g., bone marrow cells, blood cells, skin cells, intestinal cells. At present all the research on gene therapy is directed to correct the genetic defects in somatic cells. In essence, somatic cell gene therapy involves the insertion of a fully functional and expressible gene into a target somatic cell to correct a genetic disease permanently.

Several somatic cell gene transfer experiments are currently in clinical trials with varied success. Over 600 clinical trials utilizing somatic cell therapy are underway in the United States. Most of these trials focus on treating severe genetic disorders, including immunodeficiencies, haemophilia, thalassaemia, and cystic fibrosis. These disorders are good candidates for somatic cell therapy because they are caused by single gene defects. While somatic cell therapy is promising for treatment, a complete correction of a genetic disorder or the replacement of multiple genes in somatic cells is not yet possible. Only a few of the many clinical trials are in the advanced stages.[27]

2. Germ cell gene therapy:

The reproductive (sex) cells of an organism constitute germ cell line. Gene therapy involving the introduction of DNA into germ cells is passed on to the successive generations. For safety, ethical and technical reasons, germ cell gene therapy is not being attempted at present.

The genetic alterations in somatic cells are not carried to the next generations while as if a germ cell is genetically modified then all the cells in the organism will contain the modified gene. Therefore somatic cell gene therapy is preferred and extensively studied with an ultimate objective of correcting human diseases.

A large number of genetic disorders and other diseases are currently at various stages of gene therapy trials. A selected list of some important ones is given in table below:

Table 1 : Summary of approved and published current clinical gene therapy protocols[28]

Disorder	Objective	Target cells	Mode of delivery	Countries with protocols
Adenosine DeAminase deficiency (ADA)	ADA replacement	Blood	Retrovirus	Italy, Netherlands, United States
Alpha-1-antitrypsin deficiency	Alpha-1-antitrypsin replacement	Respiratory epithelium	Liposome	United States
AIDS	Antigen presentation HIV inactivation	Blood, marrow	Retrovirus	United States
Cancer	Immune function enhancement	Blood, marrow, tumour	Retrovirus, liposome, electroporation, cell mediated transfer	Austria, China, France, Germany, Italy, Netherlands, United States
	Tumour ablation	Tumour	Retrovirus, non-complexed DNA, cell-mediated transfer	United States
	Chemoprotection	Blood, marrow	Retrovirus	United States
	Stem-cell marking	Blood, marrow, tumour	Retrovirus	Canada, France, Sweden, United States
Cystic fibrosis	Cystic fibrosis transmembrane regulatory enzyme replacement	Respiratory epithelium	Adenovirus, liposome	United Kingdom, United States
Hemophilia B	Factor IX replacement	Skin fibroblasts	Retrovirus	China
Rheumatoid arthritis	Cytokine delivery	Synovium	Retrovirus	United States

Gene therapy: Methods

There are two methods of gene therapies:

1. *Ex-vivo gene therapy*: This involves the transfer of genes in cultured cells (e.g bone marrow cells) which are then reintroduced into the patient.
2. *In-vivo gene therapy*: The direct delivery of genes into the cells of a particular tissue is referred to as in-vivo gene therapy.

Ex-vivo gene therapy

The technique of ex-vivo gene therapy involves the following steps (Fig. 1)[29]:

- Isolation of cells with genetic defect from a patient.
- Growth of the cells in a culture medium.
- Insertion of the therapeutic gene to correct the gene defect.
- Selection of the genetically corrected cells (stable transformants) and growth of the same.
- Transplantation of the modified cells to the patient.

The procedure basically involves the use of the patient's own cells for culture and genetic correction and then their return back to the patient.

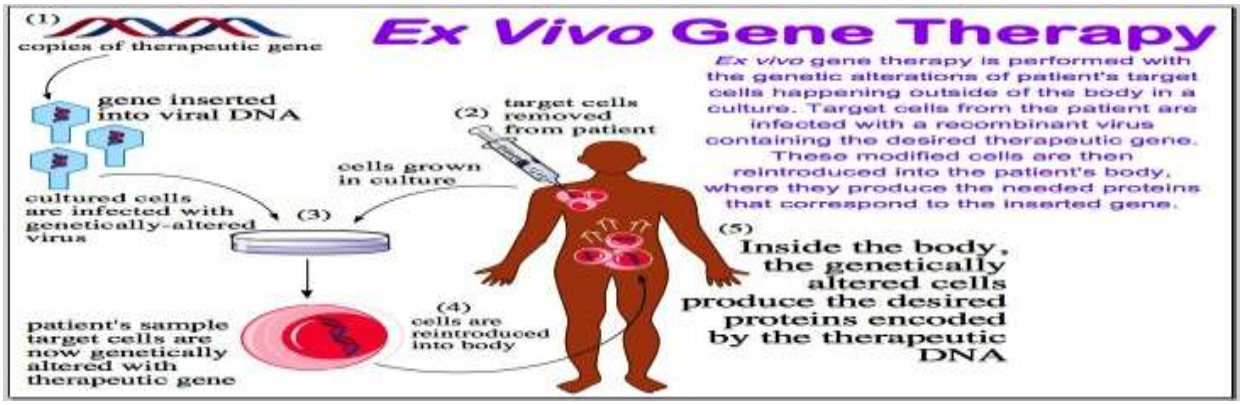

Fig.1: Flow chart depicting ex-vivo gene therapy [29]

In-vivo gene therapy

The direct delivery of the therapeutic gene into the target cells of a particular tissue of a patient constitutes in-vivo gene therapy (Fig.2)[30]. Many tissues are the potential candidates of this approach. These include liver, muscle, skin, spleen, lung, brain and blood cells. The success of the in-vivo gene therapy mostly depends on the following parameters:

- The efficiency of the uptake of the remedial (therapeutic genes) by the target cells.
- Intracellular degradation of the gene and its uptake by nucleus.
- The expression capability of the gene.

Fig.2: Flow chart depicting in-vivo gene therapy [30]

Gene delivery:

It suffices to say that the success of gene therapy largely relies on an efficient and safe delivery system. Currently, gene delivery can be divided into two categories: viral and non-viral. Successful gene delivery requires an efficient way to get the DNA into cells and to make it work. Scientists refer to these DNA delivery "vehicles" as **vectors**.

There is no "perfect vector" that can treat every disorder. Like any type of medical treatment, a gene therapy vector must be customized to address the unique features of the disorder. Part of the challenge in gene therapy is choosing the most suitable vector for treating the disorder.

To be successful, a vector must:

- *TARGET* the right cells. So that, it shouldn't wind up in the big toe.
- *INTEGRATE* the gene in the cells. So as to ensure that the gene integrates into, or becomes part of, the host cell's genetic material, or that the gene finds another way to survive in the nucleus without being trashed.

- *ACTIVATE* the gene. A gene must go to the cell's nucleus and be "turned on," meaning that it is transcribed and translated to make the protein product it encodes. For gene delivery to be successful, the protein must function properly.
- *AVOID* harmful side effects. Any time an unfamiliar biological substance is introduced into the body, there is a risk that it will be toxic or that the body will mount an immune response against it.

