INDO AMERICAN JOURNAL OF PHARMACEUTICAL RESEARCH

GENE THERAPY IN CANCER

Manik Islam [1*], Humaiara Shahrin[2]

[1]*Department of pharmacology, Jawaharlal Nehru Technological University, Hyderabad, India.*

[2]*Department of Pharmaceutics, Asia Pacific University (APU).*

ARTICLE INFO

Article history

Received 15/02/2017

Available online

08/03/2017

Keywords

Gene Therapy,

Basic Requirements for Gene Therapy,

Gene Therapy Vectors,

Gene Therapy for Cancer.

ABSTRACT

Gene therapy is a kind of experimental technique that uses genes to treat or prevent disease. In the future, these methods may allow doctors to treat a disorder by inserting a gene into a patient's as a alternative way of surgery**.** Although gene therapy is a promising treatment option for a number of diseases (including inherited disorders, some types of cancer, and certain viral infections), the technique remains risky and is still under study to make sure that it will be safe and effective. Gene therapy is currently only being tested for the treatment of diseases that have no other cures. At first gene treatment was imagined for treating genetic problems, but is presently being researched in an array of disorders, such as cancer, peripheral vascular illness, arthritis, neurodegenerative disorders along with other procured conditions. In spite of the slow clinical progress, efforts to develop specific nontoxic cancer therapies are increasing exponentially with the result that over 500 gene therapy trials have been listed with the FDA to date .A number of strategies are currently being pursued in cancer treatment, aiming to either. In this article it has demonstrated that the rule of gene therapy for the treatment of cancer and different vectors are used to fascinate this therapy.

Corresponding author

Manik Islam

Sri Lakshmi International Hostel, RVS Nagar,

Thirupati Road,

Chittoor District, Andhra Pradesh, 517127

islammanik668@gmail.com

+919666371352

*Please cite this article in press as **Manik Islam** et al. Gene Therapy in Cancer. Indo American Journal of Pharmaceutical Research.2017:7(02).*

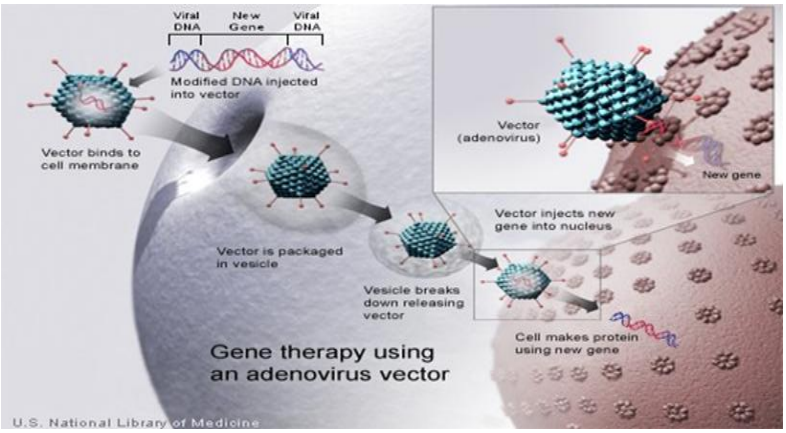
INTRODUCTION

Cancer causes one in seven deaths worldwide, making it one of most important challenges in the world of Biotechnology today [1]. Current cancer therapy including, chemotherapy, radiation therapy, have several side effects and open prove ineffective at completely eradicating malignant cell. A more selective method for targeting tumor cell is needed [2,3]. In particular, gene therapy holds great potential to selectively target cancer cells, destroying the cancer while leaving healthy tissues intact. In order to develop gene therapy treatment, two main obstacles must be overcome: a therapeutic agent must be developed a facilitate genetic changes, and a method must be optimized to deliver the therapeutic agent to the target cell. Recent advancement in both the design of the therapeutic agent and the delivery methods allow change in both genomic and in gene expression to be achieved in target cell with a high degree of accuracy and efficiency [4]. The review highlight several viral and non-viral vectors are commonly used for the functioning of gene therapy in cancer.

