BASIC AND CLINICAL CANCER REPORTS | Volume 1, Issue 2 | 2026

Cancer Gene Therapy: Strategies and Emerging Advances

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Abstract

Cancer gene therapy has developed from relatively simple strategies for replacing defective genes into a broad therapeutic discipline that includes gene addition, gene silencing, suicide-gene therapy, oncolytic virotherapy, immunomodulatory gene transfer, RNA therapeutics, programmable genome and epigenome editing, and genetically engineered immune cells. The biological rationale is to exploit molecular abnormalities that distinguish malignant cells from normal tissues while using genetic payloads to restore tumor-suppressive functions, suppress oncogenic dependencies, trigger selective cytotoxicity, remodel the tumor microenvironment, or enhance antitumor immunity. This review critically examines the major cancer gene therapy strategies, their molecular mechanisms, delivery platforms, clinical development, advantages, and limitations. Tumor-suppressor replacement, particularly adenoviral TP53 delivery, established an important proof of concept for restoring a lost cellular checkpoint, whereas antisense oligonucleotides, small interfering RNAs, microRNA-based approaches, and CRISPR interference provide increasingly programmable methods for suppressing oncogenic signaling. Suicide-gene systems such as herpes simplex virus thymidine kinase/ganciclovir and cytosine deaminase/5-fluorocytosine exploit selective intracellular conversion of prodrugs and can generate bystander killing. Oncolytic viruses combine tumor-selective replication with direct lysis, antigen release, innate immune activation, and delivery of therapeutic transgenes; recent engineering approaches increasingly arm viruses with cytokines, immune agonists, or other payloads. Immunomodulatory gene therapy seeks to increase local cytokine activity, antigen presentation, costimulation, or chemotactic recruitment while limiting systemic toxicity. RNA therapeutics and lipid nanoparticle technologies provide transient, potentially repeatable delivery, whereas CRISPR/Cas systems, base editors, prime editors, and epigenome editors enable increasingly precise modification of endogenous genetic programs. Ex vivo genetic engineering of T cells, natural killer cells, and other immune effectors has already produced clinically established cancer therapies, while in vivo genome editing remains an important translational frontier. Despite substantial progress, efficacy is constrained by tumor heterogeneity, inadequate delivery to solid tumors, extracellular and intracellular barriers, antiviral immunity, off-target activity, genomic instability, antigen escape, immune suppression, manufacturing complexity, and cost. Current development is therefore moving toward biomarker-guided patient selection, tumor-restricted expression, transient or controllable editing, multiplex engineering, improved non-viral delivery, and rational combinations with checkpoint inhibitors, radiotherapy, chemotherapy, targeted agents, and other cell therapies. Overall, cancer gene therapy is evolving from a collection of individual technologies into an integrated precision-oncology platform in which the therapeutic payload, delivery system, target cell, and treatment context are designed as a single intervention.

Keywords: cancer; gene therapy; gene replacement; gene silencing; suicide gene therapy; oncolytic viruses; RNA therapeutics; CRISPR-Cas; CAR-T cells; gene delivery; precision oncology

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1. Introduction

Cancer is fundamentally a disease of accumulated genetic, epigenetic, metabolic, and microenvironmental alterations. Genomic instability, activation of oncogenic pathways, loss of tumor-suppressor functions, defective DNA-damage responses, altered apoptosis, abnormal metabolism, and immune escape collectively permit malignant cells to proliferate, survive, invade, and adapt under therapeutic pressure [1–4]. These properties also create therapeutic vulnerabilities. Whereas conventional chemotherapy and radiotherapy act largely through pharmacologic or physical damage, gene therapy attempts to manipulate the biological information that controls malignant behavior. Consequently, cancer gene therapy is not a single modality but a family of interventions in which DNA, RNA, viral genomes, or genetically modified cells are used to alter tumor biology or antitumor immunity [5–8].

The earliest conceptual approaches focused on correcting or compensating for a genetic defect. Tumor-suppressor replacement, particularly delivery of wild-type TP53, sought to restore cell-cycle arrest and apoptosis in cancer cells carrying dysfunctional p53 signaling [9–11]. Other approaches attempted to silence oncogenes, deliver prodrug-converting enzymes, inhibit angiogenesis, or stimulate immune responses [12–18]. These strategies demonstrated that genetic manipulation could produce antitumor effects, but they also exposed a central limitation: a potent therapeutic gene is ineffective if it cannot reach a sufficiently large fraction of malignant cells at an appropriate level and duration of expression [19–22].

The development of viral vectors transformed the field. Adenoviral vectors permit high-level transient expression without requiring genomic integration, while retroviral and lentiviral vectors can provide durable expression after integration into target-cell genomes [23–27]. Adeno-associated virus (AAV) offers attractive tissue tropism and a comparatively favorable safety profile, although its limited packaging capacity and pre-existing immunity complicate some oncology applications [28,29]. More recently, non-viral systems—including lipid nanoparticles, polymeric nanoparticles, conjugates, extracellular vesicles, and cell-based carriers—have emerged as alternatives that can provide transient expression and may be easier to manufacture at scale [30–34].