Some of the vectors used in gene therapy include:

A. Viral Vector:

Mother Nature is a brilliant scientist! Over the last three billion years or so, she's developed an incredibly efficient means of delivering foreign genes into cells: **the virus**.

Usually when we think of viruses, we think of the ones that cause diseases like the common cold, the flu, and HIV/AIDS. But scientists have actually been able to use viruses to deliver DNA to cells for gene therapy. Why reinvent the wheel if there's a perfectly good one out there? If we can modify viruses to deliver genes without making people sick, we may have a good set of gene therapy tools.

Advantages of viral vectors:

- They're very good at targeting and entering cells.
- Some target specific types of cells.
- They can be modified so that they can't replicate and destroy cells.

Drawbacks of viral vectors:

- They can carry a limited amount of genetic material. Therefore, some genes may be too big to fit into some viruses.
- They can cause immune responses in patients, leading to two potential problems:
- Patients may get sick.
- The immune system may block the virus from delivering the gene to the patient's cells, or it may kill the cells once the gene has been delivered.

Some of the different types of viruses used as gene therapy vectors are described as follows:

- ***Retrovirus:***-First viruses to be used as vectors in gene therapy experiments were retroviruses[31]. They belong to a class of viruses (RNA as genetic material) which can create double stranded DNA copies with the enzyme reverse transcriptase. These copies of its genome can be integrated into the chromosome of host cell by another enzyme carried the virus called integrase. Now the host cell has been modified to contain a new gene. If such modified host cells divide later, their descendants will contain the new genes. Although retroviruses have been used in most gene therapy experiments so far, they present problems.[32] One such problem is that integrase enzyme can insert genetic material of the virus into any arbitrary position in the genome of the host, which can lead to insertional mutagenesis (if insertion is in the middle of the gene) or uncontrolled cell division (if gene happens to be one regulating cell division) leading to cancer. This problem has recently begun to be addressed by utilizing zinc finger nuclease[33] or by including certain sequences such as beta globin locus control region to direct the site of integration to specific chromosome.
  Gene therapy trial using retroviral vector to treat X-linked severe combined immune deficiency represent the most successful application till date.[34] Also this has been tried to treat SCID due to ADA deficiency with relative success. As researchers have grown more confident, they have begun injecting altered retroviruses directly into tissues where the corrected genes are needed. For example- in cystic fibrosis (mutated gene impairs lung function), healthy genes are inserted directly to the lining of bronchial tube. Experimental animal studies are being conducted to establish the effect in muscular dystrophy.
- ***Adenoviruses***:- *(Fig. 3)* A class of viruses with double-stranded DNA genomes that cause respiratory, intestinal, and eye infections in humans. The virus that causes the common cold is an adenovirus.

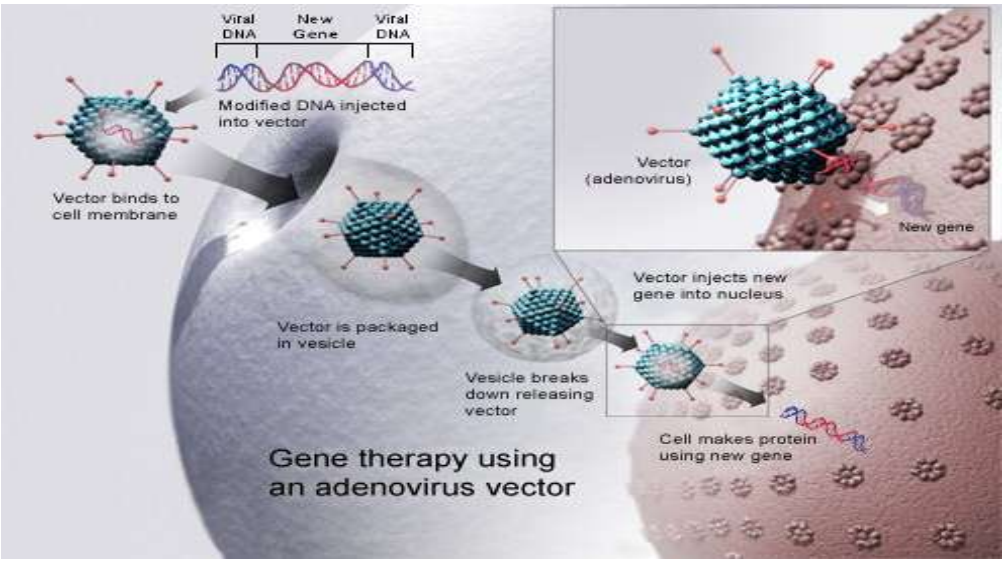

Fig. 3 : Gene therapy using an adenovirus vector[35]

- ***Adeno -associated viruses***:- A class of small, single-stranded DNA viruses that can insert their genetic material at a specific site on chromosome 19 with nearly 100% certainty. Researchers believe that most people carry AAV which do not cause disease and do not provoke an immune response. Scientists have demonstrated the animal experiments using AAV to correct genetic defects.[36]
- ***Cis and trans-acting elements*-** Replication-defective vectors always contain a "transfer construct". The transfer construct carries the gene to be transduced or "transgene". The transfer construct also carries the sequences which are necessary for the general functioning of the viral genome: packaging sequence, repeats for replication and, when needed, priming of reverse transcription. These are denominated cis-acting elements, because they need to be on the same piece of DNA as the viral genome and the gene of interest.
- ***Herpes simplex viruses*-** A class of double-stranded DNA viruses that infect a particular cell type, neurons. Herpes simplex virus type 1 is a common human pathogen that causes cold sores.[37]

B. Non-Viral Methods:

In the non-viral approach, the genetic materials are considered as chemical entities or pharmaceutical products and particulate carriers are used in their delivery. Research efforts have yielded several non-viral methods gene transfer such as electroporation (creation of electric field induced pores in plasma membrane), sonoporation (ultrasonic frequencies to disrupt cell membrane), magnetofection (use of magnetic particle complexed with DNA), gene guns (shoots DNA coated gold particles into cells by using high pressure) and receptor mediated gene transfer are being explored.[38] Each method has its own advantages and disadvantages.

In general, a non-viral gene delivery system comprises plasmid DNA complexed and condensed by a polycationic agent. The polycation can be a cationic lipid, a liposome composed of cationic lipids, a cationic polymer (eg, polylysine, polyethyleneimine) or a positively charged protein (histones). Although non-viral systems can protect DNA from nuclease degradation, the lesser ability to overcome various systemic barriers presents substantial challenges to their use in gene delivery. Additionally the ionic interaction of positively surface charged plasmid/polycation complex with plasma proteins can result in particle destabilization, characterized by aggregation of particles or premature release of DNA.

