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Review Article

Advancement of Gene Therapy in Cancer

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ABSTRACT

Gene therapy is the transfer of specific genetic material to modify the encoding of gene products or to change the biological product of tissues for the management of various diseases. This review summarizes the principles of genome editing approaches such as meganucleases, transcription activators like effector Nucleases, zinc finger Nucleases, and CRISPR-associated Nucleases with their mechanisms. This review also explains the types of gene delivery systems as viral and non-viral delivery systems. It also summarizes some gene therapy strategies that help in the treatment of cancer (suicide gene therapy, tumor suppressor gene activation and Immunotherapy). It has been observed that gene therapy in cancer treatment faces a wide range of challenges and limitations. Gene therapy is considered to be safe because it is harmless than chemotherapy. It is promising in the treatment of cancer because earlier it was very difficult to treat cancer. The FDA And EMA have approved CAR T Cell therapy for the treatment of cancers such as blood, leukemia, and multiple myeloma.

Keywords: Gene therapy, Cancer, CAR-T cell, CRISPR, Clinical Trials, Oncolytic Virotherapy.

INTRODUCTION

The uncovering of DNA helical structures was a landmark that opened the way for gene therapy in clinical practice. In recent times, molecular techniques have been progressing to review DNA codes and alter MRNA by post-transcriptional modifications. Gene therapy is the transfer of specific genetic material to alter the appearance of a gene or that controls the biological features of tissues. Gene therapy can solve the problem when peptides are too weak to be used as recombinant therapeutics because of its limitations such as low bioavailability, extreme toxicity, stability issues, etc. The special potential of gene therapy can specifically target the mutated gene that causes cancer (oncogenes) and come out as a successful treatment for cancer therapy. Gene therapy is dependent on different mechanisms such as modifying difficult genes with curative genes, silencing undesirable genes, or the transfection of a curative gen for the cure of a disease [1].

In the gene therapy technique, genetic material (DNA or RNA) is supplied to a target with the help of a vector that helps in the transfer of genetic material to the host cell. Gene therapy's main goal is to provide a replica of damaged genes or genes that amplify the expression of curative genes that influence disease or prevent the expression of curative genes. It evolved during the 1990s and has quickly become popular among other therapy methods nowadays. First years studies were performed mainly on hereditary diseases but nowadays many diseases like neurodegenerative disease, rheumatoid arthritis, heart disease, and infectious disease are also been researched [2]. With the recognition of genes that play a significant role in cancer formation, research and clinical studies on gene therapy for cancer have started to gain great impact [3].

Gene Therapy

Gene therapy is the transfer of genes (DNA, RNA, mRNA, etc) to the patient to fix the damaged genes that have caused the disease.[4] Medicines used for gene therapy are advanced technological medical products that contain genes that have therapeutic, prophylactic, or diagnostic properties and they are used to repair the damaged tissue and restore the deficiencies to maintain the usefulness of the body and avoid the expression of unwanted or damaged genes [5]. Gene therapy is dependent on different mechanisms like exchanging a mutated gene that is causing disease with a healthy copy, restricting or silencing the expression of an unwanted gene, suppressing the unwanted gene, and inserting a new gene to treat that disease

Gene therapy can be done in 2 types of cells *i.e.* somatic cells or germline cells. In a somatic cell, gene therapy will only affect the modified tissues whereas in germline cell gene therapy, genetic changes will be transferred to the family so due to this problem there are no clinical trials for germline gene therapy [6]. Presently, somatic cell gene therapy is harmless for the management of many diseases [7].

Gene therapy uses genetic material (DNA or RNA) which can be transferred either by viral or non-viral delivery systems, which are known as vectors that help in the transfer of foreign genetic material to the host organ. Gene therapy's main work is to transfer curative genes to target cells with the help of a safe, stable, and effective vector or carrier. Both viral and non-viral vectors can be used for the transfer of genes to targeted cells but non-viral vectors are mostly

used because it has superiorities when compared to viral vectors. The genetic material is delivered to the target organ (*in-vivo* gene therapy) or already modified cells from the host and re-administered into a host (*ex-vivo* gene therapy). Gene therapy has a big scale of applications like gene replacement and removal of genetic disorders like hemophilia, cancer, etc. Gene therapy is given to alter the expression of genes and modify the biological features of living cells for medical purposes [8].