Cancer immunology has broadened the definition of gene therapy further. Genetically engineered T cells expressing chimeric antigen receptors (CARs) or tumor-specific T-cell receptors (TCRs) can function as living therapeutic agents, while gene editing can modify endogenous receptors, checkpoint pathways, cytokine signaling, or alloreactivity [35–42]. The clinical success of CD19- and BCMA-directed CAR-T products has demonstrated that genetically modified cells can produce durable responses in selected hematologic malignancies, although cytokine-release syndrome, immune effector cell-associated neurotoxicity, antigen escape, limited persistence, and the difficulty of treating solid tumors remain major challenges [43–47].

Programmable genome editing has introduced another conceptual shift. CRISPR/Cas systems can disrupt genes, introduce sequences, regulate transcription, alter epigenetic states, or, with base and prime editors, install defined sequence changes without conventional double-strand breaks [48–53]. In oncology, these tools can be applied directly to tumor cells, but their near-term clinical development has been more advanced in ex vivo immune-cell engineering, where edited cells can be characterized before administration [54–58]. Early clinical studies have demonstrated the feasibility of multiplex editing in cancer patients and have provided valuable information about persistence, chromosomal alterations, immunogenicity, and manufacturing constraints [59–62].

This review examines the principal strategies of cancer gene therapy from a translational perspective. The discussion moves from the biological rationale and major therapeutic architectures to delivery systems, tumor targeting, combination treatment, clinical evidence, safety, manufacturing, and future directions. Particular attention is given to the distinction between approaches that alter tumor cells directly and those that genetically reprogram immune or supporting cells. This distinction is clinically important because the optimal vector, route of administration, duration of expression, and safety framework differ substantially between these settings [5,19,30,54].

2. Biological Rationale and Molecular Targets

A rational gene therapy begins with a disease mechanism that can be manipulated. Tumor suppressors such as TP53, RB1, PTEN, CDKN2A, and components of DNA-damage response pathways are frequently disrupted in cancer. Restoration or reactivation of these pathways may induce cell-cycle arrest, senescence, apoptosis, or increased susceptibility to other therapies [3,9,10,63]. However, tumor-suppressor replacement is biologically more difficult than replacing a missing protein in a monogenic disorder because cancer cells often contain multiple cooperating lesions and can bypass a restored pathway through parallel signaling circuits [4,64].

Oncogenic dependencies represent a second major class of targets. Mutant KRAS, MYC, BCL2, STAT3, survivin, VEGF, viral oncogenes, fusion proteins, and lineage-specific transcription factors have all been investigated as candidates for genetic inhibition [12,65–70]. The concept of oncogene addiction provides a rationale for targeting a

driver on which a tumor remains disproportionately dependent even after additional mutations have accumulated. CRISPR screens have expanded this concept by systematically identifying context-dependent vulnerabilities and synthetic-lethal interactions that may not be obvious from static genomic data [71–74].

DNA repair and replication vulnerabilities are particularly attractive because malignant cells frequently acquire repair defects while becoming highly dependent on compensatory pathways. BRCA1/2-deficient tumors, for example, can display dependence on PARP-mediated repair, and CRISPR functional screens can identify additional synthetic-lethal relationships [75–77]. In principle, gene therapy can exploit these dependencies by suppressing compensatory genes or selectively restoring a toxic DNA-damage response. Nevertheless, direct editing of tumor genomes remains challenging because the therapeutic system must discriminate malignant from normal cells and must reach heterogeneous tumor populations [48,78].

The tumor microenvironment is another therapeutic target. Cancer-associated fibroblasts, endothelial cells, tumor-associated macrophages, myeloid-derived suppressor cells, and exhausted lymphocytes can all contribute to immune suppression and treatment resistance [79–82]. Genetic interventions can alter cytokine gradients, chemokine production, extracellular matrix composition, angiogenesis, or immune-cell function. This ecosystem-level view is increasingly important for solid tumors, where malignant cells may constitute only a fraction of the cellular compartment and where physical barriers can prevent therapeutic payloads from reaching the cancer cells themselves [30,79,83].

Finally, cancer-specific molecular signatures can be used to improve selectivity. Tumor-specific promoters, aberrant transcription-factor activity, viral oncogene dependence, unique fusion junctions, mutant alleles, and tumor-associated surface antigens have all been explored as targeting signals [84–88]. The most effective future platforms are likely to combine several levels of selectivity—for example, a tumor-targeted nanoparticle carrying a transient editor under a cancer-selective promoter, or an oncolytic virus engineered both for tumor-restricted replication and local immune activation [89–92].

3. Major Cancer Gene Therapy Strategies

Cancer gene therapy strategies can be organized according to their primary biological objective: replacing a missing function, suppressing a harmful gene, generating a selective metabolic vulnerability, delivering a cytotoxic or immunostimulatory payload, stimulating endogenous immunity, or permanently reprogramming a cell. Increasingly, individual products combine several of these mechanisms. For example, an oncolytic virus may replicate selectively in tumor cells, lyse them, express an immune agonist, and release tumor antigens, whereas an engineered T cell may express a CAR while simultaneously carrying gene edits that reduce exhaustion or alloreactivity [35,40,89,93].

3.1 Tumor-suppressor gene replacement

Tumor-suppressor replacement introduces a functional gene into malignant cells to restore a biological activity lost during oncogenesis. TP53 has been the archetypal target because p53 integrates oncogenic stress, DNA damage, replication stress, and apoptotic signaling. When activated, p53 can induce p21-mediated cell-cycle arrest, senescence, apoptosis, and transcriptional programs that alter tumor metabolism and immune signaling [9,10,94]. Adenoviral vectors are particularly suited to this strategy because they can achieve high-level transient expression in dividing and non-dividing cells without requiring stable genomic integration [23,24].

