



## ONCOLOGY ON GENE THERAPY

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### ABSTRACT:

Cancer treatment has been the major goal of the gene therapy studies over the decades. Although there is no cancer gene therapy drug in the market yet, substantial progress has been made in defining potential targets and in developing viral and nonviral gene delivery systems recently. Numerous genes have been studied as the targets for cancer gene therapy so far. Various gene therapy strategies, including suicide gene therapy, oncolytic viral therapies, antiangiogenesis, and gene therapy vaccines have been developed.

The combination of gene therapy with conventional methods, such as chemotherapy, radiotherapy, and immunotherapy, has further improved the efficacy.

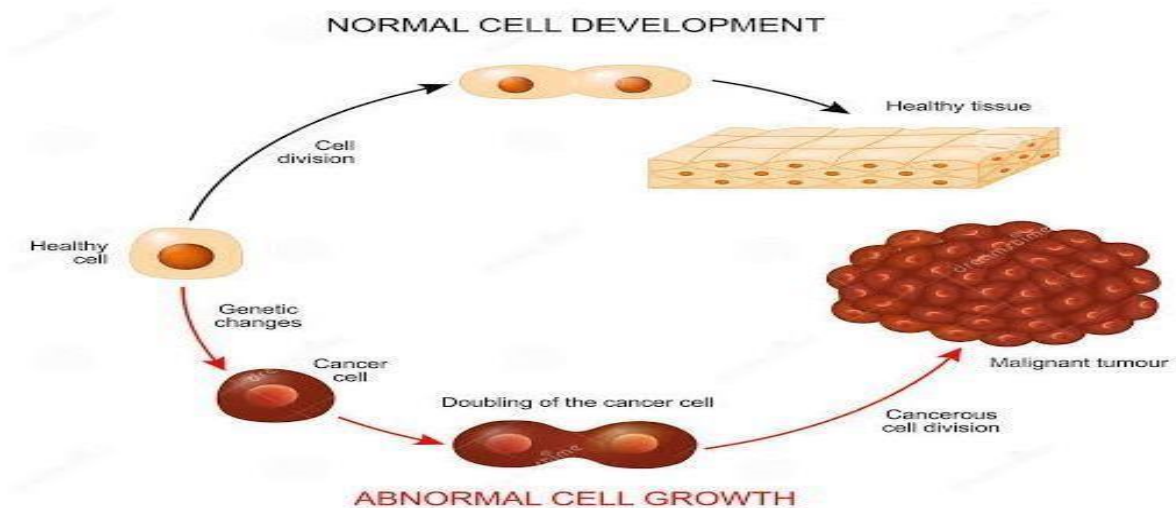
Although the preclinical and experimental studies have yielded highly encouraging results, there are still few gene therapy agents at phase III trials. In these, we will review gene transfer systems, Immunotherapy, Oncolytic agents, Target gene therapy to cancer and cancer gene therapy in clinic.

**Keywords:-** DNA, RNA, oncologic vector, Targeted vector.

### INTRODUCTION:-

Uncontrolled growth of Cancer is a disease characterized by an accelerated and cells that have the capacity to spread throughout the body and affect vital organ function. When detected at a late stage, cancer is generally fatal, therefore intensifying the search for new medication to help patients. Gene therapy appears to be an adequate antineoplastic strategy. The improvements in the past 20 years in the molecular biology have evoked optimism in the treatment of cancer and yielded a number of targeted drugs in the market. However, the curative treatment of the cancer has still been possible with only the early diagnosis and early intervention in the vast majority of the solid tumours. Almost half of the cancer

patients diagnosed each year have been dying of the disease throughout the world. In particular, the patients with distant metastasis have no hope of cure with the current treatment modalities.

