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Review

Challenges and Opportunities of Gene Therapy in Cancer

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Abstract

Gene therapy involves either the direct introduction of genetic material (DNA or RNA) into the host cell (or organ), known as *in vivo* gene therapy, the re-introduction of the modified target cells taken out of the host, or *ex vivo* gene therapy. Cancer is mainly caused by the non-functioning of genes required for normal cell proliferation, and it has emerged as the leading cause of death globally due to the absence of efficient and safe therapies as well as early diagnostic modalities. Therapeutic trials using gene therapy have shown that they considerably increase the survival rate and life expectancy of patients with cancer. There are

many potential strategies for the treatment of cancer using gene therapy currently being used, including (a) expressing a gene to induce apoptosis or increase tumor sensitivity to conventional drug/radiation therapy; (b) inserting a wild-type tumor suppressor gene to compensate for its loss/deregulation; (c) blocking the expression of an oncogene using an antisense (RNA/DNA) approach; and (d) enhancing tumor immunogenicity to stimulate immune cell reactivity. Gene therapy can employ many different genes, including anti-angiogenesis, any suicidal gene, immunotherapeutic gene, siRNA gene, pro-apoptotic gene, oncolytic gene, and gene-directed enzyme prodrug. Moreover, with advancements in gene transfer technologies, various kinds of new treatment strategies have been developed that complement conventional therapies used to treat cancer that are used to modify the DNA directly, such as zinc finger nucleases (ZFNs), transcription activator-like effector nucleases (TALENs), clustered regularly interspaced short palindromic repeats/CRISPR-associated protein 9 (CRISPR/Cas9), etc. Even though there has been a lot of progress in pre-clinical research in both better targeting and expression in a tumor-selective way, there are still a lot of problems that need to be fixed before it can be used in humans. These problems include non-specific expression, low-efficiency delivery, and biosafety. This review will highlight gene therapy's current challenges and future opportunities in cancer treatment.

Keywords
Cancer; gene therapy; tumor suppressor gene; oncogene; therapeutic; CRISPR-Cas system

1. Introduction

About 50 years ago, scientists first proposed the idea of gene therapy, which introduces recombinant genetic material into human cells to treat various diseases. As a result of the challenging and constrained development process that produced this method as an effective treatment, additional advancements were made [1]. Gene therapy is defined by the European Medicines Agency (EMA) as “a biological medication that contains an active ingredient that is a recombinant nucleic acid and is used or given to humans to alter, correct, add, replace, or delete genetic sequences, and those therapeutic, prophylactic, or diagnostic effects are directly related to that nucleic acid or to the gene expression product of that sequence.” Gene therapy drugs do not contain contagious disease vaccines [2].

The history of gene therapy (Figure 1) begins with the first mention of natural genetic transfer in 1928 when F. Griffith discovered a process called transformation through the observation that a heat-killed extract of a virulent pneumococcal strain could transform a non-virulent strain into a virulent strain [3]. Then, other natural gene transfer techniques were also discovered, such as conjugation and transduction [2]. Elisabeth and Wacław Szybalski, in 1962, successfully corrected HPRT-deficient mammalian cells using gene therapy by transferring normal DNA to diseased cells [4]. In 1966, Edward Tatum explored the potential of gene therapy through viral vectors in somatic cells [5]. A study by Rogers et al. (1968), built on Tatum's work, showed that virus-mediated gene transfer could work. They used the wild-type (WT) Shope papilloma virus to introduce the arginase

gene into two female pediatric patients with a urea cycle disorder, but the results were not positive [6].

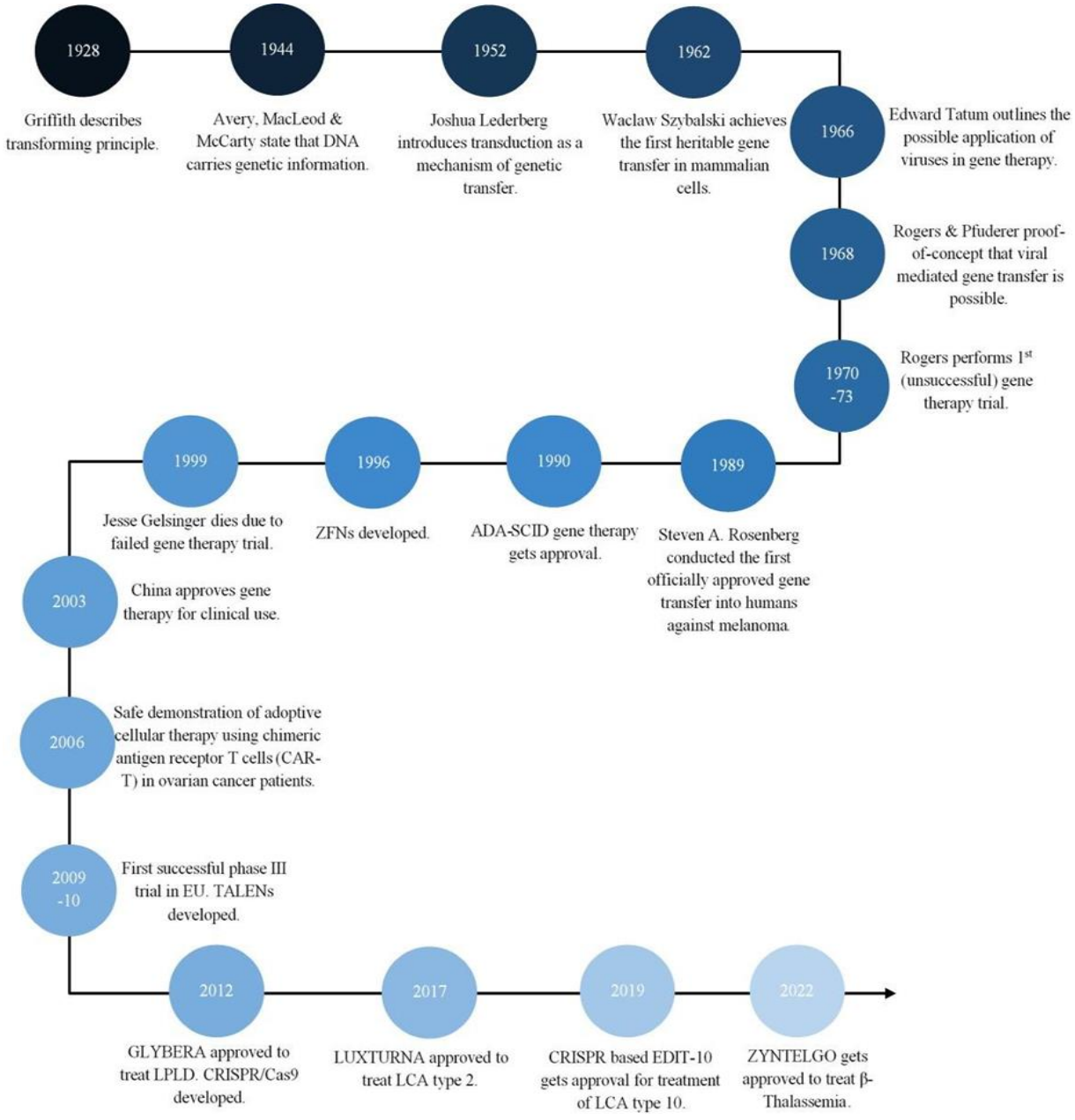

Figure 1 Systematic timeline showing the development of gene therapy over the years.

Later on, in 1989, the first clinical protocol to insert a foreign gene into human immune cells was approved by the FDA and the National Institutes of Health (NIH), and it was the first test performed on terminally ill cancer patients [7]. Many clinical trials were conducted in subsequent years. In 1990, the NIH made further advances and approved the first clinical trial of gene therapy against severe combined immunodeficiency (SCID) in a four-year-old girl by transforming the normal adenosine deaminase (ADA) gene. Despite receiving a lot of criticism from the media about the use of viruses in gene therapy, the observed results were positive, showing immense success when the four-year-old turned into a healthy 18-year-old, making this the first successful event in the history of gene therapy [8]. However, a few trials also failed, as 18-year-old Jesse Gelsinger was injected with 38

trillion recombinant adenovirus particles to treat ornithine transcarbamylase deficiency, resulting in his death [9]. Despite this tragedy, scientists are still making progress because novel genetic therapies promise to be far more effective than current approaches, such as protein therapy or pharmacotherapeutics, in treating a wide range of diseases and defects.

In 2006, Kershaw et al. demonstrated that T cells exhibiting a response to the ovarian cancer-related alpha-folate receptor (FR) were produced through genetic modification of the patient's T cells. This was achieved by introducing a chimeric gene that combined an anti-FR single-chain antibody with the signaling component of the Fc receptor gamma chain. However, the cells did not sustain themselves for over a few days [10]. Following the success of initial clinical trials of gene therapy, researchers have been delving deeper into gene therapy to find a safe, long-lasting, and hopefully permanent cure to treat fatal diseases. At present, active clinical trials to treat SCID-X1 are taking place, wherein retroviral vectors are used to deliver a functional copy of the mutated and dysfunctional genes for autologous hematopoietic stem and progenitor cells (HSPCs) [11] to promote thymopoiesis [12]. Moreover, multiple gene therapies started becoming available for clinical use, e.g., GLYBERA for Lipoprotein lipase deficiency (LPLD) [13], LUXTURNA for Leber congenital amaurosis (LCA) type 2 [14], Betibeglogene autotemcel (beti-cel) gene-therapy (ZYNTEGLO) for β-Thalassemia, and so on [15].

2. Types of Gene Therapy

Based on the target cell, gene therapy is classified into two categories, i.e., somatic cell gene therapy and germline cell gene therapy (Figure 2). A recombinant gene is placed into a healthy somatic cell in bodily cell gene therapy. In contrast, the same gene is inserted into the genome of a germ cell or stem cell in germline gene therapy to replace a defective gene that is responsible for a particular disease [16]. Based on the process, gene therapy is classified into three categories: *ex vivo*, *in vivo*, and *in situ* (Figure 2). *Ex vivo* gene therapy removes diseased cells from the patient's body. Then, they are treated outside the body by modifying genetically, allowing the disease's phenotype to be corrected. The treated (or fixed) cells that have gained normal function are subsequently infused into the patient's body.

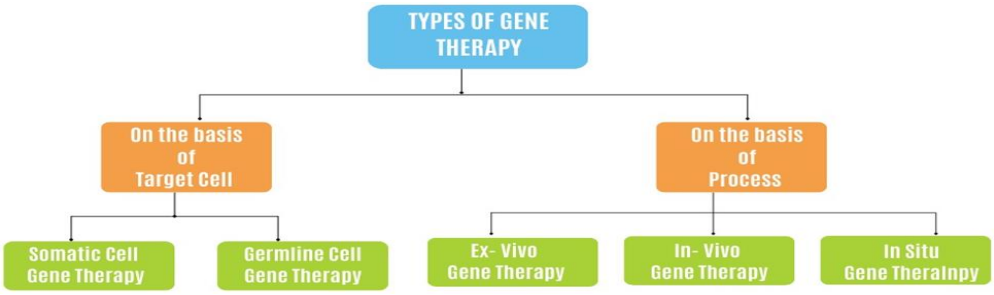

Figure 2 Types of gene therapies -On the basis of Target cells and Processes.

In vivo gene therapy, the diseased cells are treated in the patient's body without taking them out using different viral vectors, such as adenoviral vectors (AVs) or adeno-associated viral vectors (AAVs). In this approach, the recombinant vector containing the gene of interest is infused systemically into the patient's bloodstream or cerebrospinal fluid, which then attaches to the specific cells and delivers the correct version of the gene into them. *In situ,* gene therapy is similar

to *in vivo* gene therapy, but in this, the recombinant viral vector is directly injected into a patient's body at the site of diseases such as a tumor or a suitable area of the brain [17].

3. Types of Cancer Therapy

With more than 10 million fatalities caused by cancer each year, it has become one of the main reasons for death worldwide [18]. Endogenous or environmental factors continuously stress cells, resulting in DNA damage. To preserve the accuracy and fidelity of genomic DNA, a sophisticated mechanism has been developed by the host cell's i.e., DNA damage response (DDR) network, which includes DNA repair mechanisms, apoptotic machinery, and cell cycle checkpoints [19-22]. Any of these altered or mutated mechanisms trigger additional mutations in cells, which introduce genomic instability. Along with the growing DNA damage accumulation, this genomic instability is a defining characteristic of cancer [23].

The advancement of technology has led to new therapeutic methods for cancer treatment apart from the standard ones, which include surgery, chemotherapy, and radiotherapy. Other methods, like immunotherapy, hormone therapy, and stem cell therapy, have been developed recently and are undergoing trials to increase the survival chances after cancer treatment (Figure 3).

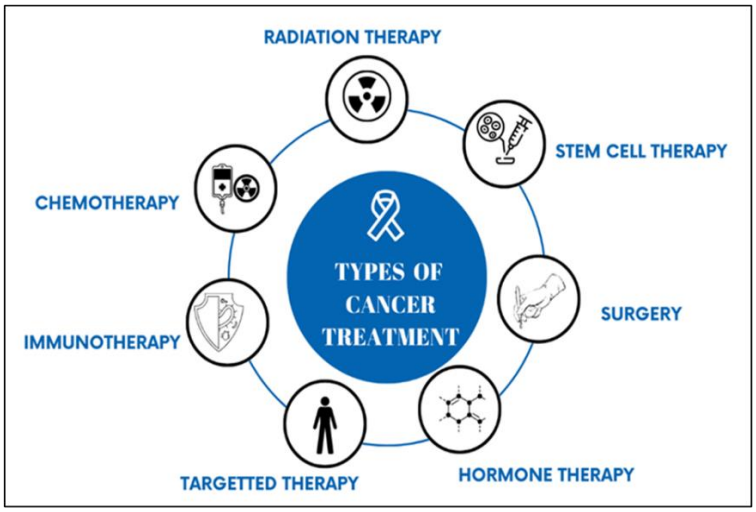

Figure 3 Types of cancer treatment - radiation therapy, stem cell therapy, surgery, hormone therapy, targeted therapy, immunotherapy, and chemotherapy.

4. Other Therapies for Cancer Treatment

4.1 Stem Cell Therapy

The patient's stem cells are used as therapeutic agents in this treatment. Various modified and unmodified patient's stem cells are transplanted to induce cancer cell death or as carriers for other medicinal drugs. Some types of stem cells, like hematopoietic stem cells, can also help enhance the body's immune system against cancerous cells to treat cancer. However, stem cell therapy can sometimes lead to tumorigenesis as it interacts with other cancerous cells, immune response-related side effects, or dysfunction of tissues or organs. Hence, this approach has been proven less effective, but these drawbacks have led to progress in cancer treatment, and now the immune

system is being utilized to treat cancer. Immunotherapy has evolved from all these therapies and is currently leading the race for cancer treatment [24].

4.2 Immunotherapy

Immunotherapy is a therapy that utilizes the immune system as a possible treatment for a disease. It can stimulate or inhibit the immune response to treat or prevent the disease. The history of immunotherapy is intertwined with that of cancer. German physicians Busch and Fehleisen were the first to notice tumor regression independently in immunotherapy patients [25]. Later on, many studies have shown the effect of immunotherapy on cancer regression [26, 27]. Immunotherapy can be used in various ways, as discussed below (Figure 4).

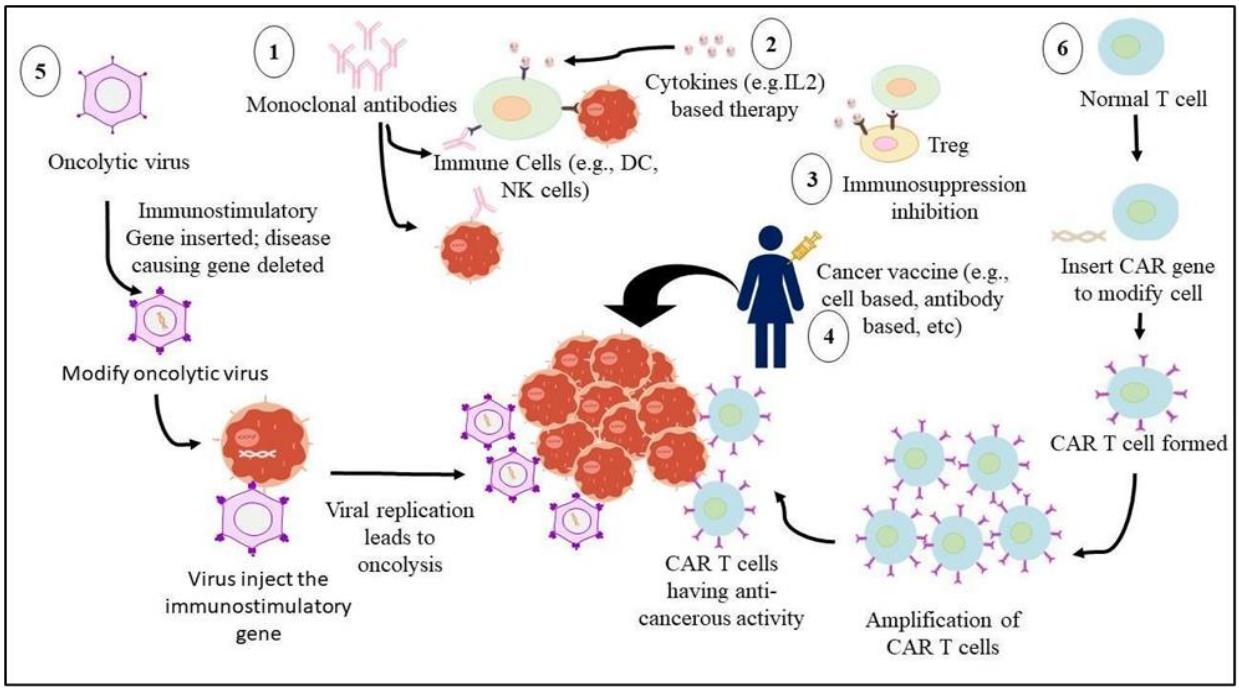

Figure 4 Various types of immunotherapies used against cancer - 1. Antibody-based immunotherapy 2. Cytokine-based immunotherapy 3. immunosuppression reducing immunotherapy 4. Cancer vaccines; 5. Viral based immunotherapy; 6. Adoptive cell therapy using CAR-T.

4.2.1 Antibody-Based Therapy

Signaling that contributes to the proliferation of cancer cells can be blocked by initiating an immune response against a particular antigen of the cell with the help of monoclonal antibodies. The development of rituximab, an FDA-approved monoclonal antibody against the CD20 cell marker of immature B cells involved in NK cell elimination, paved the road for many more monoclonal antibody-based therapies for cancer, including non-Hodgkin's lymphoma. Later, more antibody-based drugs were approved, like 4-1BB (CD137) against CD137L found on tumor cells and antigen-presenting cells, trastuzumab (Herceptin) for breast cancer, ipilimumab for blocking CTLA-4, and nivolumab for inhibition of the PD-1 molecule.

4.2.2 Cytokine-Based Therapy

In this type of therapy, various cytokines (such as interleukin 2) that have a role in immune cell proliferation are used as immunostimulatory molecules that can stimulate the immune response against cancer cells, such as metastatic kidney cancer or metastatic melanoma.

4.2.3 Immunosuppression-Reducing Therapy

It is a particular immunotherapy targeting tumor microenvironment (TME)-mediated immunosuppressive pathways. Blood vessels, fibroblasts, immune cells, and extracellular matrix comprise the TME, which can differ depending on the individual and the type of cancer. Targeting the immunosuppressive nature of the TME, especially downregulation of MHC class I and FAS/TRAIL molecules, and targeting enzymes, cytokines, and cells like Treg, can be a good strategy for cancer treatment.

4.2.4 Cancer Vaccines

Cancer vaccines like Hepatitis-B (HBV) vaccines and human papillomavirus (HPV) vaccines are the most common forms of immunotherapy being used. Cancer vaccines can be classified into two groups: autologous and allogeneic.