Gene therapy

Gene therapy is a kind of therapy that consists of inserting a number of corrective genes which have been made in the actual laboratory, to the genetic material of the patient's tissue to remedy a genetic disorder. Based on “Gene therapy” (2008), “Gene therapy is really a strategy for fixing defective genes liable for illness development.” The concept regarding gene therapy would be to target the actual genes from the cell that are the origin from the problem. A gene is comprehended to function as the building block of living creatures [5].

Gene treatment works on controlling the genes from the cells to be able to improve or restrain protein features, hence managing their department cycles. It changes the hereditary material from the cells as well as causes the actual cells in order to either return back for their initial form or damage themselves (Cross & Burmester, 2006) [6]. There are different ways gene therapy can be achieved. The three most typical types associated with gene treatment involve updating the faulty gene having a normal gene, altering the actual gene to return it to normal perform, and managing the gene’s legislation (Cross & Burmester, 2006) [7]. Gene legislation is realized as a normal process completed by your body itself. It's accountable for maintaining the correct functions from the cell. It may improve cell exercise or control it with respect to the body requirements. This regulating function is missing by cancer cells as well as gene therapy enables you to attain it.



Gene therapy using an adenovirus vector: A new gene is injected into an adenovirus vector, which is used to introduce the modified DNA into a human cell. If the treatment is successful, the new gene will make a functional protein (8,9).

Uses of gene therapy

Gene therapy is being used in many ways. For example, to: Replace missing or defective genes; Deliver genes that speed the destruction of cancer cells; Supply genes that cause cancer cells to revert back to normal cells; Deliver bacterial or viral genes as a form of vaccination; Provide genes that promote or impede the growth of new tissue; and; Deliver genes that stimulate the healing of damaged tissue. A large variety of genes are now being tested for use in gene therapy. Examples include: A gene for the treatment of cystic fibrosis (a gene called CFTR that regulates chloride); Genes for factors VIII and IX, deficiency of which is responsible for classic hemophilia (hemophilia A) and another form of hemophilia (hemophilia B), respectively; Genes called E1A and P53 that cause cancer cells to undergo cell death or revert to normal; AC6 gene which increases the ability of the heart to contract and may help in heart failure; and VEGF, a gene that induces the growth of new blood vessels (angiogenesis) of use in blood vessel disease. A short synthetic piece of DNA (called an oligonucleotide) is being used by researchers to "pre-treat" veins used as grafts for heart bypass surgery. The piece of DNA seems to switch off certain genes in the grafted veins to prevent their cells from dividing and thereby prevent atherosclerosis [10].

BASIC REQUIREMENTS FOR GENE THERAPY

POTENTIAL OF GENE THERAPY

Gene therapy provides a brand new treatment paradigm for healing human illness. Rather than changing the illness phenotype by utilizing agents which have interaction with gene items, or tend to be themselves gene items, gene treatment can theoretically alter specific genes leading to disease remedy following just one supervision (11). At first gene treatment was imagined for treating genetic problems, but is presently being researched in an array of disorders, such as cancer, peripheral vascular illness, arthritis, neurodegenerative disorders along with other procured conditions. [12].

GENE IDENTIFICATION & CLONING

Despite the fact that the variety of gene treatment strategies is very distinct, certain key components are necessary for an effective gene therapy technique (13). Probably the most fundamental of these types of is how the appropriate gene should be determined and cloned. Upon conclusion of the actual Human Genome Task, gene availability is going to be limitless, but until then your starting point for just about any gene treatment strategy continues to be gene id and cloning with regard to relevant genes associated to the condition [14].

GENE TRANSFER & EXPRESSION

When the gene may be determined and cloned, the following concern must end up being expression. Questions regarding the effectiveness of gene transport and gene phrase remain in the headlines of gene therapy research (15). Presently much discussion in the actual field associated with gene therapy orbits around the actual transfer of preferred genes to correct cells, after which getting sufficient amounts of expression with regard to disease remedy (16,17). Hopefully, future investigation on gene move and tissue-specific gene phrase will solve these issues within the vast majority of gene therapy methods. Additional important factors for the gene therapy strategy consist of: a sufficient knowledge of the pathogenesis from the targeted condition, potential unwanted effects of the actual gene treatment, and knowledge of the focus on cells to get the gene treatment [18].