Gene Editing Tools

Conventional gene therapy mainly depends on viral-based genes that are either randomly incorporated into the host genome (example: retroviruses) or stay as extrachromosomal DNA copy (example: Adeno-associated Virus) and utters a protein that is missing or altered in human disorder. Unlikely traditional gene therapy, gene editing gives more flexible tools for gene therapy *e.g.* accurately correct point variants, spot an extra, healthy gene at a secure genomic location, or interfere with a gene. The ongoing gene editing process relies on the introduction of endogenous double strands of DNA breaks (DSBs) and restores mechanisms. When DBSs happen by nucleases, cellular DNA repair mechanisms are initiated. There are two type of main mechanisms for fixing or repairing double-strand breaks, non-homologous end joining (NHEJ) and homology-directed repair (HDR). Gene editing nucleases can be modified to identify and split the genome at particular DNA sequences, causing DSBs which are effectively restored by either NHEJ or HDR [9].

NHEJ fixes damaged DNA without a homologous template. Because of this reason, NHEJ can lead to the insertion or deletion of the nucleotide in the damage center therefore it is error-prone. HDR varies from NHEJ because it repairs DNA damage using a homologous template. Mainly having used a homologous sequence, this shape of DNA has fewer chances to cause errors. From a clinical point of view, HDR is more favorable for restoring mutation or alteration in genes or for incorporating genes for therapeutic purposes. Nowadays, there are four different types of gene-editing nuclease enzymes accessible based on their structures: meganucleases, transcription activator-like effector nucleases, zinc finger nucleases and CRISPR-associated nucleases [10].

Meganucleases(MNs)

They are sequence-specific endonucleases that are aware of new unique large (14–40 bp) target sites. It has little cytotoxicity making it an alluring tool for genome editing. Active engineering techniques cover the creation of fusion protein from already known MN domains and engineering MN specificity through the direct alteration of protein residue present in the DNA binding domain. The difficulty in reengineering and short editing efficiency limits the uses of MNs [11].

Transcription activators like effector nucleases (TALANs)

They are artificial DNA nucleases created by fusing a DNA binding domain with a non-specific nuclease domain obtained from Fok I endonuclease that particularly cut the required DNA sequence. TALE effectors DNA binding domain has a similar unit of 33 to 35 preserved amino acids and each repeat is similar, other than 12 and 13 positions which are variable and have high correlation with specific nucleotide recognition. DNA cleavage domain is not specific from Fok I endonuclease. The Fok I domain functions as a dimer which needs to be constructed with special DNA binding sites in the target genome. The number of amino acids in both TALE DNA binding and the Fok I are important for better activity. TALEN is used to check genomes by inducing DSB that cells make a response to with the repair mechanisms [12].

Zinc finger nucleases

It is artificially produced by fusing site-specific zinc finger protein with another non-specific cleavage domain of the FokI restriction endonuclease. The DNA binding component from 3 to 6 zinc finger repeats and each can recognize between 9 and 18 base pairs. ZFN has three zinc fingers in which each identifies or recognizes three base pair DNA sequences to form a three-finger array that is connected to nine base pair target sites and the non-specific cleavage domain. ZFPs provide a site-specific DSB to the genome and smooth the path for local homologous recombination that boosts targeted genome editing. The ZFN encoding plasmid-based targeted administration of necessary genes reduces the limitations of the viral administration if ZFNS are not fixed at the target site then off-target break might occur. Such off-target breakage might cause DBS which causes cell damage an off-target break might ease the random integration of donor DNA [13].

