GENE THERAPY FOR ORAL CANCER - JOURNEY TO A NEW HORIZON

Arun Singh[1] Manjunath Badni[2] Anil Singh[3] Fahad M Samadi[4] Arpita Kabiraj[5]

[1]Lecturer, Department of Oral Pathology & Microbiology, Kothiwal Dental College, Moradabad
[2]Reader, [3]Prof & Head, [4]Lecturer, [5]PG Student, Department of Oral Pathology & Microbiology, Sardar Patel Post Graduate Institute of Medical & Dental Sciences, Lucknow. India

Corresponding Author: Arun Singh, Lecturer, Department of Oral Pathology & Microbiology, Kothiwal Dental College, Moradabad

Abstract

The past two decades have been golden years for the genetics of cancer. It has become clear through the work of countless laboratory groups that both inherited and sporadic cancers arise through defects or misregulations of their genomes. Despite advances in surgery, radiotherapy, and chemotherapy, the survival of patients with oral squamous cell carcinoma have not significantly improved over the past several decades. Thus, an entirely new approach to its treatment utilizing genetic aids has evolved. The majority of the head and neck cancers comprise of Oral squamous cell carcinoma (OSCC). The traditional therapies for the management of cancer and their various modifications including surgery, radiotherapy and chemotherapy have not refined the survival rates yet. Gene therapy represents a fundamentally new mode for the effective treatment of a disease. It essentially consists of the introduction of the genetic material into the target cells of an individual without producing toxic effects on surrounding tissues. The essence of gene therapy is attributed to the replacement of the defective gene with a normal gene, thus restoring the lost function in the patient's body. The aim of this review is to analyze the different modalities of gene therapy currently used to manage precancerous and cancerous lesions of the oral cavity.

Key words: Gene, Gene therapy, Oral squamous cell carcinoma, Vectors

Introduction

The past two decades have been golden years for the genetics of cancer. It has become clear through the work of countless laboratory groups that both inherited and sporadic cancers arise through defects or misregulations of their genomes. The cartography of the order, accumulation and interactions of genetic lesions during tumour initiation and progression has reasonable detail for many human tumour types. Such information is proving to be tremendously valuable in diagnosis and in grouping patients into prognostic categories. There is every reason to believe that the continuous use of increasingly sophisticated genomic and proteomic technologies will add another order of magnitude to the precision of such conclusions.[1]

Modifications of traditional cancer therapies, including surgery, radiotherapy and chemotherapy have not improved the survival rates of patients with mucosal squamous cell carcinoma. Local and/or regional tumour recurrence develops in approximately one third of patients, despite definitive treatment.[2]

It is now widely acknowledged that cancer has a genetic origin. Thus, an entirely new approach to its treatment utilizing genetic aids has evolved. DNA damage is considered to be one of the most important factors for carcinogenesis because of the insults of carcinogens or damage due to spontaneous DNA replication. Inability to correct the DNA damage due to mutated DNA repair genes or absence of functional cell cycle check-point genes may give the cell a growth advantage. These mutated genes or other downstream genes are thought to be good targets for gene therapy.[3]

The majority of the head and neck cancers comprise of Oral Squamous Cell Carcinoma (OSCC). OSCC is a disease in which the genes that control the cell growth and apoptosis are mutated. This leads the cells to invade into deeper tissues and metastasize. The traditional therapies for the management of cancer and their various modifications including

surgery, radiotherapy and chemotherapy have not refined the survival rates yet.[4,5]

However, this situation could change effectively in the coming future with the implementation of new gene with the defective one and the activation or inactivation of others may inhibit or suppress tumour growth.[6]

Gene therapy represents a funda mentally new mode for the effective treatment of a disease. It essentially consists of the introduction of the genetic material into the target cells of an individual without producing toxic effects on surrounding tissues. The essence of gene therapy is attributed to the replacement of the defective gene with a normal gene, thus restoring the lost function in the patient's body. It was conceived originally as an approach to treat autosomal recessive Mendelian disorders and it has been applied to a broad range of acquired conditions such as cancers, infections and degenerative disorders.[3]

The aim of this review is to analyze the different modalities of gene therapy currently used to manage precancerous and cancerous lesions of the oral cavity. They include addiction gene therapy, suicide gene therapy, immuno-therapy, oncolytic virus therapy, inhibition of tumour angiogenesis, gene deletion therapy and antisense RNA.