Gene delivery and activation

For many disorders, gene therapy works only if we are able to deliver a ordinary gene to a lot of cells-say a number of million-in the tissue. And they need to the proper cells, within the proper tissue. When the gene gets to its location, it should be initialized, or switched on, to help to make the proteins it encodes. And when it's switched on, it must stick to; cells possess a habit associated with shutting lower genes which are too energetic or demonstrating other uncommon behaviors [19]. Introducing changes to the wrong tissue targeting the gene towards the correct cells is vital to the achievement of any kind of gene therapy treatment method. Just like important, although, is ensuring the gene isn't integrated into the incorrect cells. Delivering the gene towards the wrong tissue will be ineffective; also it could trigger health troubles for the individual. For instance, incorrect targeting might incorporate the actual therapeutic gene right into a patient's tiniest seed line, or reproductive: cells that ultimately develop sperm as well as eggs. Ought to this occur, the individual would move the launched gene to his / her children. The effects would differ, depending about the gene [20].

Gene Therapy Successes

Researchers have been working for decades to create gene therapy towards the clinic, yet not many patients have obtained any efficient gene-therapy remedies. But that does not mean gene therapy is definitely an impossible desire. Even although gene therapy may be slow to achieve patients, its future is extremely motivating. Decades associated with research possess taught us a great deal about creating safe as well as effective vectors, targeting various kinds of cells, as well as managing as well as minimizing defense responses within patients. We've also realized a lot concerning the disorder genes on their own. Today, numerous clinical tests are underway, where scientists are very carefully testing remedies to assure that any kind of gene treatment brought to the clinic is actually both secure and helpful. Here are some gene treatment success tales. Successes represent a number of approaches-different vectors, various target cellular populations, and each in vivo as well as ex vivo approaches-to treating a number of disorders [21].

Immune deficiencies

A number of inherited immune insufficiencies have already been treated effectively with gene treatment. Most generally, blood originate cells are taken out from sufferers, and retroviruses are utilized to provide working copies from the faulty genes. Following the genes happen to be delivered, the originate cells tend to be returned towards the patient. Since the cells tend to be treated away from patient's entire body, the computer virus will invade and move the gene in order to only the preferred target tissue. Severe Mixed Immune Insufficiency (SCID) was among the first hereditary disorders to become treated effectively with gene treatment, proving how the approach might work. Nevertheless, the very first clinical tests ended once the viral vector induced leukemia (a kind of blood cancer) in certain patients. Since that time, researchers have initiated trials along with new, safer virus-like vectors which are much not as likely to trigger cancer [22].

Blood disease

Sufferers with beta-Thalassemia possess a defect within the beta-globin gene, which codes to have oxygen-carrying proteins in red blood cell. Because from the defective gene, patients do not have enough red blood cells to transport oxygen to any or all the body tissues. Many who've this disorder rely on blood transfusions with regard to survival. Within 2007, someone received gene treatment for serious beta-Thalassemia. Blood originate cells were obtained from his bone fragments marrow as well as treated having a retrovirus in order to transfer a functional copy from the beta-globin gene. The actual modified originate cells had been returned in order to his entire body, where these people gave increase to wholesome red bloodstream cells. Seven years following the procedure, he had been still succeeding without bloodstream transfusions. An identical approach might be used to deal with patients along with sickle cellular disease [23].

Cancer

Several promising gene-therapy treatments are under development for cancer. One, a modified version of the herpes simplex 1 virus (which normally causes cold sores) has been shown to be effective against melanoma (a skin cancer) that has spread throughout the body. The therapy, known as T-VEC, utilizes the virus that's been altered in order that it may (1) not cause cold sores; (2) destroy just most cancers tissue, not healthy ones; as well as (3) help to make indicators which captivate the actual person's personal defense tissue, assisting all of them learn how to identify as well as battle most cancers tissue all over your body. Herpes is actually inserted straight into the actual person's growths. This replicates (makes much more associated with itself) within the most cancers tissue till these people burst open, liberating much more infections that may invade extra most cancers tissue [24].

Parkinson's disease

Patients with Parkinson's disease gradually lose cells in the brain that produce the signaling molecule dopamine. As the disease advances, patients lose the ability to control their movements.

