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Gene therapy: Recent development, benefits and pitfall

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Abstract: *The use of gene therapy technology to treat genetic diseases has achieved great success in last few years. The advance in biotechnology field provides much hope for the future of gene therapy and made this method possible to treat and cure many genetic, infectious and chronic diseases. Furthermore the unavailability of proper treatment and cure for many diseases made gene therapy urgent need. Gene therapy has sparked great interest because it offers the possibility of a permanent cure. The recent discoveries in DNA technology like mi RNA and CRISPER/Cas9 simplify the difficulties and challenges in gene therapy like the gene vector and gene expression control. In addition, it is possible now to use gene therapy for germ-line correction. Gene therapy consists of the introduction of normal genetic material into cells with genetic abnormalities for a therapeutic purpose. A wide range of vectors have been developed and used for this biotechnology. The world predicting that in the next twenty years DNA technology and gene therapy will change the practice of medicine from a treatment-based to a prevention-based practice. Researchers are starting to move away from developing new drugs, and towards finding an ultimate solution. They hope that in the coming years, every genetic disease will have gene therapy as its treatment. Gene therapy could be the last therapy that the human race will ever need. Gene therapy has some potential risks. A gene can't easily be inserted directly into patient's cells. The very expensive therapies have a lot of unknowns and raise a number of ethical and practicality questions. This manuscript reviews the potential use of the gene therapy as well some of the pitfalls and challenges.*

Keywords*: Gene therapy, Developments, Disadvantages, Guidelines*.

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I. Introduction

Gene therapy is the therapeutic delivery of nucleic acid polymers into a patient's cells as a drug to treat disease [1].The first attempt at modifying human DNA was performed in 1980 by Martin Cline, but the first successful nuclear gene transfer in humans, approved by the National Health Institutes of Health, was performed in May 1989[2].The first therapeutic use of gene transfer as well as the first direct insertion of human DNA into the nuclear genome was performed by French Anderson in a trial starting in September 1990.Between 1989 and February 2016,over 2,300 clinical trials had been conducted, more than half of them in phase 1[3].Not all medical procedures that introduce alterations to a patient's genetic makeup can be considered gene therapy. Bone marrow transplantation and organ transplants in general have been found to introduce foreign DNA into patients [4].The first commercial gene therapy, Gendicine ,was approved in China,2003 for the treatment of certain cancers[5].In 2011 Neovasculgen was registered in Russia as first-in-class gene therapy drug for treatment of peripheral artery disease, including critical limb ischemia[6].In 2012, Glybera, a treatment for a rare inherited disorder, became the first treatment to be approved for clinical use in either in Europe ot the United States after its endorsement by the European commission[7,8].

II. Historical perspectives

As early as 1970 Friedman and Roblin authored a paper in *Science* titled "gene therapy for human genetic disease"[9].Rogers(1970) was cited for progressing the *exogenous good DNA* be used to replace the defective DNA in those who suffer from genetic defects[10].In 1980s a retrovirus vector system was designed that could efficiently insert foreign genes into mammalian chromosomes [11].First approved gene therapy clinical research in the US took place on 24 September 1990,ast the National Institute of Health(NIH),under the direction of Willian French Anderson[12].Four year-old Ashanti DeSilva received treatment for a genetic defect that left her with ADA-SCID, a severe immune system deficiency. The effects were temporary, but successful [13].