History Of Gene Therapy

Joshua Lederberg and Edward Tatum laid out the fundamental tenets for gene therapy.[7] The science leading to gene therapy took a large step forward when Michael *et al.* succeeded in transferring a gene (TK gene, which codes for thymidine kinase) into mammalian cells in 1977.[6] The idea of gene transfer for treating human diseases was put forward by Cusack and Tanabe in 1998.[8]

On September 14, 1990, the first approved gene therapy clinical trial took place when Ashanthi De Silva, a 4 year-old girl with Adenosine Deaminase (ADA)-deficiency / Severe Combined Immunodeficiency (SCID) syndrome, was given her own T cells engineered with a retroviral vector carrying a normal ADA gene by the NIH (National Institutes of Health) team of Anderson, Blaese and Rosenberg.[9]

Definition Of Gene Therapy

Gene therapy can be defined as gene transfer for the purpose of treating human disease (Cusack and Tanabe, 1998).[4] This includes the transfer of new genetic material as well as the manipulation of existing genetic material. This holds true especially for cancer cells, where dominantly activated oncogenes can be targeted.

Concept Of Gene Therapy

Gene therapy is a therapeutic intervention based on the modification of the genetic material of the living cells. The term "gene therapy" refers to replacing or repairing a defective gene in the diseased cell's genome in order to restore normal cell function and tissue integrity. It is rightly defined as "the genetic modifications of the cells of a patient in order to fight a disease".[3] Gene therapies include both the transfer of new genetic material and the manipulation of existing genetic material.[10]

A successful gene therapy requires that the:-

- Genetic malfunction/nature of a disease is clearly understood.
- Therapeutic material can be delivered to the target cells in the affected tissue or organ.
- Therapeutic material is active for the intended duration and delivers the intended benefit to the target cells.
- Harmful side effects, if any, are manageable.[6]

The most widely used gene therapy procedure includes the following steps:-

(i) Identification, isolation and amplification of the gene to be used in the treatment.
(ii) Extraction and in vitro culture of tissue cells from the patient to be treated.
(iii) Transfer of the therapeutic genes into these cells via a vector.
(iv) Transfer into the patient of selected gene-containing cells.[10]

Techniques Of Gene Transfer

Gene therapy has the potential to target cancer cells while sparing normal tissues.

It can be successful with the efficient

transfer of the genes into the cell. The gene (cDNA) is cloned into a vector so that the foreign gene is deposited into the target cell.

An ideal vector should have the following characteristics:-

(i) It should be able to protect and deliver DNA across the cell membrane into the nucleus.

(ii) It should have the ability to regulate the expression of the gene of interest and minimize the toxicity by targeting gene delivery to specific cells.

(iii) It should be easy to be produced in large amounts.

(iv) It should be inexpensive to be produced in sufficient quantities.[3]

After the cloning of the therapeutic gene is done on a specific vector with appropriate regulatory sequence (promoter/enhancer), it is submitted to the target cells. The genes are delivered either:-

- *Ex vivo*- where cells from a selected tissue of the patient are removed, exposed to the gene transfer vector, selected for the transgene using markers and then the genetically corrected cells are reintroduced into the patient's body.
- *In vivo*- where the vector DNA is injected directly into the body, generally into the tissues to be treated.[3]

Delivery Systems

Genetic material can be transferred *via* a vector that is defined as the vehicle that is used to deliver the gene of interest in the cell. The desired gene is contained in a plasmid, which is a DNA strand that can result in messenger RNA (mRNA) production and hence, protein production. The ideal vector would transfer a precise amount of genetic material into each target cell, thereby allowing for expression of the gene product without causing toxicity.

(a) Chemical Transfection

DNA is introduced firstly by calcium phosphate, lipids or protein complexes. Lipid vectors are generated by a combination of plasmid DNA and a lipid solution that result in the formation of a liposome. This fuses with the cell membranes of a variety of cell types, introducing the plasmid DNA into the cytoplasm and nucleus, where it is transiently expressed. Secondly, DNA is bound to the positively charged molecules such as DEAE-dextran or polybrene which then binds to the negatively charged cell membrane. Certain carcinoma cells like OSCC express high levels of folate receptor. Linkage of DNA or DNA-lipid complexes to folate can specifically target cancer cells.[4]