Autologous vaccines are personalized vaccines made from patients' cells that are modified and multiplied in the laboratory and re-injected into patients to evoke an immune response. The first autologous vaccine was developed to treat castration-resistant prostate cancer, called sipuleucel-T. In contrast, *allogenic vaccines* are prepared by growing the cells of other individuals in laboratories, which can trigger immune responses against cancer cells [27, 28].

4.2.5 Oncolytic Viruses

This is a unique type of immunotherapy in which viruses are genetically modified by replacing their pathogenesis genes with immunostimulatory genes that stimulate the immune response when transferred to the cells to treat cancer. One of the first oncolytic viruses approved by the FDA was the herpes simplex-1 virus T-VEC for treating metastatic melanoma. Pexa-Vec, CG0070, and G471 are a few others that have shown optimistic results in clinical trials [29].

4.2.6 Adoptive Cell Therapies

This therapy utilizes patient’s T cells, modified to target cancer cells. Though there are different methods to modify T cells, the most popular one is CART therapy [27]. Even after so much advancement, immunotherapy still has limitations, like immune-related adverse side effects, short-life, and a high dose requirement. Novel immunotherapies like those targeting programmed cell death (PD-1) or its ligand, chimeric antigen receptor (CAR)-T cell therapies, and nanomedicines can prove to overcome such limitations [30].

4.2.7 Chimeric Antigen Receptor (CAR) T Cell Therapy

Chimeric antigen receptors (CARs) T cell therapy, or chimeric immune receptor T cell therapy, is another immunotherapy-based approach to treating cancer. CAR uses recombinant techniques to

modify T cell receptors to target cancer-specific antigens by recognizing the epitopes in an MHC-independent manner [31]. These receptors have four parts: an antigen-binding domain, a hinge/space region, a transmembrane domain, and an intracellular signaling domain [32]. All parts have their specific functions. Antigen-binding domains mainly binds to the specific cell surface receptors in cancer cells via their variable heavy and light chains, like monoclonal antibodies [33].

CAR-T cell-associated toxicities are also one of the concerns. Thus, engineering the CAR structure such that its toxicity is limited to the tumor is essential. This can be done by decreasing affinity toward antigen to micromolar, having tumor-specific co-stimulatory molecules, altering the hinge and transmembrane regions to modulate cytokine secretion [34], and using human antibodies to engineer CAR rather than murine-derived antibodies [32]. Having "off-switches" in CAR can regulate the immune response (e.g., CD20 expression in CAR can help reduce CAR-T cells via rituximab treatment) [35].

5. Gene Therapy for Cancer Treatment

Even with the great potential of the therapies discussed above, each has some limitations and drawbacks. Significant limitations are their side effects, lack of a specific delivery system, lack of specificity towards cancer cells, and cancer recurrence. To treat cancer, new treatment methods are being investigated in which gene therapy can be used as an alternative that is highly targeted towards the treatment of cancer. Various strategies of gene therapy have been developed recently to treat cancer. Some of them are discussed below.

5.1 Strategies in Cancer Gene Therapy

As discussed earlier, in gene therapy, a regular or corrected version of a gene is mainly inserted into the diseased cell to treat the disease caused by mutation or non-functioning of any gene. Different gene therapy strategies have been developed to treat cancer by targeting other genes or pathways in the cell (Figure 5).

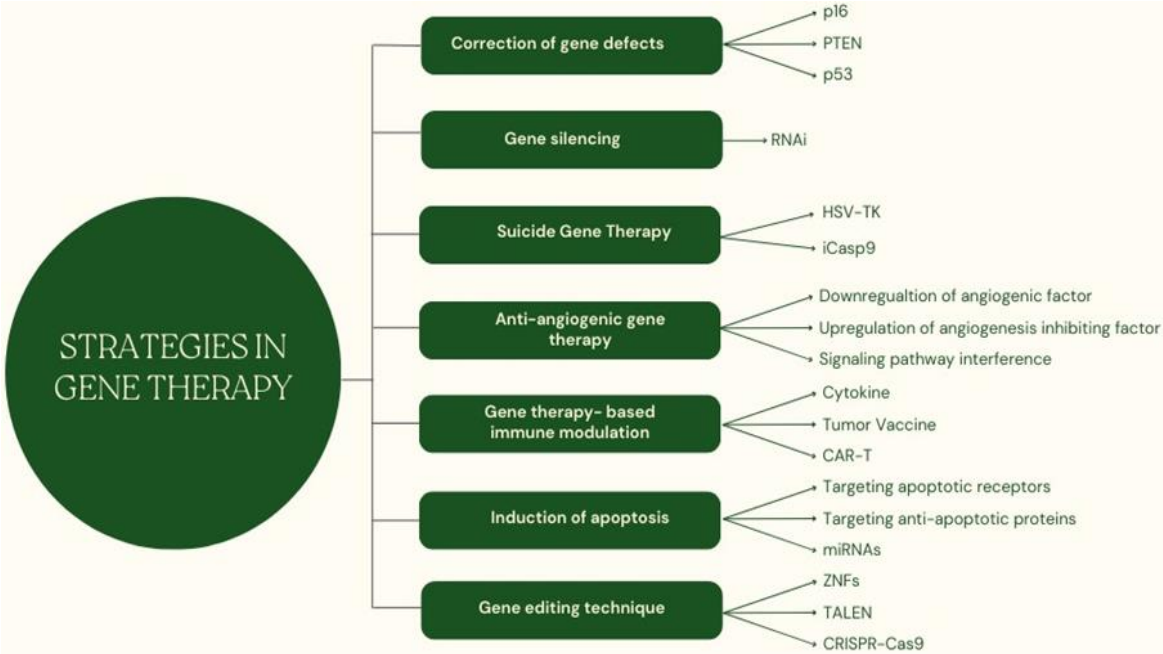

Figure 5 Strategies in gene therapy used for cancer treatment - Correction of genetic defects, Gene Silencing, Suicide Gene Therapy, Anti-angiogenic Gene therapy, Gene therapy-based immunomodulation, Induction of apoptosis, Gene editing techniques.

5.1.1 Correction of Gene Defects

Cancer is a multistage process that is mainly triggered by mutations in various genes concerned in cell cycle regulation, progression, or death. In this type of gene therapy approach, a corrected or standard version of a gene (such as the tumor suppressor gene, p53) is inserted into the cancerous cells to compensate for the loss/deregulation caused by mutation. Most tumor mutations are usually found in two types of genes: proto-oncogenes and tumor suppressor genes. Tumor suppressor genes regulate generally cell cycle, differentiation, and apoptotic machinery, such as p53, retinoblastoma gene (Rb), p16INK/CDKN2, and PTEN [36-39]. According to clinical research, the transfer of wild-type p53 genes into non-small cell lung carcinomas using retroviral vectors containing the human tumor suppressor p53 gene under the control of the beta-actin promoter results in cancer cell apoptosis and tumor regression [40]. The first gene product against neck and head squamous cell carcinoma to be approved was called Gendicine, a recombinant adenoviral vector expressing the p53 gene rather than the E1 gene developed by Shenzhen SiBiono GeneTech Co. Ltd [41]. Similar trials have been done for other tumor suppressor genes, such as Let-7. The effectiveness of conventional cancer chemotherapy may be increased by replacing these tumor suppressor miRNAs [42].

5.1.2 Gene Silencing

In certain cancers, some genes (such as oncogenes) become expressed or sometimes overexpressed to dysregulate the cell cycle and its normal progression and cause cancer. So, this strategy can be used in which genes are inserted in cells in such a way that they can initiate the RNAi pathway, which further blocks or knocks down the expression of an oncogene using an antisense miRNA or siRNA. In this strategy, small non-coding RNA (~21 nt) sequences bind to the target sequence to create a DNA-RNA hybrid, which is then degraded by the degradation machinery [43]. This process is particular to knocking down the expression of a gene. Moreover, it is beneficial to inhibit the expression of genes whose inhibitors have not yet been discovered [44]. For e.g., in many tumors, *myc* gene is involved in cancer, which mainly affects stemness and heterogeneity within tumors [45]. In melanoma and leukemia models, an encapsulated phosphorothioate c-*myc* antisense oligonucleotide decreases the expression of c-*myc*, resulting in a less aggressive tumor and increased survival [46]. Similarly, the k-*ras* gene plays an important role in expanding colorectal cancer (about 40-60%) as the k-*ras* protein became permanently activated due to mutation. Studies show that mutant-specific small interfering RNA against k-ras in pancreatic cancer cell lines decreases tumor cell proliferation, angiogenic potential, and capacity to form malignant tumors and increases tumor cell apoptosis [47, 48].

5.1.3 Suicide Gene Therapy

Suicide gene therapy, in which a transgene is introduced into the tumor and expressed, has shown to be a successful strategy. Prodrug administration may occur after transgene delivery. When

a transgene is expressed, it either produces a toxin for the cells or changes a prodrug, an inactive drug, into an active toxic form that further kills cancer cells. Thus, this therapy is also known as toxin gene therapy or gene-directed enzyme prodrug therapy (GDEPT).

In the first approach, a suicidal gene called inducible caspase 9 (iC9) causes the activation of the apoptosis machinery in cancerous cells in non-small-cell lung cancer (NSCLC) through the oncolytic or conditionally replicating adenoviruses (OAdV/CRAd) that are controlled by the mesenchymal stromal cell (MSC) delivery system [49, 50].

Another suicide gene treatment tested *in vitro* delivers the gene for the enzyme cytosine deaminase (CD) along with the prodrug 5-fluorocytosine (5-FC). One of the common medications used in chemotherapy to treat hepatocellular carcinomas (HCC) is 5-fluorouracil (5-FU), which is created when cytosine deaminase deaminates the chemical [51]. In another report, Herpes Simplex Virus (HSV)-thymidine kinase (TK)-expressing tumor cells showed higher sensitivity towards ganciclovir triphosphate. HSV-TK enzyme converts the prodrug ganciclovir into its active form, ganciclovir triphosphate [52]. This strategy has demonstrated encouraging results in patients with prostate cancer and malignant gliomas [53-55].

5.1.4 Anti-Angiogenic Therapy

Angiogenesis, also known as the development of new blood vessels, is a critical step in tumor development, growth, and metastasis. As a result, there has long been interest in using it as an anti-cancer method [56].

Interleukin-12 (IL-12), which has also been associated in several studies with an anti-angiogenic effect, can boost the immune system. Nevertheless, systemic administration of recombinant IL-12 was associated with adverse reactions in clinical trials, including toxicity. According to the phase 1 study in malignant melanoma patients, intramural electroporation of plasmid IL-12 successfully generates systemic anti-tumor immune-mediated effects without appreciable local or systemic toxicity and improved survival, making it an effective tool for DNA plasmid gene transfer with potential applications as a gene therapy that supports anti-tumor immunity [57, 58]. Various studies suggest that the expression of the endostatin gene and Vastatin (a polypeptide of the NC1 domain of type VIII collagen) gene in kidney cancer cells and hepatocellular carcinoma, respectively, demonstrated an anti-angiogeneic effect, limiting tumor growth, and metastasis, increasing overall survival [59, 60].

5.1.5 Expressing a Gene to Induce Apoptosis

Besides uncontrolled cell proliferation, invasion of apoptosis is one of the significant characteristics imparted by cancer cells. Various direct and indirect mutations regarding the expression of pro- and anti-apoptotic genes have been observed in tumors. Therefore, targeting the induction of apoptosis machinery through gene therapy is the most common approach employed in cancer gene therapy. It can be carried out by introducing genes that can code for an inducer, mediator, or executioner protein in apoptosis [61, 62].

TNF-related apoptosis-inducing ligand (TRAIL) targets only malignant cells instead of normal cells and induces apoptosis in those cancerous cells only. Therefore, TRAIL and its receptors have been extensively studied. Griffith and coworkers first reported the possibility of TRAIL gene transfer therapy [63]. The novel strategy involves genetically modifying human mesenchymal stem cells

(hMSCs) with branched polyethyleneimine (bPEI) complexes and the TRAIL gene, which is a non-viral vector strategy [64]. Another example of an apoptosis-inducer is melanoma differentiation-associated gene-7 (mda-7)/Interleukin-24 (IL-24), which selectively induces apoptosis in various cancers without negatively impacting the corresponding normal tissue. Malignant cells (such as non-small cell lung cancer) can be made more sensitive to pro-apoptotic agents like initiator Casp8 by selectively silencing anti-apoptotic genes through siRNA or microRNA [65, 66].

5.1.6 Gene Therapy-Based Immunomodulation

To eradicate the target cancer cells, immunotherapy typically involves enhancing the host immune system. Circulating antibodies are a component of one arm of the immune system that B cells (humoral immunity) secrete after membrane immunoglobulin (B cell receptors) activate them. The cellular immunity (cell-mediated by T cells) mechanism allows the second arm to engage with antigens on tumor cells [56].

Cellular therapies using techniques like chimeric antigen receptor (CAR)-T cells, engineered T cell receptors, tumor-infiltrating lymphocytes (TILs), natural killer (NK) cells, and cytotoxic T lymphocytes (CTLs) are gaining popularity. One of the first cell therapies to receive approval was CAR-T cell immunotherapy. This treatment uses T cells from the patient or a healthy donor that have been genetically modified to produce antigen-specific receptors to cancer cells and then injected back into the patient. The CAR has an intracellular domain (ICD) for signaling, a transmembrane domain (TM), and a single-chain fragment variable (scFv) to find antigens that are specific to tumors. The CD3 chain, which controls IL-2 secretion and has anti-tumor activity *in vivo*, transmits tyrosine activation signals. T cells then use these signals to activate and kill their target cells. More costimulatory molecules were added to boost the effectiveness of the second generation of CARs. The third generation of CARs added another co-stimulatory domain to the design for enhancement. The fourth-generation CARs were created to co-express some significant cytokines, such as IL-7, IL-12, IL-15, or suicide genes, for a greater capacity of T cells to increase [67, 68].

5.1.7 Genome Editing - CRISPR/Cas9

Clustered regularly interspaced short palindromic repeats (CRISPR)/Cas9 is a widely used gene editing technology for correcting genome errors and turning particular genes on or off in cells and organisms. It is a defense system first found in *E. coli* and is present in bacteria to defend against foreign DNA and RNA by recognizing and destroying them [69]. Its large laboratory and medical applications, like rapid cell generation, animal model generation, functional genomic screening, and direct imaging of cellular genomes with relative ease of handling [70, 71], make it one of the best DNA editing techniques to be discovered compared to other engineered nucleases like TALEN and Zinc-finger nucleases [72]. CRISPR/Cas system is divided into two classes. Class I systems (types I, III, and IV) consist of multi-subunit Cas-protein complexes and are found more in archaea than bacteria. Class II systems (types II, V, and VI) that consist of a single Cas-protein are extensively used in genetic engineering due to their simple structure [73]. Unlike the other DNA editing tools, CRISPR/Cas9 consists of two essential components:

Guide RNA (sgRNA) which is made of CRISPR RNA (crRNA), which recognizes the target DNA, and a trans-activating CRISPR RNA (tracrRNA) that acts like a binding scaffold for Cas-9 nuclease [74].

Cas9 (CRISPR-associated protein 9) is an endonuclease that induces double-stranded DNA breaks, allowing genome modifications. It can bind sgRNA and specific target DNA with the help of its protospacer adjacent motif (PAM) domain and create a double-strand break (DSB) in the target sequence [72].

Two processes can repair the DSB generated by cas9. The first one is non-homologues end joining (NHEJ), which can potentially cause insertion or deletion mutations (indels). However, this mechanism is error-prone and leads to either loss of function, genomic rearrangements, or NHEJ-mediated homology-independent knock-in [75]. The other method is homology-directed repair (HDR), which utilizes assisted recombination of DNA donor templates (can be altered) to repair the DSB. HDR leads to gain-of-function mutations and can be used to insert tags, reporters, etc. HDR can be utilized for the introduction of [75].

Many scientists are researching to develop a therapy against HIV-1/AIDS based on CRISPR/Cas9 technology [76], but many other studies have demonstrated its use to target various genes causing tumors [77, 78]. For example, PD1 was either singly targeted or in conjunction with the native TCR (T cell receptor)-alpha constant chain (TRAC) and T cell receptor-beta constant chain (TRBC) genes utilizing CRISPR-Cas9 gRNA-mediated KO [79]. A different team employed Cas9 to enhance CD19 CAR targeting to the TRAC locus in T cells [80]. This led to uniform CAR expression and boosted CAR-T cell potency compared to CARs randomly inserted into the genome.

In vivo CRISPR therapies have also shown potential in preclinical studies. A study focused on oncogenic gene fusions, which provided cancer cell selectivity due to the fusion, as well as the disruption of a genetic defect that promotes tumor growth [81]. Another preclinical example used nuclear factor-B (NF-B), which is only active in cancer cells, to trigger the transcription of CRISPR-Cas13a components and cause oncogene silencing specific to cancer cells [82].

Many clinical trials have been done using various approaches to gene therapy to treat cancer (Table 1).

Table 1 Number of gene therapy clinical trials in cancer treatment regarding targeted gene, vector, toxicity issue, and clinical outcome.

Type of Gene Therapy	Cancer Targeted	Gene Targeted	Vector	Trial ID	Reference
Immunotherapy	Pancreatic	CEA and MUC-1	Vaccinia and fowl pox virus	NCT00088660	[83]
Immunotherapy	Kidney	CD-80	Adenovirus	NCT00040170	[83]
Gene transfer	Glioblastoma	*HSVtk*	Mouse retrovirus	NCT00001328	[83]
Gene transfer	Head and neck	P53	Adenovirus	NCT00041613	[83]
Antibody-Based	BREAST	Osteoactivin/ GPNMB	Rat Hepatoma cells	NCT01156753	[84]
Antibody-Based	Breast and ovarian	HER2/Erb2	mice	Multiple	[84]

6. Vectors for Therapeutic Gene Delivery

Gene therapy introduces genetic material into cancer cells without damaging non-cancer cells. Targeted therapy in cancer is of utmost importance for successfully implementing gene therapy. For the success of gene therapy, the delivery of the therapeutic gene to the targeted cell/tissue is crucial. The therapeutic gene can be delivered into the cells by various means. The simplest way is to provide nude DNA directly into the cells. However, the method has many limitations, such as more amount and large size of DNA that can be provided. Moreover, nude DNA is more prone to nuclease digestion. These limitations can be overcome using some carriers, usually known as 'vectors' [85]. These vectors are used in the CRISPER/Cas9 system, which lets scientists make big changes to specific cells, like deletion of large DNA and genomic rearrangement in hematopoietic progenitors and embryonic stem cells [86]. Additionally, it has also come with certain side effects, such as nucleus structural flaws, micronuclei, and chromothripsis, a type of chromosomal rearrangement that is flawed [87]. Overall, Cas9-expressing cell lines that were transported by vectors showed an increased amount of DNA repair. This finding opens a new avenue for studying tumor heterogeneity and cancer genomics, as well as potential therapeutic vulnerabilities [88, 89].

A vector should ideally be injectable, have *in vivo* target specificity, be regulatable, have the ability to maintain prolonged gene expression, and be nonimmunogenic [90]. There are two main strategies used in gene delivery: viral-based vectors and non-viral-based vectors (Figure 6) [91].