CRISPR-Cas

It is a heritable, versatile immune system of bacteria that supplies them with memory of former virus infections and protects against re-infection. Dissimilarity to the human adaptive immune system, CRISPR is passed on to the next generation of bacteria providing the colony immune to future virus infections. CRISPR immunity depends on the incorporation of the invader's DNA (i.e. virus or plasmid) into the bacterial genome [14]. CRISPR assists the bacterium in recognizing the viral sequences and breaking them. CRISPR stands for clustered regularly interspaced short palindromic repeats that are broken by "spacer" sequence. These "spacer" sequences are viral sequences incorporated during past viral infections when reproduced into short RNA sequences, which are capable of leading the Cas endonuclease to complementary sequences of viral DNA. Upon target recognition, Cas binds to viral DNA and cleaves it, and safeguards the prokaryotic cell from infection. CRISPR's immune system alters to create a gene editing tool that can target changes to DNA. The ultimate common is CRISPR/Cas9, which has the Cas9 endonuclease and a small noncoding guide RNA (gRNA) that contains two elements: a target-specific CRISPR RNA (crRNA) and a co-worker trans-activating RNA(tracrRNA). The gRNA unit escorts Cas9 to a specific genomic locus by base pairing between the crRNA sequence and the target sequence [30]. CRISPR-Cas-mediated gene repair, insertion or deletion and disruption so helpful in finding applications in many areas of biomedical research, agriculture, medicine and biotechnology [15].

Gene Transfer Technologies

After the arrival of recombinant DNA technology which helps in gene therapy and how effective and safely administered gene products are has become a big challenge. A vector is a vehicle that is used to deliver the gene of interest. An ideal vector can deliver a gene to a specific tissue, put up enough foreign gene size and attain the level

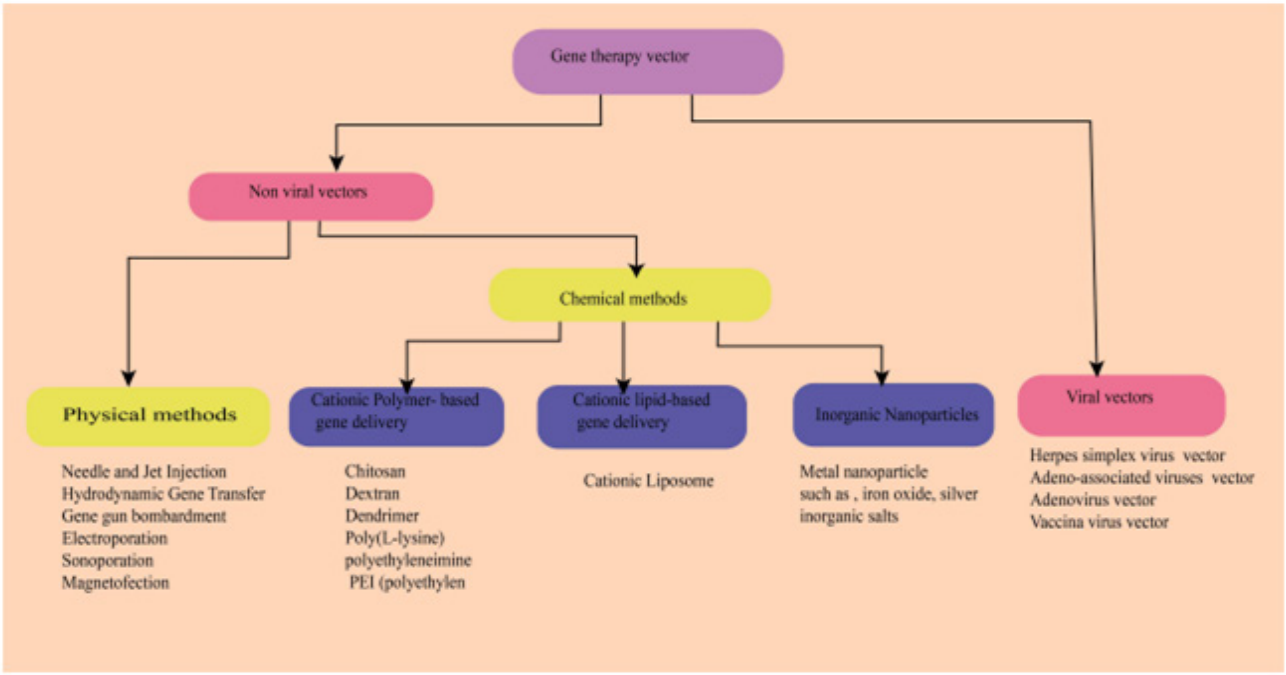

Figure 1: Overview of delivery systems which are used in gene therapy

and the time of transgenic expression which is enough to correct the defect gene. Transfer of the gene products is done by the viral vector, autofiction and non-viral vector (ie physical and chemical) methods as given in Figure 1 [16]. The important step of gene therapy is choosing of suitable vector.