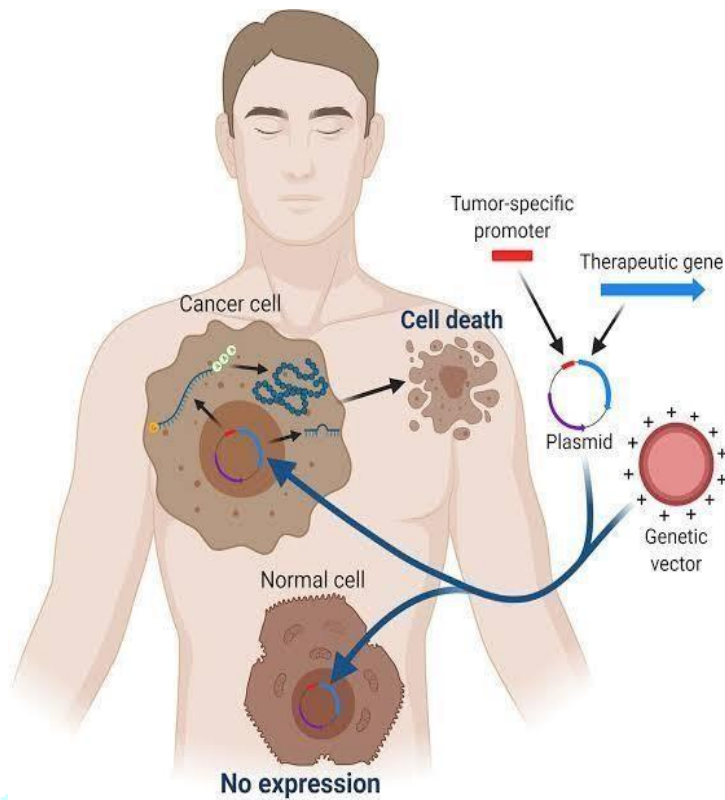


## What is Gene Therapy

Gene therapy is the treatment or prevention of a disease that is carried out through the insertion of nucleotide sequences (DNA or RNA) into the cell. Genes that carry the information necessary to create a protein within the cell are usually introduced. The purpose of this transference of genetic material or of genes is to reestablish a cellular function that had been abolished or become defective, to introduce a new function or to interfere in an existing function.

A simple example would be the use of gene therapy in treating a disease caused by a defective gene in a patient's cells. This defective gene would produce a defective protein incapable of carrying out a certain function. With gene therapy, a normal gene could be introduced into the patient's cells that would produce the adequate protein and thus cure the disease. However, it is presently very difficult to substitute the function of a defective gene by replacing it with a new gene.

Different gene therapy strategies are based on a combination of three key elements: the genetic material to be transferred, the transference method and the type of target cell. Cancer gene-therapy is the transfer of nucleic acids into tumour or normal cells to eliminate or reduce tumour burden by direct cell-killing, immunomodulation, or correcting genetic errors to reverse the malignant state. Genes may also be incorporated into normal tissues to enhance resistance to conventional cancer treatments. DNA can be introduced into target cells *ex vivo* and placed back into the host or directly into target cells *in vivo*. Agents that facilitate the transfer of genes to recipient cells are called vectors. Replication-defective viruses, liposomes, or a delivery complex consisting of nucleic acid conjugated to ligands are examples of vectors. Genes are transduced into cells by viruses or transfected into cells by non-viral vectors.



## METHODS OF GENE

### A] Gene Transfer Therapy:-

The challenge in gene therapy is to deliver an adequate amount of genetic material into target cells or tissues and to maintain gene expression for a desired period of time. Genetic material can be to their target cells or tissues via different methods of delivery.

In principle, we can group them into:

- physical:-
  - Examples -Electroporation
  - Ultrasound
  - Gene gun deliveries
- viral:-
  - Example-Adenovirus
  - Retrovirus
  - Vaccina virus
  - Adeno associated virus

(3) non-viral methods:-

- Example-Liposomes  
Nanoparticles

(4) Bacterial or yeast.

#### □ **Viral Vector:-**

The most commonly used viral vectors used for gene transfer are adenoviruses, lenti- and retroviruses (including the human immunodeficiency virus (HIV)), vaccinia viruses, adeno associated viruses (AAV), and baculoviruses. These vectors differ from each other regarding their cell tropisms, expression profiles, transgene capacities, immunogenicity, as well as different duration of transgene expression.

In addition to their origin, viral vectors can be divided into integrating and non-integrating vectors. Adenoviruses and baculoviruses are examples of non-integrating vectors. They lack the ability to integrate their genome (and, hence, with it also the transgene) into the host genome. Lenti- and retroviruses, as well as AAVs, on the contrary, are examples of vectors that do integrate into the host genome. While the expression of the transgene is transient in case of nonintegrating viral vectors (diminishing in a few weeks), integrating vectors commonly results in long-term expression (months, up to years). This integration of the transgene into the host genome has raised concerns about the safety of these vectors. This is due to the fact that integration has been observed with retroviral vectors to occur occasionally in actively expressed sites (i.e., insertional mutagenesis)