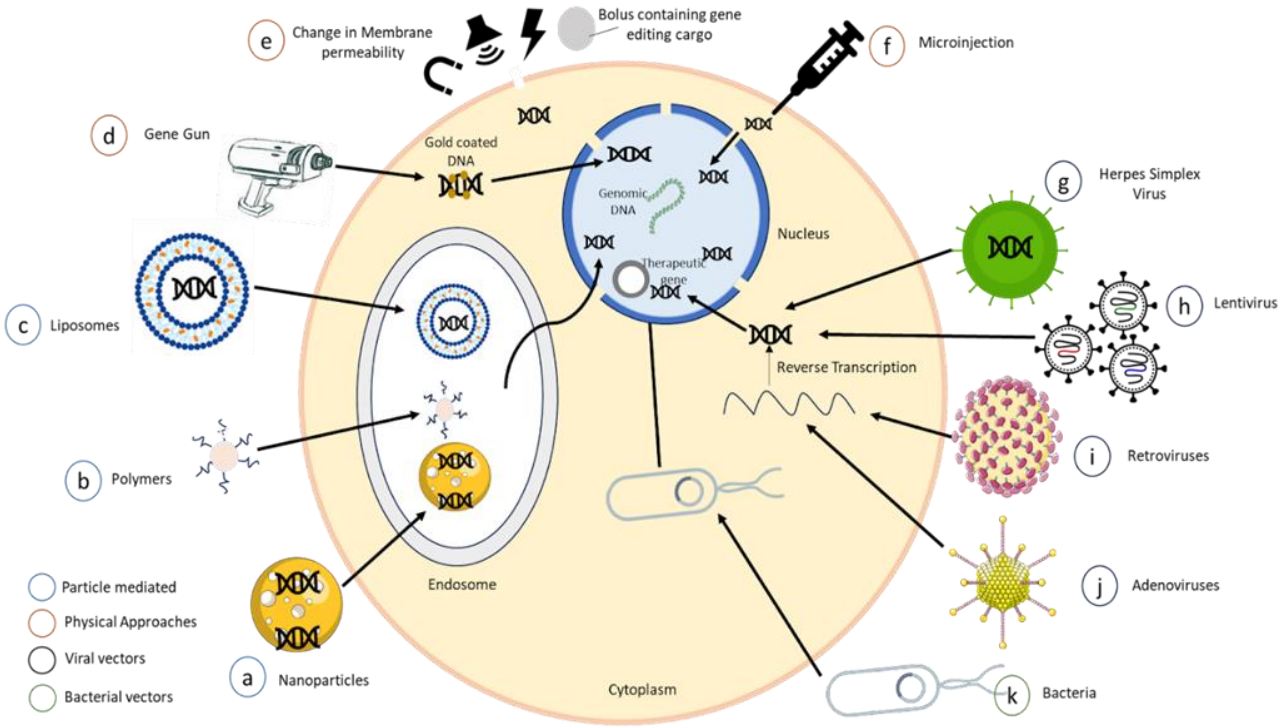

Figure 6 Schematic Diagram of Gene Delivery Methods. Non-viral based - a. Nanoparticles, b. Polymer material vectors, c. Liposomes, d. Gene gun, e. Change in membrane permeability due to electric field, magnetic field, ultrasound frequency, and hydrodynamic pressure, f. Microinjection, Viral based - g. Herpes Simplex virus, h. Lentivirus, i. Retrovirus, j. Adenovirus, k. Bacteria based.

6.1 Viral Based Vectors

Compared to other methods currently used, the viral-based gene delivery method is one of the most effective ways to transfer genes. Recombinant DNA technology helps design viral-based vectors by removing the disease-causing genes from viral vectors and replacing them with the desired gene. Different viruses, including lentiviruses, retroviruses, adeno-associated viruses (AAV), and adenoviruses, can be used as vectors to transport genes selectively to cancer cells. The selection of a specific vector is influenced by several variables, such as its ability to package, host specificity, gene expression profile, and propensity to trigger immunological responses, mainly when repeated administrations are necessary [92].

Adenoviruses (AV) are non-enveloped viruses that can package up to 7.5 kb of foreign DNA in their double-stranded DNA (dsDNA) genome. These viruses are most commonly used as delivery systems for genes and other therapeutic agents. Many AV-based recombinant vectors have been engineered to accommodate up to 14 kb of foreign DNA [93]. However, they tend to display short-term expression and immunogenicity [94]. Another related virus known as AAV has been used frequently. AAVs are small, non-enveloped viruses with a single-stranded DNA (ssDNA) genome. Their packaging capacity is ~4 kb of foreign DNA. However, AAV vectors do not result in harmful or pathogenic reactions. However, the effectiveness of delivery and transgenic expression has decreased due to the significant immune responses caused by recurrent administration of AAV vectors [92].

Another virus that can be used as a delivery system is the herpes simplex virus (HSV), a dsDNA-enveloped virus responsible for latent infection in the brain ganglia. However, modifying HSV expression vectors has made long-lasting transgene expression possible. These modifications resulted in a circularized genome that stays as a viral episome instead of integrating into the host cell's genome. HSV vectors can accommodate more than 30 kb of foreign genetic material. Even 150 kb of foreign genetic material can be packaged via engineered HSV amplicons [95]. Although non-essential genes have been removed from the HSV genome, HSV vectors have been linked to rather significant cytopathogenicity [96].

One of the most widely used RNA-containing viruses as vectors is retrovirus (RV). It is a single-stranded RNA (ssRNA) enveloped retroviruses (RVs) with ~8 kb of packaging capacity. Engineered self-inactivating RV (SIN-RV) safe vectors have been developed, with no instances of leukemia or inadequate integration noted in clinical trials. However, insertional oncogenesis is uncommon during RV therapy, suggesting that adenosine deaminase-deficient severe combination immunodeficiency (ADA-SCID) differs from other inherited immunodeficiencies [97].

Lentivirus (LV) is a ssRNA-containing virus that can carry 8 kb of foreign genetic material and is similar to RVs in most aspects. Even though some unfavorable events, including insertional oncogenesis, have also been shown, LV-based vectors demonstrate low cell cytotoxicity and offer better biosafety for therapeutic applications because of their different chromosomal integration [98].

A bacteriophage-based vector known as the M13 phage has recently been developed. It is utilized as an alternative vehicle for gene therapy with programmable specificity. However, the low transduction efficiency is still a hurdle for its use in treatment. A recent *in vitro* trans-phage study showed CD16+ NK cells can efficiently kill cancer cells expressing membrane-bound fragment crystallizable (Fc) through antibody-dependent cell-mediated cytotoxicity (ADCC)-like mechanism. A xenograft mouse model confirmed the tumor growth-suppressing potential [99].

6.2 Non-Viral Based Vectors

Virus-based vectors have a few disadvantages, such as transient expression, immunological reactions, safety, size of insert, etc., that can be overcome by using non-viral vectors. Non-viral based vectors are synthetic vectors mainly made up of chemicals. These chemical carriers have many advantages, such as protecting nucleic acid from degradation, sustainable release, enhanced transfection efficiency, and efficient delivery of genes into target cells [100]. The non-viral vectors can be made up of lipids, peptides, inorganic nanoparticles, and polymers [100].

Non-viral vector-based delivery methods are easy to produce, can deliver a large genome, and are relatively safe [101]. Carriers of this delivery method can be classified as particle-based, physical-methods, or bacterial-based vectors. A few are discussed below [102].

6.2.1 Particle Mediated Delivery

In this mechanism, various particles such as carbon nanomaterials, metal/metal oxide nanomaterials, polyvinyl nanomaterials, liposomes, dendrimers, micelles, nanogels, and nanofibers are used as delivery mechanisms to carry the gene into the cancer cells. Some of them are discussed below [103].

Liposomes. Natural or manufactured lipids and surfactants are combined to create liposomes, which are effective drug transporters and have a 40 nm to 500 nm size range. Its form resembles the cell membrane in nature, and its lipid membrane and watery center allow it to transport both lipophilic and hydrophilic substances. Liposomes provide good opportunities for gene transfer with genetic materials, including DNA (antisense) oligonucleotides, siRNAs, DNAzymes, aptamers, and ribozymes [104].

Lipoplexes. The lipids and polymers usually carry positively charged groups that interact with the negatively charged DNA/RNA and form complexes known as lipoplexes and polyplexes [105]. The positively charged groups on lipoplexes and polyplexes help compact DNA, provide protection from nucleases, and enhance the uptake of DNA by the cells due to the negatively charged nature of cell membranes [85, 106].

Mainly, three kinds of lipids have been used to form lipoplexes: cationic, anionic, and neutral. The cationic lipoplexes are primarily used as vectors, either mono-valent cationic lipids or multi-valent cationic lipids. In the gene therapy approach, N-[1-(2,3-dioleyloxy) propyl]-N,N,N-trimethylammonium chloride (DOTMA) is the most widely used monovalent cationic lipids [107]. Other catioininc lipids are 1,2-bis(oleoyloxy)-3-(trimethylammonio) propane (DOTAP) [108] and 3β [N-(N',N'-dimethylaminoethane)-carbamoyl] cholesterol (DC-Chol) [109] were synthesized for higher transfection efficiency.

While DOSPA, (2,3-dioleyloxy-N-[2(sperminecarboxamido) ethyl]-N,N-dimethyl-l propanaminium trifluoroacetate), is a multivalent cationic lipid that was derived from DOTMA. Its structure is related to DOTMA except for a spermine group bound to the hydrophobic chains via a peptide bond [110].

The presence of spermine group DNA became more compact, which enhanced stability [110]. Both the lipids are still used commercially. The combination of DOSPA with DOPE at a 3:1 ratio is commercialized as a transfection reagent called Lipofectamine®. Since its launch in 1993, it has

become the most commonly used transfection reagent in gene therapy, cited in more than 50,000 scientific papers as the “Gold Standard” for non-viral gene delivery [111].

The gold standard status was granted for the highlighted “high transfection” of DNA, siRNA, and miRNA. The transfection was into a broad range of cells, including difficult-to-transfect cells [112], compared to alternative transfection reagents (DC-Chol/DOPE formulation), as demonstrated by Fiume et al. [113].

These lipoplexes face drawbacks, like short half-life and low transfection efficiency [85]. Many modifications have been made to overcome these limitations. One of the modifications is PEGylation, which reduces aggregation of particle aggregation and increases their half-time [114].

Another modification is incorporating a cholesterol domain in PEGylated lipoplexes, which multifold enhances the transfection efficiency [115]. Some studies show that including helper lipids such as DOPE also improves the transformation efficiency as it helps endosomal escape [116].

Polyplexes: When positive-charged cationic polymers are complexed with negatively charged DNA/RNA, the complexes are known as polyplexes. These polyplexes are usually more stable and compact DNA more efficiently than lipoplexes. The main polymers used for gene delivery are the cationic and the amphiphilic polymers [85, 106]. Many kinds of polymers are used to prepare polyplexes, such as Poly-l-Lysine (PLL), polyethyleneimine (PEI), polyamidoamine (PAMAM Poly lactic-co-glycolic acid (PLGA). Polyethylenimine (PEI) is one of the most widely used cationic polymers in gene delivery due to its high transfection efficiency, but it is non-biodegradability and has high toxicity limit [85, 106]. These drawbacks can be overcome either by the addition of acrylate (PLA) to branched PEI [117] or by adding amino acids histidine and lysine to low molecular weight PEI (600 Da) [118].

Other strategies include developing novel dendriplexes, which improve transfection efficiency and reduce toxicity levels [119]. He synthesized a dendriplex using amine-terminated carbosilane dendriplexes via Huisgen *cyclo*-addition with an ammonium group per branch in its structure.

Nanoparticles: Various anticancer therapies successfully employ chemical medicines and siRNA delivery systems based on polymer nanoparticles, as they enhance the efficacy of drugs and aid in the delivery process. For instance, the poor stability of mTOR siRNA under biological circumstances limits its ability for lung cancer treatment. Still, encapsulated in modified poly (amino-ether) (mPAE) polymer while creating stable and biodegradable nanoparticles can knock down genes and cause apoptosis in A549 and H460 lung cancer cells. The effective utilization of various cationic polymers is based on electrostatic interactions [120].

In addition to polymer nanoparticles, lipids can be used to produce nanoparticles. Lipid-based nanoparticles mainly consist of solid cores known as Solid-liquid core nanoparticles (SLNs). These has been developed around the 1990s to deliver the DNA into cells [121]. These particles' average size ranges from 40 to 1000 nm and are spherical in shape [122]. SLNs contain solid fat (0.1-30% w/w) dispersed in an aqueous phase. Various lipids can be used to prepare SLNs such as triglycerides (compritol), partial glycerides, fatty acids (stearic acid, palmitic acid), steroids (cholesterol), and waxes (cetyl palmitate) along with emulsifiers which help in dispersion of the lipid component [123].

Peptides and Inorganic Nanoparticles: Apart from lipids and polymers, some peptides and inorganic nanoparticles also used as vectors. Amino acids condense to form short chains ~10-20 residues are known as peptides. Synthetic peptides, usually made up of positively charged amino

acids such as lysine or arginine, have shown role in condensing DNA and DNA delivery [124] used TGN peptide in brain targeting. Inorganic nanoparticles are another category of chemical or non-viral-based vectors for gene targeting. These inorganic nanoparticles can be easily prepared to cross the cell membrane to deliver nucleic acids into the cells [125]. These nanoparticles usually comprise gold, calcium phosphate, silica, magnetic compounds, and quantum dots [126-128].

Dendrimers: Nanoscale molecules known as dendrimers self-assemble and have three shells: an outer shell, a symmetric center, and an inner shell. They act as suitable carriers for siRNA administration because they have properties like minimal cytotoxicity, electrostatic interactions, polyvalency, chemical stability, water solubility, and ease of surface modification [104].

Novel Type to Non-Viral Based Vectors: In gene therapy, vectors/carriers are delivered into the body used to treat a disease. So, the carriers must be designed in such a way that they can respond to or withstand the biological changes going on in the body. These vectors or carriers are bio-responsive or innovative carriers that can respond to a natural change [100]. These vectors are designed to respond to regular physical changes, such as ATP concentration, pH, temperature, and redox potential [127, 129-133].

These vectors' physiochemical properties like DNA compaction, size, integrity, shape, transfection efficiency etc, are significantly enhanced in response to the change in environmental conditions [134]. A comparative analysis of the advantages and disadvantages of viral vectors vs non-viral vectors is given in Table 2.

Table 2 Advantages and disadvantages of viral vectors vs non-viral vectors.

	Viral based vectors	**Non-viral based vectors**
Advantages	Ability to escape from endosomes High transfection efficiency	Less toxic and immunogenic Less expensive, comparatively safe Easy to implement
Disadvantages	High immunogenicity and cytotoxicity insertional mutagenesis, disrupting the expression of tumor suppression gene or activating an oncogene leading to the malignant transformation of cells.	Low efficiency, less ability to target gene expression to the required area Require numerous doses It can damage certain types of cells

6.3 Bacterial Vectors

Another promising medium that has emerged is bacterial-mediated cancer therapy. It has shown increased survival rates post-treatment in *in vivo* tumor models. Bacterial vectors have many unique features, like specificity for a broad range, including the activation of immune responses at tumor sites and easy removal due to antibiotic sensitivity. Bacterial vectors can be systemically administered and provide tumor-cell-specific delivery of either DNA or a protein of interest [135]. Escherichia, Listeria, and Salmonella have commonly used bacteria for this purpose [136-139].

7. Perspective of Vector Design

For a vector to be effectively utilized in gene therapy, its design and the mode of delivery must be considered. Designing appropriate vectors for gene therapy requires overcoming several limitations. Characteristics of a suitable vector while designing [100, 105].

The following points must be considered for designing a vector that can successfully deliver the gene into the target cell.

1. Safe
2. Specific delivery of a gene to the target
3. High transfection efficiency
4. Can cross gene delivery barriers such as blood, tissue, endosomal, and nuclear
5. Non-immunogenic
6. Robust in design
7. Low toxicity
8. Can express transgene in targeted cells
9. Do not have off-target expression
10. Do not have overexpression-associated cytotoxicity
11. Can be produced in large amount
12. Low production cost

Each type of vector has its own advantages and limitations. There are several limitations of non-viral vectors, such as extracellular stability of the delivery complex, internalization and the cellular trafficking of the vector, and the level and the sustainability of expression of the therapeutic gene. For instance, it was observed that systemic administration of nude plasmid DNA in mice's bloodstream proved inefficient, as the exogenous DNA is degraded by nucleases [140]. The remaining DNA accumulates in the non-parenchymal endothelial cells of the liver. However, no detectable levels of transgene expression could be observed.

In contrast, using a hydrodynamic injection method (intravenous administration of plasmid DNA in a large volume of saline solution at high pressure), significant expression of the transgene was seen in the liver due to an enlargement in the liver fenestrae and generation of membrane pores or forced vesicular internalization [141]. However, the hydrodynamic method is not feasible with human gene transfer because of these morphological changes in cell membranes.

Considering the issue with the level of therapeutic gene expression, it depends on the type of promoter used to drive expression. It is directly correlated with the efficiency of gene transfer *in vivo*. Studies have indicated that tissue-specific promoters might be advantageous for targeted transcription, but their utility is limited due to low gene transcription levels. Further development using tissue-specific promoters, enhancers, and introns substantially increased the long-term expression to therapeutic levels [142].

The immune system's capacity to identify CpG unmethylated motifs in bacterial DNA, to which the immune cells respond by releasing cytokines and inciting inflammation, is another factor that may restrict the effectiveness of gene expression. It has been observed that transgenic expression levels can be raised by removing CpG motifs from bacterial genes expressed in mammalian systems [143].

Similarly, various conditions must be met for the use of viral vectors in gene delivery. When using adenoviral vectors for therapeutic gene delivery, it is implied that the virion must not undergo its typical lysogenic life cycle after the gene is inserted into the target cell. Cell lysis would ensue from this, impairing the transgene's expression. Making deletions in the E1 and E3 sections of the viral

genome is one method; nevertheless, this has raised safety concerns. The outcome is replication-defective viral particles, or first-generation adenoviral vectors [144].

Because systemic or local administration of adenoviral vectors triggers the immune response, methods have been developed to get around this restriction on using these vectors for therapeutic gene delivery. Modifying the adaptive immune response to the adenoviral vectors is one such tactic. Tolerance to adenoviral vectors has been successfully induced in one pre-clinical research using a dendritic cell-based approach [145]. The absence of pre-existing immunological memory against non-human adenoviruses has been exploited in other ways, making such vectors appealing instruments for gene therapy.

Limitations on insertional mutagenesis, cell type infectivity, internal promoter selection, and the effects of vector elements on virus titer and transduction are all significant factors to consider while developing a viral vector [140].

Hence, non-viral gene therapy vectors are less efficient at transduction and have restricted specificity, which relies on functional groups linked to the delivery complex. This necessitates laborious structural adjustments to attain a reliable and effective gene therapy delivery system. In contrast to viral vectors, which primarily rely on helper cell lines to produce infectious particles, non-viral vectors have a comparatively better safety profile and can be made on a massive scale due to their chemical makeup. Furthermore, getting high-quality viral vectors at good titers might be costly and complex. Compared to non-viral methods, viral vector transduction is significantly more efficient. Their specificity, meanwhile, is limited to cells expressing the appropriate receptors needed for the viral particle to internalize. One of the main issues restricting the therapeutic use of viral vectors is their immunogenic and genotoxic effect in the case of integrative vectors.

Therefore, a "perfect" gene therapy vector would only be able to efficiently transduce a small subset of target cells. A vector of this kind should also have a high safety profile, enabling systemic administration without the risk of cytotoxic or genetic side effects.

8. Sequence of Gene

The deliberate selection and modification of genetic material to accomplish particular therapeutic goals is a necessary part of the rational design of gene sequences for gene therapy [146]. Gene therapy aims to treat or prevent diseases by introducing, replacing, or modifying genetic material within a patient's cells. Various genes of different pathways, such as repair, apoptosis, cell cycle regulation, etc., have been exploited for gene therapy. All have distinct advantages and disadvantages (Table 3).

Table 3 The advantages or disadvantages of different target genes used for clinical or treatment outcomes.