Recombinant adenoviral p53 therapy provided one of the earliest clinically meaningful demonstrations that a tumor-suppressor gene could be delivered as an anticancer medicine. Gendicine, an rAd-p53 product, was approved in China in 2003 for head and neck cancer and has been used primarily in combination with radiotherapy or other modalities [11,95,96]. The long clinical history of this product is informative: the therapeutic concept is feasible, but the magnitude and durability of benefit depend strongly on tumor accessibility, transduction efficiency, tumor genotype, immune context, and combination treatment. More recent analyses continue to regard rAd-p53 as an important historical milestone in cancer gene therapy rather than a universal solution for TP53-mutant tumors [95–97].

Other replacement strategies have targeted PTEN, RB-pathway components, or genes that restore differentiation and apoptotic competence. In principle, restoration of a tumor suppressor should be most effective when the downstream signaling architecture remains sufficiently intact to respond. In advanced tumors with multiple pathway defects, however, reintroducing a single gene may not overcome compensatory signaling. This is one reason why modern strategies increasingly combine gene replacement with chemotherapy, radiotherapy, targeted inhibitors, or immune activation [63,98,99].

3.2 Oncogene inhibition and gene silencing

Gene silencing attempts to reduce the abundance or activity of oncogenic transcripts without necessarily altering genomic DNA. Antisense oligonucleotides can hybridize to target RNA and promote degradation or alter RNA processing, while siRNAs recruit the endogenous RNA-induced silencing complex to catalyze sequence-specific transcript degradation [13,14,100]. MicroRNA therapeutics operate at a broader regulatory level: tumor-suppressive microRNAs may be restored with mimics, whereas oncogenic microRNAs can be inhibited with antagomirs or related inhibitors [101–103].

The principal advantage of RNA-based suppression is reversibility. A transient reduction in an oncogenic protein can be advantageous when permanent genomic disruption is unnecessary or undesirable. RNA molecules can also be redesigned relatively rapidly as new molecular targets are identified. However, systemic delivery is constrained by nuclease degradation, renal clearance, innate immune sensing, limited tissue penetration, endosomal sequestration, and heterogeneous intracellular uptake [30,100,104]. Consequently, delivery chemistry is often as important as sequence design.

Lipid nanoparticles have become a particularly important platform for nucleic-acid delivery. Their composition can be tuned to protect RNA, facilitate cellular uptake, and promote endosomal escape. Tumor-directed nanoparticles may incorporate antibodies, peptides, aptamers, or other ligands to improve cell specificity, although receptor heterogeneity can limit the benefit of a single targeting ligand [31,105–107]. Extracellular vesicles and exosomes provide another biologically derived carrier with potential advantages in membrane interaction and cargo protection, but standardized manufacturing, loading efficiency, biodistribution, and regulatory characterization remain unresolved [34,108].

CRISPR interference (CRISPRi) and RNA-targeting CRISPR systems such as Cas13 extend the concept of reversible gene suppression. Catalytically inactive Cas proteins can be fused to transcriptional repressors or activators, allowing endogenous gene expression to be tuned without cutting DNA, while Cas13 systems can directly target RNA [50,109]. These approaches may be particularly useful when transient pathway modulation is preferred, although efficient and selective delivery remains a major translational bottleneck.

3.3 Suicide-gene therapy and gene-directed enzyme prodrug therapy

Suicide-gene therapy creates a selective vulnerability by introducing an enzyme that converts a relatively non-toxic prodrug into a cytotoxic metabolite. The herpes simplex virus thymidine kinase (HSV-TK)/ganciclovir system is a classic example. HSV-TK phosphorylates ganciclovir, and subsequent cellular phosphorylation produces metabolites that interfere with DNA synthesis and can induce cell death [15,110]. Cytosine deaminase/5-fluorocytosine is another extensively studied system, in which cytosine deaminase converts 5-fluorocytosine to 5-fluorouracil [111].

An important theoretical advantage is the bystander effect. Because only a fraction of tumor cells may be transduced after local administration, therapeutic efficacy can extend to neighboring cells through diffusion of active metabolites, intercellular transfer, gap junctions, or secondary immune mechanisms [112–114]. This phenomenon can partially compensate for incomplete gene transfer, although its magnitude varies among tumor types and vector systems. Tumor-selective promoters and regulatory elements can further restrict transgene expression to malignant cells [84,115].

Clinical development has demonstrated feasibility but also exposed the central limitation of the approach: gene-transfer efficiency. A phase I study of intraprostatic adenoviral HSV-TK followed by ganciclovir provided early clinical evidence that prodrug activation could be performed in human tumors [116]. Later generations have attempted to improve vector distribution, enzyme activity, promoter selectivity, and combination with radiotherapy or immune checkpoint blockade [111–114]. The strategy remains attractive when the tumor is accessible for intratumoral administration or when a delivery vehicle can achieve sufficiently broad distribution.

3.4 Oncolytic virotherapy

Oncolytic viruses (OVs) are among the most clinically advanced gene-therapy platforms in oncology. They are selected or genetically engineered to preferentially infect and replicate within malignant cells, leading to direct lysis and release of tumor antigens and danger-associated molecular patterns [89,90,117]. Their therapeutic mechanism is therefore dual: viral replication produces direct cytotoxicity, while tumor-cell destruction can initiate or amplify antitumor immunity. Modern OV engineering can add a third function by encoding cytokines, immune agonists, chemokines, bispecific proteins, or other therapeutic payloads [89–92,118].