In the recent past, several chemical methods like use of synthetic oligonucleotides (to inactivate defective genes by using antisense specific to target gene), lipoplexes (made up of anionic and neutral lipids) and polyplexes (complex of polymers with DNA) have been used to facilitate delivery of the DNA into cell.[39] Recently there have been some hybrid methods developed that combine two or more techniques. For example-virosomes that combine liposomes with an inactivated HIV or influenza virus. This has been shown to have more efficient gene transfer in respiratory epithelial cell than either viral or liposomal method alone. Other hybrid methods involve mixing viral vectors with cationic lipids. Researchers are also experimenting with

introducing a 47th (artificial human) chromosome into target cells. This chromosome would exist autonomously alongside the standard 46, not affecting their workings or causing any mutation. It would be a large vector capable of carrying substantial amount of genetic code, and scientists anticipate that, because of its construction and autonomy, the body's immune system would not attack it. The advantage of direct transfers of non-complexes or protein- complexed DNA by chemical, mechanical, electrical, particle bombardment method or artificial chromosome method include the possibility of transferring relatively large DNA fragments. However, these processes are still inefficient, are limited to ex-vivo gene transfer and have undefined cytotoxic effects.[40]

Development of Gene Therapy technology:

- **1970s and earlier**

In 1972 Friedmann and Roblin authored a paper in *Science* titled "Gene therapy for human genetic disease". Rogers (1970) was cited for proposing that *exogenous good DNA* be used to replace the defective DNA in those who suffer from genetic defects.[41]

- **1990s**

The first approved gene therapy case in the United States took place on 14 September 1990, at the National Institute of Health, under the direction of Professor William French Anderson.[42] It was performed on a four year old girl named Ashanti De Silva. It was a treatment for a genetic defect that left her with ADA-SCID, a severe immune system deficiency. The effects were only temporary, but successful.[43]

New gene therapy approach repairs errors in messenger RNA derived from defective genes. This technique has the potential to treat the blood disorder thalassaemia, cystic fibrosis, and some cancers.[44] Researchers at Case Western Reserve University and Copernicus Therapeutics are able to create tiny liposomes 25 nanometers across that can carry therapeutic DNA through pores in the nuclear membrane.[45]

Sickle-cell disease is successfully treated in mice.[46] The mice – which have essentially the same defect that causes sickle cell disease in humans – through the use a viral vector, were made to express the production of fetal hemoglobin (HbF), which normally ceases to be produced by an individual shortly after birth. In humans, the use of hydroxyurea to stimulate the production of HbF has long been shown to temporarily alleviate the symptoms of sickle cell disease. The researchers demonstrated this method of gene therapy to be a more permanent means to increase the production of the therapeutic HbF.[47]

In 1992 Doctor Claudio Bordignon working at the Vita-Salute San Raffaele University, Milan, Italy performed the first procedure of gene therapy using hematopoietic stem cells as vectors to deliver genes intended to correct hereditary diseases.[48] In 2002 this work led to the publication of the first successful gene therapy treatment for adenosine deaminase-deficiency (SCID). The success of a multi-center trial for treating children with SCID (severe combined immune deficiency or "bubble boy" disease) held from 2000 and 2002 was questioned when two of the ten children treated at the trial's Paris center developed a leukemia-like condition. Clinical trials were halted temporarily in 2002, but resumed after regulatory review of the protocol in the United States, the United Kingdom, France, Italy, and Germany.[49]

In 1993 Andrew Gobea was born with severe combined immunodeficiency (SCID). Genetic screening before birth showed that he had SCID. Blood was removed from Andrew's placenta and umbilical cord immediately after birth, containing stem cells. The allele that codes for ADA was obtained and was inserted into a retrovirus. Retroviruses and stem cells were mixed, after which the viruses entered and inserted the gene into the stem cells' chromosomes. Stem cells containing the working ADA gene were injected into Andrew's blood system via a vein. Injections of the ADA enzyme were also given weekly. For four years T cells (white blood cells), produced by stem cells, made ADA enzymes using the ADA gene. After four years more treatment was needed.

The 1999 death of Jesse Gelsinger in a gene therapy clinical trial resulted in a significant setback to gene therapy research in the United States.[50][51] As a result, the U.S. FDA suspended several clinical trials pending the re-evaluation of ethical and procedural practices in the field.[52]

- **2003**

In 2003 a University of California, Los Angeles research team inserted genes into the brain using liposomes coated in a polymer called polyethylene glycol. The transfer of genes into the brain is a significant achievement because viral vectors are too big to get across the blood–brain barrier. This method has potential for treating Parkinson's disease.[53]

RNA interference or gene silencing may be a new way to treat Huntington's disease. Short pieces of double-stranded RNA (short, interfering RNAs or siRNAs) are used by cells to degrade RNA of a particular sequence. If a siRNA is designed to match the RNA copied from a faulty gene, then the abnormal protein product of that gene will not be produced.[54]

- **2006**

In March 2006 an international group of scientists announced the successful use of gene therapy to treat two adult patients for X-linked chronic granulomatous disease, a disease which affects myeloid cells and which gives a defective immune system. The study, published in Nature Medicine, is believed to be the first to show that gene therapy can cure diseases of the myeloid system.[55]

In May 2006 a team of scientists led by Dr. Luigi Naldini and Dr. Brian Brown from the San Raffaele Telethon Institute for Gene Therapy (HSR-TIGET) in Milan, Italy reported a breakthrough for gene therapy in which they developed a way to prevent the immune system from rejecting a newly delivered gene.[56] Similar to organ transplantation, gene therapy has been plagued by the problem of immune rejection. So far, delivery of the 'normal' gene has been difficult because the immune system recognizes the new gene as foreign and rejects the cells carrying it. To overcome this problem, the HSR-TIGET group utilized a newly uncovered network of genes regulated by molecules known as microRNAs. Dr. Naldini's group reasoned that they could use this natural function of micro RNA to selectively turn off the identity of their therapeutic gene in cells of the immune system and prevent the gene from being found and destroyed. The researchers injected mice with the gene containing an immune-cell micro RNA target sequence, and the mice did not reject the gene, as previously occurred when vectors without the micro RNA target sequence were used. This work will have important implications for the treatment of hemophilia and other genetic diseases by gene therapy.