Viral vectors used in gene transfer or gene delivery

Viruses were the earliest and most popularly used vectors to administer genes into target tissue. Viral vectors make sure that almost all cells can be infected without affecting the viability of the cell. Viruses have unique features that make them suitable for the delivery of genes in clinical practice. Surface proteins on viruses interconnect with host receptors which activate endocytosis. Once it enters, viruses deliver their genome into the nucleus for viral gene expression. Adeno-associated virus (AAV), herpes simplex virus (HSV), adenovirus (Ad) and lentivirus (LV) are extremely important viral vectors [17].

Bacterial-mediated gene transfer (Bactofection)

Some of the bacteria specifically target tumor cells which leads to RNA interference (RNAi) and gene silencing by stopping RNA activity like protein synthesis. Many *in vivo* and *in vitro* studies have shown that intracellular bacteria such as *Listeria monocytogenes, salmonella* spp, *shigellaflexneri, E.coli, Bifidobacteriumlongum,* and *Yersinia enterocolitica* use to transfer plasmid prodrug converting enzymes and tumor causing agents (cytotoxic agents) into the target cell. Phase 1 trials are done by using *listeria, salmonella, shigella, Bifidobacterium* and *clostridium* gene therapy for cancer and other clinical trials are going on the effects of lactococcus synthesizing interleukin 10 against the disease called colitis in phase 2 [18].

Chemical-based non-viral vectors

Viral vectors are based on gene transfer and display better and longer gene encoding but have some limitations like carcinogenicity, immunogenicity, less specific to target cell, high cost, and inability to deliver large genome size. Non-viral methods give better advantages because it is safe, easy to produce, and can deliver large genomes. Chemical vectors also called non-viral vectors which are grouped as organic and inorganic vectors. The organic vector is made of cationic lipid-based vectors: peptide-based vectors and synthetic cationic polymer-based vectors. These cationic organic vectors form complexes with DNA which is negatively charged with the help of an electrostatic bond. The complexes keep the genomic material safe and increase cell uptake and intracellular delivery. Normally, non-viral vectors help to transfer Large DNA (plasmid DNA), small DNA and RNA (Si RNA, mRNA) into target tissue [19]. Physical methods use distinctive mechanical forces to ease the administration of gene material into the host tissues [47]. It is different from viral and chemical methods to decrease barriers that restrict DNA transfer into host tissues. It is achievable to deliver genes into host tissues with the help of mechanical forces. There are several methods and most of them have the same mode of gene transfer *i.e.* physically formed transient pores into the cell membrane through which the genetic material can enter the host cell [48,49]. Hydrodynamic gene transfer, needle and jet injection, sonoporation, electroporation, magnetofection and gene gun bombardment are some of the examples of physical DNA delivery methods [20].

Gene therapy strategies for cancer treatment

Cancer is a diverse and complex disease in which some cells of the body grow rapidly which are uncontrollable and invade other tissues,

with notable individual differences. Progress in cancer therapy needs a new therapeutic agent with a new mode of action, with many mechanisms of cell death and synergy with conventional management. Cancer is a serious public health issue worldwide. In November 2017, more than 2597 clinical trials were conducted in gene therapy in the whole world. Among these trials, more than 65% were associated with cancers which were followed by monogenic and cardiovascular diseases. Till August 2019, only 22 gene products were approved for the treatment of different diseases. It has been reported that cancer has caused about 10 million deaths in 2020[21].

The treatment choice is made according to the type of cancer, grade, prognosis and the individual. Treatment choices for cancer are chemotherapy, radiotherapy, surgery, hormone treatment, immunotherapy, hyperthermia, photodynamic therapy, stem cell transplantation and targeted therapy. Gene therapy is different from traditional methods because it can be used without showing many side effects [22].