Gene targeted	Advantage	Disadvantage	Reference
p53	● p53 mutations are seen in at least 50% of the cancers. ● The processes of apoptosis and	● Heterogeneity of p53 causes different mutations within the same tumor,	[147-149]

	the cell cycle are crucial in halting the growth of tumors. ● p53 also controls biological functions, such as DNA repair, metabolism, and the antioxidant response.	affecting therapy and clinical course. ● Some cells may develop chemo-resistance, bypassing p53-mediated cell death	
RB1	● RB1 mutation is found in several cancers such as retinoblastoma, osteosarcoma, bladder cancer and small-cell lung cancer. ● RB1 halts cell division by binding to and inactivating E2F transcription factors, preventing them from promoting cell growth.	● RB1 is a critical protein for normal development and differentiation, requiring cautious consideration in therapeutic targeting.	[150, 151]
PTEN	● PTEN mutation occurs in 20-40% cancers including endometrial cancer, glial tumors, prostate cancer, melanoma, non-small cell lung cancer, and breast cancer. ● It is involved in P13/ATK pathway, which regulates cell survival and proliferation. Mutation in PTEN leads to the dysregulation of the pathway. ● Loss of PTEN function may also lead to therapeutic resistance.	● PTEN is involved in different cellular functions such as metabolic pathways, cell motility and angiogenesis ● Overexpression of PTEN may lead to dysregulation such as increased energy expenditure. ● PTEN functions in different subcellular compartments. Making it challenging to deliver therapeutic genes effectively.	[152-155]
KRAS	● KRAS protein regulates MAPK and PI3K/AKT pathways which are essential in cell growth and differentiation. ● KRAS also regulates cell growth and survival through the activation of RALGDS and RAL GTPases.	● The heterogeneity of KRAS mutation decreases therapeutic efficacy due to varying tumor cell subpopulations.	[156, 157]
BRAF	● BRAF mutations commonly present in human cancers with an 8% occurrence in all human	● The lack of mutant specificity makes it difficult to determine the dose	[158, 159]

	cancers, primarily in hairy cell leukemia (100%), melanoma tumors (40%-50%) and thyroid carcinoma (10%-70%) ● BRAF participates in MAPK/ERK pathway signalling which regulates cell growth, migration, and proliferation. Loss in BRAF signalling leads to continuous and unregulated activation of the pathway.	● BRAF V600E mutation inhibition may lead to paradoxical activation which causes enhanced cell proliferation.

The key steps and considerations in the rational design of gene sequences for gene therapy are:

- Gaining a thorough understanding of the disease: identify genetic defects and disease pathways.
- Target Identification: Determine specific genes or pathways for intervention.
- Therapeutic Gene Selection: Choose genes to replace, supplement, or inhibit.
- Promoter and Enhancer Elements: Select elements for precise gene regulation.
- Vector Design: Choose viral or non-viral vectors for gene delivery.
- Immune Response: Minimize immune reactions to the gene or vector.
- Codon Usage Optimization: Optimize for host codon usage for efficient translation.

With knowledge of molecular biology, genetics, virology, immunology, and other pertinent domains, a multidisciplinary approach is necessary to design gene sequences for gene therapy rationally. Technological developments in gene editing, like CRISPR-Cas9, also significantly improve gene therapy treatments' specificity and accuracy.

The general design of genes has:

1. **Enhancer and Promoter:** Selecting a potent, externally induced, tissue-specific promoter guarantees high transgene expression in the targeted cells. Moreover, enhancers can increase transgene expression, especially in tissue with low transcriptional activity. For instance, the expression of the reporter gene increased 5.83 times as a result of the hyperthermia-inducible hsp70 promoter. Due to the system's cell-type dependence, head and neck cancer gene therapy clinical applications should be carefully considered [160].
2. **Codon optimization:** maximizing the amount of protein produced and translation efficiency by adjusting the transgene's usage to match the preferred codons of the target cells [161].
3. **Gene insulator sequences:** Encircle the transgene with insulator sequences to prevent it from being silenced or from affecting neighboring genes, ensuring safety [162].
4. **Intron inclusion:** By stabilizing the mRNA transcript and improving its export from the nucleus, introns can boost the synthesis of proteins [163].
5. **Targeting sequences:** By including targeting sequences in the vector or transgene, it becomes possible to attach to particular target cell receptors, promoting effective delivery and cell uptake [164].

a) **Codon harmonization:** Reducing the likelihood of an immunological reaction against the foreign protein can be achieved by coordinating the transgene's codon usage with the host genome [165].
b) **Immunogenic epitope removal:** Removing putative immunogenic epitopes from the transgene sequence can help lower the likelihood of immunological rejection even more [166].
c) **Immune-modulatory sequences:** By incorporating immune-modulatory sequences into the vector, the immune system's reaction to the transgene and vector components can be inhibited [167].

9. Methods to Deliver Genes

Many methods have been used to transfer genes into cells for gene therapy purposes. All the methods can be divided into biological, chemical, mechanical and physical [168, 169]. Each method has its advantage and disadvantages. Physical methods use various physical forces, such as mechanical, electrical, sound, magnetic, etc., to introduce gene into the cells. Some of the methods are discussed below.

9.1 Microinjection

Microinjection is a mechanical method that directly introduces DNA (or gene) and drugs into the cells [170]. Over the past three decades, it has been one of the most widely used methods of physical delivery methods to deliver nucleic acids [171].

This method uses microneedles made of glass or silica to inject specific DNA (or genes) directly into the cytoplasm and nuclei [172].

The advantages are that it has better reproducibility is simple, painless, and safe. At the same time, it can easily transport genetic material (such as DNA and siRNA) and macromolecule drugs (like proteins and antibodies) [173]. This technique has a few challenges, like the syringe getting contaminated and degraded when DNA vaccines cover the surface of these metal microneedles and the generation of an immune response [174].

In a similar kind of method which uses high pressure to eject a liquid substance through the skin is known as *Jet injection method*. There are various physical means to generate the instantaneous energy to propel injection, depending on the desired injection depth or injection site, such as compressed air or gas, mechanical, or pyrotechnic propulsion using gunpowder ignition. The disadvantages include moderate efficiency and tissue damage [175].

Another technique known as *Hydrodynamic gene transfer* uses a large volume of gene editing cargo rapidly injected in the bloodstream, increasing the hydrodynamic pressure of the cells in multiple organs, which causes a temporary boost in membrane permeability and fixes cargo into cells. This technique carries a high risk due to the use of large volumes of genetic material [176].

9.2 Ballistic Gene Delivery (Gene Gun)

It is another mechanical method of gene delivery into the cells in which DNA or genes are transferred into the cells in a needle-free mechanism using a pressurized ballistic device [177]. It was first developed by Sanford et al. to deliver DNA into plant tissues [178]. In this method, DNA or

nucleic acid is coated on gold or tungsten nano- to micron-sized particles, which are then delivered directly into the cells using a pressurized ballistic device (known as a gene gun). The pressure is generated using either a helium discharge or a high-voltage electric spark [179]. Though this technique has low cytotoxicity and causes less tissue damage, it has low efficiency [180]. Gene gun delivery method has a great potential to deliver DNA/RNA vaccine genes into various tissues such as the stratum corneum of the skin, epidermis, skeletal muscle fibers, neurons, and liver tissue *in vitro* as well as *in vivo* [181-184].

9.3 Electroporation

This technique increases permeability in the plasma membrane by creating unstable aqueous pores with the help of an electric field. This technique is handy due to its efficiency and capacity to transfer large amount of DNA. A few of the disadvantages are its complexity and the requirement for skilled technicians [185]. In this method, electric field is applied across the cell membrane to change its permeability by creating transient pores through which any material can be transported inside the cells. Neumann first developed this method in 1982 for transfecting the mouse lyoma cells. Electroporation is used in many applications in biological science, such as delivery of drugs, plasmids, and DNA [186]. The change in permeability is directly related to the electric field's intensity (or strength) and duration of its exposure. Depending on the duration and electric field intensity, the effect on permeability can be divided into four different phases- 1) no poration is detected, 2) reversible poration, 3) non-thermal irreversible poration, and 4) thermal irreversible poration [187].

Gene therapy is usually done in the reversible phase of poration, where pores are created in the cell membrane reseal after the removal of the electric field [188]. This method is most widely used to deliver genes (or DNA) *in vitro* as well as *in vivo* in various tissues or cells such as skin, liver, muscle, tumor, mouse retinal cells, neurons, etc [189-191].

Recently, Wang et al. (2014) developed an electroporation based in vivo gene delivery system for injecting DNA vaccines in which a minicircle DNA carrying a HIV-a-gag gene was transferred into cells [192].

9.4 Magnetoporation

Magnetoporation is a similar physical approach used to transfer DNA (or genes) into the cells by altering their porosity [186]. Chan first proposed it in 1996, and it was later presented by many other authors [193-195]. In this method, when mixed with magnetofection reagent, DNA forms a biomolecule/magnetic reagent complex, which is then transferred into the cells by applying a magnetic field. It has been observed that under the influence of the magnetic field, the rate of endocytosis and pinocytosis across the cell membrane became enhanced [179].

The efficiency of magnetoreception mainly depends on the type of mangnetofection reagent (such as Fe_3O_4, γ-Fe_2O_3, $CoFe_2O_4$, $NiFe_2O_4$, and $MnFe_2O_4$) and the stability of DNA-reagent complex [196, 197]. While the technique is highly efficient and has low toxicity, transfection can be unstable [198]. The advantages and Disadvantages of non-viral-based gene delivery mechanisms are summarized in Table 4.

Table 4 Advantages and Disadvantages of non-viral based gene delivery mechanisms [186, 199].

Method of gene delivery	Advantages	Disadvantages
Microinjection	Delivery material directly into cytoplasm or nucleus It can be used for many tissues Any amount of DNA can be injected No complications due to immunogenicity Specificity	Highly inefficient for single needles Smaller-sized cells are difficult to transfect Need specialized training
Gene gun	DNA and other therapeutic agents can be delivered directly into cells Excellent method for skin, cornea and epidermis transfection Avoid inflammation	Lack of specificity Limited amount of DNA can be delivered Can infect only a limited number of cells Variable amount of DNA delivered in cells
Electroporation	Simplicity; Lower cost; No need for vector, can transfect both dividing and non-dividing cells.	Invasiveness, short-term pain, tissue damage Many parameters affect the transfection efficiency Low cell viability Risk of inflammation in in vivo applications
Magnetoporation	Non-invasive transfection reagents increase the efficiency	Lower efficiency for nude DNA, transfection reagents aggregation
Sonoporation	Non-invasive, ultrasound contrast reagents increase the efficiency, Lower transfection efficiency, Less destructive	Lower precision, lower reproducibility, tissue damage, Poorly controlled
Optoporation	Less dependent on cell type, single cell poration, Precise and efficient, Non-invasive, Less cell death, Controlled process, Can be used for the inaccessible sites in body	low irradiation area, low penetration capacity, Less efficient for large population of cells, costly, Need specialized training

10. Cancers Treated with Gene Therapy

10.1 Breast Cancer

Breast cancer is the most prevalent cancer type among women worldwide, as evidenced by the fact that in Asia, every 1 in 8 women suffers due to it [198]. Many genes controlling pathways such as metastasis, apoptosis, and cell cycle regulation are mutated or overexpressed in breast cancer [200].

The ERBB2 protein is an oncoprotein of the EGFR family that is overexpressed in 20% of invasive breast cancers. It is known to increase breast cancer invasion and metastasis and has been linked to poor patient survival. The discovery of ERBB2 pathway regulatory dysfunction in breast cancer pathogenesis has caused the creation of ERBB2-targeted therapies. There was a study in which breast cancer cells were transfected with the HSV1-tk gene under the transcriptional control of the ERBB2 251 bp promoter (p256-TK).

This resulted in increased ganciclovir sensitivity without impacting normal cells [201, 202]. Even though MDA-MB-231 cell line damage was noted, liposome administration employing a combination of EGFR siRNA with other EGFR small molecule inhibitor(s) is potentially helpful for treating triple-negative breast cancer [203]. Phase 1 clinical studies were conducted on a retrovirus (MetXia-P450) that encodes the human cytochrome P450 gene and was injected into metastatic cutaneous tumor nodules. Cyclophosphamide was then administered orally as a prodrug, and anti-tumor activity was seen in a subset of patients. High expression of MUC-1 has been indicative of a poor prognosis for breast cancer. An adenoviral-mediated suicide gene therapy is a promising option using an enhancer region of 114 bp that can control the transcription of a heterologous promoter [204]. Moreover, the release of anti-miR-155 in C57BL/6 mice with MDA-MB-231 cells has inhibited tumor growth [205]. Another study discovered that an adeno-associated virus that encodes soluble TRAIL could effectively inhibit the development of human-origin breast cancer in nude mice. Chemotherapies and TRAIL gene therapy had additive and synergistic anti-tumor effects [206].

10.2 Hepatocellular Carcinoma

Hepatocellular carcinoma (HCC) is among the leading cancers with the most significant mortality rate due to late diagnosis and ineffective treatments, along with treatments having adverse effects. Target gene therapy seems like a treatment method with much potential [207]. In HCC gene therapy, a tumor-specific promoter AFP promoter is most commonly used for the expression of sodium/iodide symporter (NIS)-like genes to improve radiotherapy efficiency [208] and increase tumor sensitivity to chemotherapy due to the HSV1-tk gene [209].

Another study shown that the use of targeted gene therapy against HCC is human telomerase reverse transcriptase (hTERT) and arginine deaminase (ADI) gene shows promising results in inhibiting cancer progression [210, 211]. Several oncogenes have proven effective targets for siRNA-mediated knockdown in various studies. These targets include Sphk2, Midkine, YAP, VEGF, Bmi, AEG-1, and Notch1 [212]. Also, long non-coding RNAs (lncRNA) and micro-RNAs (miRNAs) function as tumor suppressor genes. miR-214 is one example demonstrating how it inhibits the growth and migration of HCC cells by targeting PDK2 and PHF6, suggesting a possible therapeutic target for HCC patients [213].

10.3 Lung Cancer

Lung cancer was the leading cause of mortality due to cancer, according to data from the WHO [214], even though there have been several improvements in chemotherapy, surgery, and radiotherapy. A study observed that, in the absence of other viral components, the vesicular stomatitis virus (VSV) matrix protein (MP) promotes apoptosis in tumor cells. Hence, with the use of the wild-MP gene, a construct pVAX-M recombinant plasmid was prepared. It induced apoptosis causing suppression of malignant tumor growth *in vivo* and *in vitro* assays. Then, a phTERTM plasmid was constructed, which encoded VSV MP under transcriptional control of the hTERT promoter. This construct displayed anti-tumor activity, specifically against lung adenocarcinoma [215].

An example of a tissue-specific oncogene present in the case of lung cancer is thyroid transcription factor 1 (TTF-1), a member of the Nkx2 transcription factors. Their expression levels are associated with patient prognosis [216]; hence, they are a potential target for gene therapy. A potent strategy against the expression levels is the use of the miR-7 expression vector under TTF-1 promoter transcriptional control (p-T-miR-7), which resulted in reduced tumor growth rate, migration, and metastasis of lung cancer cells both in vivo and in vitro [217, 218].

Instead of the earlier mention, a promising approach is suicide gene therapy. A study was conducted to understand herpes simplex virus-thymidine kinase/human interleukin-12 (HSV-TK/hIL-12) fusion gene's targeted anticancer impact, which is the human non-small cell lung cancer (hNSCLC) promoter, also known as hSLPI, controls gene expression. The results indicated a targeted antitumor effect on the regulation of hNSCLC by the fusion gene. Also, a more potent antitumor effect was observed due to suicide gene and immune gene therapy instead of single gene therapy [219]. In another study, a recombinant adenovirus (Ad-EC) that targets the EGFR and expresses active revCASP3 while being driven by the tumor-specific SLPI promoter caused effective inhibition of cancer cells. It was observed to effectively reduce EGFR expression and prevent Hep-2 cell growth [220, 221].

Carcinoembryonic antigen (CEA) is a prognostic marker used in lung cancer and is a member of the cell-surface glycoprotein family [222]. A study was carried out to direct the bacteriophage E gene (pCEA-E) towards lung cancer cells (A-549 human and LL2 mouse cell lines), but not normal lung cells (L132 human embryonic lung cell line). CEA was used as a tumor-specific promoter along with paclitaxel (PTX) using cell culture, tumor spheroid models (MTS), subcutaneously generated tumors, and lung cancer stem cells (CSCs). It was observed that pCEA-E induced significant inhibition of cell proliferation and decrease in volume growth of A-549 and LL2 MTS, leading to intense apoptosis compared to L132 MTS. Also, pCEA-E was observed to enhance the antitumor effects of PTX when combined, which was also seen in A-549 CSCs that are pertaining to the cancer recurrence. Hence, CEA promoters can be used to regulate lung cancer cells' production of the E gene, particularly, and improve the efficacy of PTX against this type of tumor [223]. In another study, a recombinant plasmid with the double suicide genes thymidine kinase (TK) and cytosine deaminase, as well as the CEA promoter (CD), was constructed (pCEA-TK/CD). The study demonstrated that pCEA-TK/CD transfection in the presence of prodrugs 5-flucytosine and ganciclovir reduced inhibitory concentration 50 and promoted apoptosis and cyclomorphosis, making it a promising gene therapy approach for treating lung cancer [224].

It has been found that human Wnt inhibitory factor-1 (hWIF-1) effectively works as an anti-oncogenic for NSCLC gene therapy. Given the failure of viral vectors, development was focused on

targeting NSCLC cells specifically, using SP5-2 peptide coated on PEI and branched PEI1800. When administered to A549 cells, the vehicle had a 50% success rate for transfection, highlighting it as a potential genetic vehicle for delivering therapeutic nucleic acids to cancer cells [225]. Due to features like enhanced chemical stability, increased nucleic acid loading capacity, decreased cytotoxicity, and controlled release, nanocarriers like nanostructured lipid carriers (NLC) have emerged to show potential for non-viral vehicle-mediated gene therapy [226].

10.4 Pancreatic Cancer

The prognosis for pancreatic cancer is quite dismal, and it is very aggressive. Pancreatic cancer was the seventh leading cause of cancer-related death worldwide because of a lack of proper treatments, with 458,918 new cases and 432,242 deaths in 2018 [227]. Comparing pancreatic cancer cells to normal cells reveals that the cholecystokinin type A receptor (CCKAR) promoter is relatively more active. In the nude mouse xenograft model, the modified CCKAR promoter was employed to direct the expression of a powerful pro-apoptotic gene called BikDD. Therefore, this CCK/Mpd-Bik-DD/liposome can be a potential therapeutic for treating pancreatic cancer. Following this, another group of researchers demonstrated a significantly enhanced antitumor effect and increased patient survival without any substantial amount of toxicity in vivo pancreatic cancer using an expression vector called “VISA” (VP16-GAL4-WPRE integrated systemic amplifier) using CCKAR that targets the expression of BikDD [228, 229].

In pancreatic cancer, various mucins like MUC1, MUC4, MUC5AC, and MUC16 are overexpressed and contribute to the disease's poor prognosis. Mucins are highly O-glycosylated proteins. Mucin1 (MUC1) and mesothelin (MSLN) are two proteins that are more common in pancreatic ductal adenocarcinoma (PDA) than in normal pancreatic cells. These proteins are linked to how aggressive pancreatic tumors are. Therefore, MUC1 is a potential target for therapeutic studies to treat PDA. Diphtheria toxin (DTA) inhibits cell protein synthesis, resulting in cytotoxic function. DTA was transfected using the MUC1 promoter-driven luciferase construct and showed a cytotoxic effect targeting only tumor cells. Further, the efficacy of the drug was improved by combining MUC1 with MSLN-targeted DTA [230, 231]. The limitation of MUC1-based therapy is that MUC1 along with MUC3 is usually expressed in gastrointestinal, colorectal, and breast epithelia; therefore, it may impart some side effects after treatment [232].

A super promoter with improved and specific activity against pancreatic cancer using the human insulin promoter was created. This promoter, called SHIP1 (synthetic human insulin super-promoter) is used to target the expression of Pancreatic and duodenal homeobox 1 (PDX1), which is found to be overexpressed in PDAC and insulinoma. PDX1 is a transcription factor that regulates vital functions within the pancreas, like the expression of the insulin gene β-cell maturation, and maintains its function. SHIP1 is regulated by viral thymidine kinase followed by ganciclovir (SHIP1-TK/GCV), resulting in a cytotoxic effect on PDAC [233, 234].