In August 2006, scientists at the National Institutes of Health (Bethesda, Maryland) successfully treated metastatic melanoma in two patients using killer T cellsgenetically retargeted to attack the cancer cells. This study constitutes one of the first demonstrations that gene therapy can be effective in treating cancer.[57]

In November 2006 Preston Nix from the University of Pennsylvania School of Medicine reported on VRX496, a gene-based immunotherapy for the treatment ofhuman immunodeficiency virus (HIV) that uses a lentiviral vector for delivery of an antisense gene against the HIV envelope. In the Phase I trial enrolling five subjects with chronic HIV infection who had failed to respond to at least two antiretroviral regimens, a single intravenous infusion of autologous CD4 T cells genetically modified with VRX496 was safe and well tolerated. All patients had stable or decreased viral load; four of the five patients had stable or increased CD4 T cell counts. In addition, all five patients had stable or increased immune response to HIV antigens and other pathogens. This was the first evaluation of a lentiviral vector administered in U.S. Food and Drug Administration-approved human clinical trials for any disease.[58] Data from an ongoing Phase I/II clinical trial were presented at CROI 2009.[59]

- **2007**

On 1 May 2007 Moorfields Eye Hospital and University College London's Institute of Ophthalmology announced the world's first gene therapy trial for inherited retinal disease. The first operation was carried out on a 23 year-old British male, Robert Johnson, in early 2007.[60] Leber's congenital amaurosis is an inherited blinding disease caused by mutations in the RPE65 gene. The results of a small clinical trial in children were published in New England Journal of Medicine in April 2008.[61]They researched the safety of the subretinal delivery of recombinant adeno-associated virus (AAV) carrying RPE65 gene, and found it yielded positive results, with patients having modest increase in vision, and, perhaps more importantly, no apparent side-effects.

- **2008**

Main article: Gene therapy of the human retina

In May 2008, two more groups, one at the University of Florida and another at the University of Pennsylvania, reported positive results in independent clinical trials using gene therapy to treat Leber's congenital amaurosis.

In all three clinical trials, patients recovered functional vision without apparent side effects. These studies, which used adeno-associated virus, have spawned a number of new studies investigating gene therapy for human retinal disease.

- **2009**

In September 2009, the journal Nature reported that researchers at the University of Washington and University of Florida were able to give trichromatic vision tosquirrel monkeys using gene therapy, a hopeful precursor to a treatment for color blindness in humans.[62] In November 2009, the journal *Science* reported that researchers succeeded at halting a fatal genetic disorder called adrenoleukody strophy in two children using a lentivirus vector to deliver a functioning version ofABCD1, the gene that is mutated in the disorder.[63]

- **2010**

A paper by Komáromy *et* al. published in April 2010, deals with gene therapy for a form of achromatopsia in dogs. Achromatopsia, or complete color blindness, is presented as an ideal model to develop gene therapy directed to cone photoreceptors. Cone function and day vision have been restored for at least 33 months in two young dogs with achromatopsia. However, the therapy was less efficient for older dogs.[64]

In September 2010, it was announced that an 18 year old male patient in France with beta-thalassemia major had been successfully treated with gene therapy.[65]Beta-thalassemia major is an inherited blood disease in which beta haemoglobin is missing and patients are dependent on regular lifelong blood transfusions.[66] A team directed by Dr. Phillipe Leboulch (of the University of Paris, Bluebird Bio and Harvard Medical School[67]) used a lentiviral vector to transduce the human ß-globin gene into purified blood and marrow cells obtained from the patient in June 2007.[68] The patient's haemoglobin levels were stable at 9 to 10 g/dL, about a third of the hemoglobin contained the form introduced by the viral vector and blood transfusions had not been needed. Further clinical trials were planned.[69]Bone marrow transplants are the only cure for thalassemia but 75% of patients are unable to find a matching bone marrow donor.

- **2011**

In 2007 and 2008, a man being treated by Gero Hütter was cured of HIV by repeated Hematopoietic stem cell transplantation (see also Allogeneic stem cell transplantation, Allogeneic bone marrow transplantation, Allotransplantation) with double-delta-32 mutation which disables the CCR5 receptor; this cure was not completely accepted by the medical community until 2011.[70] This cure required complete ablation of existing bone marrow which is very debilitating.

In August 2011, two of three subjects of a pilot study were confirmed to have been cured from chronic lymphocytic leukemia (CLL). The study carried out by the researchers at the University of Pennsylvania used genetically modified T cells to attack cells that expressed the CD19 protein to fight the disease. In 2013, the researchers announced that 26 of 59 patients had achieved complete remission and the original patient had remained tumor-free.[71]

Human HGF plasmid DNA therapy of cardiomyocytes is being examined as a potential treatment for coronary artery disease as well as treatment for the damage that occurs to the heart after myocardial infarction.[72][73]

- **2012**

The FDA approves clinical trials of the use of gene therapy on thalassemia major patients in the US. Researchers at Memorial Sloan Kettering Cancer Center in New York begin to recruit 10 participants for the study in July 2012.[74] The study is expected to end in 2014.[75]

In July 2012, the European Medicines Agency recommended approval of a gene therapy treatment for the first time in either Europe or the United States. The treatment, called Alipogene tiparvovec (Glybera), compensates for lipoprotein lipase deficiency, which can cause severe pancreatitis.[76] The recommendation was endorsed by the European Commission in November 2012 and commercial rollout is expected in late 2013.[77]

In December 2012, it was reported that 10 of 13 patients with multiple myeloma were in remission "or very close to it" three months after being injected with a treatment involving genetically engineered T cells to target proteins NY-ESO-1 and LAGE-1 which exist only on cancerous myeloma cells. This procedure had been developed by a company called Adaptimmune.

- **2013**

In March 2013, Researchers at the Memorial Sloan-Kettering Cancer Center in New York, reported that three of five subjects who had acute lymphocytic leukemia(ALL) had been in remission for five months to two years after being treated with genetically modified T cells which attacked cells with CD19 genes on their surface, i.e. all B-cells, cancerous or not. The researchers believed that the patients immune systems would make normal T-cells and B-cells after a couple of months however they were given bone marrow to make sure. One patient had relapsed and died and one had died of a blood clot unrelated to the disease.

Following encouraging Phase 1 trials, in April 2013, researchers in the UK and the US announced they were starting Phase 2 clinical trials (called CUPID2 and SERCA-LVAD) on 250 patients[78] at several hospitals in the US and Europe to use gene therapy to combat heart disease. These trials were designed to increase the levels of SERCA2a protein in the heart muscles and improve the function of these muscles.[79] The FDA granted this a Breakthrough Therapy Designation which would speed up the trial and approval process in the USA.[80]

In July 2013 the Italian San Raffaele Telethon Institute for Gene Therapy (HSR-TIGET) reported that six children with two severe hereditary diseases had been treated with a partially deactivated lentivirus to replace a faulty gene and after 7–32 months the results were promising. Three of the children had metachromatic leukodystrophy which causes children to lose cognitive and motor skills.[81] The other children had Wiskott-Aldrich syndrome which leaves them to open to infection, autoimmune diseases and cancer due to a faulty immune system.[82]

In October 2013, the Great Ormond Street Hospital, London reported that two children born with adenosine deaminase severe combined immunodeficiency disease (ADA-SCID) had been treated with genetically engineered stem cells 18 months previously and their immune systems were showing signs of full recovery. Another three children treated since then were also making good progress. ADA-SCID children have no functioning immune system and are sometimes known as "bubble children."

In October 2013, Amit Nathswani of the Royal Free London NHS Foundation Trust in London reported that they had treated six people with haemophilia in early 2011 using genetically engineered adeno-associated virus. Over two years later all six were still producing blood plasma clotting factor.