As given in Figure 2, gene therapy strategies for the treatment of cancer can be classified as suicide gene therapy, tumor suppressor gene activation, inhibition of oncogene activation, immunotherapy and antiangiogenic gene therapy.

Suicide gene therapy

A toxic agent is in which is chemotherapeutic given to cells in the form of a prodrug. A gene that codes enzymes that start up the prodrug is transferred to cancer cells. Thus the start-up drug with harmful effects kills the cancer cells. The most investigated suicide gene or prodrug system in the sector is the herpes simplex virus thymidine kinase or ganciclovir system. The enzyme catalyzed by the phosphorylation of ganciclovir causes the transformation of ganciclovir to ganciclovir. This product becomes ganciclovir triphosphate with the help of cellular enzymes. The end product causes the death of the cell by binding to DNA and causing the end of a chain. Ganciclovir triphosphate cannot pass the cell membrane while ganciclovir can pass and stay in the cell. In current studies, radio frequency hyperthermia and the herpes simplex virus thymidine kinase /ganciclovir (HSV-TK/GCV) gene therapy combination stand out as a new technique of therapy. It has proved that it is effective and promising anticancer efficiency in mice and rats [23].

A recent study has shown that a combination of cyclophosphamide and CYP26TM-RED suicide genes have main effects on tumor treatment. Despite recent developments mentioned here, the point that is forgotten is that this therapy option that the active drug might also affect neighboring cells [24].

Tumor suppressor gene activation

Other genes responsible for the transformation of healthy cells into cancer cells are recessive suppressor genes. As the name suggests, these genes play a function in the inactivation of cancer pathogenesis. Rb gene, p53gene, CDK inhibitors, and BRCA-1/2 are chief tumor suppressor genes. They prevent the uncontrollable proliferation of cells by triggering apoptosis or inhibiting the cell cycle [59, 60]. p53 gene is a more vital and highly studied tumor suppression gene. it is responsible for the induction of apoptosis and detection of DNA damage. The p53 protein interrupts cell cycle and differentiation, that is the biochemical pathways of IAP gene families bcl-2 and capase. It was concluded from a result of meta-analysis that p53 is effective in cervical cancer [25].

The anti-tumor effect of the combination of cisplatin which is used for the treatment of bladder cancer with p53.p16 and PTEN gene therapies have shown superior to classical cisplatin treatments [26].

Immunotherapy

Most of the cancer therapies nowadays have been conducted with immunotherapy. Cancer immunotherapy depends on the immune system of patients to identify and attack cancer cells. Cancer antigens are intercellular molecules and cancer cells are immunogenic. Therefore, T-cell-mediated cellular immunity is much more important than B-cell-mediated humoral immunity. Nevertheless, a normal immune response is not enough to remove tumor cells. The potential of cancer cells to escape the immune system relies on the secretion of immunosuppressive factors, downregulation of major histocompatibility complex molecules, and antigen expression. Taking into consideration these genes therapy approaches were formed that target the synthesis of major histocompatibility complexes, multiple genes that code for immunostimulant factors and costimulatory molecules [64]. Cellular toxicity is somewhat considered to play an important role in anticancer immunity. In