Talimogene laherparepvec (T-VEC), an engineered HSV-1 expressing GM-CSF, established a landmark clinical proof of concept for oncolytic immunotherapy in melanoma [119]. Its success demonstrated that local viral therapy can generate systemic immune effects and that genetic modification of a viral backbone can produce a clinically useful

cancer medicine. Subsequent HSV-1 platforms have explored deletion of neurovirulence or immune-evasion genes, altered receptor interactions, tumor-selective promoters, and insertion of immunostimulatory transgenes [120–122].

Adenoviruses, vaccinia viruses, reoviruses, measles viruses, Newcastle disease virus, vesicular stomatitis virus, and other viral backbones have also been investigated [89,117,123]. Each platform has distinct advantages in genome capacity, tropism, pre-existing immunity, manufacturing, and safety. Adenovirus is attractive for its engineering flexibility and high transgene expression; HSV-1 can accommodate large inserts and can be controlled with antiviral agents; vaccinia offers a large genome and cytoplasmic replication; and some RNA viruses exhibit intrinsic tropism for malignant cells with activated antiviral pathways [120,123–125].

The major barriers are delivery and host immunity. Pre-existing neutralizing antibodies can limit systemic viral exposure, while innate immune clearance can shorten the period available for tumor infection. Dense extracellular matrix, abnormal vasculature, high interstitial pressure, and spatial heterogeneity can further restrict intratumoral spread [83,126]. Current engineering strategies therefore increasingly emphasize dual targeting, capsid or envelope modification, immune evasion during delivery, and payloads that remodel the tumor microenvironment [89,90,118].

Combination therapy is especially promising. Viral infection can increase antigen presentation and inflammatory signaling, potentially sensitizing previously non-inflamed tumors to PD-1/PD-L1 or CTLA-4 blockade [118,127]. Current clinical development includes engineered adenoviruses expressing IFN-β and CD40 ligand, and other armed viruses designed to combine direct oncolysis with immune stimulation [128]. Recent reviews emphasize that the field is moving from simple tumor-selective replication toward multifunctional viral platforms that integrate oncolysis, immune modulation, and tumor-microenvironment engineering [89,90].

3.5 Immunomodulatory gene therapy

Immunomodulatory gene therapy seeks to strengthen antitumor immunity rather than relying exclusively on direct tumor-cell killing. Candidate genes include IL-2, IL-12, IL-15, GM-CSF, interferons, chemokines, costimulatory ligands, and other immune agonists [16,129–131]. Local gene expression can provide high concentrations at the tumor site while reducing the systemic exposure that limits recombinant cytokine therapy. This approach is particularly attractive for cytokines such as IL-12, whose systemic administration can cause substantial toxicity [132].

A second strategy is to improve antigen presentation. Dendritic cells can be genetically modified to express tumor antigens, costimulatory molecules, or cytokines, creating cellular vaccines that stimulate tumor-specific T cells [133–135]. Tumor cells can also be engineered to express immunostimulatory factors, although direct tumor-cell modification is generally more practical for accessible lesions than for disseminated disease. Personalized neoantigen approaches further integrate tumor sequencing with nucleic-acid or cell-based delivery, allowing genetic information from an individual tumor to guide antigen selection [136,137].

Immunomodulatory gene therapy can also be designed to counter suppressive components of the tumor microenvironment. Examples include engineering immune cells to resist TGF-β signaling, modifying chemokine receptors to improve tumor infiltration, or deleting inhibitory pathways such as PD-1, CISH, or other intracellular checkpoints [58,138–141]. These strategies illustrate the convergence between gene therapy and immunotherapy: the therapeutic gene is not necessarily directed at the tumor cell, but instead reprograms the immune compartment that must ultimately eliminate it.

3.6 Antiangiogenic gene therapy

Tumor growth and metastasis require vascular support, and VEGF-driven angiogenesis is one of the best-characterized mechanisms of tumor vascularization [142,143]. Gene therapy can inhibit angiogenesis by delivering soluble receptor fragments, endogenous angiogenesis inhibitors, RNA molecules, or regulatory factors that suppress proangiogenic signaling [144–146]. Compared with repeated protein administration, local expression of an antiangiogenic factor could theoretically provide prolonged exposure from a single intervention.

The biological adaptability of tumor vasculature is the main limitation. Blocking VEGF can activate alternative angiogenic pathways, alter pericyte coverage, increase hypoxia, or select for more invasive tumor phenotypes [147]. Consequently, antiangiogenic gene therapy is unlikely to be optimal as a single-pathway intervention in most advanced cancers. Combination with cytotoxic therapy, immune modulation, or multi-target gene regulation may be more effective.

3.7 RNA-based gene therapy

RNA-based therapeutics encompass siRNA, miRNA mimics and inhibitors, antisense oligonucleotides, long non-coding RNA modulation, and messenger RNA (mRNA) delivery. Unlike integrating DNA vectors, RNA generally

produces transient effects and therefore offers a useful balance between potency and reversibility [100–103]. mRNA can encode cytokines, tumor antigens, engineered receptors, or genome-editing proteins, allowing one platform to support several therapeutic architectures [30,31,104].