- **2014**

In January 2014, researchers at the University of Oxford reported that six people suffering from choroideremia had been treated with a genetically engineered adeno-associated virus with a copy of a gene REP1. Over a six month to two year period all had improved their sight. Choroideremia is an inherited genetic eye disease for which in the past there has been no treatment and patients eventually go blind.[83][84]

In March 2014 researchers at the University of Pennsylvania reported that 12 patients with HIV had been treated since 2009 in a trial with a genetically engineered virus with a rare mutation known to protect against HIV (CCR5 deficiency). Results were promising.[85][86]

Applications of Gene therapy:

Diverse applications of gene therapy are being pursued- mostly in experimental animals, but some have been tried clinically. Inherited single gene disorders appear simpler to correct or cure. In addition several innovative strategies against cancer,viral diseases, acquired life style diseases, etc are being applied. The prominent ones are:

- *Cystic fibrosis:* by insertion of cystic fibrosis transport regulator (CFTR) gene into respiratory epithelial cells. This gene regulates expression of an apical chloride channel which is dysfunctional in cystic fibrosis. The limitation is that the airway epithelial cells are rapidly shed off.
- *Severe combined immunodeficiency disease (SCID):* by introducing gene for adenosine deaminase which is deficient.
- *Growth hormone deficiency:* by implanting cultured myoblasts transfected with GH gene.
- *Familial hypercholesterolemia:* by introducing LDL receptor gene into hepatocytes.
- *Lesch-Nyhan syndrome:* by introducing hypoxanthine phosphoribosyl transferase gene to correct deficiency of this enzyme in the CNS which causes severe neuropsychiatric disorder.
- *Parkinsonism:* by introducing the gene for tyrosine hydroxylase to augment dopamine production in basal ganglia.
- *Alzheimer's disease, huntington's chorea, familial amyotrophic lateral sclerosis, Gaucher's disease:* by supplementing the defective genes.
- *Stroke, head injury, multiple sclerosis:* by delivering nerve growth factor gene.
- *Duchenne muscular dystrophy:* by administering muscle dystropin gene.

- **Cancer:**

i. By genetic introduction of an enzyme (viral thymidine kinase) into tumour cells followed by a prodrug that is converted to the toxic metabolite- tumour cells are selectively killed.
ii. By inserting TNFα, IL-2 and other cytokine genes into tumour cells to increase their immune recognition and destruction by tumour infiltrating lymphocytes.
iii. By introducing promoter "antisense" gene or "suppressor" gene which negatively regulate tumour growth.
iv. By introducing multidrug resistant MDR-1 gene into bone marrow cells and render them less susceptible to destruction by myelosuppressant drugs . Thus a limiting toxicity of many anticancer drugs can be overcome.

- *Prevention of restenosis of grafted coronary vessel:* by introducing genes which inhibit growth of intimal cells.
- *Anaemia:* by myoblast mediated introduction of human erythropoietin gene.
- *Haemophilia:* by introducing factor VIII gene.
- *Insulin dependent diabetes mellitus*: by introducing insulin-1 gene into liver to act as ectopic site for insulin production.
- *HIV infection*: by injecting fibroblasts expressing HIV envelop glycoprotein gene to augment immunity against HIV.

Advantages and disadvantages of gene therapy :

Advantages of gene therapy

- In case of 'silencing' a gene. In the case of someone with HIV, which had not yet developed into AIDS, scientists could save them the pain and suffering of the disease by using gene therapy to 'silence' the disease before its onset.
- Gene therapy has the potential to eliminate and prevent hereditary diseases such as cystic fibrosis and is a possible cure for heart disease, AIDS and cancer.
- Very effective when delivered to tissues correctly.
- Side effects of drugs can be avoided.
- It fixes the problem at its source.

Disadvantages of Gene Therapy

- Short lived nature of gene therapy.
- Immune response - Genes injected with a virus may trigger an immune response against the virus. Problems with viral vectors (once inside the patient, the viral vector could recover its ability to cause disease).
- Multigene disorders - The genetic material might not get into the right cell, or the right place in the cell's DNA.
- Hard to deliver genes efficiently throughout a tissue or system.
- It can be quite expensive.
- The long term effects of gene therapy are unknown.

Ethical issues surrounding gene therapy:

Because gene therapy involves making changes to the body's set of basic instructions, it raises many unique ethical concerns. The ethical questions surrounding gene therapy include:

- How can "good" and "bad" uses of gene therapy be distinguished?
- Who decides which traits are normal and which constitute a disability or disorder?
- Will the high costs of gene therapy make it available only to the wealthy?
- Could the widespread use of gene therapy make society less accepting of people who are different?
- Should people be allowed to use gene therapy to enhance basic human traits such as height, intelligence, or athletic ability?

Current gene therapy research has focused on treating individuals by targeting the therapy to body cells such as bone marrow or blood cells. This type of gene therapy cannot be passed on to a person's children. Gene therapy could be targeted to egg and sperm cells (germ cells), however, which would allow the inserted gene to be passed on to future generations. This approach is known as germline gene therapy.

The idea of germline gene therapy is controversial. While it could spare future generations in a family from having a particular genetic disorder, it might affect the development of a fetus in unexpected ways or have long-term side effects that are not yet known. Because people who would be affected by germline gene therapy are not yet born, they can't choose whether to have the treatment. Because of these ethical concerns, the U.S. Government does not allow federal funds to be used for research on germline gene therapy in people.

The future of Gene Therapy:

Theoretically, gene therapy is the permanent solution for genetic diseases. But it is not as simple as it appears, since gene therapy has several inbuilt complexicities. Gene therapy broadly involves isolation of a specific gene, making its copies, inserting them into target tissue cells to make the desired protein. The story does not end here. It is absolutely essential to ensure that the gene is harmless to the patient and it is appropriately expressed (too much or too little will be no good). Another concern in gene therapy is the body's immune system which reacts to the foreign proteins produced by the new genes.

The public in general have exaggerated expectations on gene therapy. The researchers at least for the present, are unable to satisfy them. As per the records, by 2000 about 1000 Americans had undergone clinical trials involving various gene therapies. Unfortunately, the gene therapists are unable to categorically claim that gene therapy has permanently cured any one of these patients! Some people in the media (leading newspapers and magazines) have openly questioned whether it is worth to continue research on gene therapy!!

It may be true that as of now, gene therapy de to several limitations has not progressed the way it should, despite intensive research. But a breakthrough may come anytime, and of course this is only possible with persistent research in the field of gene therapy. And a day may come (it might take some years) when almost ever disease will have a gene therapy, as one of the treatment modalities. And gene therapy will revolutionize the practice of medicine!!