#### • **Non-Viral Vector:**

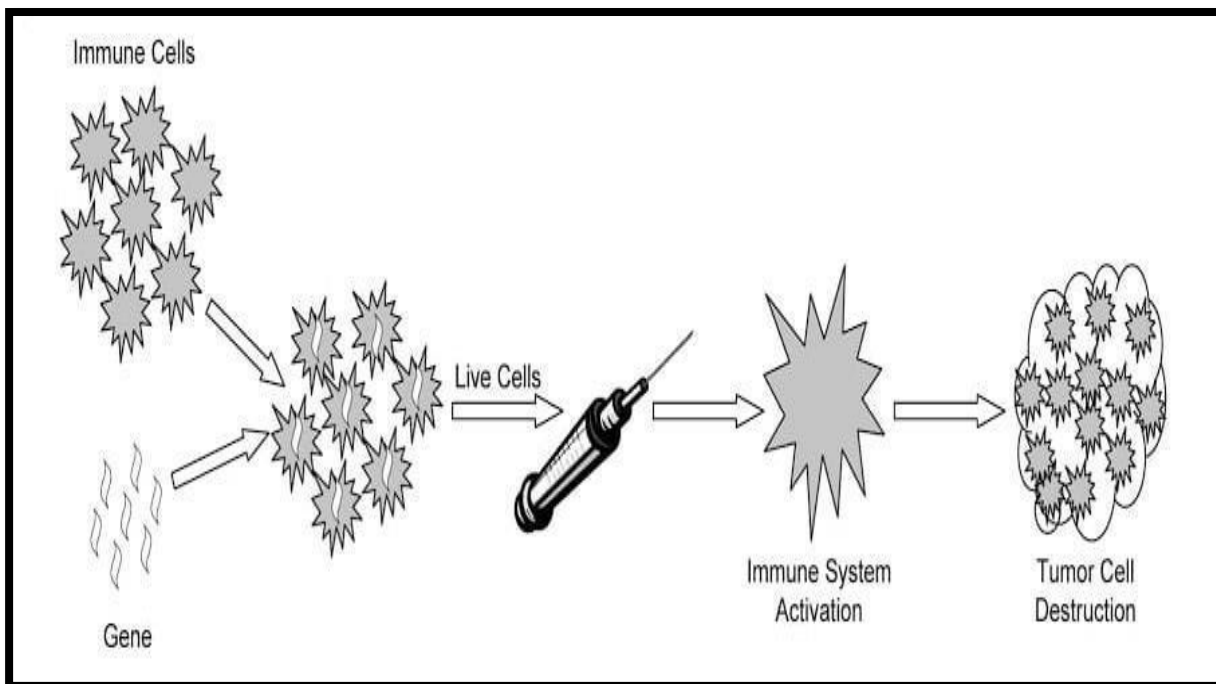
Viral vectors have been shown to be efficient gene transfer tools. Nevertheless, drawbacks such as rapid clearance of viral vectors from the bloodstream (when injected systemically), their immunogenic and inflammatory potential, has urged the development of new synthetic gene delivery vectors.

In fact, non-viral gene delivery systems are a topic that is currently being studied extensively as alternatives for viral delivery systems. The simplest form of a non-viral system is naked plasmid DNA. The advantage of naked plasmid is that it poses the lowest form of toxicity or other unwanted reactions.

In addition, it is easy to formulate and inexpensive to produce. However, its disadvantage is the low transfection efficiency compared to viral-mediated gene transfer. As a result, to improve transfection efficiency, cationic polymers, or lipids formulations have been developed to condense plasmid DNA to protect the degradation of DNA and to enhance uptake and transfection of plasmids. The advantage with those formulations is that polymers or lipids can comparatively easily be designed to attain certain properties.

For example, non-viral vectors can easily be targeted to a target tissue or cell by coupling of cell- or tissue-specific targeting moieties on the carrier. Furthermore, by determining the size of the micro- or nanoparticle the bio distribution, cellular internalization, and intracellular trafficking of the micro- or nanoparticle can be influenced.

Unfortunately, the success of non-viral delivery systems in clinical applications in gene therapy has been limited. Compared to viral vectors, non-viral vectors have not gone through the evolution and process of time that viruses have, which typically can be seen as low transduction efficiencies in vivo.