Lipid nanoparticles have substantially accelerated the development of mRNA and siRNA technologies because they can protect nucleic acids from degradation and facilitate cytosolic delivery [31,105]. In cancer, however, the challenge is more demanding than in hepatocyte-dominant delivery systems because solid tumors exhibit heterogeneous perfusion, extracellular-matrix barriers, variable endocytosis, and immunosuppressive niches. Tumor-targeted or stimulus-responsive nanoparticles are therefore being developed to improve selectivity and endosomal escape [30,105–107].

The transient nature of RNA can also be exploited therapeutically. Repeated dosing may permit adaptive modulation of oncogenic pathways without permanent genomic alteration. Conversely, short intracellular persistence can require frequent administration, and innate immune recognition of RNA can either enhance antitumor immunity or reduce tolerability depending on the molecular design [100,104]. Chemical modification, sequence optimization, purification, and formulation are therefore central determinants of clinical performance.

3.8 CRISPR/Cas genome editing and programmable regulation

CRISPR/Cas technology has expanded gene therapy from gene addition toward direct manipulation of endogenous genomic programs. Cas9 can generate targeted double-strand breaks that are repaired predominantly by non-homologous end joining, producing gene disruption, or can be combined with donor templates to support homology-directed repair [48–53]. In cancer, this enables direct disruption of oncogenes, viral oncogenes, or genes that support tumor survival, while ex vivo applications permit editing of immune cells before infusion.

The therapeutic possibilities extend beyond conventional Cas9. CRISPRa and CRISPRi use catalytically inactive Cas proteins fused to transcriptional regulators to activate or repress genes without cutting DNA [50,51]. Base editors couple a Cas-derived targeting system to a deaminase to produce defined nucleotide conversions, whereas prime editors use a reverse-transcriptase-based mechanism guided by a specialized pegRNA to install substitutions, small insertions, or deletions [52,53,148]. These technologies may reduce some risks associated with double-strand breaks, although they introduce their own off-target and bystander-editing considerations [149–151].

Direct tumor editing is conceptually attractive because a driver mutation could become the molecular Achilles heel of a cancer cell. Studies have explored selective targeting of mutant KRAS alleles, fusion oncogenes, HPV E6/E7, and other cancer-specific sequences [65,152–155]. However, tumor heterogeneity, delivery barriers, and the risk of editing normal cells remain substantial obstacles. A strategy that works in a homogeneous cell line may be much less effective in a genetically diverse tumor containing subclones with distinct mutations and variable expression of the target [4,156].

CRISPR is also powerful as a discovery platform. Genome-scale screens can identify essential genes, synthetic-lethal interactions, mechanisms of drug resistance, and determinants of immune-cell function [71–74,157]. This research function indirectly strengthens gene therapy by improving target selection. Rather than editing genes simply because they are frequently mutated, investigators can identify dependencies that are functionally necessary in a particular molecular context.

Ex vivo immune-cell engineering is currently one of the most compelling applications. CRISPR can disrupt endogenous TCR loci, PDCD1, CISH, HLA-related genes, or other pathways, while simultaneously inserting tumor-specific receptors [54,58–62,138]. A first-in-human study demonstrated the feasibility of multiplex CRISPR-edited T cells in patients with refractory cancer, with persistent edited cells detected after infusion [59]. More recent studies have evaluated allogeneic CRISPR-engineered CAR-T cells and CRISPR-edited T cells targeting intracellular checkpoints, illustrating the rapid clinical maturation of this area [60,61].

The next generation of edited cellular therapies is moving toward base and prime editing. These approaches may allow more precise engineering of antigen receptors, removal of endogenous TCRs, modulation of HLA expression, or introduction of resistance traits without relying exclusively on double-strand breaks [148,158]. Their clinical potential is significant, but manufacturing complexity, editing efficiency, unintended edits, chromosomal abnormalities, immunogenicity, and long-term clonal behavior require careful surveillance [59,149,159].

3.9 Epigenetic gene therapy

Cancer-associated epigenetic alterations can silence tumor-suppressor genes or activate malignant transcriptional programs without changing DNA sequence. DNA methylation, histone modification, nucleosome positioning, and enhancer activity can therefore become targets for gene regulation [160,161]. CRISPR-dCas9 fused to epigenetic

enzymes provides a programmable method for local transcriptional activation or repression, potentially allowing tumor-suppressor reactivation without permanent sequence editing [162].

Epigenome editing is attractive because it may be reversible and locus-specific, but the stability of the induced epigenetic state, the possibility of spreading effects to neighboring regulatory elements, and the durability of gene expression remain incompletely defined [162,163]. Combining epigenetic editing with immune or targeted therapies may be especially useful where transcriptional plasticity contributes to drug resistance.

4. Gene Delivery Platforms

Delivery is arguably the central engineering problem in cancer gene therapy. A therapeutic construct must reach the relevant tissue, cross extracellular barriers, enter the appropriate cells, release its cargo intracellularly, and maintain an adequate therapeutic window while minimizing exposure of normal tissues [19–22,30]. The optimal delivery system depends on whether the target is a disseminated hematologic malignancy, an accessible solid tumor, the liver, the central nervous system, or an ex vivo cell product.

Adenoviral vectors offer high transgene expression and broad tropism, but pre-existing immunity and inflammatory responses can restrict systemic administration [23,24]. Their non-integrating nature is advantageous when transient expression is desired. HSV-1 vectors provide a large genome that can accommodate multiple transgenes and can be engineered for tumor-selective replication [120–122]. Retroviral and lentiviral vectors are particularly useful for stable ex vivo engineering of immune cells because integration supports durable transgene expression [25–27].