Conclusion:

Most scientists believe the potential for gene therapy is the most exciting application of DNA science, yet undertaken. How widely this therapy will be applied, depends on the simplification of procedure. As gene therapy is uprising in the field of medicine, scientists believe that after 20 years, this will be the last cure of every genetic disease. Genes may ultimately be used as medicine and given as simple intravenous injection of gene transfer vehicle that will seek our target cells for stable, site-specific chromosomal integration and subsequent gene expression. And now that a draft of the human genome map is complete, research is focusing on the function of each gene and the role of the faulty gene play in disease. Gene therapy will ultimately play Copernican part and will change our lives forever.

References:

1. Pearson H (2006). "Genetics: what is a gene?" *Nature* 441 (7092): 398–401.
2. Elizabeth Pennisi (2007). "DNA Study Forces Rethink of What It Means to Be a Gene". *Science* 316 (5831): 1556–1557.
3. Miller DA. "Human gene therapy comes of age". Nature 1992;375:455-460.
4. Verma IM, Weitzman MD. "Gene therapy: Twenty-first century medicine". Annu Rev Biochem 2005; 74: 711-738.
5. Knoell DM, Yiu IM. "Human gene therapy for hereditary diseases: a review of trials". *Am J Health Syst Pharma* 1998;55:899-904.
6. Ginter EK. "Gene therapy of hereditary disease". *Vopr Med Khim* 2000;46:265-78.
7. Friedmann T. "A brief history of gene therapy". *Nature Genetics* 1992;2:93-98.
8. Sheridan C (2011). "Gene therapy finds its niche". *Nature Biotechnology* 29(2): 121–128.
9. (January 2014) Gene Therapy Clinical Trials Worldwide Database *The Journal of Gene Medicine*, wiley.com., Retrieved 28 April 2014.
10. Maguire AM, Simonelli F, Pierce EA, Pugh EN, Mingozzi F, Bennicelli J, Banfi S, Marshall KA, Testa F, Surace EM, Rossi S, Lyubarsky A, Arruda VR, Konkle B, Stone E, Sun J, Jacobs J, Dell'Osso L, Hertle R, Ma JX, Redmond TM, Zhu X, Hauck B, Zelenaia O, Shindler KS, Maguire MG, Wright JF, Volpe NJ, McDonnell JW, Auricchio A, High KA, Bennett J (2008). "Safety and Efficacy of Gene Transfer for Leber's Congenital Amaurosis". *New England Journal of Medicine* 358 (21): 2240–2248.
11. Simonelli F, Maguire AM, Testa F, Pierce EA, Mingozzi F, Bennicelli JL, Rossi S, Marshall K, Banfi S, Surace EM, Sun J, Redmond TM, Zhu X, Shindler KS, Ying GS, Ziviello C, Acerra C, Wright JF, McDonnell JW, High KA, Bennett J, Auricchio A (2009). "Gene Therapy for Leber's Congenital Amaurosis is Safe and Effective Through 1.5 Years After Vector Administration". *Molecular Therapy* 18(3): 643–650.
12. Cideciyan AV, Hauswirth WW, Aleman TS, Kaushal S, Schwartz SB, Boye SL, Windsor EA, Conlon TJ, Sumaroka A, Roman AJ, Byrne BJ, Jacobson SG (2009). "Vision 1 Year after Gene Therapy for Leber's Congenital Amaurosis".*New England Journal of Medicine* 361 (7): 725–727.
13. Bainbridge JW, Smith AJ, Barker SS, Robbie S, Henderson R, Balaggan K, Viswanathan A, Holder GE, Stockman A, Tyler N, Petersen-Jones S, Bhattacharya SS, Thrasher AJ, Fitzke FW, Carter BJ, Rubin GS, Moore AT, Ali RR (2008). "Effect of Gene Therapy on Visual Function in Leber's Congenital Amaurosis". *New England Journal of Medicine* 358 (21): 2231–2239.
14. Fischer A, Hacein-Bey-Abina S, Cavazzana-Calvo M (2010). "20 years of gene therapy for SCID". *Nature Immunology* 11 (6): 457–460..
15. Ferrua F, Brigida I, Aiuti A (2010). "Update on gene therapy for adenosine deaminase-deficient severe combined immunodeficiency". *Current Opinion in Allergy and Clinical Immunology* 10 (6): 551–556.
16. Geddes, Linda (30 October 2013) 'Bubble kid' success puts gene therapy back on track' The New Scientist, Retrieved 2 November 2013
17. Cartier N, Aubourg P (2009). "Hematopoietic Stem Cell Transplantation and Hematopoietic Stem Cell Gene Therapy in X-Linked Adrenoleukodystrophy". *Brain Pathology* 20 (4): 857–862.
18. Ledford, H. (2011). "Cell therapy fights leukaemia". *Nature*.
19. Coghlan, Andy (26 March 2013) "Gene therapy cures leukaemia in eight days" The New Scientist, Retrieved 15 April 2013.
20. Coghlan, Andy (11 December 2013) " Souped-up immune cells force leukaemia into remission" New Scientist, Retrieved 15 April 2013.