Mesenchymal stem cells (hMSCs) have recently been created and used as a novel, non-viral delivery system for anti-cancer therapy. It has been shown that genetically altered MSCs cause solid tumors to undergo more apoptosis and restrict growth and angiogenesis [64]. Tumor necrosis factor (TNF)-related apoptosis-inducing ligand (TRAIL), a member of the TNF cytokine superfamily, is one of the most potent anti-cancer therapeutic targets. The hMSCs are modified using TRAIL and bPEI

(branched polyethyleneimine) to increase their transfection efficiency, induce apoptosis in cancer cells, and increase internalization, respectively [235, 236].

10.5 Colorectal Cancer

The second deadliest and third most prevalent cancer, colorectal cancer, has higher mortality rates than all other cancers combined. Initially formed as adenomatous polyps, they progressively form malignant tumors due to successive mutations [214, 237].

Fibroblast growth factor 18 is required during the embryonic development of cartilage and bone. This growth factor is a part of the canonical Wnt signaling pathway, which tends to be heightened in colon cancer cases. Consequently, there is an observed elevation of FGF18 expression in colorectal cancers (CRCs). The presence of FGF18 appears to enhance CRCs' advancement and aggressive behavior, thereby promoting their progression. In SW480 and HCT116 colon carcinoma cell lines, the activity of FGF18 has been found to be elevated in contrast to normal human umbilical cord colon cells. Furthermore, the targeted effectiveness of the FGF18 promoter within tumor cells was validated by introducing the HSV1-tk (thymidine kinase) gene into CRC cells. This intervention notably suppressed the growth of these cells upon exposure to ganciclovir treatment by inducing apoptosis [238, 239].

Further, another modified promoter, pUCUPARTK (HSV1-tk under urokinase plasminogen activator receptor), was used to target RAS signaling in CRC, another one of the most altered mutated (KRAS) pathways besides Wnt signaling. RAS signaling is a positive regulator for uPAR, a gene that encodes a serine protease. This protease aids in plasminogen's transformation into plasmin, which is the active form. Similarly, following the administration of ganciclovir, an enhanced cytotoxic effect was observed using Annexin-propidium iodide staining [240, 241].

COX-2 stands for cyclooxygenase-2, an enzyme responsible for initiating the oxidation of arachidonic acid during the synthesis of prostaglandins. This process plays a critical role in the emergence of cancer and the growth of tumors. In colon cancer, COX-2 is found to be excessively active, being overexpressed in around 93% of cases, and similarly in rectal cancer, where it's over-expressed in about 87% of cases. Studies have shown that the degree of overexpression of COX-2 correlates with the advancement of cancer and the mortality rates among patients diagnosed with colorectal cancer [242-244]. Cox-2-mediated prostaglandin E2 (PGE2) overexpression plays a significant role in tumor progression and aggressiveness. The engineered promoters, like COX-2-PGDH (hydroxyprostaglandin dehydrogenase) and VEGFR1/flt-1 (vascular endothelial growth factor receptor 1), decreased the proliferation and migration of colon cancer cells [245].

10.6 Prostate Cancer

Prostate cancer is the fifth leading cause of death among men on a global scale and is the second most commonly diagnosed cancer. This disease claims the lives of 358,989 individuals annually and poses a significant threat to older men [246].

As already mentioned numerous times, the HSV1-TK suicide gene has been exploited to construct many promoters as a potent anti-tumor agent. Another synthetic promoter uses the glucose-regulated protein 78 (GRP78) promoter, a promoter found to be active only in cancer cells instead of normal cells. This GRP78 protein functions as a potent anti-apoptotic factor; this protein assumes a pivotal role in safeguarding tumor cells against apoptosis. Moreover, it significantly contributes to

the advancement of tumors, angiogenesis, metastasis, and the development of resistance to therapeutic interventions. Under the regulatory action of the GRP78 promoter, the HSV1-TK suicide gene expresses, and cells become susceptible to ganciclovir and its metabolites. Specifically, GCV-diphosphate shows cytotoxic effects against prostate cancer [247-249].

Prostate cancer generally metastasizes preferentially to the bone during the initial aggressiveness of the cancer. Therefore, targeting the human osteonectin promoter (hON-522E) is gaining attention. This promoter regulates the expression of the osteonectin protein, which plays a crucial role in cell adhesion, proliferation, and migration, and its expression is upregulated in metastatic prostate cancers. A synthetic vector was engineered utilizing the hON-522E promoter to control the transcription of an HSV1-TK suicide gene. This setup showcased the initiation of cell death in vitro (specifically in PC3M cells) and exhibited a deceleration in the growth of prostate tumors within a xenograft model. Notably, this effect was achieved without causing toxicity in other organs [250, 251].

Prostate-specific antigen (PSA) is a cytoplasmic protein found within the cells of the prostate gland as well as the epithelial cells lining the prostatic ducts. While its presence has been confirmed in healthy prostate tissue, PSA is observed to be significantly elevated in prostate cancer cells. In contrast, prostate-specific membrane antigen (PSMA) is an integral protein located on the membranes of prostatic epithelial cells. Notably, PSMA expression is increased in prostate cancer, particularly in cases involving metastasis. Hence, the promoters associated with these two proteins emerge as promising contenders for guiding gene therapy in cases of prostate cancer. One strategy involved the creation of a plasmid containing the thymidine kinase suicide gene, with its transcriptional control governed by a fragment of the human PSA enhancer/promoter. To enhance specificity, JC polyomavirus virus-like particles were employed as carriers of the recombinant plasmid, benefiting from their affinity for androgen receptor-positive prostate cancer cells. In vitro experiments revealed that PSAtk-VLPs, the constructed plasmid's capability to induce cell death in 22Rv1 prostate cancer cells, also demonstrated growth inhibition effects in a xenograft mouse model. Similarly, a recombinant plasmid was engineered utilizing regulatory components from PSA and PSMA (prostate-specific membrane antigen) to oversee the transcription of apoptin. The transfection of the human prostatic adenocarcinoma cell line LNCaP with this plasmid notably reduced cell viability by inducing apoptosis [252-255].

10.7 Bladder Cancer

Bladder cancer is a global burden, with an estimated 500,000 new cases and 200,000 deaths annually. It encompasses a range of severity, from chronic non-invasive tumors to aggressive, advanced stages requiring intensive treatment [256]. Bladder cancer is the 10th most common cancer worldwide, being more common in men than women [257].

Mutations in the p53 gene are often observed during the early onset of the tumor, contributing to the unregulated growth of cells [258]. A mutation in exons of the FGFR3 gene upstream of RAS genes activates the RAS-MAPK pathway, producing more cell growth signals, and is often present in 70% of early tumors [259]. 13-27% of bladder tumors were found to have somatic mutations in the PIK3CA oncogene, which codes for the catalytic subunit p110α of class-IA PI3-kinase [260]. RAS oncogenes also showed mutations in about 13% of the cancers [261].

Regardless of suitable cellular receptors for the chosen vector, transfection of the urothelium posed the greatest obstacle to intravesical gene therapy, as indicated in the preceding sections. The glycocalyx, which includes the glycosaminoglycan (GAG) layer and protects the urothelium, performs numerous tasks, such as preventing bladder infections and, in the case of gene therapy, preventing bladder infections caused by viral vectors [262]. A hopeful alternative has been made available with the approval of the first gene therapy for genitourinary cancers, nadofaragene firadenovec (Adstiladrin®) and interferon-α (IFNα) [263]. The Phase 3 trial validated the safety and effectiveness of nadofaragene firadenovec. Its effectiveness can be further increased by selecting patients based on traits or biomarkers that indicate sensitivity or resistance, such as the induction of systemic anti-adenoviral antibodies, which means a durable positive clinical response to nadofaragene firadenovec [264]. Finding substitute vectors that increase transfection efficiency in order to provide more long-lasting therapeutic responses presents another development opportunity. The transitory transgenic expression of IFNα owing to adenoviral immunogenicity is one of the potential drawbacks of rAdIFNα/Syn3. Lentiviral vectors (LV) were examined in preclinical models as a potential solution to this potential issue since they offer more consistent transgene expression and are less immunogenic than adenoviruses. It has been demonstrated that LV vectors expressing IFNα or β-gal may transduce normal bladder urothelium and murine bladder cancer cell lines in a stable manner. Additionally, there is potential to enhance IFNα gene therapy to create innovative combination approaches that target resistance mechanisms. Numerous therapeutically relevant targets, such as PD-L1 and EGFR, were found in the trials assessing LV-IFNα gene therapy and should be investigated further in conjunction with interferon gene therapy [265].

Gene delivery is essential as explained in the following example of bladder cancer treatment using gene therapy. Adenoviral transgene expression is known to face several challenges. One of them is that adenovirus entry is blocked by an anti-adherence layer composed of secreted glycosaminoglycans (GAG) present on the luminal epithelial surface of the bladder. To overcome this, a gene-based drug was developed, comprising a recombinant adenovirus encoding IFN (rAd-IFN) and a novel small molecule excipient Syn3 for treating superficial bladder cancer [266]. Treatment with Syn3 produced consistently high gene transfer and expression in the urinary bladders of rodents and pigs [267]. Also, studies have proved that Ad-IFNA (adenoviruses encoding interferon-A) can overcome resistance to IFN-A protein both in vitro and in vivo and support evaluation of intravesical Ad-IFNA/Syn3 for the treatment of superficial bladder cancer [263].

11. Limitations of Gene Therapy

Despite the broad utility of gene therapy, it has many limitations, such as specific delivery of genes to the target cells, cleavage by nucleases present in the cells, impairment of cellular normal function, homogenous expression level in all cells, off-target mutagenicity, and stability of the recombinant vector [86-89].

12. Challenges in Gene Therapy

To unlock the potential of gene therapy, like a long-term therapeutic benefit or optimally a cure, it is essential to understand the obstructions to therapeutic intervention and develop approaches to bypass these difficulties. The most effective transgenic expression for suppressing a cancer-associated gene, delivery of therapeutic genes to diseased tissue, and identification of suitable

therapeutic gene(s) that can staunch disease progression are necessary for the success of cancer-related gene therapy.

For gene therapy to be effective, precise regulation of therapeutic transgenes is essential, and unwanted side effects need to be restricted. Gene expression also needs to be completely switched off to avoid adverse effects. As a result, promoters and enhancers are essential components that are crucial when determining the length and intensity of the best transgenic expression in particular cells or tissues. Promoters come in two varieties: constitutive and inducible. Constitutive promoters enable the ongoing transcription of the genes they are associated with. In certain instances, malignant melanoma has been targeted by inserting a 200 bp enhancer element upstream of a human tyrosinase promoter specific to pigment cells [268]. Limiting expression in healthy cells is also crucial; for this purpose, silencers are utilized to keep the vector dormant in healthy normal cells [269]. Yet, depending on the nature of the encoded product and the needs of the cell, not every gene may require the regulation of transgenic expression.

Target-specific delivery of therapeutic genes is essential for the efficacy of a treatment. Therefore, the intention of selecting a suitable vector for the delivery of therapeutic genes is fundamental. Both the viral and non-viral delivery strategies have their share of complications. A notable challenge with viral vectors, like adenoviruses, is the existence of prior immunity to specific serotypes among humans stemming from natural infections or vaccinations. This can complicate their systemic use. One possible approach to addressing this challenge is the implementation of a heterologous prime-boosting regimen, which entails administering similar antigens using different vectors [270]. Repeated viral infection can influence treatment's therapeutic response, so immunosuppression must be considered in some cases [271]. Virus-based gene therapy primarily depends on the strong binding between viral fiber proteins and specific host receptors associated with particular virus strains or serotypes. When the target tissue has low receptor expression or lacks it altogether, infection efficiency decreases. Additionally, using viruses as vectors raises safety concerns about therapeutic genes being taken up by non-targeted cells or tissues. To address these limitations, transductional retargeting is a common approach in viral gene therapy. This technique involves modifying viral surface proteins to include ligands that selectively or exclusively bind to receptors found on tumor cells [92].

Although nonviral approaches offer some benefits, such as safety and less immunotoxicity, they are nevertheless thought to be less efficient than viral methods for delivery, including passing through in vivo physiological barriers, cellular/nuclear absorption, and endosomal release. Behavior within the physiological environment poses the primary challenge for vectors. While advancements in nonviral gene delivery have occurred, unresolved concerns remain. In many studies related to target gene delivery, researchers have shown silencing or expression of target proteins primarily at the in vitro cellular level. Additional in vivo data is needed to establish it as a viable alternative approach comparable to viral vectors [108]. Achieving the essential therapeutic effect relies on DNA transportation into the nucleus, and thus, a relatively larger particle size introduces an extra challenge concerning efficacy. Unlike DNA, RNA doesn't need nucleus entry for expression but is comparatively less stable. Moreover, the interaction between the cell and the vector fluctuates under different conditions, significantly impacting transfection efficiency. To date, an ideal vector system to address all these issues has yet to emerge [272]. Understanding the physicochemical and biological properties of the non-viral vectors, including their behavior under different physiological

conditions, is essential for developing an ideal delivery system. A few recent gene transfer clinical trials are cited in Table 5 [273].

Table 5 Recent gene transfer clinical trials [273].

Gene	Targeted disease	Vector	Trial
p53	Solid tumor	adenovirus	Phase 2
Anti-CD40 antibody	Metastatic cancer, epithelial tumor	adenovirus	Phase 1
RB1	Refractory retinoblastoma	adenovirus	Phase 1
GM-CSF	Breast Cancer	HSC	Phase 1

The choice of a therapeutic gene that maximizes therapeutic efficacy while minimizing toxicity is essential for successful gene therapy. The current study aims to discover novel genes differently expressed in cancer cells that may control altered characteristics. In this regard, cancer genomic data is an effective tool for identifying molecular alterations in cancer cells. In these circumstances, the capacity to conduct extensive molecular profiling of tumors, which aids in detecting target genes, offers the potential to find novel targets for future therapeutic intervention [92]. Some approved gene therapies products for medicinal use are mentioned in Table 6.

Table 6 Gene Therapies Products Approved for Therapeutic Use.

Gene therapy	Targeted disease	Characteristics	Mode of action	References
Gendicine	Head and neck squamous cell carcinoma	Modified adenovirus that delivers the p53 gene	Expresses tumor suppressor gene and induces apoptosis	[274]
Adstiladrin	high-risk non-muscle invasive bladder cancer (NMIBC) that is immune to Bacillus Calmette-Guérin (BCG)	Adenovirus based, IFN-α2b transfection	Anticancer, immunostimulatory, antiangiogenic and apoptotic effects	[83]
Tecartus	Mantle cell lymphoma (MCL), Acute lymphoblastic leukemia (ALL)	CD19-directed chimeric antigen receptor (CAR) T-cell therapy	Anti-cancer	[275]
Provenge	Prostate cancer	Prostatic acid phosphatase (PAP), an antigen present in prostate cancer tissue, and granulocyte-macrophage colony-stimulating factor (GM-CSF), a stimulant of immune cell activity,	Stimulate a patient's own immune system against cancer.	[276]

		have been combined to form the human protein PAP-GM-CSF.		
Kymriah	B-cell acute lymphoblastic leukemia	CD19-directed genetically modified autologous T cell immunotherapy Initiates the anti-tumor effect through CD3 domain	Anti-cancer CAR-T cell-based immunotherapy	[275]
Oncorine (rAd5-H101)	nasopharyngeal cancer	Oncorine adenovirus's complete depletion of the E1B-55 KD gene is what causes p53 to become inactive.	Whereas the E1b-55KD-deficient adenovirus is unable to replicate in normal cells, oncorine grows in P53-deficient cancer cells.	[248, 277]
Rexin-G	pancreatic cancer	Rexin-G is a retroviral vehicle that carries a cytocidal cyclin G1 construct.	Rexin-G causes cancer cells to undergo apoptosis and cell death by inhibiting the G1 phase of the cell cycle.	[278]
Imlygic	melanoma	An advanced HSV-1 oncolytic virus, created through double modifications involving removal of the γ34.5 and α47 segments and insertion of the human granulocyte-macrophage colony-stimulating factor (GM-CSF) gene	The α47 gene antagonizes the host cell's antigen presentation transporter. Removing this gene reduces the regulation and expression of MHC class I, boosting antitumor immune responses. The virus contains two copies of the human GM-CSF gene, driven by the CMV promoter, resulting in robust gene expression. Imlygic generates local GM-CSF, which stimulates immune system responses.	[279]
Yescarta	non-Hodgkin lymphoma	CD19-directed ex-vivo modulated autologous T cells transfected with gamma-retroviral	Genetically modified autologous CAR-T cells can target and eliminate CD19-positive cells when	[280, 281]

	vector.	reintroduced into the patient.

13. Conclusion and Future Perspectives

The paper aims to present a clear picture of the ongoing progress in gene therapy to cancer treatment. The paper deals with the employment of gene therapy in clinical trials for treating the most widespread and lethal kinds of cancers. Evidencing a constant evolution, gene therapy is a beacon of modernity, harboring the potential to herald a cutting-edge solution in the multifaceted tumor treatment realm, spanning diverse malignancies. The current trend of gene therapy revolves around techniques such as the revolutionary CAR-T cell therapy and the remarkable CRISPR-Cas9 gene-editing method. Although CRISPR-Cas9 allows to correct oncogenes without affecting normal genes, as far as current research goes, none of the currently utilized methods offer a permanent cure. Despite the tremendous strides made in harnessing the power/potential of gene therapy, the elusive goal of a permanent solution to the complexities of various cancers remains beyond our current grasp. The future of this application centers around extensive research to develop methods such as targeting immune cytokines to build a long-lasting effect. The amalgamation of gene therapy with other techniques, such as combination therapy and epigenetic therapies, may show a large potential towards effective treatment. Working together, these approaches aim to improve the results of treatment while also finding ways to overcome the formidable challenges presented by cancer's ability to adapt and resist treatment. By harnessing the inherent characteristics of nanotechnology, we can significantly refine the precision and efficiency of delivering genes to their intended cellular destinations, potentially alleviate concerns related to unintended effects on non-target cells, ultimately leading to an intensified therapeutic influence. Exerting control over suppressing the expression of a mutated gene can offer valuable insights into effectively managing the progression of cancer and the intricate mechanisms of metastasis. As research and development progress, the potential of gene therapy to profoundly influence the landscape of cancer treatment continues to expand. This ongoing advancement paves the path for a promising future where the substantial effects of cancer could potentially be significantly alleviated, and the development of potential long-term cancer treatments becomes more achievable.

Author Contributions

Milky Mittal: Writing, preparation of figure 3 & 5 and compilation of data table 1 & 6, review & editing. Annu Kumari: Writing, preparation of figure 4 and helps in compilation of data table 1, review & editing. Bhashkar Paul: Writing, preparation of figure 6 and compilation of data table 2, 4 & 6, review & editing. Adya Varshney: Writing, preparation of figure 1 and compilation of data table 3, review & editing. Bhavya: Writing, preparation of figure 2 & 3 and compilation of data table 5, review & editing.: Chaitenya Verma: Writing, preparation of graphical abstract, review & editing. Ashok Saini and Indra Mani: Conceptualization, supervision, writing – review, editing and final approval of the submitted version.

Competing Interests

The authors have declared that no competing interests exist.