A small group of patients with advanced Parkinson's disease were treated with a retroviral vector to introduce three genes into cells in a small area of the brain. These genes gave cells that don't normally make dopamine the ability to do so. After treatment, all of the patients in the trial had improved muscle control [25].

Gene Therapy Types

Gene therapy may be classified into the following types:

Germ line gene therapy

Regarding germ collection gene treatment, germ tissue, i. at the., sperm or even eggs, are modified through the introduction associated with functional genetics, which tend to be ordinarily built-into their genomes (26). Consequently, the change because of therapy will be heritable and will be passed onto later decades. This brand new approach, in theory, should be impressive in counteracting hereditary disorders as well as hereditary illnesses. However, many jurisdictions stop this with regard to application in people, at least for that present, for a number of technical as well as ethical factors (27). Although it rarely has already been tested upon humans, some various transgenic techniques happen to be used upon other varieties, which range from the following [28]

(1)Gene delivery to the nuclei taken from somatic cells at metaphase stage.

(2)Ex vivo alteration of egg cells, following in vitro fertilization.

(3)Manipulation of embryonic stem cells of mouse during in vitro culture by different gene delivery systems.

(4)Pronuclear microinjection of exogenous DNA solution by a glass needle.

(5)Transgenic delivery into sperm cells by direct or indirect injection to testis or other parts of the genital system.

Somatic gene therapy

Regarding somatic gene treatment, the therapeutic genes are transferred into the somatic cells of a patient. Any adjustments and effects is going to be restricted towards the individual only, and won't be inherited through the patient's children or later on generations [29].

There are 3 types of somatic gene therapy

Ex vivo delivery

With this system the actual genetic materials is explanted in the target cells or bone fragments marrow, grown and altered in vitro, after which transduced and/or transfected to the target cells. There tend to be no immunologic difficulties in by doing this but just the method is used where the focus on cells behave as protein release sources (like treating ADA or even hemophilia) or like a vaccine with regard to cancer remedy, so you will find major restrictions on using ex vivo shipping. In add-on, at existing only half the normal commission of replanted tissue remains practical. [30].

In situ delivery

The management of the actual genetic material immediately into the prospective tissue is within situ shipping. As the majority of the recent delivery techniques need no efficient targeting, the way in which is correct. The system may be employed in the actual delivery associated with CFTR gene through lipid as well as adenoviral vectors to some specific site within the respiratory tract and it is used in treating different cancer. However, low effectiveness of transduction may be the main problem of the system, simply because in most cancers therapy one cancerous cell may re-establish the actual tumor once again [31].

In vivo delivery

The transport of hereditary material with an proper vector, which may be a virus-like or non-viral vector, to the target tissue is within vivo shipping. This method is minimal advanced technique at existing but potentially it may be the best. The problem of the way is inadequate targeting associated with vectors towards the correct cells sites; nevertheless, enhancement in focusing on and vector improvement will resolve the issue [32].

Table no 1: Ideal gene transfer vector.

Sr. No.	Insert size	One or more genes
1	Titer	High concentration/ stable end product
2	Targeted	Yes, target either entry into specific cells or limit expression to target cells
3	Immune Response	None, safe for recipient and environment
4	Stable	Yes, free of insertional mutagenesis
5	Production	Easy / reproducible
6	Regulatable	Yes, levels of transgene expression can be up or down regulated as needed

Features of viral vector systems for their application in gene therapy

Viral vector: Advantages of Retroviral vectors

Insert capacity for transgene <7-8kb, stable integration into host DNA, recombinant virus titers of 106-107 pfu/ml, broad cell tropism of infectivity, relatively easy manipulation of viral genome for vector engineering.

Disadvantages of Retroviral vectors

Difficult targeting of viral infection, no infection of non- dividing cells, random integration into host genome, instability of vectors.