(b) Physical Transfection

This can be accomplished by electroporation, microinjection, or use of ballistic particles. Electroporation therapy with intralesional bleomycin has been reported to be a technically simple outpatient technique where high-voltage electric impulses can be delivered into a neoplasm by transiently increasing cell membrane permeability to large molecules, including cytotoxic agents, thus causing localized progressive necrosis. Electroporation can treat bulky tumours (>2cm) with complete penetration.[11]

Viral Vectors For Gene Transfer

There are several methods for the accurate transfer of gene to the target cells. However, viruses are considered to be the most efficient mode of transmission of the genes. Viruses have evolved over the years to enter the cell and efficiently arrogate the cellular machinery to make its own viral proteins.[3] Viruses commonly used in cancer gene therapies include retroviruses, adenoviruses and herpesviruses. These viruses when used as vectors are generally disabled such that they are unable to replicate on their own. However, recently replication competent viruses as gene therapy vectors are also being tested.[3]

Retroviruses

(RNA viruses), integrate their genome into the host DNA as a provirus and then replicate to make multiple copies of infective particles, which are released outside the cell. Retroviruses are RNA viruses that splice their genes permanently into the chromosomes of the cells they invade. This gene is then passed on to all future generations of these cells. Therefore, if a retrovirus is used as the vector, the therapeutic gene would be a permanent part of the patient's genome. Retroviruses lack specificity for the target cell. The most commonly used retroviral vector is the mouse

moloney murine leukaemia virus which is made replication- deficient by replacing the structural genes- *gag, pol* and *env*, with the therapeutic gene. These genes are supplied in rans by either a helper virus or a packaging cell line expressing the proteins in order to package the virus infect dividing cells and integrate the therapeutic gene into the target cells.[3,12]

Adenovirus

(DNA virus), found during infection in upper respiratory tract epithelium. These vectors are utilized because they are usually safe. Adenoviruses infect a cell, lose their protein coat, and transfer DNA into the nucleus, where it is transcribed (Fig 1). They are made replication incompetent by deletion of all or part of their E1A and E1B regulatory genes.[3,12]

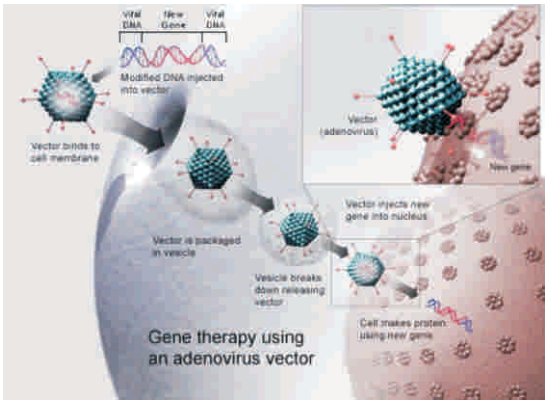

Fig. 1 Adenovirus vectors used in Gene therapy [Courtesy: US National Library of Medicine[19]]

Lipid Vectors

Viruses have several limitations and risk factors, so the use of potentially infectious agent is avoided. Some vectors like adenovirus require multiple administrations; the use of potentially infectious agent is avoided and the use of a less immune-inducing, noninfectious vector is desirable. One such nonviral vector is the liposome.[12]

Liposomes are spheres composed of a lipid membrane on the surface surrounding an aqueous core. The lipid surface is similar to that of human cells and can transport the aqueous core of material into the cell. By using a cationic-lipid complex, these structures can be made large enough to transport large plasmids into the cell. Lipid vectors lack cell specificity and there is minimal control as to where the genetic material will insert. Cationic-lipid vectors contain the DNA on the interior and the lipid bilayer on the surface. Thus, as with the viral vectors there is the potential to insert the ligands on the lipid surface to target these vectors to specific tumour cells. Cationic-lipid vectors are able to insert genetic material in both dividing and non-dividing cells.[13]

	Advantages	Disadvantages
Non viral		
Electroporation DEAE dextran Calcium phosphate Liposomes	No replication risk; transfect dividing and quiescent cells; less immunogenicity	Limited transfection energy
Naked DNA	No replication risk	Moderate efficiency; non specific cell targeting
Virus		
Retroviruses	Randomly integrate into host genome; long term genetic alterations	Infect only dividing cells; low transduction energy
Adenoviruses	Infect both dividing and quiescent cells; high transduction efficiency	Transient expression; anti-adenoviral immunity can lessen effect
Adeno-associated viruses	High transduction efficiency; low immunogenicity	Difficult to manufacture; low titre
Herpes viruses	Express thymidine kinase; high gene transfer efficiency; low immunogenicity	Toxicity related to lytic infection