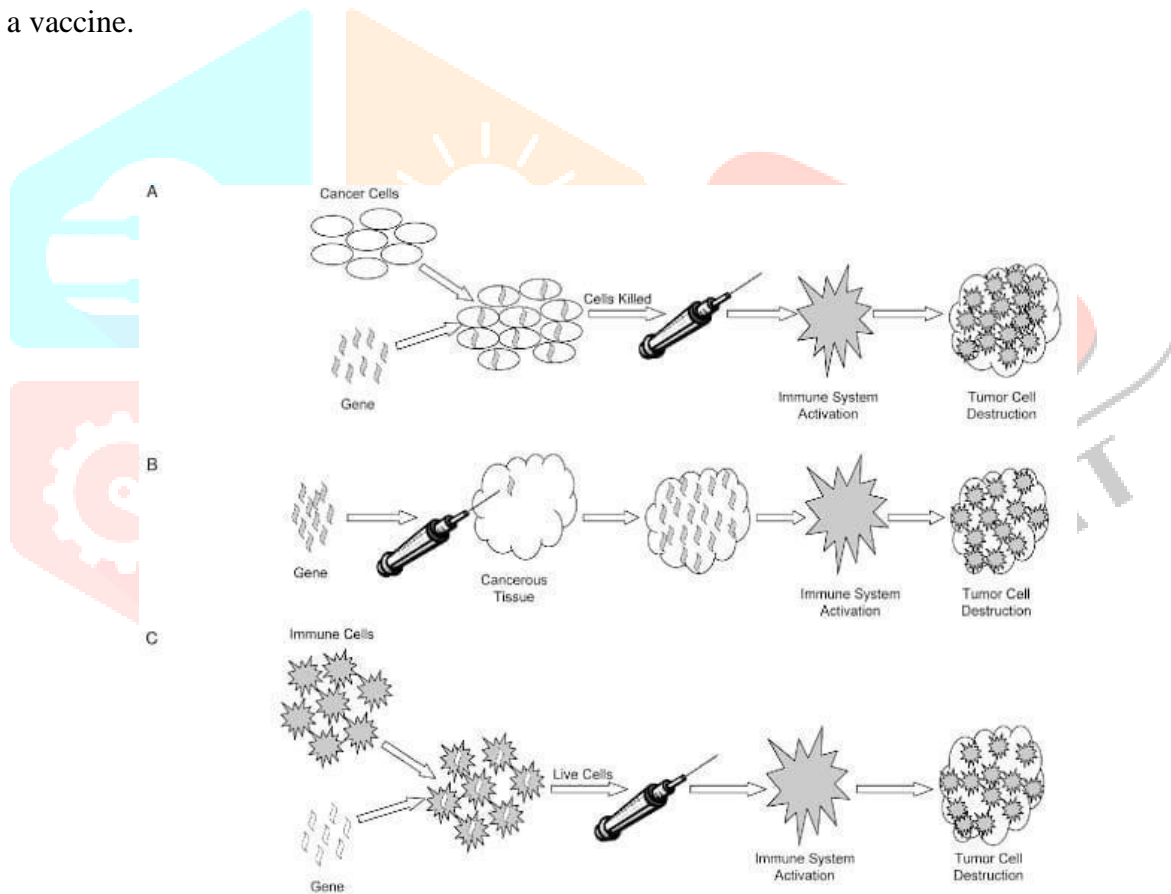


## B] Immunotherapy:-

The term “immunogene therapy” can be defined as genetically manipulating tumor cells or dendritic cells in order to stimulate antitumor immunity; the genes can be transferred in situ or ex vivo as part of the preparation of an anticancer vaccine. Immunogene therapy is emerging as one of the promising treatment modalities for malignant tumors.

On the other hand, adoptive transfer of antigen-specific T lymphocytes is an alternative of immunotherapy for cancer. In this case the strategy relies on cloned T cell receptor TCR genes that can be used to produce T lymphocyte populations of desired specificity to recognize cancer antigens and mediate cancer regression in vivo.

Currently gene therapy is being used to create recombinant cancer vaccines. Unlike vaccines for infectious agents, these vaccines are not meant to prevent disease, but to cure or contain it by training the patient's immune system to recognize the cancer cells by presenting it with highly antigenic and immunostimulatory cellular debris. Initially cancer cells are harvested from the patient (autologous cells) or from established cancer cell lines (allogeneic) and then are grown in vitro. These cells are then engineered to be more recognizable to the immune system by the addition of one or more genes, which are often cytokine genes that produce proinflammatory immune stimulating molecules, or highly antigenic protein genes. These altered cells are grown in vitro and killed, and the cellular contents are incorporated into a vaccine.