Advantage of Lentiviral vectors

Infect dividing and non-dividing cells stable gene expression, insert capacity of 10kb

Disadvantage of Lentiviral vectors

Potential insertion mutagenesis, presence of regulatory (tat, rev) and of accessory protein sequences in the packaging constructs

Advantage of Adenovirus vectors

Generation of high virus titers of 1010 pfu/ml, high level gene expression, large Generation of high virus titers of 1010 pfu/ml, high level gene expression, large insert capacity (7-8kb), infects dividing and non-dividing cells

Disadvantage of Adenovirus vectors

Immune response to viral proteins, no integration into host genome, transient gene expression

Advantage of AAV (adeno-associated virus**) vectors**

Infect dividing and non-dividing cells, broad cell tropism, potential of targeted integration, low immunogenicity and nonpathogenic

Disadvantage of AAV vectors

Limited capacity for transgenes (4kb), difficult generation of high virus titers, requirement of adenovirus or herpes virus for AAV replication.

Advantage of Herpes virus vectors

Infects a wide variety of cell types, high insertion capacity (up to 50 kb), natural tropism to neuronal cells stable viral particles allow generation of high virus titers (1012 pfu/ml).

Disadvantage of Herpes virus vectors

Possible toxicities, risk of recombination, no viral integration into host DNA.

Advantage of Poxvirus vectors

High insertion capacity, insertion of large DNA fragments possible high transgene expression level, suited for live recombinant vaccine.

Disadvantage of Poxvirus vectors:

Potential cytopathic effects

Advantage of Epstein-Barr virus vectors
Infects dividing and non-dividing cells with preference for B-cells, high insert capacity (<150kb)

Disadvantage of Epstein-Barr virus vectors:
Difficult access to packaging cell lines

Non-viral methods

Non-viral techniques can existing certain benefits over viral strategies, for example large scale generation and reduced host immunogenicity. Formerly, low amounts of transfection as well as expression from the gene kept non-viral methods in a disadvantage; nevertheless, recent improvements in vector technologies have produced molecules as well as techniques that strategy the transfection efficiencies associated with viruses. There are many techniques for non-viral gene treatment, including the actual injection of exposed DNA, electroporation, the actual gene weapon, nonoperation, magnetofection, and using oligonucleotides, lipoplexes, dendrites, as well as inorganic nanoparticles [33].

Table No 2: Comparison of Commonly Used Vectors for Gene Transfer.

Vehicle	Advantages	Disadvantages
Nonviral		
Naked DNA	Ease of production	Low efficiency
	No DNA size limitation	Transient expression
Liposome-DNA	Ease of production	Low efficiency
	Low efficiency	Transient expression
	Low immune reaction	
Viral		
Retrovirus	Ease of production	Transfer to dividing cells only
	Efficient DNA transfer	Random DNA integration
	Stable expression	DNA transfer size limited
Adenovirus	Low immune reaction	Host immune reaction
	Ease of production	Transient expression
	Efficient DNA transfer	DNA transfer size limited
	Transfer to no dividing cells	
Adeno-associated	Prolonged expression	Difficult production
	Transfer to no dividing cells	Limited insert size

The potential power of gene therapy

Due to the fact of its reliability, gene therapy has got the potential to remove cancer tissue without harmful normal, wholesome tissue. In addition, cancer gene treatments may supply alternatives whenever a disease doesn't respond in order to other old treatments. The possible of gene therapy is excellent but, in comparison to its promise, the outcomes to date continue to be quite restricted. Nevertheless, the advantages of gene treatment are thought to be about the near horizon. Gene therapy is among the hottest regions of medical investigation today. (And gene treatment companies happen to be among the greatest in the stock exchange.) The actual remarkable improvements in inherited genes, including the actual human genome task, have exposed new doors for that exploration associated with gene treatment. New systems are required to pace the improvement of gene treatment. As these types of new technologies like the "bionic chip" appear, we think that, without any doubt, gene treatment will play an ever more important as well as prominent component in medicine within the many years to arrive [34].