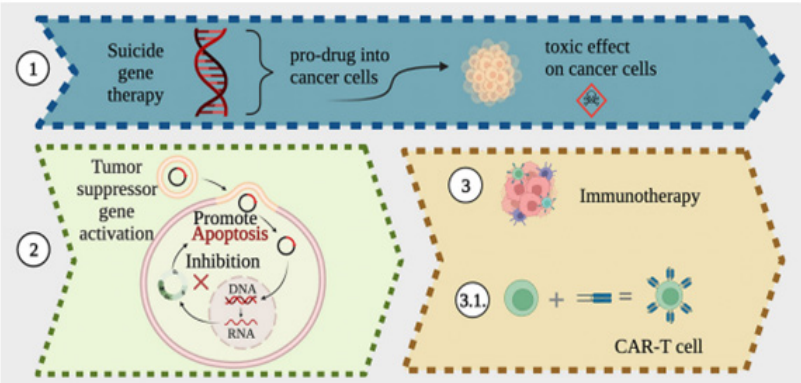

Figure 2: Gene therapy strategies in cancer treatment are briefly summarized. (1) Suicide gene therapy: In this strategy, prodrugs which turn into an active form and show cytotoxic effects in the cells are transferred into cancer cells. (2) Tumor suppressor gene activation: The cell cycle is inhibited or apoptosis is promoted by transferring tumor suppressor genes into cancer cells. (3) Immunotherapy: Cancer immunotherapy's are designed to induce or enhance T cell reactivity against tumor antigens. CAR-T is a T cell transduced with a chimeric antigen receptor specific for a tumor-associated antigen.

cancer, different immunological molecules are used to obtain an antitumoral immune response that is helpful in gene immunotherapy. For instance, genes that code for different cytokines *in vivo* or *ex vivo* are transferred to cancer cells [27]. Cancer cells synthesize proteins that are coded by the genes and are transferred to the tumor microenvironment. These immune system stimulating factors regulate the tumor microenvironment.

In ovarian cancer, successful therapy with the right conventional surgery methods is hard. Even after applying immunotherapy for an active immune response against this cancer, the lack of an effective *in vivo* gene delivery system is still a carrier in clinical practice. To control these problems, Jing *et al.* have invented a non-viral vector that codes for the anti-EpCAM/CD3 bispecific antibody. Particularly gene expression was achieved in the peritoneal cavity. It was found that the cancer cell's growth is delayed and the survival rate of rate is prolonged with limited toxicity. The results of this study show that CaPO4 Nano-needle-based non-viral gene therapy may have a great future in clinical practice. These days with improvements in immunotherapy, the discovery of tumor-related antigens and mechanism of antitumor immune response are being clarified. One of the important steps in developing gene therapy for a disease of cancer is cloning tumor-related antigens for human tumor cells. In many cases, tumor cells can be recognized by $CD8^+$ or $CD4^+$ T lymphocytes [28].

Challenges and Limitations of Gene Therapy

- Enhancing the difficulty of CAR designs may expand the risks related to CAR-T cell therapy.
- Alteration of CAR-T cells could raise the effect of these unfavorable events.
- In a particular variety of blood cancers, there is a possibility of CAR-T cell patricide and reduction caused by common antigens in the middle of leukemic and CAR-T cells like CD7 or CD5.
- The therapeutic techniques can cause latent complications such as cytokine release syndrome and neurotoxicity.
- Less bioequivalence, toxicity, expensive, and reliability problems [29].
- The process including isolation, alteration, and increasing the size of T cells exterior to the body prior to re-administration takes a longer time.
- Effectively transferring CAR Transgenes to targeted T cells internally to the body.
- Supplying risk and high production costs that are related to viral vector-based therapies nanocarrier platforms are present to be highly favorable for targeting T cells *in vivo* [30].
- Chemotherapy is not suitable for the treatment of cancer especially, breast and prostate cancer, because it leads to many adverse reactions.
- Gene therapy minimizes the challenges that are related to the recombinant therapeutic use of peptides which include low bioavailability, extreme toxicity, instability and more production costs.
- Due to ramification and low editing efficiency in re-engineering reduce the use of Meganucleases (MNs) [31].
- Viral vectors associated with gene therapy has some challenges like immunogenicity, carcinogenicity, less specific to target cells and inadequate release of big genome size.