- Pathway A -represents immunotherapy with altered cancer cell
- Pathway B-represents immunotherapy with genes in vivo
- PathwayC -represents immunotherapy

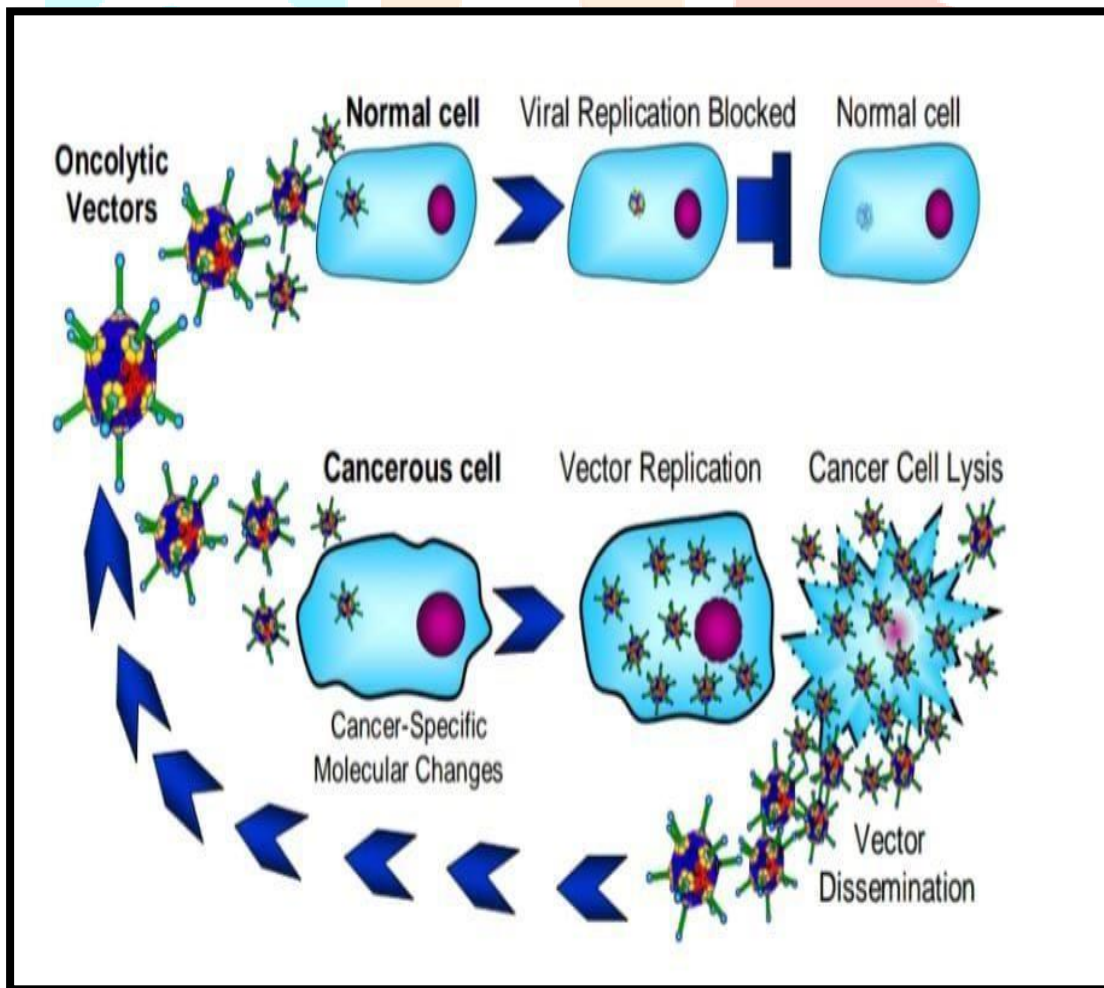


### C]Oncolytic Agents:-

The principal short-comings of gene therapy with replication- deficient viral vectors is limited intratumoral dissemination. For the purpose of overcoming this limitation there was a new therapeutic strategy boom called virotherapy or oncolytic viral therapy

Virotherapy uses a wide variety of viral vectors but the most frequently used are those derived from adenoviruses, vaccinia virus and HSV. Neoplastic cell death occurs from the viral replication effect itself

The main characteristic of virotherapy is the utilization of viral vectors that can selectively replicate themselves in tumor tissue under very specific and exclusive molecular conditions of the neoplastic cell . This characteristic allows for the elimination of tumor cells through an infectious process limited to the tumor and with few side effects, always when the dose used is within the therapeutic range that has been determined for each vector. In addition, replication amplifies the entrance dose of oncolytic viruses facilitating better dissemination on the part of the agent towards neighboring tumor cells, with the possibility of reaching metastasis. The oncolytic effect can also be strengthened by the creation of an immune response against the vector and the cancerous cells infected by it.