AAV vectors have become highly successful in genetic medicine because of their favorable tissue tropism and relatively low pathogenicity. In oncology, however, their small packaging capacity, neutralizing antibodies, and the need for adequate tumor penetration limit some applications [28,29]. AAV may be especially useful for localized delivery to tissues in which direct administration is feasible, and emerging oncology trials are evaluating multi-payload AAV gene therapy for high-grade glioma [164].

Non-viral delivery systems include lipid nanoparticles, polymeric nanoparticles, inorganic nanoparticles, peptides, aptamers, and extracellular vesicles [30–34,105]. Their advantages include transient expression, lower manufacturing complexity for some formulations, and the possibility of repeated administration. Their disadvantages include variable biodistribution, uptake by the reticuloendothelial system, endosomal trapping, and challenges in achieving selective tumor accumulation. Stimulus-responsive nanoparticles that respond to pH, enzymes, hypoxia, or redox conditions are being developed to improve tumor specificity [105,165].

Extracellular vesicles and exosomes are particularly interesting because they participate naturally in intercellular communication and can transport proteins and nucleic acids. Engineered extracellular vesicles have been investigated for delivery of CRISPR components and other therapeutic cargoes [34,108]. Nevertheless, batch-to-batch heterogeneity, loading efficiency, purification, biodistribution, and standardized potency assays remain important barriers to clinical translation.

5. Tumor Targeting and Selective Expression

Selectivity can be achieved at several levels: vector tropism, receptor-mediated targeting, tumor-specific promoters, conditional replication, mutation-specific recognition, local administration, and tumor-restricted immune activation [84–88]. No single level is sufficient for most solid tumors, and layered selectivity is increasingly favored.

Tumor-specific promoters can exploit transcription factors that are highly active in malignant cells. Examples include promoters responsive to telomerase activity, hypoxia, tissue-specific transcriptional programs, or oncogenic signaling [84,115]. Conditional replication-competent viruses can use the same logic at the level of viral replication, restricting productive infection to cells with particular molecular abnormalities [89,120].

Surface targeting can be achieved with antibodies, antibody fragments, nanobodies, peptides, aptamers, or engineered receptors. This approach is central to CAR-T and TCR therapies and is increasingly used for nanoparticle targeting [35–42,105]. However, antigen heterogeneity creates a major risk of treatment escape. Multi-antigen recognition, tandem CARs, bispecific receptors, and logic-gated designs are therefore being developed to reduce the probability that antigen-negative clones survive [45,166,167].

6. Combination of Gene Therapy with Other Cancer Treatments

Cancer gene therapy rarely needs to function in isolation. Combination therapy can increase tumor-cell susceptibility, improve immune priming, reduce resistance, or compensate for incomplete gene delivery. The most rational combinations are those in which each component addresses a distinct biological bottleneck [98,127,168].

Radiotherapy can complement gene replacement and oncolytic virotherapy. Radiation may increase viral replication or transgene expression in selected systems, enhance antigen release, and induce inflammatory signaling. Conversely, restoring p53 or introducing a suicide gene can increase the susceptibility of tumor cells to DNA damage [95–99,169].

Checkpoint blockade is particularly compatible with oncolytic viruses and immunomodulatory gene therapy. Viral infection can increase tumor antigen presentation and T-cell recruitment, potentially converting an immunologically cold tumor into one that responds more effectively to PD-1/PD-L1 blockade [118,127]. Similar principles apply to cytokine-armed viruses and gene-engineered immune cells.

Targeted therapy and gene editing can also be combined. CRISPR screens can identify resistance mechanisms, after which gene editing can test whether disabling those mechanisms restores sensitivity to a targeted drug [71,74,157]. Nanoparticles can co-deliver a genome-editing system and a pharmacologic inhibitor, creating a synchronized intervention against parallel pathways [170].

For CAR-T therapy, combination approaches include checkpoint inhibition, cytokine support, metabolic modulation, radiotherapy, antibody-drug conjugates, bispecific antibodies, and additional genetic engineering. The objective is often to improve persistence and tumor infiltration or to prevent antigen escape rather than simply increasing immediate cytotoxicity [43–47,166,171].

7. Clinical Translation and Current Therapeutic Landscape

Cancer gene therapy has moved beyond proof-of-concept experiments into a heterogeneous clinical landscape that includes approved products, late-stage programs, and early-phase experimental platforms. The FDA currently lists multiple licensed cellular and gene-therapy products, including several CAR-T products, the bladder cancer gene therapy nadofaragene firadenovec, and other cellular therapies [172]. This clinical maturity is uneven: genetically modified immune-cell therapies are substantially more established than direct in vivo genome editing of solid tumors.

CAR-T therapy has produced particularly strong outcomes in selected B-cell malignancies and multiple myeloma. Current products target antigens such as CD19 or BCMA and use different costimulatory architectures that influence expansion and persistence [43–47,173]. Nevertheless, manufacturing requires collection, activation, genetic modification, expansion, quality control, lymphodepletion, and infusion. Toxicities such as cytokine-release syndrome and immune effector cell-associated neurotoxicity require specialized monitoring [174].