Cancer gene therapy was introduced in 1992/93(Trojan *et al*.1993)[14].The treatment of glioblastoma multiforme ,the malignant brain tumor whose outcome is always fatal, was done using a vector expressing antisense IGF-1RNA(clinical trial approved by NIH protocol no 1602 November 24 1993,and by FDA in 1994[15].This therapy also represents the beginning of cancer immonogene therapy, a treatment which proves to be effective due to the anti-tumor mechanism of IGF-I antisense, which is related to strong immune and apoptotic phenomena[15].In 1992, Claudio Bordignon, working at the Vita-Salute San Raffaele University, performed the first gene therapy procedure using hematopoietic stem cells as vectors to deliver genes intended to correct hereditary diseases[16].In 2002 this work led to the publication of the first successful gene therapy treatment for adenosine deaminase deficiency(ADA-SCID).The success of a multi-center trial for treating children with SCID(severe combined immune deficiency or "Bubble boy" disease).The study was questioned when two of ten children in the trial's developed leukemia-like condition. Clinical trials were halted temporarily in 2002,but resumed after regulatory review of the protocol in the US, the United Kingdom, France, Italy and Germany[17].Bottai and associates while working on non-viral gene therapy for breast cancer, advocated that several challenges need to be addressed before considering gene therapy as an actual option for the treatment of breast cancer[18].

In March 2014, researchers reported that 12 HIV patients had been treated since 2009 in a trial with a genetically engineered virus with a rare mutation (CCR5 deficiency) known to protect against HIV with promising results[19].Clinical trials of gene therapy for sickle cell disease were started in 2014[20].There is a need for high quality randomized controlled trials assessing the risks and benefits involved with gene therapy for people with sickle cell disease[21].

In February 2015,LentiGlobin BB 305,a gene therapy treatment undergoing clinical trials for treatment for beta thalassemia gained FDA " breakthrough" status after several patients were able to forgo the frequent blood transfusions usually required to treat the disease[22].In March researchers delivered a recombinant gene encoding a broadly neutralizing antibody into monkeys infected with simian HIV, the monkeys' cells produced the antibody, which cleared them of HIV. The technique is named immunoprophylaxis by gene transfer(IGT).The animal tests for antibodies to Ebola, malaria, influenza and hepatitis were underway[23].In 0ctober,researchers announced that they have treated a baby girl, Layla Richard, with an experimental treatment using donor T-cells genetically engineered using TALEN to attack cancer cells. One year after the treatment she was still free of her cancer (a highly aggressive form of acute lymphoblastic leukemia (ALL)[24].Children with highly aggressive ALL, normally have a very poor prognosis and Layla's disease had been regarded terminal before the treatment[25].In December 2015,scientists of major world academies called for a moratorium on inheritable human genome edits, including those related to CRISPR-Cas9 technologies, but that basic research including embryo gene editing should continue[26,27].

In April 2016 the Committee for Medicinal Products for human Use of the European Medicines Agency endorsed a gene therapy treatment Strimvelis[28],and the European Commission approved in in June[29].This treats children born with ADA-SCID and who have no functioning immune system, sometimes called "bubble baby" disease. This was the second gene therapy treatment to be approved in Europe[30]
.In October, Chinese scientists reported they had started a trial to genetically modify T-cells from 10 adult patients with lung cancer and can reinject the modified T-cell back into their bodies to attack cancer cells. The T-cells had the PD-I protein (which stops or slows the immune response) removed using CRISPR-Cas9[31].A 2016 Cochrane systematic review looking at data from four trials on topical cystic fibrosis transmembrane conductance regulator(CFTR) gene therapy does not support it clinical use as a mist inhaled into the lungs to treat cystic fibrosis patients with lung infections. One of the four trials did find weak evidence that liposome based CFTR gene transfer may lead to a small respiratory improvement for people with cystic fibrosis.This weak evidence is not enough to make a clinical recommendation for routine CFTR gene therapy[32].In February 2017, Kite Pharma announced results from a clinical trial of CAR-T cells in around a hundred people with advanced Non-Hodgkin lymphoma [33].In March, French scientists reported on clinical research of gene therapy to treat sickle- cell disease[34].In August, the FDA approved tisagenlecleucel for acute lymphoblastic lymphoma. The first gene therapy approved in the United States[35].