Viral replication is a process that can be manipulated. When a virus enters a cell only some “key” replication genes are initially activated in its genome. Then proteins created by these key genes activate the rest of the viral genes to begin the viral replication process. If these key genes are not activated there is no replication. A viral vector is replication deficient if these key genes are eliminated from its genome. Likewise if the expression of these key genes is controlled, vector replication can be controlled.

The most frequently used strategy for creating controlled replication in neoplastic cells is obtained by having the key gene expression regulate or controlled by a cancer-specific or tissue-specific promoter.

In this way the key gene will only be expressed in cells where the promoter is active and if it is only active exclusively in cancer cells there will only be replication in cancerous cells. These key genes are controlled, vector replication can be controlled. The most frequently used strategy for creating controlled replication in neoplastic cells is obtained by having the key gene expression regulated or controlled by a cancer-specific or tissue-specific promoter. In this way the key gene will only be expressed in cells where the promoter is active and if it is only active exclusively in cancer cells there will only be replication in cancerous cells.

#### **Example-**

In the case of adenoviruses, the key replication gene is called E1A, although the E1B also participates but to a lesser degree. Prostate-specific antigen promoter was used to control E1A expression in one of the first oncolytic vectors. It showed E1A gene activation and viral replication only in prostate cells.

#### **D] Targeted Gene Therapy Cancer (Target Cell):-**

Neoplastic cells have molecular characteristics that distinguish them from normal cells. They have an elevated activity of genes in charge of:

- accelerating growth and/or inhibiting apoptosis (cell death)
- degrading the extracellular matrix (to spread itself)
- accelerating angiogenesis
- regulating the immune system to evade

In a similar way, cancerous cells may possess no activity or low activity of genes in charge of the functions contrary to those just mentioned. Their cellular membrane may also have changes in properties and types of receptors. These alterations are consequences of changes in the patterns of gene expression or “activation”. These molecular peculiarities are taken advantage of to create specific gene therapy strategies against cancer. The idea is to eliminate the cancer by modifying cellular and molecular functions that characterize and maintain the life of neoplastic cells.



One of the advantages of gene therapy with respect to traditional chemotherapy or radiotherapy is the capacity to selectively eliminate neoplastic cells while causing the least possible damage to healthy tissue. Ideally it should also be capable of acting at the systemic level to attack both the primary tumor and metastatic deposits. Molecular characteristics cancer can serve as a flag to mark the neoplastic cell so that it can preferentially be attacked by gene therapy vectors (targeting).