TCR-engineered T cells broaden the target repertoire because TCRs can recognize intracellular antigens presented by HLA molecules. This creates opportunities to target oncogenic driver mutations and cancer-testis antigens that are inaccessible to conventional CARs [175,176]. Recent clinical work has demonstrated the feasibility of KRAS-directed TCR therapy in pancreatic cancer, highlighting the potential for mutation-specific cellular gene therapy in solid tumors [177].

Oncolytic viruses have also reached clinical practice, most notably T-VEC in melanoma, while numerous engineered viruses remain in clinical development [119,128]. Contemporary trials increasingly test viruses in combination with checkpoint blockade or other immunotherapies. Early-phase studies of engineered adenoviruses expressing immune agonists illustrate how viral gene therapy is evolving toward multifunctional therapeutic platforms [128].

Direct in vivo CRISPR therapy for cancer remains comparatively immature. The field is constrained by the requirement for precise delivery, sufficient editing efficiency, control of exposure, and comprehensive assessment of off-target and structural genomic changes [48,149,159]. By contrast, ex vivo editing provides the opportunity to characterize the product before administration and to remove or select cells with undesirable properties. This distinction explains why clinical translation has advanced more rapidly for CRISPR-engineered immune cells than for direct tumor editing [54–62].

8. Challenges and Limitations

Tumor heterogeneity is perhaps the most fundamental biological obstacle. A solid tumor can contain genetically distinct subclones with different antigen expression, receptor density, viral permissiveness, DNA-repair capacity, and immune sensitivity [4,156]. A gene therapy directed at one driver may therefore eliminate one population while selecting for another. Multiplex targeting and combination therapy can reduce this problem, but they increase manufacturing and safety complexity.

Delivery to solid tumors remains a major physical barrier. Abnormal vasculature, elevated interstitial pressure, extracellular matrix density, stromal compartments, and heterogeneous perfusion can all prevent uniform distribution [19–22,83]. Systemic delivery introduces additional barriers, including serum stability, renal clearance, liver uptake, complement activation, and sequestration by phagocytic cells [30,105]. Intratumoral administration improves local exposure but is limited by tumor accessibility and does not necessarily treat distant metastases.

Immunogenicity is a double-edged problem. Immune recognition of viral vectors can limit repeated dosing, but in oncolytic virotherapy immune activation is part of the desired mechanism [89,118]. For CRISPR, pre-existing or induced immunity against Cas proteins and other components may affect efficacy and safety [149]. In engineered cell therapy, immune recognition of non-self HLA or transgene products can limit persistence, while excessive immune activation can cause serious toxicity [174].

Genome editing introduces additional safety considerations. Off-target cleavage, large deletions, chromosomal rearrangements, translocations, unintended base conversions, and clonal selection have all been described or remain plausible risks depending on the platform [59,149–151,178]. Importantly, the absence of a predicted off-target site in computational analysis does not establish biological safety; comprehensive genomic assays and long-term follow-up are necessary.

Manufacturing is another major determinant of accessibility. Autologous CAR-T products can require individualized manufacturing for every patient, while viral-vector production demands highly controlled cell culture, purification, potency testing, and replication-competent virus assessment [43–47,179]. Allogeneic and off-the-shelf products may reduce turnaround time and cost but require strategies to minimize graft-versus-host disease, host rejection, and immune-mediated elimination [60,166].

Regulatory and ethical considerations become increasingly important as editing becomes more permanent and multiplexed. Product characterization must include not only identity and potency but also genomic integrity, vector copy number where relevant, residual editing components, and the potential for abnormal clonal expansion [59,159,178]. Long-term surveillance is particularly important for integrating vectors and permanent genome-editing approaches.

9. Future Perspectives

The next generation of cancer gene therapy will probably be defined less by individual platforms than by integrated therapeutic design. A successful product may combine a tumor-specific targeting mechanism, a transient or controllable genetic payload, an immune-modulatory component, and a biomarker-based selection strategy [5,30,89]. This systems-level approach is especially important for solid tumors, where no single mechanism is likely to overcome genetic heterogeneity, physical barriers, and immune suppression simultaneously.

Delivery technologies are expected to remain a major area of innovation. Ligand-directed nanoparticles, biomimetic particles, engineered extracellular vesicles, tumor-penetrating peptides, and stimulus-responsive systems may improve the ratio of tumor to normal-tissue exposure [30,34,105,165]. In parallel, capsid engineering and rational vector evolution may produce viral vectors with altered tropism and reduced susceptibility to pre-existing neutralizing antibodies.

CRISPR 2.0 technologies—including high-fidelity nucleases, base editors, prime editors, epigenetic editors, and RNA-targeting systems—may broaden the range of clinically useful genetic interventions while reducing dependence on double-strand breaks [148–151,180]. The key objective will not simply be higher editing efficiency but an improved therapeutic index: adequate activity in the intended cells with minimal unintended modification elsewhere.

Engineered immune cells are likely to become increasingly programmable. Future CAR-T and TCR-T products may incorporate multiple antigen-recognition modules, logic-gated activation, cytokine payloads, metabolic adaptations, resistance to suppressive signals, and safety switches [35–42,166,171]. Base and prime editing could facilitate more precise engineering of these products and may help move the field toward standardized allogeneic therapies [158,180].

Personalized cancer gene therapy is another major direction. Tumor sequencing, transcriptomics, single-cell analysis, spatial profiling, and functional CRISPR screens can identify the vulnerabilities and antigens that are most relevant to an individual tumor [71,74,157]. This information can then guide selection of a therapeutic gene, receptor, editor, or viral payload. Such an approach transforms gene therapy from a one-size-fits-all technology into a molecularly adaptive intervention.