III. Beginning of Gene Therapy

The first attempt, an unsuccessful one, at gene therapy(as well as the first case of medical transfer of foreign gene into humans not counting organ transplantation) was performed by Martin Cline on 10 July 1980[36].Cline claimed that one of the genes in his patients was active six months later, though he never published his data or had it verified[37].After extensive research on animals throughout the 1980s and a 1989 bacterial gene tagging trial on humans, the first gene therapy widely accepted as a success was demonstrated in a trial that started on 14 September 1990,when Ashi DeSilva was treated for ADA-SCID[38].

The first somatic treatment that produced a permanent genetic change was performed in 1993[39].This procedure was referred to sensationally and somewhat inaccurately in the media as a" three parent baby",

though mtDNA is not the primary human genome and has little effect on an organism's individual characteristics beyond empowering their cells[39]Early clinical failures led to dismissal of gene therapy. Clinical successes since 2006 regained researchers' attention, although ss of 2014,it was still largely an experimental technique[7].These include treatment of retinal diseases Leber's congenital amaurrosis [40],and chorioderemia[41],X-linked SCID[42],ADA-SCID [43], adenoleukodystrophy [44],chronic lymphocytic leukemia(CLL)[45],acute lymphocytic leukemia (ALL{46],multi myeloma [47],hemophilia[48],and Parkinson's disease[49].Between 2013 and April 2014,US companies invested over 600 million in the field[5].
The first commercial gene therapy,Gendicine,was approved in China for the treatment of certain cancers[5].In 2011 Neovasculgen was registered in Russia as first-in-class gene- therapy drug for treatment of peripheral artery disease, including critical limb ischemia[6,].In 2012, Glybera,a treatment for a rare inherited disorder became the first treatment to be approved for clinical use in either in Europe or United States after its endorsement by the European Commission[7].

IV. Application of Gene Therapy and Type of Cell

Following early advances in genetic engineering of bacteria, cells, and small animals, scientists startedconsidering how to apply it to medicine. Two main approaches were considered-replacing or disrupting defective genes [28].Scientists focused on diseases caused by single gene defect, such as cystic fibrosis, haemophilia, muscular atrophy, thassemia, and sickle cell anemia. Glybera treats on such disease, caused by a defect in lipoprotein lipase[8].DNA must be administered, reach the damaged cells, center the cell and either express or disrupt a protein[50].Multiple delivery techniques have been explored. The initial approach incorporated DNA in to an engineered virus to deliver the DNA into a chromosome [51].Naked DNA approaches have also been explored, especially in the context of vaccine development[52].
Generally efforts focused on administering a gene that causes a needed protein to be expressed. More recently, increased understanding of nuclease function has led to more direct DNA editing, using techniques such as zinc finger nuclease and CRISPR.The vector incorporates genes into chromosomes. The expressed nuclease then knock out and replace genes in the chromosome.AS of 2014 these approaches involve removing cells from patients, editing a chromosome and returning the transformed cells to patients [53].Gene editing a potential approach to alter the human genome to treat genetic diseases[54],viral diseases and cancer[55,56].As of 2016 these approaches were still years from being medicine[57].

Types of cell: Gene therapy may be classified into two types, Somatic and Germline.
Somaticcell: In somatic cell therapy (SCGT),the therapeutic genes are transferred into any cell other than a gamete, germ cell, gametocyte or undifferentiated stem cell. Any modifications affect the individual patients only and are not inherited by offspring. Somatic gene therapy represents mainstream basic and clinical research, in which therapeutic DNA (either integrated in the genome or as an external episode or plasmid) is used to treat disease. Over 600 clinical trials utilizing SCGT are underway in the US. Most focus on severe genetic disorders, including immunodeficiencies, hemophilia, thalassemia and cystic fibrosis. Such single gene disorders are food candidates for somatic cell therapy. The complete correction of a genetic disorder or the replacement of multiple genes is not yet possible. Only a few of the trials are in the advanced stages [58].