References

1. Dunbar CE, High KA, Joung JK, Kohn DB, Ozawa K, Sadelain M. Gene therapy comes of age. Science. 2018; 359: eaan4672.
2. Wirth T, Parker N, Ylä-Herttuala S. History of gene therapy. Gene. 2013; 525: 162-169.
3. Fink GR. A transforming principle. Cell. 2005; 120: 153-154.
4. Dulak J. Gene therapy. The legacy of Wacław Szybalski. Acta Biochim Pol. 2021; 68: 359-375.
5. Tatum EL. Molecular biology, nucleic acids, and the future of medicine. Perspect Biol Med. 1966; 10: 19-32.
6. Rogers S, Pfuderer P. Use of viruses as carriers of added genetic information. Nature. 1968; 219: 749-751.
7. Roberts L. Human gene transfer test approved. Science. 1989; 243: 473.
8. Scheller EL, Krebsbach PH. Gene therapy: Design and prospects for craniofacial regeneration. J Dent Res. 2009; 88: 585-596.
9. Somia N, Verma IM. Gene therapy: Trials and tribulations. Nat Rev Genet. 2000; 1: 91-99.
10. Kershaw MH, Westwood JA, Parker LL, Wang G, Eshhar Z, Mavroukakis SA, et al. A phase I study on adoptive immunotherapy using gene-modified T cells for ovarian cancer. Clin Cancer Res. 2006; 12: 6106-6115.
11. Blanco E, Izotova N, Booth C, Thrasher AJ. Immune reconstitution after gene therapy approaches in patients with X-linked severe combined immunodeficiency disease. Front Immunol. 2020; 11: 608653.
12. Howe SJ, Mansour MR, Schwarzwaelder K, Bartholomae C, Hubank M, Kempski H, et al. Insertional mutagenesis combined with acquired somatic mutations causes leukemogenesis following gene therapy of SCID-X1 patients. J Clin Invest. 2008; 118: 3143-3150.
13. Melchiorri D, Pani L, Gasparini P, Cossu G, Ancans J, Borg JJ, et al. Regulatory evaluation of Glybera in Europe-two committees, one mission. Nat Rev Drug Discov. 2013; 12: 719.
14. Schimmer J, Breazzano S. Investor outlook: Focus on upcoming LCA2 gene therapy phase III results. Hum Gene Ther Clin Dev. 2015; 26: 144-149.
15. Locatelli F, Thompson AA, Kwiatkowski JL, Porter JB, Thrasher AJ, Hongeng S, et al. Betibeglogene autotemcel gene therapy for non-β^0/β^0 genotype β-thalassemia. N Engl J Med. 2022; 386: 415-427.
16. Bank A. Human somatic cell gene therapy. Bioessays. 1996; 18: 999-1007.
17. Papanikolaou E, Bosio A. The promise and the hope of gene therapy. Front Genome Ed. 2021; 3: 618346.
18. Zaimy MA, Saffarzadeh N, Mohammadi A, Pourghadamyari H, Izadi P, Sarli A, et al. New methods in the diagnosis of cancer and gene therapy of cancer based on nanoparticles. Cancer Gene Ther. 2017; 24: 233-243.
19. Brown JS, Sundar R, Lopez J. Combining DNA damaging therapeutics with immunotherapy: More haste, less speed. Br J Cancer. 2018; 118: 312-324.
20. Chabanon RM, Rouanne M, Lord CJ, Soria JC, Pasero P, Postel-Vinay S. Targeting the DNA damage response in immuno-oncology: Developments and opportunities. Nat Rev Cancer. 2021; 21: 701-717.
21. Huang R, Zhou PK. DNA damage repair: Historical perspectives, mechanistic pathways and clinical translation for targeted cancer therapy. Signal Transduct Target Ther. 2021; 6: 254.

22. Pearl LH, Schierz AC, Ward SE, Al-Lazikani B, Pearl FM. Therapeutic opportunities within the DNA damage response. Nat Rev Cancer. 2015; 15: 166-180.
23. Surova O, Zhivotovsky B. Various modes of cell death induced by DNA damage. Oncogene. 2013; 32: 3789-3797.
24. Chu DT, Nguyen TT, Tien NL, Tran DK, Jeong JH, Anh PG, et al. Recent progress of stem cell therapy in cancer treatment: Molecular mechanisms and potential applications. Cells. 2020; 9: 563.
25. Oiseth SJ, Aziz MS. Cancer immunotherapy: A brief review of the history, possibilities, and challenges ahead. J Cancer Metastasis Treat. 2017; 3: 250-261.
26. McCarthy EF. The toxins of William B. Coley and the treatment of bone and soft-tissue sarcomas. Iowa Orthop J. 2006; 26: 154-158.
27. Dobosz P, Dzieciątkowski T. The intriguing history of cancer immunotherapy. Front Immunol. 2019; 10: 2965.
28. Lin MJ, Svensson-Arvelund J, Lubitz GS, Marabelle A, Melero I, Brown BD, et al. Cancer vaccines: The next immunotherapy frontier. Nat Cancer. 2022; 3: 911-926.
29. Pham T, Roth S, Kong J, Guerra G, Narasimhan V, Pereira L, et al. An update on immunotherapy for solid tumors: A review. Ann Surg Oncol. 2018; 25: 3404-3412.
30. Ma X, Li SJ, Liu Y, Zhang T, Xue P, Kang Y, et al. Bioengineered nanogels for cancer immunotherapy. Chem Soc Rev. 2022; 51: 5136-5174.
31. Fonkoua LA, Sirpilla O, Sakemura R, Siegler EL, Kenderian SS. CART cell therapy and the tumor microenvironment: Current challenges and opportunities. Mol Ther Oncolytics. 2022; 25: 69-77.
32. Sterner RC, Sterner RM. CAR-T cell therapy: Current limitations and potential strategies. Blood Cancer J. 2021; 11: 69.
33. Chailyan A, Marcatili P, Tramontano A. The association of heavy and light chain variable domains in antibodies: Implications for antigen specificity. FEBS J. 2011; 278: 2858-2866.
34. Sterner RM, Sakemura R, Cox MJ, Yang N, Khadka RH, Forsman CL, et al. GM-CSF inhibition reduces cytokine release syndrome and neuroinflammation but enhances CAR-T cell function in xenografts. Blood. 2019; 133: 697-709.
35. Philip B, Kokalaki E, Mekkaoui L, Thomas S, Straathof K, Flutter B, et al. A highly compact epitope-based marker/suicide gene for easier and safer T-cell therapy. Blood. 2014; 124: 1277-1287.
36. Matlashewski G, Lamb P, Pim D, Peacock J, Crawford L, Benchimol S. Isolation and characterization of a human p53 cDNA clone: Expression of the human p53 gene. EMBO J. 1984; 3: 3257-3262.
37. Shanker M, Jin J, Branch CD, Miyamoto S, Grimm EA, Roth JA, et al. Tumor suppressor gene-based nanotherapy: From test tube to the clinic. J Drug Deliv. 2011; 2011: 465845.
38. Vogelstein B, Lane D, Levine AJ. Surfing the p53 network. Nature. 2000; 408: 307-310.
39. Wiman KG. The retinoblastoma gene: Role in cell cycle control and cell differentiation. FASEB J. 1993; 7: 841-845.
40. Roth JA, Nguyen D, Lawrence DD, Kemp BL, Carrasco CH, Ferson DZ, et al. Retrovirus-mediated wild-type P53 gene transfer to tumors of patients with lung cancer. Nat Med. 1996; 2: 985-991.
41. Zhang WW, Li L, Li D, Liu J, Li X, Li W, et al. The first approved gene therapy product for cancer Ad-p53 (Gendicine): 12 years in the clinic. Hum Gene Ther. 2018; 29: 160-179.

42. Broderick JA, Zamore PD. MicroRNA therapeutics. Gene Ther. 2011; 18: 1104-1110.
43. Esquela-Kerscher A, Slack FJ. Oncomirs-microRNAs with a role in cancer. Nat Rev Cancer. 2006; 6: 259-269.
44. Parashar D, Rajendran V, Shukla R, Sistla R. Lipid-based nanocarriers for delivery of small interfering RNA for therapeutic use. Eur J Pharm Sci. 2020; 142: 105159.
45. Vita M, Henriksson M. The Myc oncoprotein as a therapeutic target for human cancer. Semin Cancer Biol. 2006; 16: 318-330.
46. Putney SD, Brown J, Cucco C, Lee R, Skorski T, Leonetti C, et al. Enhanced anti-tumor effects with microencapsulated c-myc antisense oligonucleotide. Antisense Nucleic Acid Drug Dev. 1999; 9: 451-458.
47. Fleming JB, Shen GL, Holloway SE, Davis M, Brekken RA. Molecular consequences of silencing mutant K-ras in pancreatic cancer cells: Justification for K-ras-directed therapy. Mol Cancer Res. 2005; 3: 413-423.
48. Krens LL, Baas JM, Gelderblom H, Guchelaar HJ. Therapeutic modulation of k-ras signaling in colorectal cancer. Drug Discov Today. 2010; 15: 502-516.
49. Hoyos V, Del Bufalo F, Yagyu S, Ando M, Dotti G, Suzuki M, et al. Mesenchymal stromal cells for linked delivery of oncolytic and apoptotic adenoviruses to non-small-cell lung cancers. Mol Ther. 2015; 23: 1497-1506.
50. Mohseni-Dargah M, Akbari-Birgani S, Madadi Z, Saghatchi F, Kaboudin B. Carbon nanotube-delivered iC9 suicide gene therapy for killing breast cancer cells in vitro. Nanomedicine. 2019; 14: 1033-1047.
51. Zhang B, Chen M, Zhang Y, Chen W, Zhang L, Chen L. An ultrasonic nanobubble-mediated PNP/fludarabine suicide gene system: A new approach for the treatment of hepatocellular carcinoma. PLoS One. 2018; 13: e0196686.
52. Sheikh S, Ernst D, Keating A. Prodrugs and prodrug-activated systems in gene therapy. Mol Ther. 2021; 29: 1716-1728.
53. Colombo F, Barzon L, Franchin E, Pacenti M, Pinna V, Danieli D, et al. Combined HSV-TK/IL-2 gene therapy in patients with recurrent glioblastoma multiforme: Biological and clinical results. Cancer Gene Ther. 2005; 12: 835-848.
54. Freeman SM, Abboud CN, Whartenby KA, Packman CH, Koeplin DS, Moolten FL, et al. The “bystander effect”: Tumor regression when a fraction of the tumor mass is genetically modified. Cancer Res. 1993; 53: 5274-5283.
55. Nasu Y, Saika T, Ebara S, Kusaka N, Kaku H, Abarzua F, et al. Suicide gene therapy with adenoviral delivery of HSV-tK gene for patients with local recurrence of prostate cancer after hormonal therapy. Mol Ther. 2007; 15: 834-840.
56. Singh V, Khan N, Jayandharan GR. Vector engineering, strategies and targets in cancer gene therapy. Cancer Gene Ther. 2022; 29: 402-417.
57. Daud A, Takamura KT, Diep T, Heller R, Pierce RH. Long-term overall survival from a phase I trial using intratumoral plasmid interleukin-12 with electroporation in patients with melanoma. J Transl Med. 2015; 13: O3.
58. Li T, Kang G, Wang T, Huang HE. Tumor angiogenesis and anti-angiogenic gene therapy for cancer. Oncol Lett. 2018; 16: 687-702.
59. Sun E, Han R, Lu B. Gene therapy of renal cancer using recombinant adeno-associated virus encoding human endostatin. Oncol Lett. 2018; 16: 2789-2796.

60. Shen Z, Yao C, Wang Z, Yue L, Fang Z, Yao H, et al. Vastatin, an endogenous antiangiogenesis polypeptide that is lost in hepatocellular carcinoma, effectively inhibits tumor metastasis. Mol Ther. 2016; 24: 1358-1368.
61. Lebedeva IV, Su ZZ, Sarkar D, Fisher PB. Restoring apoptosis as a strategy for cancer gene therapy: Focus on p53 and mda-7. Semin Cancer Biol. 2003; 13: 169-178.
62. Opalka B, Dickopp A, Kirch HC. Apoptotic genes in cancer therapy. Cells Tissues Organs. 2002; 172: 126-132.
63. Griffith TS, Stokes B, Kucaba TA, Earel Jr JK, VanOosten RL, Brincks EL, et al. TRAIL gene therapy: From preclinical development to clinical application. Curr Gene Ther. 2009; 9: 9-19.
64. Serakinci N, Cagsin H. Programming hMSCs into potential genetic therapy in cancer. Crit Rev Eukaryot Gene Expr. 2019; 29: 343-350.
65. Jia LT, Chen SY, Yang AG. Cancer gene therapy targeting cellular apoptosis machinery. Cancer Treat Rev. 2012; 38: 868-876.
66. Chattopadhyay S, Sarkar SS, Saproo S, Yadav S, Antil D, Das B, et al. Apoptosis-targeted gene therapy for non-small cell lung cancer using chitosan-poly-lactic-co-glycolic acid-based nano-delivery system and CASP8 and miRs 29A-B1 and 34A. Front Bioeng Biotechnol. 2023; 11: 1188652.
67. Lee DW, Kochenderfer JN, Stetler-Stevenson M, Cui YK, Delbrook C, Feldman SA, et al. T cells expressing CD19 chimeric antigen receptors for acute lymphoblastic leukaemia in children and young adults: A phase 1 dose-escalation trial. Lancet. 2015; 385: 517-528.
68. Xu D, Jin G, Chai D, Zhou X, Gu W, Chong Y, et al. The development of CAR design for tumor CAR-T cell therapy. Oncotarget. 2018; 9: 13991-14004.
69. Rodríguez-Rodríguez DR, Ramírez-Solís R, Garza-Elizondo MA, Garza-Rodríguez MD, Barrera-Saldaña HA. Genome editing: A perspective on the application of CRISPR/Cas9 to study human diseases. Int J Mol Med. 2019; 43: 1559-1574.
70. Hsu PD, Lander ES, Zhang F. Development and applications of CRISPR-Cas9 for genome engineering. Cell. 2014; 157: 1262-1278.
71. Yin H, Xue W, Chen S, Bogorad RL, Benedetti E, Grompe M, et al. Genome editing with Cas9 in adult mice corrects a disease mutation and phenotype. Nat Biotechnol. 2014; 32: 551-553.
72. Zhan T, Rindtorff N, Betge J, Ebert MP, Boutros M. CRISPR/Cas9 for cancer research and therapy. Semin Cancer Biol. 2019; 55: 106-119.
73. Koonin EV, Makarova KS. Origins and evolution of CRISPR-Cas systems. Philos Trans R Soc B. 2019; 374: 20180087.
74. Asmamaw M, Zawdie B. Mechanism and applications of CRISPR/Cas-9-mediated genome editing. Biologics. 2021; 15: 353-361.
75. Jiang F, Doudna JA. CRISPR-Cas9 structures and mechanisms. Annu Rev Biophys. 2017; 46: 505-529.
76. Xiao Q, Guo D, Chen S. Application of CRISPR/Cas9-based gene editing in HIV-1/AIDS therapy. Front Cell Infect Microbiol. 2019; 9: 69.
77. Choi BD, Yu X, Castano AP, Darr H, Henderson DB, Bouffard AA, et al. CRISPR-Cas9 disruption of PD-1 enhances activity of universal EGFRvIII CAR T cells in a preclinical model of human glioblastoma. J Immunother Cancer. 2019; 7: 304.

78. Zhao Z, Shi L, Zhang W, Han J, Zhang S, Fu Z, et al. CRISPR knock out of programmed cell death protein 1 enhances anti-tumor activity of cytotoxic T lymphocytes. Oncotarget. 2018; 9: 5208-5215.
79. Stadtmauer EA, Fraietta JA, Davis MM, Cohen AD, Weber KL, Lancaster E, et al. CRISPR-engineered T cells in patients with refractory cancer. Science. 2020; 367: eaba7365.
80. Eyquem J, Mansilla-Soto J, Giavridis T, van der Stegen SJ, Hamieh M, Cunanan KM, et al. Targeting a CAR to the TRAC locus with CRISPR/Cas9 enhances tumour rejection. Nature. 2017; 543: 113-117.
81. Martinez-Lage M, Torres-Ruiz R, Puig-Serra P, Moreno-Gaona P, Martin MC, Moya FJ, et al. In vivo CRISPR/Cas9 targeting of fusion oncogenes for selective elimination of cancer cells. Nat Commun. 2020; 11: 5060.
82. Gao J, Luo T, Lin N, Zhang S, Wang J. A new tool for CRISPR-Cas13a-based cancer gene therapy. Mol Ther Oncolytics. 2020; 19: 79-92.
83. Cross D, Burmester JK. Gene therapy for cancer treatment: Past, present and future. Clin Med Res. 2006; 4: 218-227.
84. Sapra P, Shor B. Monoclonal antibody-based therapies in cancer: Advances and challenges. Pharmacol Ther. 2013; 138: 452-469.
85. Al-Dosari MS, Gao X. Nonviral gene delivery: Principle, limitations, and recent progress. AAPS J. 2009; 11: 671-681.
86. Kosicki M, Tomberg K, Bradley A. Repair of double-strand breaks induced by CRISPR-Cas9 leads to large deletions and complex rearrangements. Nat Biotechnol. 2018; 36: 765-771.
87. Leibowitz ML, Papathanasiou S, Doerfler PA, Blaine LJ, Sun L, Yao Y, et al. Chromothripsis as an on-target consequence of CRISPR-Cas9 genome editing. Nat Genet. 2021; 53: 895-905.
88. Enache OM, Rendo V, Abdusamad M, Lam D, Davison D, Pal S, et al. Cas9 activates the p53 pathway and selects for p53-inactivating mutations. Nat Genet. 2020; 52: 662-668.
89. Katti A, Diaz BJ, Caragine CM, Sanjana NE, Dow LE. CRISPR in cancer biology and therapy. Nat Rev Cancer. 2022; 22: 259-279.
90. Nabel GJ. Development of optimized vectors for gene therapy. Proc Natl Acad Sci. 1999; 96: 324-326.
91. Mali S. Delivery systems for gene therapy. Indian J Hum Genet. 2013; 19: 3-8.
92. Das SK, Menezes ME, Bhatia S, Wang XY, Emdad L, Sarkar D, et al. Gene therapies for cancer: Strategies, challenges and successes. J Cell Physiol. 2015; 230: 259-271.
93. Lundstrom K. Viral vectors in gene therapy: Where do we stand in 2023? Viruses. 2023; 15: 698.
94. Xie Z, Zeng X. DNA/RNA-based formulations for treatment of breast cancer. Expert Opin Drug Deliv. 2017; 14: 1379-1393.
95. Holmes KD, Cassam AK, Chan B, Peters AA, Weaver LC, Dekaban GA. A multi-mutant herpes simplex virus vector has minimal cytotoxic effects on the distribution of filamentous actin, α-actinin 2 and a glutamate receptor in differentiated PC12 cells. J Neurovirol. 2000; 6: 33-45.
96. Wu N, Watkins SC, Schaffer PA, DeLuca NA. Prolonged gene expression and cell survival after infection by a herpes simplex virus mutant defective in the immediate-early genes encoding ICP4, ICP27, and ICP22. J Virol. 1996; 70: 6358-6369.
97. Pai SY. Built to last: Gene therapy for ADA SCID. Blood. 2021; 138: 1287-1288.
98. Ciuffi A. Mechanisms governing lentivirus integration site selection. Curr Gene Ther. 2008; 8: 419-429.