21. LeWitt PA, Rezai AR, Leehey MA, Ojemann SG, Flaherty AW, Eskandar EN, Kostyk SK, Thomas K, Sarkar A, Siddiqui MS, Tatter SB, Schwalb JM, Poston KL, Henderson JM, Kurlan RM, Richard IH, Van Meter L, Sapan CV, During MJ, Kaplitt MG, Feigin A (2011). "AAV2-GAD gene therapy for advanced Parkinson's disease: A double-blind, sham-surgery controlled, randomised trial". *The Lancet Neurology* 10 (4): 309–319.
22. "Gene therapy deserves a fresh chance". *Nature* 461 (7268): 1173–2009. 2009.
23. Kolata, G. (6 November 2009). "After Setbacks, Small Successes for Gene Therapy". New York Times.
24. Herper, Matthew (26 March 2014) "Gene Therapy's Big Comeback Forbes magazine", Retrieved 28 April 2014
25. Gallagher, James. (2 November 2012) BBC News – Gene therapy: Glybera approved by European Commission. Bbc.co.uk. Retrieved on 15 December 2012.
26. Richards, Sabrina. "Gene Therapy Arrives in Europe". The Scientist. Retrieved 16 November 2012.
27. Mavilio F, Ferrari G (2008). "Genetic modification of somatic stem cells. The progress, problems and prospects of a new therapeutic technology". *EMBO Rep*. 9 Suppl 1: S64–9.
28. Dube ID,Cournoyer D. "Gene therapy: here to stay". *Can Med Assoc J* 1995;152:1605-1613.
29. Ex-vivo Gene therapy (http://en.wikipedia.org/wiki/file:gene_therapy.Jpg)
30. In-vivo Gene therapy (http://en.wikipedia.org/wiki/file:gene_therapy.Jpg)
31. Rochat T, Morris MA. "Viral vector for gene therapy". *J Aerosol Med* 2002;15:229-235.
32. Baun C, Dullman J, Li Z, et al. "Side effects of retroviral gene transfer into hematopoietic stem cells". *Blood* 2003;101:2099-114.
33. Durai S, Mani M, Kandavelou K, Wu J, Porteus MH, Chandrasegaran S. " Zinc finger nuclease: custom designed molecular scissors for genome engineering of plants and mammalian cells". *Nucleic Acid Res* 2005;33:5978-90.
34. Gasper HB, Thrasher AJ. "Gene therapy for severe combined immunodeficiencies". *Expert Opin Biol Ther* 2005;9:1175-82.
35. Gene therapy using an adenovirus vector (http://en.wikipedia.org/wiki/file:gene_therapy.Jpg)
36. Sibbald B. "Death but one unintended consequence of gene therapy trial". *CMAJ* 2001;164:1612.
37. Friedmann T., Roblin R. (1972) *Science* 175 (25), 949.
38. Wagner E, Curiel D, Cotton M. "Delivery of drugs, proteins and genes using transferring as a ligand for receptor-mediated endocytosis". *Adv Drug Deliv Rev* 1994;14:113-135.
39. Pierce EA, Liu Q, Igouchera O, Dmarrudin R, Ma H, Diamond SL, et al. "Oligonucleotide-directed single base DNA alteration in mouse embryonic stem cells". *Gene Therapy* 2003;10:24-33.
40. Wolff JA, Malone RW, Williams P, et al. "Direct gene transfer into mouse muscle in vivo". *Science* 1990;247:1465-68
41. Rogers S, New Scientist 1970, p. 194
42. "The first gene therapy". Life Sciences Foundation. 2011-06-21. Retrieved 2014-01-07.
43. Blaese RM, Culver KW, Miller AD, Carter CS, Fleisher T, Clerici M, Shearer G, Chang L, Chiang Y, Tolstoshev P, Greenblatt JJ, Rosenberg SA, Klein H, Berger M, Mullen CA, Ramsey WJ, Muul L, Morgan RA, Anderson WF (1995). "T lymphocyte-directed gene therapy for ADA- SCID: Initial trial results after 4 years". *Science* 270 (5235): 475–480.
44. Penman, Danny (11 October 2002). "Subtle gene therapy tackles blood disorder". New Scientist. Retrieved 17 August 2010.
45. "DNA nanoballs boost gene therapy". New Scientist. 12 May 2012. Retrieved 17 August 2010.
46. Wilson, Jennifer Fisher (18 March 2002). "Murine Gene Therapy Corrects Symptoms of Sickle Cell Disease". *The Scientist – Magazine of the Life Sciences*. Retrieved 17 August 2010.
47. St. Jude Children's Research Hospital (4 December 2008). "Gene Therapy Corrects Sickle Cell Disease In Laboratory Study". ScienceDaily. Retrieved 29 December 2012.
48. Abbott A (1992). "Gene therapy. Italians first to use stem cells". *Nature* 356(6369): 465–199.
49. Cavazzana-Calvo M, Thrasher A, Mavilio F (Feb 2004). "The future of gene therapy". *Nature* 427 (6977): 779–81.
50. Stein, Rob (11 October 2010). "First patient treated in stem cell study". Washington Post. Retrieved 10 November 2010.
51. "Death Prompts FDA to Suspend Arthritis Gene Therapy Trial". Medpage Today. 2007-07-27. Retrieved 10 November 2010.
52. Stolberg, Sheryl Gay (2000-01-22). "Gene Therapy Ordered Halted At University". New York Times. Retrieved 10 November 2010.

53. Ananthaswamy, Anil (20 March 2003). "Undercover genes slip into the brain". New Scientist. Retrieved 17 August 2010.
54. Holmes, Bob (13 March 2003). "Gene therapy may switch off Huntington's". New Scientist. Retrieved 17 August 2010.
55. Ott MG, Schmidt M, Schwarzwaelder K, Stein S, Siler U, Koehl U, Glimm H, Kühlcke K, Schilz A, Kunkel H, Naundorf S, Brinkmann A, Deichmann A, Fischer M, Ball C, Pilz I, Dunbar C, Du Y, Jenkins NA, Copeland NG, Lüthi U, Hassan M, Thrasher AJ, Hoelzer D, von Kalle C, Seger R, Grez M (2006). "Correction of X-linked chronic granulomatous disease by gene therapy, augmented by insertional activation of MDS1-EVI1, PRDM16 or SETBP1". *Nature Medicine* 12 (4): 401–409.
56. Brown BD, Venneri MA, Zingale A, Sergi Sergi L, Naldini L (2006). "Endogenous microRNA regulation suppresses transgene expression in hematopoietic lineages and enables stable gene transfer". *Nature Medicine* 12 (5): 585–591.
57. Morgan RA, Dudley ME, Wunderlich JR, Hughes MS, Yang JC, Sherry RM, Royal RE, Topalian SL, Kammula US, Restifo NP, Zheng Z, Nahvi A, de Vries CR, Rogers-Freezer LJ, Mavroukakis SA, Rosenberg SA (2006). "Cancer Regression in Patients After Transfer of Genetically Engineered Lymphocytes".*Science* 314 (5796): 126–129.
58. Levine BL, Humeau LM, Boyer J, MacGregor RR, Rebello T, Lu X, Binder GK, Slepushkin V, Lemiale F, Mascola JR, Bushman FD, Dropulic B, June CH (2006)."Gene transfer in humans using a conditionally replicating lentiviral vector".*Proceedings of the National Academy of Sciences* 103 (46): 17372–17377.
59. "Penn Medicine presents HIV gene therapy trial data at CROI 2009". EurekAlert!. 10 February 2009. Retrieved 19 November 2009.
60. "Gene therapy first for poor sight". *BBC News*. 1 May 2007. Retrieved 3 May 2010.
61. A. M. Maguire, F. Simonelli, and F. Simonelli, "Safety and efficacy of gene transfer for Leber's congenital amaurosis," New England Journal of Medicine, vol. 358, no. 21, pp. 2240–2248, 2008.
62. Dolgin, E. (2009). "Colour blindness corrected by gene therapy". *Nature*.
63. Cartier N, Hacein-Bey-Abina S, Bartholomae CC, Veres G, Schmidt M, Kutschera I, Vidaud M, Abel U, Dal-Cortivo L, Caccavelli L, Mahlaoui N, Kiermer V, Mittelstaedt D, Bellesme C, Lahlou N, Lefrère F, Blanche S, Audit M, Payen E, Leboulch P, l'Homme B, Bougnères P, Von Kalle C, Fischer A, Cavazzana-Calvo M, Aubourg P (2009). "Hematopoietic stem cell gene therapy with a lentiviral vector in X-linked adrenoleukodystrophy". *Science* 326 (5954): 818–23.
64. Komáromy AM, Alexander JJ, Rowlan JS, Garcia MM, Chiodo VA, Kaya A, Tanaka JC, Acland GM, Hauswirth WW, Aguirre GD (2010). "Gene therapy rescues cone function in congenital achromatopsia". *Human Molecular Genetics* 19 (13): 2581–2593.
65. Cavazzana-Calvo M, Payen E, Negre O, Wang G, Hehir K, Fusil F, Down J, Denaro M, Brady T, Westerman K, Cavallesco R, Gillet-Legrand B, Caccavelli L, Sgarra R, Maouche-Chrétien L, Bernaudin F, Girot R, Dorazio R, Mulder GJ, Polack A, Bank A, Soulier J, Larghero J, Kabbara N, Dalle B, Gourmel B, Socie G, Chrétien S, Cartier N, Aubourg P, Fischer A, Cornetta K, Galacteros F, Beuzard Y, Gluckman E, Bushman F, Hacein-Bey-Abina S, Leboulch P (2010). "Transfusion independence and HMGA2 activation after gene therapy of human β-thalassaemia". *Nature* 467 (7313): 318.
66. Galanello R, Origa R (2010). "Beta-thalassemia". *Orphanet Journal of Rare Diseases* 5: 11.
67. Leboulch P (20 March 2013). "Five year outcome of lentiviral gene therapy for human beta-thalassemia, lessons and prospects". *Proceedings of the 3rd Pan-European Conference on Haemoglobinopathies and Rare Anaemias* 3 (1s).
68. Beals, Jacquelyn K. (16 September 2010). Gene Therapy Frees Beta-Thalassemia Patient From Transfusions for 2+ Years. Medscape.com (16 September 2010). Retrieved on 2012-12-15.
69. (11 July 2012) ß-Thalassemia Major With Autologous CD34+ Hematopoietic Progenitor Cells Transduced With TNS9.3.55 a Lentiviral Vector Encoding the Normal Human ß-Globin Gene ClinicalTrials.gov, Clinical trial NCT01639690 at the Memorial Sloan-Kettering Cancer Center, Retrieved 12 February 2014.
70. Rosenberg, Tina (29 May 2011) The Man Who Had HIV and Now Does Not, New York Magazine.
71. "Gene Therapy Turns Several Leukemia Patients Cancer Free. Will It Work for Other Cancers, Too?". Singularity Hub. Retrieved 2014-01-07.
72. Yang ZJ, Zhang YR, Chen B, Zhang SL, Jia EZ, Wang LS, Zhu TB, Li CJ, Wang H, Huang J, Cao KJ, Ma WZ, Wu B, Wang LS, Wu CT (2008). "Phase I clinical trial on intracoronary administration of Ad-hHGF treating severe coronary artery disease". *Molecular Biology Reports* 36 (6): 1323–1329.