Finally, combination therapy will likely become increasingly rational rather than empirical. Computational modeling and functional genomics can identify synthetic interactions between genetic interventions and approved drugs, allowing therapies to be selected based on mechanism rather than convenience [73,157]. The most successful future trials are therefore likely to incorporate biomarker-defined cohorts, pharmacodynamic measures of target engagement, longitudinal immune profiling, and molecular analysis of treatment-resistant lesions.

10. Conclusion

Cancer gene therapy has matured from an experimental concept centered on gene replacement into a diverse therapeutic field that can manipulate tumor cells, immune cells, viral vectors, and the tumor microenvironment.

Tumor-suppressor replacement demonstrated that restoration of a lost genetic function could be clinically feasible, while gene silencing and suicide-gene approaches established alternative mechanisms for selective tumor control. Oncolytic viruses subsequently combined gene delivery with direct cytolysis and immune activation, and engineered immune cells transformed genetic modification into a living therapeutic platform [9–18,89,119].

The current frontier is increasingly programmable. RNA technologies permit transient pathway modulation, CRISPR systems allow targeted genome and epigenome manipulation, and base and prime editors provide progressively finer control over genetic information. At the same time, clinical experience has clarified that molecular potency alone is insufficient. Delivery, selectivity, tumor heterogeneity, immune barriers, genomic safety, manufacturing, and cost determine whether a promising laboratory strategy can become a practical cancer treatment [19–22,48,149,179].

Future progress will therefore depend on integrating genetic engineering with precision oncology, advanced delivery, immunology, and functional genomics. Rather than seeking a universal gene-therapy platform, the field is moving toward context-specific therapeutic architectures in which the payload, vector, target cell, route of administration, and combination partner are selected according to the biology of the individual cancer. This convergence may ultimately allow gene therapy to complement existing standards of care and, for selected malignancies, become a central component of curative cancer treatment.

Declarations

Competing Interests

The authors declare that they have no known financial conflicts of interest or personal relationships that could have influenced the work presented in this manuscript.

Ethical approval

This review article does not include any new studies involving human participants or animals conducted by the authors.

Funding

The authors received no specific financial support for the research, authorship, or publication of this review.

Availability of data and materials

Data sharing is not applicable because no new datasets were generated or analyzed for this review.

Table 1. Major cancer gene therapy strategies and their principal characteristics

Strategy	Representative payload/target	Primary mechanism	Major advantage	Key limitation
Gene replacement	TP53, PTEN	Restore tumor-suppressive function	Direct biological correction	Requires efficient tumor transduction; pathway bypass
Gene silencing	siRNA, ASO, miRNA	Reduce oncogenic RNA/protein	Transient and programmable	Delivery, stability, off-target effects
Suicide gene therapy	HSV-TK, cytosine deaminase	Convert prodrug to cytotoxin	Bystander killing	Low transduction efficiency
Oncolytic viruses	HSV-1, adenovirus, vaccinia	Selective replication + oncolysis	Combines cytotoxicity and immune activation	Neutralizing immunity; delivery barriers
Immunomodulatory gene therapy	IL-12, IL-15, GM-CSF	Local immune stimulation	Potentially converts cold tumors	Cytokine toxicity; heterogeneity
Antiangiogenic gene therapy	VEGF pathway inhibitors	Suppress tumor vascularization	Sustained local expression	Pathway redundancy
RNA/mRNA therapy	siRNA, miRNA, mRNA	Transient gene regulation or expression	Flexible, repeatable dosing	Endosomal escape; innate sensing
CRISPR genome editing	Cas9, Cas12, Cas13	Gene knockout, correction or RNA	High programmability	Off-target/structural genomic risk

		targeting		
Base/prime editing	ABE/CBE, prime editors	Precise sequence modification	Reduced dependence on DSBs	Delivery and editing-window constraints
Engineered immune cells	CAR-T, TCR-T, NK cells	Genetic reprogramming of immunity	Living drugs with expansion	Toxicity, manufacturing, antigen escape

Table 2. Principal delivery platforms used in cancer gene therapy

Platform	Typical cargo	Strengths	Limitations	Common oncology applications
Adenovirus	DNA/transgenes	High expression; non-integrating	Inflammation; pre-existing immunity	TP53 therapy; oncolytic adenovirus
HSV-1	Large DNA payloads	Large cargo capacity; oncolysis	Antiviral immunity; neurovirulence engineering	Oncolytic virotherapy
Lentivirus/retrovirus	Stable transgenes	Durable expression	Integration-associated risks; manufacturing	CAR-T/TCR-T; engineered immune cells
AAV	DNA transgenes	Tissue tropism; favorable safety profile	Small cargo; neutralizing antibodies	Localized gene delivery; CNS research
Lipid nanoparticles	RNA/RNP	Transient expression; scalable formulation	Biodistribution; endosomal trapping	siRNA, mRNA, CRISPR delivery
Polymeric nanoparticles	RNA/DNA/protein	Chemical tunability	Complex formulation and toxicity concerns	Experimental tumor targeting
Extracellular vesicles	RNA/protein/RNP	Biomimetic transport	Heterogeneity; manufacturing	Experimental CRISPR/RNA delivery
Cell-based carriers	Viruses/nucleic acids	Active tumor homing possible	Complexity and regulatory burden	Experimental solid-tumor delivery

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