Clinical trials and current Applications

Approved Gene Therapies for Cancer

- Suicide gene therapy is considered to be one of the most efficient treatments for breast cancer.
- At present, gene therapy utilizes four types of gene-editing nuclease enzymes which are Meganucleases, Zinc-finger nucleases, Transcription activator-like effectors nucleases, CRISPR- associated nucleases [32].
- CAR T cell therapy is applied in the treatment of myeloid and lymphoid leukemia.
- Oncolytic Virotherapy is a favorable application in tumor immunotherapy.
- In the year 2003, Gendicine was the foremost gene product that was approved by the Chinese State Food and Drug Administration (SFDA) for the treatment of neck and head squamous cell carcinoma [33].
- Currently, more than 30000 patients are being recovered by GENDICINE.
- Oncorine is approved for the management of refractory nasopharyngeal cancer.
- Many antiangiogenic monoclonal antibodies that stop the VEGF response have been approved commonly BEVACIZUMAB in the last 20 years.
- IMLYGIC was the first approved viral Oncolytic gene therapy medicine in the management of melanoma in the USA in the year 2015.
- FDA has approved KYMRIAH as a CD19 CAR T cell, gene product in the management of B-cell acute lymphoblastic leukemia
- YESCARTA can be used in the management of adult B cell lymphoma and aggressive non-Hodgkin lymphoma.
- The Philippines Food and Drug Bureau in the year 2007 approved REXIN-G in the management of soft tissue sarcoma and osteosarcoma.
- In 2017, the FDA and EMA approved CAR T cell established therapy in the management of blood cancers, lymphoma leukemia, and multiple myeloma.

Clinical trials and their outcomes

1. TALEN has been included in clinical trials to establish CAR for specific types of blood cancer like acute myeloid leukemia, lymphoid malignancy, refractory B cell, multiple myeloma, and B cell acute lymphoblastic leukemia and is involved in increasing gene editing outcomes [34].
2. Various microorganisms such as *Listeria, Bifidobacterium, Salmonella, Shigella*, and *Clostridium* gene therapy are ongoing under the phase 1 clinical trial against cancer.
3. In phase 2 of the clinical trial, events of lactococcus producing IL-10 are ongoing against colitis.
4. In the year 2017 Nov, more than 2597 clinical trials have been performed on gene therapy universally [35].
5. Amid particular trials more than 65% are related to cancer ahead of monogenetic and cardiovascular diseases.
6. Patients suffering from prostate cancer were studied in phase 1

clinical trials by utilizing the Oncolytic adenovirus CD/HSV-1 TK gene [36].

Future directions and innovations

In recent decennary, gene therapy in cancer treatment has been encouraged faster but for now, some drugs are profitably accessible, while leftovers are in clinical trials [37].

Gene therapy is more harmful than chemotherapy, hence it can considered as a safe and effective treatment of cancer in the future.

Gene therapy can modify the upcoming cancer therapy by generalized cancer management tactics, depending upon nature, location size of the tumor as well as immune status and genetic profile of the patient.

The death rate of cancer has been reduced due to early detection by various therapies.

Various gene therapy techniques are used which are suicide and toxin gene therapy, Immunotherapy, SiRNa with antisense technology, MDRI-transduced cells, etc. [38].

Except for the above strategies, gene therapy also reduces some challenges like the alteration of viral vectors to decrease toxicity and immunogenicity, but enhances the transportation efficiency of non-viral vectors. Gene therapy also minimizes the harmful events related to retrovirus vectors for gene therapy in humans. Treatment of cancer by gene therapy is more effective and favorable.

CONCLUSION

Gene therapy for cancer treatment represents a promising frontier in oncology, offering potential solutions for previously untreatable or hard-to-treat cancers. By targeting the genetic and molecular underpinnings of tumors, gene therapy aims to repair, replace, or silence faulty genes within cancer cells, enhance the immune system's ability to recognize and destroy cancer, or directly deliver therapeutic genes to the tumor site. This approach is still in its early stages, with significant progress in clinical trials demonstrating potential, especially in personalized and targeted treatments.

However, challenges remain such as ensuring safe and effective gene delivery, minimizing off-target effects, and overcoming resistance mechanisms. Furthermore, the high cost of developing gene therapies and the complexity of tailoring treatments to individual genetic profiles pose hurdles. Despite these obstacles, ongoing research and technological advancements, particularly in CRISPR and viral vectors, continue to enhance the feasibility and precision of gene therapy.

In conclusion, while gene therapy for cancer holds immense promise, it is not yet a mainstream treatment option. Continued innovation and refinement will be essential to address current challenges and bring gene-based treatments into broader clinical use. As research progresses, it is likely that gene therapy will become an integral part of the cancer treatment landscape, offering more targeted, effective, and personalized options for patients [39].

CONFLICT OF INTEREST

None

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