Germline: In germline gene therapy(GGT),germ cells(Sperm or egg) are modified by introduction of functional genes into their genomes. Modifying a germ cell causes all the organism's cells to contain the modified gene. The change is heritable and passed on to later generations. Australia, Canada, Germany, Israel, and the Netherland prohibit GGT for application in human beings for technical and ethical reasons, including insufficient knowledge about possible risks to future generations[59],and higher risks versus SCGT[60].The US has no federal controls specifically addressing human genetic modification(beyond FDA regulations for therapies in general)[59,61].
Viral vectors: In order to replicate, viruses introduce their genetic materials into host cell, tricking the host's cellular machinery inti using it as blueprints for viral proteins. Retroviruses go a stage further by having their genetic material copied into the genome of host cell. Scientists exploit this by substituting a virus's genetic material with therapeutic DNA(The term DNA' may be an oversimplification, as some viruses contain RNA, and gene therapy could take this form as well).A number of viruses have been used for human gene therapy, including retrovirus, adenoviruses, herpes simplex, vaccinia and adeno-associated virus[3].Non-viral gene therapy for breast cancer also have been used[18].

V. Pitfall and Mortality

Gene therapy is not free of obstacles and mortality. Frequent hindrances are:

a).Some therapies may breach the Weismann barrier(between soma and germ- line) protecting the testes, potentially modifying the germline, falling afoul of regulations in countries that prohibit the latter practice[62].

b).Insertional mutagenesis-If DNA is integrated in sensitive spot in the genome, for example in a tumor suppressor gene, the therapy could induce a tumor. This has occurred in a chemical trials for X-linked severe combined immunodeficiency (X-SCID) patients, in which hemato poietic stem cells were transduced with a corrective transgene using a retrovirus,and this led to the development of T cell lekumia in 3 of 20 patients[63].One possible solution is to add a functional tumor suppressor gene to the DNA to be integrated. This may be problematic since the longer the DNA is, harder it is to integrate into cell genome.CRISPR technology allows researchers to make much more precise genome changes at exact location [64].

c).Cost-Alipogene tiparvovec or Glybera,for example, at a cost of $1.6 million per patient, was reported in 2013 to be world's most expensive drug [65].

Mortality: Three patients' deaths have been reported in gene therapy trials, putting the field under close scrutiny.the first was that of Jesse Gelsinger in 1999.Jesse Gelsinger was dead because of immune rejection response [66].One X-SCID patient died of leukemia in 2003[38].In 2007,a rheumatoid arthritis patient died from an injection, the subsequent investigation concluded that the death was not related to gene therapy[67].

VI. Gene therapy in clinical practice

Frequently uses of gene therapy include**:**

Fertility: Gene therapy techniques have the potential to provide alternative treatments for those with infertility. Recent, successful experimentation on mice has proven that fertility can be restored by using the gene therapy method, CRISPR[68].Spermatogenical stem cells from another organism were transplanted into the testes of an infertile mouse. The stem cells re-established spermatogenesis and fertility[69].

Gene doping: Athletes might adopt gene therapy technologies to improve their performance[70].Gene doping is not known to occur, but multiple gene therapies may have such effects. Kayser *et al*. argue that gene doping could level the playing field if all athletes receive equal access. Critics complain that any therapeutic intervention for non-therapeutic/enhancement compromises the ethical foundations of medicine and sports [71].

Genetic engineering: Genetic engineering could be used to change physical appearance, metabolism, and even improve physical capabilities and mental facilities such as memory and intelligence. Ethical claims about germline engineering include beliefs that every fetus has right to remain genetically unmodified, that parents hold the right to genetically modify their offspring, and that every child has right to be born free of preventable diseases[72].For parents, genetic engineering could be seen as another child enhancement technique to add to diet, exercise, education, training, cosmetic and plastic surgery[73].Another theorist claims that moral concern limit but not prohibit germline engineering[74].

Possible regulatory schemes include a complete ban, provision to everyone, or professional self-regulation. The American Medical Association's Council on Ethical and Judicial Affairs stated that "genetic interventions to enhance traits should be considered permissible only in severely restricted situations (1) clear and meaningful benefits to the fetus or child (2) no trade-off with other characteristics or traits, and (3) equal access to the genetic technology, irrespective of income or other socioeconomic characteristics"[75].