Gene Therapy Strategies

Gene therapy serves to be very apt for Carcinoma because primary and recurrent lesions are readily accessible for injection or application for the agent. The recent advancements in the various approaches to gene therapy includes:-

Defective Gene replacement:

Tumour suppressor genes e.g. p53 is mostly defective in 50% cases.[5] The percentage of epithelial cells expressing mutated p53 is usually higher with greater severity of the epithelial disorder. Thus, one of the tumour suppressor genes most commonly used in gene therapy is the p53 gene, and numerous viral vectors, especially adenoviral vectors, have been developed for its application.[1] Certain other examples of the defective genes include regulators of apoptosis, papillomaviruses and the gene that encodes the receptor for Epidermal Growth Factor. Suppression of these genes can be done using antisense RNA, ribozymes and siRNA and delivery methods have included direct transfer, lipofection and adenovirus vectors. These studies have shown to reduce the potential for tumour growth in vitro cases and not in animals or patients.[3,14]

Suicide Gene Therapy':

"Suicide Gene Therapy" for cancer involves the introduction of a gene into a cell that will convert a nontoxic prodrug into a toxic substance.[6] Another term for this is *genetic prodrug activation therapy.*[12]

The first gene therapy to be described and investigated about was the Herpes Simplex Virus Thymidine Kinase (HSV- TK). This gene encodes a viral enzyme that phosphorylates ganciclovir into a monophosphate form, which is then further phosphorylated by intracellular enzymes into ganciclovir triphosphate compound that terminates DNA synthesis (Matthews and Boehme, 1988). Ganciclovir triphosphate inhibits DNA polymerase and is incorporated into DNA, causing chain termination and cell death. Ganciclovir (GCV) is an excellent substrate for HSV-TK and a poor substrate for mammalian thymidine kinase. This selectivity of ganciclovir facilitates the achievement of cytotoxic levels in transfected cells leaving the normal cells unharmed.[4,5]

Another form of suicide gene therapy uses the cytosine deaminase gene. The prodrug for this system is the minimally toxic 5 fluorocytosine, which is converted in the tumour by cytosine deaminase into highly toxic 5-fluorouracil. This system permits higher levels of 5-fluorouracil to accumulate at the tumour than would be possible with systemic therapy. In immunodeficient mice in which human-derived head and neck squamous cell carcinomas were grown, HSV-*tk*/ganciclovir gene therapy resulted in tumour regression and extended survival (Fig:2). Additional investigations in immune-competent mice with head and neck cancer confirmed the efficacy of this treatment but detected the HSV-*tk* gene in distant organs when the viral dose was high.[12]

Another example is the gene CYP2B1 which activates cyclophosphamide to a toxic form. This approach can kill oral cancer cells efficiently in culture and reduce the growth of tumours in animals but no human clinical trial has demonstrated its efficacy.[5,14]

It appeared initially that suicide gene therapy was an impossible attempt but however, it has been found that cells are modified by the suicide gene are also killed. This local killing has been termed *"the bystander effect".*[4]

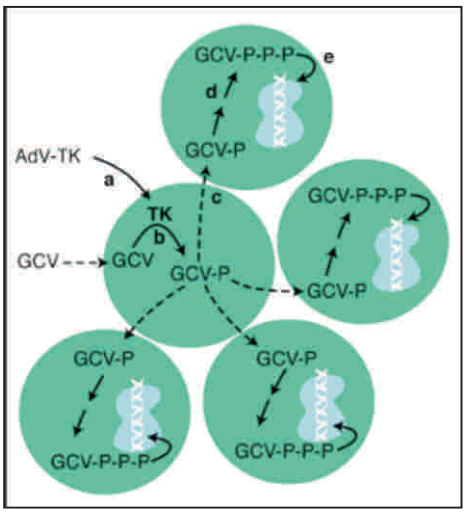

Fig:2 *Suicide Gene Therapy. An adenoviral vector (AdV) delivers the Herpes simplex thymidine kinase (TK) gene to its target tumour cell.*
(b) The expressed TK phosphorylates the antiviral drug ganciclovir (GCV),a nucleoside analogue, to its nucleotide (GCV-P), a biochemical reaction.
(courtesy: Expert Review in Molecular Medicine; Cambridge University Press)[20]