• **Targeted delivery:-**

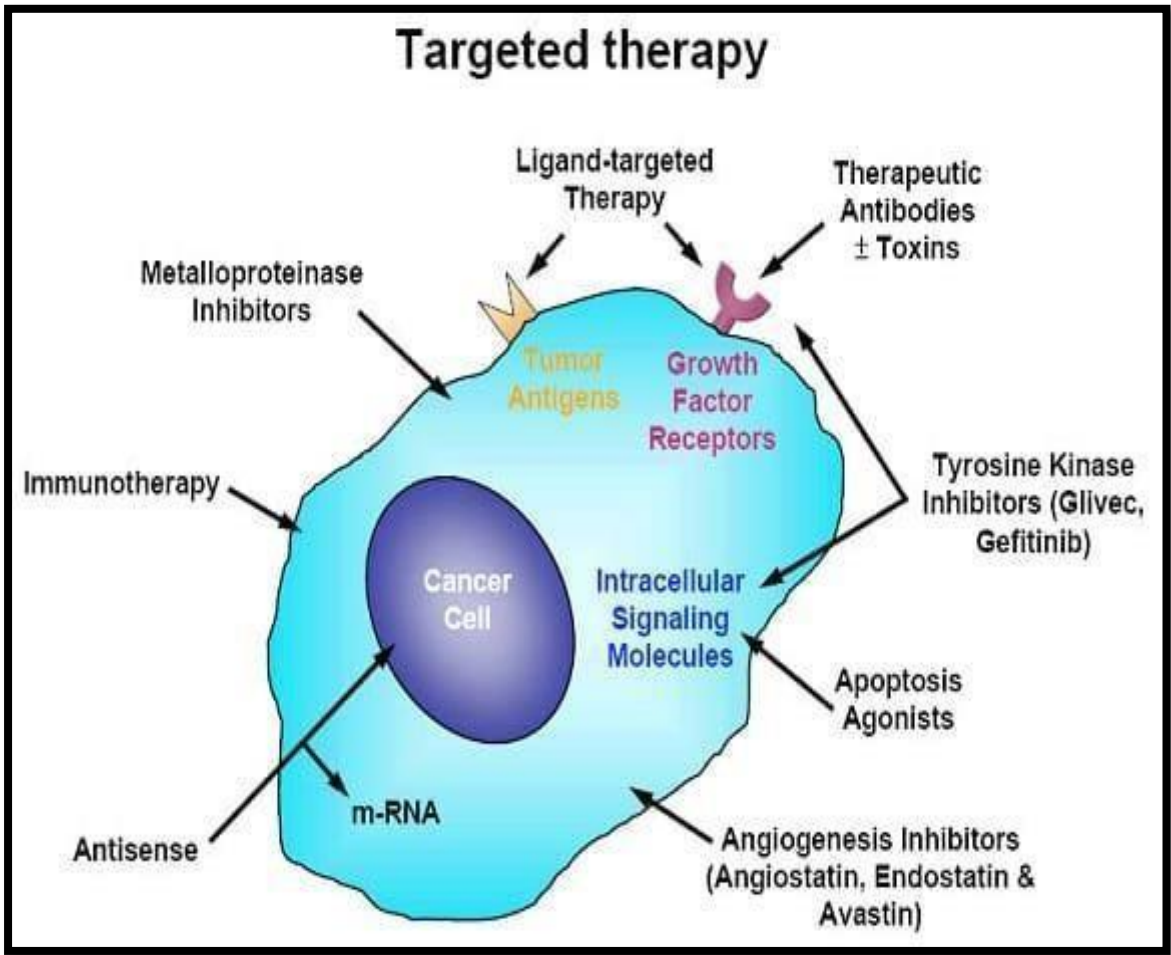
Delivery of the vector directly to the tumor site by intratumoral injection is the simplest manner to direct therapy towards the cancer and thereby largely avoids normal tissues. This option is not useful in systemic treatments or when the tumor is not visible, as in metastasis.

The transfer of genes is entirely dependent on the interaction between the vector and target cell surface. There are differences in the efficiency of each vector for entering into cells.

Another simple strategy includes the exploitation of natural viral tropisms, such as those exhibited by adenoviruses to target lung epithelium cancer or by herpes simplex virus to target the nervous system.