GENE THERAPY FOR CANCER

Most cancers cells would be the most substantially considered target with regard to gene treatment, because many malignancies are unresponsive to conventional therapy and rapidly fatal. In comparison to inherited diseases, cancer-related gene therapy isn't specifically directed towards correction associated with genetic mutations but additionally uses gene supply to focus on a healing biological agent towards the cancer cellular [35]. A number of highly innovative therapeutic methods designed for gene treatment for most cancers include: (1) addition of the wild-type growth suppressor gene to check a mutant growth suppressor gene; (2) antisense RNA ways of ‘‘turn off’’ expression of the oncogene; (3) transfer of the gene to improve immunogenicity from the tumor through expression of the immunomodulation gene or cytokine gene in the tumor; (4) transfer of a gene coding for a ‘‘prodrug’’ to the tumor, leading to tumor- specific cell killing by production of a toxic metabolite; (5) inhibition of tumor angiogenesis; and (6) chemo protective genes transferred to save patients’ hematopoietic cells from chemotherapy-induced toxicity[36]. Numerous gene therapy methodologies to day have focused upon remedies for most cancers.

Though numerous cancers possess a genetic predisposition, all of them include acquired mutations, & because they advance their tissue become much less differentiated & much more heterogeneous with regards to the mutations these people carry. In common cancers have a minimum of one mutation to some proto-oncogene (yielding a good oncogene) & a minimum of one to the tumor suppressor gene, permitting the most cancers to proliferate. The number of various cancers experienced & the actual mutations these people carry, have resulted in a number of methods for gene treatment namely; immunopotentiation, oncogene inactivation, tumor suppressor gene alternative, molecular chemotherapy & medication resistance genetics [37]. The purpose of immunopotentiation would be to increase the response from the immune in order to cancers, thereby resulting in their devastation. Passive immunotherapy aims to improve the pre-existing immune reaction to the actual cancer although active immunotherapy triggers a defense response towards an unrecognized or even poorly antigenic tumor. Passive immunotherapy usually involves harvesting tumor infiltrating lymphocytes & treating them to express increased cytokines e. g. IL-2 & TNF-alpha.

The cell population is then expanded in vitro & returned to the patient. Tumor cells are used for active immunotherapy, genetically modifying them to increase expression of antigen presenting molecules/stimulatory molecules, local concentrations of cytokines (e. g. IL-2; Lemig et al, 1996) or tumor antigens (erbB2 oncoprotein; Disis et al, 1994). The cells are then irradiated prior to being returned to the patient, preventing the reintroduction of replication competent tumor cells. These approaches have been termed cancer vaccines [38]. Oncogene inactivation uses the same techniques employed for dominantly inherited monogenic diseases. The oncogene may be targeted at the level of the DNA, RNA transcription or protein product. Oligodeoxynucleotides are short single stranded pyrimidine rich DNA sequences that form a triple helix with purine rich double stranded DNA sequence. Oligodeoxynucleotides are designed in a sequence specific manner to target the promoter regions of oncogenes. At the RNA level antisense techniques prevent transport & translation of the oncogene mRNA by providing a complementary RNA molecule (e. g. c-myc; Collins et al, 1992). Ribozymes, antisense oligoribonucleotides with a cleavage action, will also reduce the stability of oncogene mRNA. Transport of the oncogene product to the cell surface can be prevented by a single chain antibody with specificity for the oncogene product & a localization signal for the endoplasmic reticulum. Despite multiple genetic abnormalities, restoration of the tumor suppressor gene, such as p53, can be sufficient to cause cellular apoptosis & arrest tumor growth.

Moreover, expression of p53 is synergistic with chemotherapeutic drugs such as cisplastin & adjacent tumor cells that have not been transduced are killed, in what is termed the bystander effect. The p53 gene has been identified as important in a range of cancers & though less elegant than oncogene inactivation techniques, this method has proved robust enough to be included in a number of clinical trials. Other tumor suppressor genes may also prove useful e. g. BRCA1sv [39]. An alternative means of killing a tumor cell is to transduce a gene coding for a toxic product, known as molecular chemotherapy. The gene of choice is usually herpes simplex virus thymidine kinase (HSV/TK) which converts the prodrug ganciclovir into toxic metabolites. The effected cell is supported via the gap junctions of adjacent cells, until the toxin burden is too great killing both the affected cell & its neighbors. An advantage to this system is that all the transduced cells will be killed, allowing allogeneic tumor cells to be prepared in advance, Oldfield & Ram, 1995). HSV/TK is also used in other gene therapy protocols, allowing the treatment to be aborted at any time. Many cancer chemotherapy regimens are limited by their low therapeutic index, as determined by hematopoietic cells. By harvesting precursor bone marrow cells & transducing them with the gene MDR1, coding for the drug efflux protein p170, a population of resistant cells can be cultured. When returned to the patient, much higher doses of chemotherapy may be used [40].