Immunologic gene therapy

The immunologic gene therapy approach to oral cancer involves either increasing the immunogenic potential of tumour cells or augmenting the patient's immune response to a tumour. Biological molecules produced by tumour cells are found to elicit strong immune response. T-cells are the major immune cells involved in anti tumour immunity. Patients with OSCC present altered function of immune cells, including NK cells, T lymphocytes and numerous cytokines.[4,12]

Mechanisms to increase the sensitivity of the tumour to normal therapeutic processes are under investigation. Radiosensitivity to γ radiation and chemosensitivity to 5-fluorocil (5-FU) were reported in OSCC after suppression of NK-kB activity, which activates the antiapoptotic proteins TNF, TRAF-1, TRAF-2 and cIAP-1. NF-kB also increases the expression of proinflammatory cytokines, e.g., IL-1α, IL-6 and IL-8, and of enzymes that degrade matrix metalloproteinase-9 (MMP-9). NF-kB appears to contribute towards the progression and metastasis of various cancers, including OSCC, therefore, its inhibition may be a useful coadjuvant treatment in oral cancer therapy. Other studies have addressed the transduction of IL-2 gene, which appears to have an anti-tumour effect, by using the mutated fibroblast of an adenovirus and an RGD peptide (Adv-F/RGD). The intratumoural injection of Adv-F/RGD showed a high anti-tumour effect due to increased mononuclear cell infiltration and major necrotic changes. It also achieved local control of the disease, an essential objective since most deaths result from metastasis.[12]

Another therapeutic approach may be to use the monoclonal antibody Anti-ICAM-2 alongside the intratumoural gene transfer of interleukin-12. ICAM-2 is a glycosylated protein with surface adhesion that is expressed in endothelial cells and activates lymphocytes. Recent studies found that systemic administration of Anti-ICAM-2 induced the complete regression of OSCC lesions. However, the tumour regression is dependent on the immune system function and the induction of specific tumour toxins by the action of CD8 lymphocytes.[4]

Apoptosis - inducing genes

One of the major problems in treating solid tumours by either radiation therapy or chemotherapy is that the tumour cells are often resistant to apoptosis and therefore do not succumb to the conventional treatment. Hence, the therapeutic approaches have been aimed at killing cancer by inducing apoptosis. At the molecular level, mutation of the p53 tumour suppressor gene is found in greater than 50% of human tumours. p53 plays a major role by inducing apoptosis in cells carrying damaged DNA. Wild type p53 has been used either alone or in combination with other apoptosis inducing genes or in combination with radiotherapy. Overexpression of pro-apoptotic molecules such as Bax favour death of cells resistant to ionizing radiation. This expression could sensitize to ionizing radiation. Caspase-8, a member of the family of Caspases is also involved in bringing about apoptosis. Studies have reported that caspase-8 effectively induced cell death in gliomas and could be a useful strategy for gene therapy of gliomas.[3]

Blocking angiogenesis

Tumours require a constant supply of oxygen, nutrients, hormones and growth factors for their existence and dissemination. This is provided by formation of new blood vessels or angiogenesis. Experimental tumours have been shown to regress by inhibiting angiogenesis which is proven to be suitable for gene therapy. The two popular inhibitors of angiogenesis are angiostatin and endostatin. These are generated by proteolysis of larger proteins such as plasminogen (for angiostatin) and collagen XVII (for endostatin). However, for continuous administration of the protein gene therapy approaches are preferred. However, for continuous administration of the protein gene therapy approaches are preferred. Genes coding for the angiogenesis inhibitors can be introduced either directly into the patient's cells ex vivo or through generic cells that have been genetically modified to overexpress the protein of interest.[3]

Combination therapy

Cancer gene therapy approaches are often designed as single gene therapy; however, greater therapeutic effect might be obtained if

combined with an established conventional treatment regimen such as chemotherapy or radiotherapy. Normal bone marrow cells highly susceptible to killing by chemotherapeutic drugs. Hence, marrow toxicity is a major complication of high dose chemotherapy. In order to protect the bone marrow multi drug resistance (MDR-1) gene has been introduced into the bone marrow cells. MDR-1 gene encodes a 170 kDa P- glycoprotein which is an energy dependant cellular pump which actively effluxes the drugs like paclitaxel and anthracycline.[15]