As early in the history of biotechnology as 1990, there have been scientists opposed to attempts to modify the human germline using these tools[76], and such concerns have continued as technology progressed[77].With the advent of new techniques like CRISPR, in March 2015 a group of scientists urged a worldwide moratorium on clinical use of gene editing technologies to edit the human genome in a way that can be inherited[78].In April 2015,researchers sparked controversy when they reported results of basic research to edit the DNA of non-viable human embryos using CRISPR[68].A committee of the American National Academy of Sciences and National Academy of Medicine gave qualified support to human genome editing in 2017[79], once answers have been found and efficiency problems "but for serious conditions under stringent oversight"[80].

Gene therapy in cancer: Introduction of tumor suppressor genes into rapidly dividing cells has been thought to slow down or arrest tumor growth. Adenoviruses are commonly utilized vector for this purpose. Much research has been focused on the use of adenoviruses that cannot reproduce, or reproduce to a limited extent, within the patient to ensure safety via avoidance of cytolytic destruction of noncancerous cells infected with the vector. However, new studies focus on adenoviruses that can be permitted to reproduce, and destroy the cancerous cells in the process, since the adenoviruses' ability to infect normal cells is substantially impaired, potentially resulting in a far more effective treatment [81].

Microorganisms in cancer therapy: Chemotherapeutic drugs have a hard time penetrating tumors to kill them at their core because these cells may lack a good blood supply. Researchers have been using anaerobic bacteria such as *Clostridium novyi*, to consume the interior of oxygen-poor tumors. These should die when they come in contact with the tumor's oxygenated sides, meaning they would be harmless to the rest of the body .A major problem has been that bacteria do not consume all parts of the malignant tissue. However, combining the therapy with chemotherapeutic treatment can help to solve this problem. Another strategy is to use anaerobic bacteria that have been transformed with an enzyme that can convert a non-toxic prodrug into toxic drug. With the proliferation of bacteria in the necrotic and hypoxic areas of the tumor, the enzyme is expressed solely in the tumor. Thus, asystematically applied prodrug is metabolized to the toxic drug only in the tumor. This has been demonstrated to be effective with the nonpathogenic an aerobe *Clostridium sporogenes* [82].

VIII. Guidelines for human gene research and therapy

The Helsinki Declaration (Ethical Principles for Medical Research Involving Human Subjects)was amended by the world Medical Association's General Assembly in 2008.This document provides principles physicians and researchers must consider when involving human as research subjects. The Statement on Gene Therapy Research initiated by the Human Genome Organization (HUGO) in 2001 provides a legal baseline for all countries. Hugo's document emphasizes human freedom and adherence to human rights, and offers recommendations for cosmetic gene therapy, including the importance of recognizing public concern about such research [83].

An NIH advisory committee published a set of guidelines on gene manipulation[84].The guidelines discuss lab safety as well as human test subjects and various experimental types that involve genetic changes. Several sections specifically pertain to human genetic engineering, including Section III-C-I. This section describes required review processes and other aspects when seeking approval to begin clinical research involving transfer into a human patient[85].The protocol for a gene therapy clinical trial must be approved by the NIHs Recombinant DNA Advisory Committee prior to any clinical trial beginning this is different from any other kind of clinical trial[84].As with other kinds of drugs, the FDA regulates the quality and safety of gene therapy products and supervises how these products are used clinically. The therapeutic alteration of human genome falls under the same regulatory requirements as any other medical treatment. Research involving human subjects, such as clinical trials, must be reviewed and approved by FDA and institutional Review Board[86].

XI. Conclusions

The current available advanced technology made gene therapy possible and successful. It is hoped that in the coming year's gene treatment will be available for every disease like cancer, diabetes mellitus, and infectious diseases etc. Researchers look forward that soon all difficulties and pitfalls using gene therapy like vector selection, gene expression control as well as ethical issues will be resolved resulting in successful treatment and cure for most of the human diseases.

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