99. Kao CY, Pan YC, Hsiao YH, Lim SK, Cheng TW, Huang SW, et al. Improvement of gene delivery by minimal bacteriophage particles. ACS Nano. 2023; 17: 14532-14544.
100. Helal NA, Osami A, Helmy A, McDonald T, Shaaban LA, Nounou MI. Non-viral gene delivery systems: Hurdles for bench-to-bedside transformation. Die Pharmazie Int J Pharm Sci. 2017; 72: 627-693.
101. Belete TM. The current status of gene therapy for the treatment of cancer. Biologics. 2021; 15: 67-77.
102. Shim G, Kim D, Le QV, Park GT, Kwon T, Oh YK. Nonviral delivery systems for cancer gene therapy: Strategies and challenges. Curr Gene Ther. 2018; 18: 3-20.
103. Afrin H, Geetha Bai R, Kumar R, Ahmad SS, Agarwal SK, Nurunnabi M. Oral delivery of RNAi for cancer therapy. Cancer Metastasis Rev. 2023; 42: 699-724.
104. Etheridge ML, Campbell SA, Erdman AG, Haynes CL, Wolf SM, McCullough J. The big picture on nanomedicine: The state of investigational and approved nanomedicine products. Nanomedicine. 2013; 9: 1-14.
105. Santana-Armas ML, de Ilarduya CT. Strategies for cancer gene-delivery improvement by non-viral vectors. Int J Pharm. 2021; 596: 120291.
106. Sung YK, Kim SW. Recent advances in the development of gene delivery systems. Biomater Res. 2019; 23: 8.
107. Felgner PL, Ringold GM. Cationic liposome-mediated transfection. Nature. 1989; 337: 387-388.
108. Leventis R, Silvius JR. Interactions of mammalian cells with lipid dispersions containing novel metabolizable cationic amphiphiles. Biochim Biophys Acta Biomembr. 1990; 1023: 124-132.
109. Gao X, Huang L. A novel cationic liposome reagent for efficient transfection of mammalian cells. Biochem Biophys Res Commun. 1991; 179: 280-285.
110. Zhang Y, Satterlee A, Huang L. In vivo gene delivery by nonviral vectors: Overcoming hurdles? Mol Ther. 2012; 20: 1298-1304.
111. Ondrej V, Lukásová E, Falk M, Kozubek S. The role of actin and microtubule networks in plasmid DNA intracellular trafficking. Acta Biochim Pol. 2007; 54: 657-663.
112. Cardarelli F, Digiacomo L, Marchini C, Amici A, Salomone F, Fiume G, et al. The intracellular trafficking mechanism of lipofectamine-based transfection reagents and its implication for gene delivery. Sci Rep. 2016; 6: 25879.
113. Fiume G, Di Rienzo C, Marchetti L, Pozzi D, Caracciolo G, Cardarelli F. Single-cell real-time imaging of transgene expression upon lipofection. Biochem Biophys Res Commun. 2016; 474: 8-14.
114. Harvie P, Wong FM, Bally MB. Use of poly (ethylene glycol)-lipid conjugates to regulate the surface attributes and transfection activity of lipid-DNA particles. J Pharm Sci. 2000; 89: 652-663.
115. Xu L, Wempe MF, Anchordoquy TJ. The effect of cholesterol domains on PEGylated liposomal gene delivery in vitro. Ther Deliv. 2011; 2: 451-460.
116. Xu Y, Szoka FC. Mechanism of DNA release from cationic liposome/DNA complexes used in cell transfection. Biochemistry. 1996; 35: 5616-5623.
117. Ding GB, Meng X, Yang P, Li B, Stauber RH, Li Z. Integration of polylactide into polyethylenimine facilitates the safe and effective intracellular siRNA delivery. Polymers. 2020; 12: 445.
118. Wu XR, Zhang J, Zhang JH, Xiao YP, He X, Liu YH, et al. Amino acid-linked low molecular weight polyethylenimine for improved gene delivery and biocompatibility. Molecules. 2020; 25: 975.

119. Arnaiz E, Doucede LI, Garcia-Gallego S, Urbiola K, Gomez R, Tros de Ilarduya C, et al. Synthesis of cationic carbosilane dendrimers via click chemistry and their use as effective carriers for DNA transfection into cancerous cells. Mol Pharm. 2012; 9: 433-447.
120. Abbasi E, Aval SF, Akbarzadeh A, Milani M, Nasrabadi HT, Joo SW, et al. Dendrimers: Synthesis, applications, and properties. Nanoscale Res Lett. 2014; 9: 247.
121. Müller RH, Radtke M, Wissing SA. Solid lipid nanoparticles (SLN) and nanostructured lipid carriers (NLC) in cosmetic and dermatological preparations. Adv Drug Deliv Rev. 2002; 54: S131-S155.
122. Thatipamula RP, Palem CR, Gannu R, Mudragada S, Yamsani MR. Formulation and in vitro characterization of domperidone loaded solid lipid nanoparticles and nanostructured lipid carriers. Daru. 2011; 19: 23-32.
123. Das S, Chaudhury A. Recent advances in lipid nanoparticle formulations with solid matrix for oral drug delivery. AAPS PharmSciTech. 2011; 12: 62-76.
124. Boulikas T. Encapsulation of plasmid DNA (lipogenes™) and therapeutic agents with nuclear localization signal/fusogenic peptide conjugates into targeted liposome complexes. Athens, Greece: Regulon Inc.; 2016.
125. Jin L, Zeng X, Liu M, Deng Y, He N. Current progress in gene delivery technology based on chemical methods and nano-carriers. Theranostics. 2014; 4: 240-255.
126. Loh XJ, Lee TC, Dou Q, Deen GR. Utilising inorganic nanocarriers for gene delivery. Biomater Sci. 2016; 4: 70-86.
127. Ramamoorth M, Narvekar A. Non viral vectors in gene therapy-an overview. J Clin Diagn Res. 2015; 9: GE01-GE06.
128. Tian H, Chen J, Chen X. Nanoparticles for gene delivery. Small. 2013; 9: 2034-2044.
129. Twaites BR, de las Heras Alarcón C, Cunliffe D, Lavigne M, Pennadam S, Smith JR, et al. Thermo and pH responsive polymers as gene delivery vectors: Effect of polymer architecture on DNA complexation in vitro. J Control Release. 2004; 97: 551-566.
130. Sethuraman VA, Na K, Bae YH. pH-responsive sulfonamide/PEI system for tumor specific gene delivery: An in vitro study. Biomacromolecules. 2006; 7: 64-70.
131. Zhang Y, He J, Cao D, Zhang M, Ni P. Galactosylated reduction and pH dual-responsive triblock terpolymer Gal-PEEP-a-PCL-ss-PDMAEMA: A multifunctional carrier for the targeted and simultaneous delivery of doxorubicin and DNA. Polym Chem. 2014; 5: 5124-5138.
132. Mo R, Jiang T, Gu Z. Enhanced anticancer efficacy by ATP-mediated liposomal drug delivery. Angew Chem Int Ed Engl. 2014; 126: 5925-5930.
133. Yu M, Zhao K, Zhu X, Tang S, Nie Z, Huang Y, et al. Development of near-infrared ratiometric fluorescent probe based on cationic conjugated polymer and CdTe/CdS QDs for label-free determination of glucose in human body fluids. Biosens Bioelectron. 2017; 95: 41-47.
134. Dincer SE, Türk M, Pişkin E. Intelligent polymers as nonviral vectors. Gene Ther. 2005; 12: S139-S145.
135. Forbes NS. Engineering the perfect (bacterial) cancer therapy. Nat Rev Cancer. 2010; 10: 785-794.
136. Ahmad S, Casey G, Cronin M, Rajendran S, Sweeney P, Tangney M, et al. Induction of effective antitumor response after mucosal bacterial vector mediated DNA vaccination with endogenous prostate cancer specific antigen. J Urol. 2011; 186: 687-693.

137. Byrne WL, Murphy CT, Cronin M, Wirth T, Tangney M. Bacterial-mediated DNA delivery to tumour associated phagocytic cells. J Control Release. 2014; 196: 384-393.
138. Paglia P, Medina E, Arioli I, Guzman CA, Colombo MP. Gene transfer in dendritic cells, induced by oral DNA vaccination with salmonella typhimurium, results in protective immunity against a murine fibrosarcoma. Blood. 1998; 92: 3172-3176.
139. Van Pijkeren JP, Morrissey D, Monk IR, Cronin M, Rajendran S, O'Sullivan GC, et al. A novel listeria monocytogenes-based DNA delivery system for cancer gene therapy. Hum Gene Ther. 2010; 21: 405-416.
140. Kawabata K, Takakura Y, Hashida M. The fate of plasmid DNA after intravenous injection in mice: Involvement of scavenger receptors in its hepatic uptake. Pharm Res. 1995; 12: 825-830.
141. Kobayashi N, Kuramoto T, Yamaoka K, Hashida M, Takakura Y. Hepatic uptake and gene expression mechanisms following intravenous administration of plasmid DNA by conventional and hydrodynamics-based procedures. J Pharmacol Exp Ther. 2001; 297: 853-860.
142. Wooddell CI, Reppen T, Wolff JA, Herweijer H. Sustained liver-specific transgene expression from the albumin promoter in mice following hydrodynamic plasmid DNA delivery. J Gene Med. 2008; 10: 551-563.
143. Chevalier-Mariette C, Henry I, Montfort L, Capgras S, Forlani S, Muschler J, et al. CpG content affects gene silencing in mice: Evidence from novel transgenes. Genome Biol. 2003; 4: R53.
144. Goverdhana S, Puntel M, Xiong W, Zirger JM, Barcia C, Curtin JF, et al. Regulatable gene expression systems for gene therapy applications: Progress and future challenges. Mol Ther. 2005; 12: 189-211.
145. Kushwah R, Oliver JR, Duan R, Zhang L, Keshavjee S, Hu J. Induction of immunological tolerance to adenoviral vectors by using a novel dendritic cell-based strategy. J Virol. 2012; 86: 3422-3435.
146. Arita M, Kobayashi S. DNA sequence design using templates. New Gener Comput. 2002; 20: 263-277.
147. Chen J. The cell-cycle arrest and apoptotic functions of p53 in tumor initiation and progression. Cold Spring Harb Perspect Med. 2016; 6: a026104.
148. Ozaki T, Nakagawara A. Role of p53 in cell death and human cancers. Cancers. 2011; 3: 994-1013.
149. Monti P, Menichini P, Speciale A, Cutrona G, Fais F, Taiana E, et al. Heterogeneity of TP53 mutations and P53 protein residual function in cancer: Does it matter? Front Oncol. 2020; 10: 593383.
150. Linn P, Kohno S, Sheng J, Kulathunga N, Yu H, Zhang Z, et al. Targeting RB1 loss in cancers. Cancers. 2021; 13: 3737.
151. Chinnam M, Goodrich DW. RB1, development, and cancer. Curr Top Dev Biol. 2011; 94: 129-169.
152. Molinari F, Frattini M. Functions and regulation of the PTEN gene in colorectal cancer. Front Oncol. 2014; 3: 326.
153. Samuels Y, Ericson K. Oncogenic PI3K and its role in cancer. Curr Opin Oncol. 2006; 18: 77-82.
154. Milella M, Falcone I, Conciatori F, Cesta Incani U, Del Curatolo A, Inzerilli N, et al. PTEN: Multiple functions in human malignant tumors. Front Oncol. 2015; 5: 125824.
155. Bononi A, Pinton P. Study of PTEN subcellular localization. Methods. 2015; 77-78: 92-103.
156. Tidyman WE, Rauen KA. The RASopathies: Developmental syndromes of Ras/MAPK pathway dysregulation. Curr Opin Genet Dev. 2009; 19: 230-236.

157. Ferreira A, Pereira F, Reis C, Oliveira MJ, Sousa MJ, Preto A. Crucial role of oncogenic KRAS mutations in apoptosis and autophagy regulation: Therapeutic implications. Cells. 2022; 11: 2183.
158. Maitre E, Cornet E, Troussard X. Hairy cell leukemia: 2020 update on diagnosis, risk stratification, and treatment. Am J Hematol. 2019; 94: 1413-1422.
159. Śmiech M, Leszczyński P, Kono H, Wardell C, Taniguchi H. Emerging BRAF mutations in cancer progression and their possible effects on transcriptional networks. Genes. 2020; 11: 1342.
160. Schmidt M, Heimberger T, Gruensfelder P, Schler G, Hoppe F. Inducible promoters for gene therapy of head and neck cancer: An in vitro study. Eur Arch Otorhinolaryngol. 2004; 261: 208-215.
161. Gustafsson C, Govindarajan S, Minshull J. Codon bias and heterologous protein expression. Trends Biotechnol. 2004; 22: 346-353.
162. Wei W, Brennan MD. The gypsy insulator can act as a promoter-specific transcriptional stimulator. Mol Cell Biol. 2001; 21: 7714-7720.
163. Chow LT, Gelinas RE, Broker TR, Roberts RJ. An amazing sequence arrangement at the 5′ ends of adenovirus 2 messenger RNA. Cell. 1977; 12: 1-8.
164. Beck C, Uramoto H, Borén J, Akyurek LM. Tissue-specific targeting for cardiovascular gene transfer. Potential vectors and future challenges. Curr Gene Ther. 2004; 4: 457-467.
165. Inouye S, Sahara-Miura Y, Sato JI, Suzuki T. Codon optimization of genes for efficient protein expression in mammalian cells by selection of only preferred human codons. Protein Expr Purif. 2015; 109: 47-54.
166. Nagata S, Pastan I. Removal of B cell epitopes as a practical approach for reducing the immunogenicity of foreign protein-based therapeutics. Adv Drug Deliv Rev. 2009; 61: 977-985.
167. Annoni A, Gregori S, Naldini L, Cantore A. Modulation of immune responses in lentiviral vector-mediated gene transfer. Cell Immunol. 2019; 342: 103802.
168. Mehier-Humbert S, Guy RH. Physical methods for gene transfer: Improving the kinetics of gene delivery into cells. Adv Drug Deliv Rev. 2005; 57: 733-753.
169. Kim TK, Eberwine JH. Mammalian cell transfection: The present and the future. Anal Bioanal Chem. 2010; 397: 3173-3178.
170. Donnelly RF, Singh TR, Woolfson AD. Microneedle-based drug delivery systems: Microfabrication, drug delivery, and safety. Drug Deliv. 2010; 17: 187-207.
171. Zhang Y, Yu LC. Microinjection as a tool of mechanical delivery. Curr Opin Biotechnol. 2008; 19: 506-510.
172. Yamamoto F, Furusawa M, Furusawa I, Obinata M. The ‘pricking’ method: A new efficient technique for mechanically introducing foreign DNA into the nuclei of culture cells. Exp Cell Res. 1982; 142: 79-84.
173. Zhi D, Yang T, Zhang T, Yang M, Zhang S, Donnelly RF. Microneedles for gene and drug delivery in skin cancer therapy. J Control Release. 2021; 335: 158-177.
174. Zhu T, Zhang W, Jiang P, Zhou S, Wang C, Qiu L, et al. Progress in intradermal and transdermal gene therapy with microneedles. Pharm Res. 2022; 39: 2475-2486.
175. Chang CY, Tai JA, Sakaguchi Y, Nishikawa T, Hirayama Y, Yamashita K. Enhancement of polyethylene glycol-cell fusion efficiency by novel application of transient pressure using a jet injector. FEBS Open Bio. 2023; 13: 478-489.

176. Mandip KC, Steer CJ. A new era of gene editing for the treatment of human diseases. Swiss Med Wkly. 2019; 149: w20021.
177. Yang NS, Burkholder J, Roberts B, Martinell B, McCabe D. In vivo and in vitro gene transfer to mammalian somatic cells by particle bombardment. Proc Natl Acad Sci. 1990; 87: 9568-9572.
178. Sanford JC, Klein TM, Wolf ED, Allen N. Delivery of substances into cells and tissues using a particle bombardment process. Part Sci Technol. 1987; 5: 27-37.
179. Herrero MJ, Sendra L, Miguel A, Aliño SF. Physical methods of gene delivery. In: Safety and efficacy of gene-based therapeutics for inherited disorders. Cham: Springer; 2017. pp. 113-135.
180. Slivac I, Guay D, Mangion M, Champeil J, Gaillet B. Non-viral nucleic acid delivery methods. Expert Opin Biol Ther. 2017; 17: 105-118.
181. Udvardi A, Kufferath I, Grutsch H, Zatloukal K, Volc-Platzer B. Uptake of exogenous DNA via the skin. J Mol Med. 1999; 77: 744-750.
182. Zelenin AV, Kolesnikov VA, Tarasenko OA, Shafei RA, Zelenina IA, Mikhailov VV, et al. Bacterial β-galactosidase and human dystrophin genes are expressed in mouse skeletal muscle fibers after ballistic transfection. FEBS Lett. 1997; 414: 319-322.
183. Bittman KS, Panzer JA, Balice-Gordon RJ. Patterns of cell-cell coupling in embryonic spinal cord studied via ballistic delivery of gap-junction-permeable dyes. J Comp Neurol. 2004; 477: 273-285.
184. Kettunen P, Demas J, Lohmann C, Kasthuri N, Gong Y, Wong RO, et al. Imaging calcium dynamics in the nervous system by means of ballistic delivery of indicators. J Neurosci Methods. 2002; 119: 37-43.
185. Potter H, Heller R. Transfection by electroporation. Curr Protoc Mol Biol. 2018; 121: 9.3.1-9.3.13.
186. Du X, Wang J, Zhou Q, Zhang L, Wang S, Zhang Z, et al. Advanced physical techniques for gene delivery based on membrane perforation. Drug Deliv. 2018; 25: 1516-1525.
187. Yarmush ML, Golberg A, Serša G, Kotnik T, Miklavčič D. Electroporation-based technologies for medicine: Principles, applications, and challenges. Annu Rev Biomed Eng. 2014; 16: 295-320.
188. Kotnik T, Frey W, Sack M, Meglič SH, Peterka M, Miklavčič D. Electroporation-based applications in biotechnology. Trends Biotechnol. 2015; 33: 480-488.
189. Wagstaff PG, Buijs M, van den Bos W, de Bruin DM, Zondervan PJ, de la Rosette JJ, et al. Irreversible electroporation: State of the art. Onco Targets Ther. 2016; 9: 2437-2446.
190. Suzuki T, Shin BC, Fujikura K, Matsuzaki T, Takata K. Direct gene transfer into rat liver cells by in vivo electroporation. FEBS Lett. 1998; 425: 436-440.
191. Miyazaki JI, Aihara H. Gene transfer into muscle by electroporation in vivo. Nat Biotechnol. 1998; 16: 867-870.
192. Bugeon S, de Chevigny A, Boutin C, Coré N, Wild S, Bosio A, et al. Direct and efficient transfection of mouse neural stem cells and mature neurons by in vivo mRNA electroporation. Development. 2017; 144: 3968-3977.
193. Wang Y, Cui H, Li K, Sun C, Du W, Cui J, et al. A magnetic nanoparticle-based multiple-gene delivery system for transfection of porcine kidney cells. PLoS One. 2014; 9: e102886.
194. Chan DCF. Magneto-biolistic methods. Boulder, CO: University of Technology Corp; 1998.
195. Plank C, Scherer F, Schillinger U, Anton M. Magnetofection: Enhancement and localization of gene delivery with magnetic particles under the influence of a magnetic field. J Gene Med. 2000; 2: S24.