Excision gene therapy

This therapy involves the removal of oncogenes, thereby inhibiting the growth of the tumour cells. Thus, the efficacy of using akadaic acid to suppress Egr-1 (early growth factor1) protein expression is being studied in the OSCC setting. Okadaic acid is a highly toxic polyether that inhibits phosphorylation of types 1 and 2A proteins, reducing the expression of Egr-1. This helps to trigger the inhibition of tumour activity, since it is related to cell proliferation and division. The genes that control growth and cell cycle progression, including some of the tissue factors like- TGF-β1, PDGF-A and PTEN are regulated by the expression of the protein Egr-1. Thus, inhibiting this protein represents a good therapeutic approach for the tumour cells. Some studies demonstrated that inhibition of the protein kinase C reduces the expression of this gene, triggering higher sensitivity of the tumour to radiotherapy.[10,16]

Antisense RNA and Ribozymes

Gene expression can usually be inhibited by RNA that is complementary to the strand of DNA expressing the gene. This "antisense" RNA can prevent the activity of several known oncogenes, including myc, fos, and ras, and can inhibit viruses such as HSV-1, HPV, and HTLV-1. Conventional usage of this technique is restricted due to the difficulty of introducing a sufficient quantity of antisense molecules to inhibit tumour growth. New strategies using strong promoters are being developed to address this potential limitation. An antisense RNA approach has been developed regarding the interference with an autocrine pathway in oral cancer involving epidermal growth factor receptor and (EGFR) and its ligand, transforming growth factor(TGF-α). A Phase I study in patients with advanced oral cancer is under way to determine the safety and biologic effects of liposome-mediated intra-tumoural EGFR antisense gene therapy. Results have been positive, showing a low toxicity and high efficacy.[10,17]

Other strategies

Alternative cancer therapy approaches have been based on oncolytic viruses which selectively attack tumour but not normal cells. ONYX-015 is a mutant adenovirus which lacks the E1B-55 kD protein and is thus incapable of replication. However, it was observed that ONYX-015 could replicate in and cause cytopathogenicity in tumour cells which carry p53 mutations. Selective intratumoural replication and tumour specific tissue destruction has been reported in phase I and II clinical trials in patients with recurrent, refractory squamous cell carcinoma of the head and neck. However, <15% of these patients showed any clinical benefit. The combination of replication competent viruses along with chemotherapeutic drugs (intratumoural ONYX-015 used along with cisplastin and 5-fluorouracil) was well tolerated by patients and showed tumour selective augmentation of chemotherapy efficacy.[3]

Future prospects of gene therapy

Gene transfer, while a radical new type of treatment, is also the only gene therapy product to obtain regulatory approval in any global market, as demonstrated by China's 2003 approval of Gendicine for clinical use. Gene transfer technology allows an incredible diversity of treatment possibilities. This diversity can be used to complement traditional therapies, as well as provide radically new frontiers for treatment. Gene transfer therapy can rely on the current information known about the genetics of cancer formation, bringing a more sophisticated and personalized approach to therapy.

The various advancements in understanding and manipulating the genes have given the scientists and researchers to alter patient's genetic material to fight or prevent disease. Several approaches to gene therapy of oral

cancer now exist in the laboratory and some have been tested in patients also. It is widely accepted though that any conceptually new therapeutic mode of treatment would take a longer time to be established as a routine treatment. History reveals that it has always taken a longer time for the acceptance of any new means of treatment, be it the use of antibiotics, immunization procedures or Gene therapy, which is merely a decade old. Scientists and researchers have been working on this aspect and improvement is noticed from the ongoing human clinical trials. It is therefore believed that Gene therapy will have a greater impact in this century.

The success of this treatment modality will ultimately depend on the ability to target every cancer cell, express the gene of interest at high levels and minimize toxicity by targeting gene delivery to specific cells. The ongoing research in the field of molecular biology combined with the unfolding of the mystery of the human genome has made cancer-based gene therapy more viable and promising.

Thus, we can consider gene therapy to be the thin rim of ray at the fringe of the eclipse but it surely needs to have improvements and constant ongoing research to prevent the topic from fading into the category of appealing legends.[3,10,14,18]

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