73. Hahn W, Pyun WB, Kim DS, Yoo WS, Lee SD, Won JH, Shin GJ, Kim JM, Kim S (2011). "Enhanced cardioprotective effects by coexpression of two isoforms of hepatocyte growth factor from naked plasmid DNA in a rat ischemic heart disease model". *The Journal of Gene Medicine* 13 (10): 549–555.
74. On Cancer: Launch of Stem Cell Therapy Trial Offers Hope for Patients with Inherited Blood Disorder | Memorial Sloan-Kettering Cancer Center. Mskcc.org (16 July 2012). Retrieved on 15 December 2012.
75. ß-Thalassemia Major With Autologous CD34+ Hematopoietic Progenitor Cells Transduced With TNS9.3.55 a Lentiviral Vector Encoding the Normal Human ß-Globin Gene. ClinicalTrials.gov. Retrieved on 15 December 2012.
76. Pollack, Andrew (20 July 2012) European Agency Backs Approval of a Gene Therapy, New York Times.
77. First Gene Therapy Approved by European Commission. UniQure (2 November 2012). Retrieved on 15 December 2012.
78. Bosely, Sarah (30 April 2013) Pioneering gene therapy trials offer hope for heart patients The Guardian, Retrieved 28 April 2014.
79. (8 September 2013) First gene therapy trial for heart failure begins in UK The Physicians Clinic, Retrieved 28 April 2014.
80. (10 April 2014) Celladon Receives Breakthrough Therapy Designation From FDA for MYDICAR(R), Novel, First-in-Class Therapy in Development to Treat Heart Failure New York Times, Retrieved 28 April 2014.
81. Biffi A, Montini E, Lorioli L, Cesani M, Fumagalli F, Plati T, Baldoli C, Martino S, Calabria A, Canale S, Benedicenti F, Vallanti G, Biasco L, Leo S, Kabbara N, Zanetti G, Rizzo WB, Mehta NA, Cicalese MP, Casiraghi M, Boelens JJ, Del Carro U, Dow DJ, Schmidt M, Assanelli A, Neduva V, Di Serio C, Stupka E, Gardner J, von Kalle C, Bordignon C, Ciceri F, Rovelli A, Roncarolo MG, Aiuti A, Sessa M, Naldini L (2013). "Lentiviral hematopoietic stem cell gene therapy benefits metachromatic leukodystrophy". *Science* 341 (6148): 1233158.
82. Aiuti A, Biasco L, Scaramuzza S, Ferrua F, Cicalese MP, Baricordi C, Dionisio F, Calabria A, Giannelli S, Castiello MC, Bosticardo M, Evangelio C, Assanelli A, Casiraghi M, Di Nunzio S, Callegaro L, Benati C, Rizzardi P, Pellin D, Di Serio C, Schmidt M, Von Kalle C, Gardner J, Mehta N, Neduva V, Dow DJ, Galy A, Miniero R, Finocchi A, Metin A, Banerjee PP, Orange JS, Galimberti S, Valsecchi MG, Biffi A, Montini E, Villa A, Ciceri F, Roncarolo MG, Naldini L (2013). "Lentiviral hematopoietic stem cell gene therapy in patients with Wiskott-Aldrich syndrome". *Science* 341 (6148): 1233151.
83. MacLaren RE, Groppe M, Barnard AR, Cottriall CL, Tolmachova T, Seymour L, Clark KR, During MJ, Cremers FP, Black GC, Lotery AJ, Downes SM, Webster AR, Seabra MC (2014). "Retinal gene therapy in patients with choroideremia: initial findings from a phase 1/2 clinical trial". *Lancet* 383 (9923): 1129–37.
84. Beali, Abigail(25 January 2014) "Gene therapy restores sight in people with eye disease" The New Scientist, Retrieved 25 January 2014
85. Tebas P, Stein D, Tang WW, Frank I, Wang SQ, Lee G, Spratt SK, Surosky RT, Giedlin MA, Nichol G, Holmes MC, Gregory PD, Ando DG, Kalos M, Collman RG, Binder-Scholl G, Plesa G, Hwang WT, Levine BL, June CH (2014). "Gene Editing ofCCR5in Autologous CD4 T Cells of Persons Infected with HIV". *New England Journal of Medicine* 370 (10): 901.
86. Dvorsky, George (6 March 2014) "Scientists Create Genetically Modified Cells That Protect Against HIV", io9, Biotechnology, Retrieved 6 March 2014.

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