196. Mah C, Zolotukhin I, Fraites TJ, Dobson J, Batich C, Byrne BJ. Microsphere-mediated delivery of recombinant AAV vectors in vitro and in vivo. Mol Ther. 2000; 1: S293.
197. Arora S, Gupta G, Singh S, Singh N. Advances in magnetofection &- magnetically guided nucleic acid delievery: A Review. J Pharm Technol Res Manage. 2013; 1: 19-29.
198. Das AK, Gupta P, Chakraborty D. Physical methods of gene transfer: Kinetics of gene delivery into cells: A Review. Agric Rev. 2015; 36: 61-66.
199. Dastjerd NT, Valibeik A, Rahimi Monfared S, Goodarzi G, Moradi Sarabi M, Hajabdollahi F, et al. Gene therapy: A promising approach for breast cancer treatment. Cell Biochem Funct. 2022; 40: 28-48.
200. Mellott AJ, Forrest ML, Detamore MS. Physical non-viral gene delivery methods for tissue engineering. Ann Biomed Eng. 2013; 41: 446-468.
201. de Ruijter TC, Veeck J, de Hoon JP, van Engeland M, Tjan-Heijnen VC. Characteristics of triple-negative breast cancer. J Cancer Res Clin Oncol. 2011; 137: 183-192.
202. Delacroix L, Begon D, Chatel G, Jackers P, Winkler R. Distal ERBB2 promoter fragment displays specific transcriptional and nuclear binding activities in ERBB2 overexpressing breast cancer cells. DNA Cell Biol. 2005; 24: 582-594.
203. Maeda T, Matsubara H, Asano T, Ochiai T, Sakiyama S, Tagawa M. A minimum c-erbB-2 promoter-mediated expression of herpes simplex virus thymidine kinase gene confers selective cytotoxicity of human breast cancer cells to ganciclovir. Cancer Gene Ther. 2001; 8: 890-896.
204. Al-Mahmood S, Sapiezynski J, Garbuzenko OB, Minko T. Metastatic and triple-negative breast cancer: Challenges and treatment options. Drug Deliv Transl Res. 2018; 8: 1483-1507.
205. Jing X, Liang H, Hao C, Yang X, Cui X. Overexpression of MUC1 predicts poor prognosis in patients with breast cancer. Oncol Rep. 2019; 41: 801-810.
206. Dai Y, Zhang X. MicroRNA delivery with bioreducible polyethylenimine as a non-viral vector for breast cancer gene therapy. Macromol Biosci. 2019; 19: 1800445.
207. Zheng L, Weilun Z, Minghong J, Yaxi Z, Shilian L, Yanxin L, et al. Adeno-associated virus-mediated doxycycline-regulatable TRAIL expression suppresses growth of human breast carcinoma in nude mice. BMC Cancer. 2012; 12: 153.
208. Montaño-Samaniego M, Bravo-Estupiñan DM, Méndez-Guerrero O, Alarcón-Hernández E, Ibáñez-Hernández M. Strategies for targeting gene therapy in cancer cells with tumor-specific promoters. Front Oncol. 2020; 10: 605380.
209. Ma XJ, Huang R, Kuang AR. AFP promoter enhancer increased specific expression of the human sodium iodide symporter (hNIS) for targeted radioiodine therapy of hepatocellular carcinoma. Cancer Invest. 2009; 27: 673-681.
210. Park JH, Kim KI, Lee KC, Lee YJ, Lee TS, Chung WS, et al. Assessment of α-fetoprotein targeted HSV1-tk expression in hepatocellular carcinoma with in vivo imaging. Cancer Biother Radiopharm. 2015; 30: 8-15.
211. Jiang H, Guo S, Xiao D, Bian X, Wang J, Wang Y, et al. Arginine deiminase expressed in vivo, driven by human telomerase reverse transcriptase promoter, displays high hepatoma targeting and oncolytic efficiency. Oncotarget. 2017; 8: 37694-37704.
212. Ni Y, Schwaneberg U, Sun ZH. Arginine deiminase, a potential anti-tumor drug. Cancer Lett. 2008; 261: 1-11.
213. Reghupaty SC, Sarkar D. Current status of gene therapy in hepatocellular carcinoma. Cancers. 2019; 11: 1265.

214. Yu Q, Zhou J, Jian Y, Xiu Z, Xiang L, Yang D, et al. MicroRNA-214 suppresses cell proliferation and migration and cell metabolism by targeting PDK2 and PHF6 in hepatocellular carcinoma. Cell Biol Int. 2020; 44: 117-126.
215. World Health Organization. Cancer [Internet]. Geneva, Switzerland: World Health Organization; 2022. Available from: https://www.Who.Int/En/News-Room/Fact-Sheets/Detail/Cancer.
216. Zhang P, Tan J, Yang DB, Luo ZC, Luo S, Chen P, et al. Gene therapy using the human telomerase catalytic subunit gene promoter enables targeting of the therapeutic effects of vesicular stomatitis virus matrix protein against human lung adenocarcinoma. Exp Ther Med. 2012; 4: 859-864.
217. Puglisi F, Barbone F, Damante G, Bruckbauer M, Di Lauro V, Beltrami CA, et al. Prognostic value of thyroid transcription factor-1 in primary, resected, non-small cell lung carcinoma. Mod Pathol. 1999; 12: 318-324.
218. Lei L, Chen C, Zhao J, Wang H, Guo M, Zhou Y, et al. Targeted expression of miR-7 operated by TTF-1 promoter inhibited the growth of human lung cancer through the NDUFA4 pathway. Mol Ther Nucleic Acids. 2017; 6: 183-197.
219. Xu L, Wen Z, Zhou Y, Liu Z, Li Q, Fei G, et al. MicroRNA-7-regulated TLR9 signaling-enhanced growth and metastatic potential of human lung cancer cells by altering the phosphoinositide-3-kinase, regulatory subunit 3/Akt pathway. Mol Biol Cell. 2013; 24: 42-55.
220. Hao S, Du X, Song Y, Ren M, Yang Q, Wang A, et al. Targeted gene therapy of the HSV-TK/hIL-12 fusion gene controlled by the hSLPI gene promoter of human non-small cell lung cancer in vitro. Oncol Lett. 2018; 15: 6503-6512.
221. Chen P, Zhang SD, Lin Y, Cao J, Chen J, Yang BB. The construction and characterization of a novel adenovirus vector of artificial microRNA targeting EGFR. Int J Clin Exp Pathol. 2019; 12: 1968-1974.
222. Yan M, Chen J, Jiang H, Xie Y, Li C, Chen L, et al. Effective inhibition of cancer cells by recombinant adenovirus expressing EGFR-targeting artificial microRNA and reversed-caspase-3. PLos One. 2020; 15: e0237098.
223. Shao Y, Sun X, He Y, Liu C, Liu H. Elevated levels of serum tumor markers CEA and CA15-3 are prognostic parameters for different molecular subtypes of breast cancer. PLoS One. 2015; 10: e0133830.
224. Rama Ballesteros AR, Hernandez R, Perazzoli G, Cabeza L, Melguizo C, Velez C, et al. Specific driving of the suicide E gene by the CEA promoter enhances the effects of paclitaxel in lung cancer. Cancer Gene Ther. 2020; 27: 657-668.
225. Qiu Y, Peng GL, Liu QC, Li FL, Zou XS, He JX. Selective killing of lung cancer cells using carcinoembryonic antigen promoter and double suicide genes, thymidine kinase and cytosine deaminase (pCEA-TK/CD). Cancer Lett. 2012; 316: 31-38.
226. Yan LJ, Guo XH, Wang WP, Hu YR, Duan SF, Liu Y, et al. Gene therapy and photothermal therapy of layer-by-layer assembled AuNCs/PEI/miRNA/HA nanocomplexes. Curr Cancer Drug Targets. 2019; 19: 330-337.
227. Üner M, Yener G, Ergüven M. Design of colloidal drug carriers of celecoxib for use in treatment of breast cancer and leukemia. Mater Sci Eng C. 2019; 103: 109874.
228. Chen C, Yue D, Lei L, Wang H, Lu J, Zhou Y, et al. Promoter-operating targeted expression of gene therapy in cancer: Current stage and prospect. Mol Ther Nucleic Acids. 2018; 11: 508-514.

229. Li Z, Ding Q, Li Y, Miller SA, Abbruzzese JL, Hung MC. Suppression of pancreatic tumor progression by systemic delivery of a pancreatic-cancer-specific promoter driven Bik mutant. Cancer Lett. 2006; 236: 58-63.
230. Xie X, Xia W, Li Z, Kuo HP, Liu Y, Li Z, et al. Targeted expression of BikDD eradicates pancreatic tumors in noninvasive imaging models. Cancer Cell. 2007; 12: 52-65.
231. Torres MP, Chakraborty S, Souchek J, Batra SK. Mucin-based targeted pancreatic cancer therapy. Curr Pharm Des. 2012; 18: 2472-2481.
232. Tholey RM, Lal S, Jimbo M, Burkhart RA, Blanco FF, Cozzitorto JA, et al. MUC1 promoter-driven DTA as a targeted therapeutic strategy against pancreatic cancer. Mol Cancer Res. 2015; 13: 439-448.
233. Cao Y, Blohm D, Ghadimi BM, Stosiek P, Xing PX, Karsten U. Mucins (MUC1 and MUC3) of gastrointestinal and breast epithelia reveal different and heterogeneous tumor-associated aberrations in glycosylation. J Histochem Cytochem. 1997; 45: 1547-1457.
234. Liu SH, Yu J, Sanchez R, Liu X, Heidt D, Willey J, et al. A novel synthetic human insulin super promoter for targeting PDX-1-expressing pancreatic cancer. Cancer Lett. 2018; 418: 75-83.
235. Yu J, Liu SH, Sanchez R, Nemunaitis J, Rozengurt E, Brunicardi FC. PDX1 associated therapy in translational medicine. Ann Transl Med. 2016; 4: 214.
236. Naji A, Eitoku M, Favier B, Deschaseaux F, Rouas-Freiss N, Suganuma N. Biological functions of mesenchymal stem cells and clinical implications. Cell Mol Life Sci. 2019; 76: 3323-3348.
237. Nieddu V, Piredda R, Bexell D, Barton J, Anderson J, Sebire N, et al. Engineered human mesenchymal stem cells for neuroblastoma therapeutics. Oncol Rep. 2019; 42: 35-42.
238. Kuipers EJ, Grady WM, Lieberman D, Seufferlein T, Sung JJ, Boelens PG, et al. Colorectal cancer. Nat Rev Dis Primers. 2015; 1: 15065.
239. Chen T, Gong W, Tian H, Wang H, Chu S, Ma J, et al. Fibroblast growth factor 18 promotes proliferation and migration of H460 cells via the ERK and p38 signaling pathways. Oncol Rep. 2017; 37: 1235-1242.
240. Teimoori-Toolabi L, Azadmanesh K, Zeinali S. Selective suicide gene therapy of colon cancer cell lines exploiting fibroblast growth factor 18 promoter. Cancer Biother Radiopharm. 2010; 25: 105-116.
241. Mahmood N, Mihalcioiu C, Rabbani SA. Multifaceted role of the urokinase-type plasminogen activator (uPA) and its receptor (uPAR): Diagnostic, prognostic, and therapeutic applications. Front Oncol. 2018; 8: 24.
242. Teimoori-Toolabi L, Azadmanesh K, Amanzadeh A, Zeinali S. Selective suicide gene therapy of colon cancer exploiting the urokinase plasminogen activator receptor promoter. BioDrugs. 2010; 24: 131-146.
243. Chang J, Tang N, Fang Q, Zhu K, Liu L, Xiong X, et al. Inhibition of COX-2 and 5-LOX regulates the progression of colorectal cancer by promoting PTEN and suppressing PI3K/AKT pathway. Biochem Biophys Res Commun. 2019; 517: 1-7.
244. Kosumi K, Hamada T, Zhang S, Liu L, da Silva A, Koh H, et al. Prognostic association of PTGS2 (COX-2) over-expression according to BRAF mutation status in colorectal cancer: Results from two prospective cohorts and CALGB 89803 (Alliance) trial. Eur J Cancer. 2019; 111: 82-93.
245. Lech G, Słotwiński R, Słodkowski M, Krasnodębski IW. Colorectal cancer tumour markers and biomarkers: Recent therapeutic advances. World J Gastroenterol. 2016; 22: 1745-1755.

246. Kaliberova LN, Kusmartsev SA, Krendelchtchikova V, Stockard CR, Grizzle WE, Buchsbaum DJ, et al. Experimental cancer therapy using restoration of NAD+-linked 15-hydroxyprostaglandin dehydrogenase expression. Mol Cancer Ther. 2009; 8: 3130-3139.
247. World Health Organization. Global cancer rates could increase by 50% to 15 million by 2020 [Internet]. Geneva, Switzerland: World Health Organization; 2003. Available from: https://www.who.int/News/Item/03-04-2003-Global-Cancer-Rates-Could-Increase-by-50-to-15-Million-by-2020.
248. Azatian A, Yu H, Dai W, Schneiders FI, Botelho NK, Lord RV. Effectiveness of HSV-tk suicide gene therapy driven by the Grp78 stress-inducible promoter in esophagogastric junction and gastric adenocarcinomas. J Gastrointest Surg. 2009; 13: 1044-1051.
249. Liang L, Bi W, Chen W, Lin Y, Tian Y. Combination of MPPa-PDT and HSV1-TK/GCV gene therapy on prostate cancer. Lasers Med Sci. 2018; 33: 227-232.
250. Ni M, Zhang Y, Lee AS. Beyond the endoplasmic reticulum: Atypical GRP78 in cell viability, signalling and therapeutic targeting. Biochem J. 2011; 434: 181-188.
251. Chen N, Ye XC, Chu K, Navone NM, Sage EH, Yu-Lee LY, et al. A secreted isoform of ErbB3 promotes osteonectin expression in bone and enhances the invasiveness of prostate cancer cells. Cancer Res. 2007; 67: 6544-6548.
252. Sung SY, Chang JL, Chen KC, Yeh SD, Liu YR, Su YH, et al. Co-targeting prostate cancer epithelium and bone stroma by human osteonectin-promoter–mediated suicide gene therapy effectively inhibits androgen-independent prostate cancer growth. PLoS One. 2016; 11: e0153350.
253. Cai Z, Lv H, Cao W, Zhou C, Liu Q, Li H, et al. Targeting strategies of adenovirus-mediated gene therapy and virotherapy for prostate cancer. Mol Med Rep. 2017; 16: 6443-6458.
254. Lin MC, Wang M, Chou MC, Chao CN, Fang CY, Chen PL, et al. Gene therapy for castration-resistant prostate cancer cells using JC polyomavirus-like particles packaged with a PSA promoter driven-suicide gene. Cancer Gene Ther. 2019; 26: 208-215.
255. Mohammadi V, Behbahani AB, Rafiee GR, Hosseini SY, Zarei MA, Okhovat MA, et al. The effects of specific expression of apoptin under the control of PSES and PSA promoter on cell death and apoptosis of LNCaP cells. Iran J Basic Med Sci. 2017; 20: 1354-1359.
256. Tamura RE, de Luna IV, Lana MG, Strauss BE. Improving adenoviral vectors and strategies for prostate cancer gene therapy. Clinics. 2018; 73: e476s.
257. Xiang Z, Ye Z, Ma J, Lin Y, Zhou Y. Temporal trends and projections of bladder cancer burden in China from 1990 to 2030: Findings from the global burden of disease study. Clin Epidemiol. 2022; 14: 1305-1315.
258. Saginala K, Barsouk A, Aluru JS, Rawla P, Padala SA, Barsouk A. Epidemiology of bladder cancer. Med Sci. 2020; 8: 15.
259. Sidransky D, Von Eschenbach A, Tsai YC, Jones P, Summerhayes I, Marshall F, et al. Identification of p53 gene mutations in bladder cancers and urine samples. Science. 1991; 252: 706-709.
260. Homami A, Kachoei ZA, Asgarie M, Ghazi F. Analysis of FGFR3 and HRAS genes in patients with bladder cancer. Med J Islam Repub Iran. 2020; 34: 108.
261. López-Knowles E, Hernández S, Malats N, Kogevinas M, Lloreta J, Carrato A, et al. PIK3CA mutations are an early genetic alteration associated with FGFR3 mutations in superficial papillary bladder tumors. Cancer Res. 2006; 66: 7401-7404.

262. Jebar AH, Hurst CD, Tomlinson DC, Johnston C, Taylor CF, Knowles MA. FGFR3 and Ras gene mutations are mutually exclusive genetic events in urothelial cell carcinoma. Oncogene. 2005; 24: 5218-5125.
263. Nickel JC, Downey J, Morales A, Emerson L, Clark J. Relative efficacy of various exogenous glycosaminoglycans in providing a bladder surface permeability barrier. J Urol. 1998; 160: 612-614.
264. Benedict WF, Tao Z, Kim CS, Zhang X, Zhou JH, Adam L, et al. Intravesical Ad-IFNα causes marked regression of human bladder cancer growing orthotopically in nude mice and overcomes resistance to IFN-α protein. Mol Ther. 2004; 10: 525-532.
265. Mitra AP, Narayan VM, Mokkapati S, Miest T, Boorjian SA, Alemozaffar M, et al. Antiadenovirus antibodies predict response durability to nadofaragene firadenovec therapy in BCG-unresponsive non-muscle-invasive bladder cancer: Secondary analysis of a phase 3 clinical trial. Eur Urol. 2022; 81: 223-228.
266. Mokkapati S, Narayan VM, Manyam GC, Lim AH, Duplisea JJ, Kokorovic A, et al. Lentiviral interferon: A novel method for gene therapy in bladder cancer. Mol Ther Oncolytics. 2022; 26: 141-157.
267. Nagabhushan TL, Maneval DC, Benedict WF, Wen SF, Ihnat PM, Engler H, et al. Enhancement of intravesical delivery with Syn3 potentiates interferon-α2b gene therapy for superficial bladder cancer. Cytokine Growth Factor Rev. 2007; 18: 389-394.
268. Yamashita M, Rosser CJ, Zhou JH, Zhang XQ, Connor RJ, Engler H, et al. Syn3 provides high levels of intravesical adenoviral-mediated gene transfer for gene therapy of genetically altered urothelium and superficial bladder cancer. Cancer Gene Ther. 2002; 9: 687-691.
269. Nettelbeck DM, Rivera AA, Balagué C, Alemany R, Curiel DT. Novel oncolytic adenoviruses targeted to melanoma: Specific viral replication and cytolysis by expression of E1A mutants from the tyrosinase enhancer/promoter. Cancer Res. 2002; 62: 4663-4670.
270. Xie X, Mathias JR, Smith MA, Walker SL, Teng Y, Distel M, et al. Silencer-delimited transgenesis: NRSE/RE1 sequences promote neural-specific transgene expression in a NRSF/REST-dependent manner. BMC Biol. 2012; 10: 93.
271. Fournillier A, Frelin L, Jacquier E, Ahlén G, Brass A, Gerossier E, et al. A heterologous prime/boost vaccination strategy enhances the immunogenicity of therapeutic vaccines for hepatitis C virus. J Infect Dis. 2013; 208: 1008-1019.
272. Ungerechts G, Springfeld C, Frenzke ME, Lampe J, Parker WB, Sorscher EJ, et al. An immunocompetent murine model for oncolysis with an armed and targeted measles virus. Mol Ther. 2007; 15: 1991-1997.
273. Zu H, Gao D. Non-viral vectors in gene therapy: Recent development, challenges, and prospects. AAPS J. 2021; 23: 78.
274. Zhao Z, Anselmo AC, Mitragotri S. Viral vector-based gene therapies in the clinic. Bioeng Transl Med. 2021; 7: e10258.
275. Lee A. Nadofaragene firadenovec: First approval. Drugs. 2023; 83: 353-357.
276. Food and Drug Administration (FDA). Tecartus (brexucabtagene autoleucel) [Internet]. Silver Spring, MD: Food and Drug Administration (FDA); 2022. Available from: https://www.fda.gov/vaccines-blood-biologics/cellular-gene-therapy-products/tecartus-brexucabtagene-autoleucel.

277. Anassi E, Ndefo UA. Sipuleucel-T (provenge) injection: The first immunotherapy agent (vaccine) for hormone-refractory prostate cancer. P T. 2011; 36: 197-202.
278. Nemunaitis J, Tong AW, Nemunaitis M, Senzer N, Phadke AP, Bedell C, et al. A phase I study of telomerase-specific replication competent oncolytic adenovirus (telomelysin) for various solid tumors. Mol Ther. 2010; 18: 429-434.
279. Chawla SP, Bruckner H, Morse MA, Assudani N, Hall FL, Gordon EM. A phase I-II study using rexin-G tumor-targeted retrovector encoding a dominant-negative cyclin G1 inhibitor for advanced pancreatic cancer. Mol Ther Oncolytics. 2019; 12: 56-67.
280. Ahamadi M, Kast J, Chen PW, Huang X, Dutta S, Upreti VV. Oncolytic viral kinetics mechanistic modeling of Talimogene Laherparepvec (T-VEC) a first-in-class oncolytic viral therapy in patients with advanced melanoma. CPT Pharmacometrics Syst Pharmacol. 2023; 12: 250-260.
281. AlDallal SM. Yescarta: A new era for non-Hodgkin lymphoma patients. Cureus. 2020; 12: e11504.