CONCLUSION

There is no doubt that gene therapy has revolutionized the treatment process for cancer patients. Gene therapy has taken away many obstacles and side effects that were part of cancer treatment in older techniques such as chemotherapy and radiation. The information given in this paper clearly explains that gene therapy is a much more promising technique with which to approach this disease. However, there are some aspects of this technique that still need to be worked on. Some kinds of therapies still need to pass pre-clinical trials and some of them are being limited to testing on laboratory animal models. However, scientists and physicians believe that soon these therapies will be allowed to be used in clinics as part of cancer treatment. They also believe that they are at the beginning of a new era and that gene therapy will lead them to a treatment that is effective and free of side effects. This paper proposes that gene therapy is the future of cancer treatment and that humans are very close to the time when cancer can be cured effectively. Cancer patients throughout the world are counting on gene therapy to give them a new life by curing their disease.

REFERENCE

1. Wu, Xudong and Guohui Li. "Prevalent Accumulation Of Non-Optimal Codons Through Somatic Mutations In Human Cancers". *PLOS ONE* 11.8 (2016): e0160463.
2. "Gene Expression; Posttranscriptional Modifications (2C-01 - 2C-09)". *Genes & Genetic Systems* 79.6 (2004): 407-409.
3. "Somatic Mutation, Cellular Differentiation, And Cancer Causation". *JNCI: Journal of the National Cancer Institute* (1981): n. pag.
4. "412. Phase L-Lla Gene Therapy Protocol For LGMD 2C: Clinical Strategy And Implications". *Molecular Therapy* 15 (2007): S159. Web.
5. "80. Identification Of Genetic Insulator Elements To Increase The Safety Of Viral Gene Therapy Vectors". *Molecular Therapy* 17 (2009): S33.
6. Saey, Tina Hesman. "Genes & Cells: Breast Cancer Gets Gene Profile: Data Reveal Tumor Origins And May Improve Treatment". *Science News* 182.8 (2012): 8-8.
7. "Medulloblastoma Treatment Using Gene Therapy". *Journal of Gene Therapy* 1.1 (2013): n. pag.
8. Boyd, Alan and Mark Curtis. "Cell & Gene Therapy Commercial Insight – May 2016". *Cell and Gene Therapy Insights* 2.2 (2016): 153-168.
9. Brenner, Malcolm K. "Gene-Modified Cells For Stem Cell Transplantation And Cancer Therapy". *Human Gene Therapy* 25.7 (2014): 563-569.
10. "582. Mesenchymal Stem Cells Do Not Prevent Alloantibody Response Against IDUA Used To Treat MPSI". *Molecular Therapy* 21 (2013): S223.
11. Islam, Manik. "Medulloblastoma Treatment Using Gene Therapy". *Journal of Gene Therapy* 1.1 (2013): n. pag. Web.
12. Cheng, Jianjun and Alan P. Kozikowski. "We Need 2C But Not 2B: Developing Serotonin 2C (5-HT 2C) Receptor Agonists For The Treatment Of CNS Disorders". *ChemMedChem* 10.12 (2015): 1963-1967.
13. Palù, Giorgio. "Combined Strategies For Gene Therapy Of AIDS". *Gene Therapy* 4.3 (1997): 179-180. Web.
14. Khalili, Kamel, Carlos Salas, and Roberte Weinmann. "Isolation And Characterization Of Human Actin Genes Cloned In Phage Lambda Vectors". *Gene* 21.1-2 (1983): 9-17.
15. Vassaux, G, A L Manson, and C Huxley. "Copy Number-Dependent Expression Of A YAC-Cloned Human CFTR Gene In A Human Epithelial Cell Line". *Gene Therapy* 4.6 (1997): 618-623. Web.
16. "644. Differential Gene Expression After Adenovirus-Mediated P16 Gene Transfer In Human Non-Small Cell Lung Cancer Cells". *Molecular Therapy* 5.5 (2002): S211.