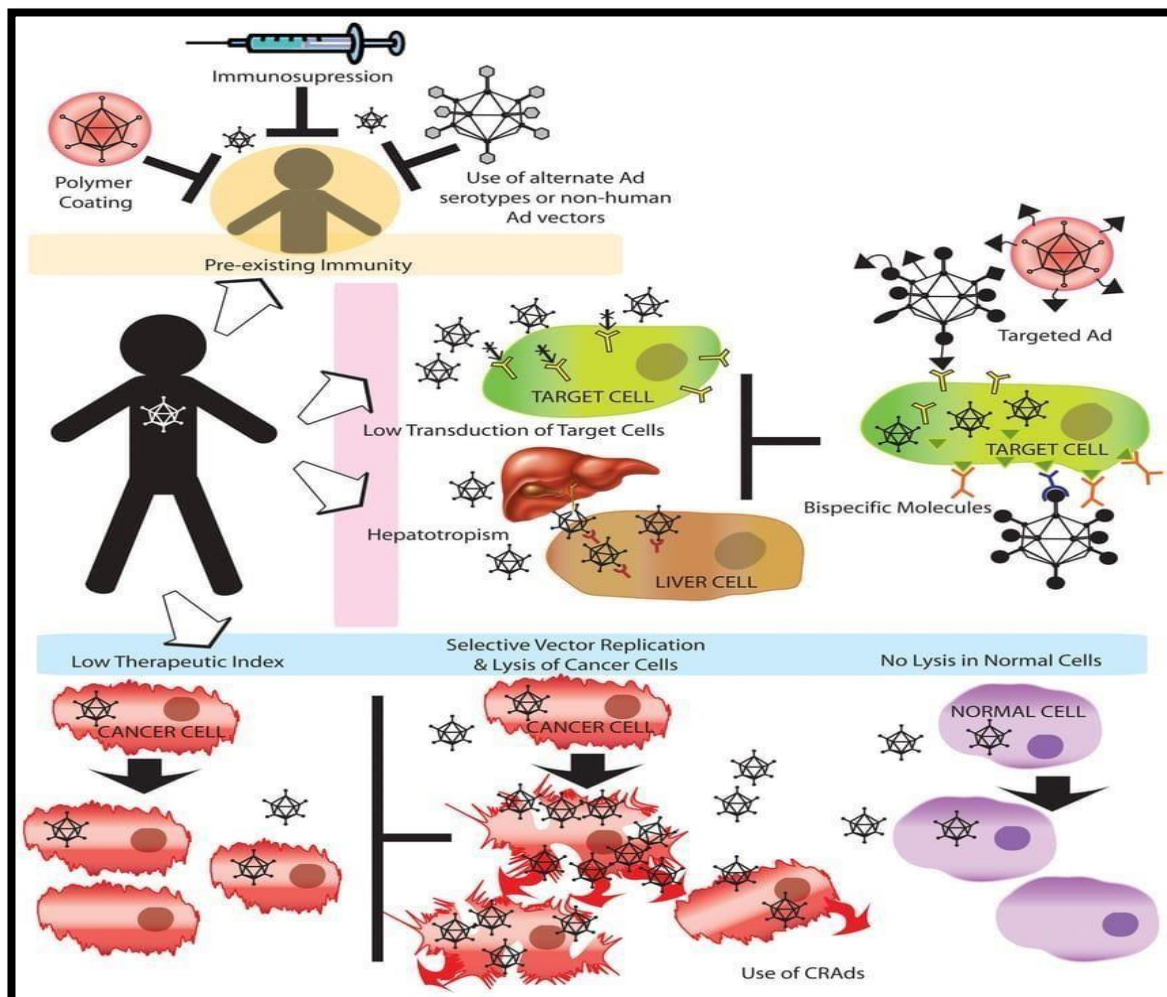
However, the interaction that naturally occurs between the vector and target cell surface can be modified in order to increase the entrance of the vectors into the cells and/or redirect their tropism. Many cancerous cells have an elevated quantity of certain types of receptors in their membranes.

Various strategies may be developed to eliminate cancerous cells by combining therapeutic genes, the type of vector and the way in which the therapy is directed towards the cancer. Not unlike car designers, only researcher creativity is the limit for creating the best gene vehicle with the best performance.



**MECHANISM OF GENE THERAPY**





### • Gene Therapy in the Clinic Future Prospects:-

The vast majority of the clinical trials of gene therapy have been devoted to the treatment of cancer so far. The gene therapy agents have been tested in many types of cancer in the clinic. Almost 1200 clinical trials (approximately 64% of all gene therapy trials) in cancer have been started, conducted, or completed. Less than 4% of those are phase II or III and only few of them are phase IV trials. Although the preclinical and experimental studies have yielded highly encouraging results, the progress in the clinic is not so remarkable. There is no gene therapy agent available in the market yet.

The most important factor that has limited the success of clinical gene therapy trials in human subjects is the delivery of the vector genetic elements or their products to the target cancer cells and their vasculature.

A second problem has been toxicity. Recent advances on improving the delivery and specificity of gene therapy vectors have suggested these trials may be more successful in the coming years.

This is especially true of the attempts to use vectors to activate the immune response against the tumor tissue. Continued testing of these strategies in the context of clinical trials may lead to new opportunities for individuals engaged in a personal struggle with cancer to control their disease.

### **Drawback-**

Indeed, the nature of the distant spread of the disease, which causes the failure of conventional treatment modalities, is also one of the main drawbacks of gene therapy of cancer.

### **Conclusion:-**

Gene therapy is an in approach to treat various diseases, including cancer. Currently most gene therapy protocols are limited to the local administration of the gene transfer. The high cost involved in viral vector manufacturing. The field of cancer gene therapy is rapidly maturing and will no doubt be part of the future of cancer therapeutics. Several very exciting cancer vaccine treatments are in late stage trials. In addition, gene transfer technology for cancer treatment holds for increasing the effectiveness of current chemotherapeutic treatment regimens. Significant advances have been made in the field of oncolytic virotherapy, and trials are in progress that incorporate this technique for precancerous, as well as cancerous treatment. Many of the past obstacles to treatment are being actively overcome and current second and third generation therapeutics are being tested. Consequently, combination therapy with existing conventional modalities or other new therapies should be considered and may offer additional benefit in cancer gene therapy.

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