17. "Further Advances In Immuno-Gene Therapy Against Human Papillomavirus By Adeno-Associated Virus Gene Transfer Into Dendritic Cells". *Molecular Therapy* 9 (2004): 222. Web.
18. Mouradian, M M and T N Chase. "Gene Therapy For Parkinson's Disease: Current Knowledge And Future Perspective". *Gene Therapy* 4.6 (1997): 504-506.
19. "Track 2C: Cellular Mechanisms And Pathophysiology - Adipose Tissue Plasticity And Gene Expression". *International Journal of Obesity* 28 (2004): S98-S104.
20. Eriksson, J.G. "2C-3 Gene Early Environment Interaction And The Metabolic Syndrome". *Early Human Development* 83 (2007): S36.
21. Gray SJ, Woodard KT, Samulski RJ. Viral vectors and delivery strategies for CNS gene therapy. *Therapeutic delivery*. 2010;1(4):517-534.
22. Emilien, G., et al. "Impact of genomics on drug discovery and clinical medicine." *Qjm* 93.7 (2000): 391-423.
23. Walters, LeRoy, and Julie Gage Palmer. *The ethics of human gene therapy*. Oxford University Press, USA, 1997.
24. Sivendran, Shanthi, et al. "Herpes simplex virus oncolytic vaccine therapy in melanoma." *Expert opinion on biological therapy* 10.7 (2010): 1145-1153.
25. Lindvall, Olle, and Anders Björklund. "Cell therapy in Parkinson's disease." *NeuroRx* 1.4 (2004): 382-393.
26. Dawkins, Richard. *The extended phenotype: The long reach of the gene*. Oxford University Press, 2016.
27. Betta, Michela. *The Moral, Social, and Commercial Imperatives of Genetic Testing and Screening*. Springer, 2006.
28. Baker, Duncan EC, et al. "Adaptation to culture of human embryonic stem cells and oncogenesis in vivo." *Nature biotechnology* 25.2 (2007): 207-215.
29. Falek, A. "Conceptual, methodological and ethical issues in genetic engineering (1989)." *Human Evolution* 5.2 (1990): 195-206.
30. Merdan, Thomas, Jindrich Kopeček, and Thomas Kissel. "Prospects for cationic polymers in gene and oligonucleotide therapy against cancer." *Advanced drug delivery reviews* 54.5 (2002): 715-758.
31. Maxted, Nigel, Brian V. Ford-Lloyd, and John Gregory Hawkes. *Plant genetic conservation: the in situ approach*. Springer Science & Business Media, 2013.
32. Nayerossadat, Nouri, Talebi Maedeh, and Palizban Abas Ali. "Viral and nonviral delivery systems for gene delivery." *Advanced biomedical research* 1.1 (2012): 27.
33. Hahn, Peter, and Elizabeth Scanlan. "Gene delivery into mammalian cells: an overview on existing approaches employed in vitro and in vivo." *Nucleic Acid Transfection*. Springer Berlin Heidelberg, 2010. 1-13.
34. Primrose, Sandy B., and Richard Twyman. *Principles of gene manipulation and genomics*. John Wiley & Sons, 2013.
35. Straw, Deborah. *The Healthy Pet Manual: A Guide to the Prevention and Treatment of Cancer*. Inner Traditions/Bear & Co, 2005.

36. Herrmann, F. "Cancer gene therapy: principles, problems, and perspectives." *Journal of molecular medicine* 73.4 (1995): 157-163.
37. Lagasse, E. "Cancer stem cells with genetic instability: the best vehicle with the best engine for cancer." *Gene therapy* 15.2 (2008): 136-142.
38. Immunother, J. "International Society for Biological Therapy of Cancer." *J Immunother* 33.8 (2010).
39. Roth, Jack A., Stephen G. Swisher, and Raymond E. Meyn. "p53 tumor suppressor gene therapy for cancer." *ONCOLOGY-WILLISTON PARK THEN HUNTINGTON*- 13 (1999): 148-154.
40. BODINE, DAVID M. "Hematopoietic Stem Cell Gene Therapy: Progress qnd Prospects." *Hematopoiesis: A Developmental Approach* (2001): 130.

5487847845**1170220**