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Gene Therapy in Glioblastoma: From Vector Engineering to Immune Microenvironment Reprogramming

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ABSTRACT

Glioblastoma (GBM) remains the most aggressive primary malignant brain tumor in adults, characterized by extensive intratumoral heterogeneity, diffuse infiltration, profound immunosuppression, and an invariably poor prognosis despite multimodal treatment strategies. Although maximal safe surgical resection followed by radiotherapy and temozolomide constitutes the current standard of care, therapeutic resistance and tumor recurrence remain nearly universal, underscoring the urgent need for innovative treatment modalities. In this context, gene therapy has emerged as a promising therapeutic paradigm capable of addressing several biological limitations associated with conventional therapies through targeted genetic modulation and immune microenvironment reprogramming. This narrative review comprehensively summarizes the advances achieved between 2016 and 2026 in GBM-directed gene therapy. Particular emphasis is placed on the engineering principles, biological characteristics, and translational applications of viral vectors including retroviral, adenoviral, and adeno-associated viral systems as well as emerging non-viral delivery platforms such as lipid nanoparticles, polymeric nanocarriers, electroporation, engineered exosomes, messenger RNA technologies, and CRISPR/Cas-based genome editing. Furthermore, the review discusses recent developments in oncolytic virotherapy, suicide gene systems, RNA interference strategies, immune-stimulatory gene delivery, and adoptive cellular gene therapies. Beyond vector technology, this review highlights the critical role of immune microenvironment remodeling in improving therapeutic efficacy. Current evidence suggests that successful gene therapy should not solely focus on tumor cell eradication but should also promote durable antitumor immunity through enhanced antigen presentation, increased lymphocyte infiltration, reversal of myeloid-mediated immunosuppression, and rational integration with immune checkpoint blockade, dendritic cell vaccines, CAR-T cell therapy, and other multimodal immunotherapeutic approaches. Finally, ongoing clinical trials, translational challenges, manufacturing considerations, biomarker-guided patient selection, and future perspectives are critically discussed to provide a comprehensive overview of the evolving landscape of gene therapy for glioblastoma.

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INTRODUCTION

One kind of brain tumor is glioblastoma. Treatment of brain cancers, including glioblastoma, is complicated by blood brain tumors. Research on the application of cutting-edge technologies like deep learning, convolutional neural networks, and extreme learning machines for brain tumor classification, segmentation, and diagnosis has been stimulated by a number of neurological and brain tumor investigations. Meningiomas, gliomas, and pituitary tumors are just a few of the brain malignancies that these technologies have demonstrated promising outcomes in precisely diagnosing and detecting.[1-3] A primary brain tumor with a dismal prognosis, glioblastoma multiforme (GBM) is made up of aggressive malignant cells.[4] GBM is separated into two subgroups according to the World Health Organization's classification system for tumors of the central nervous system.[5] These fall into two categories: isocitrate dehydrogenase (IDH)-wild type and IDH-mutant type. Instead of histological characteristics, glioblastoma multiforme exhibits molecular and cellular heterogeneity. It is also recognized for its intra-tumor and inter-patient diversity, which adds to the difficulty of managing and treating it. When it comes to clinical therapies, therapeutic approach, and prognosis classification, the definition of GBM is crucial. Nonetheless, there is ongoing discussion in the medical community over the ideal target volume in radiation therapy for GBM.[6]

Though rarely curative, current therapy is still multimodal. Tumor-treating fields may be added during maintenance therapy in certain patients, but infiltrative residual cells, glioma stem-like populations, DNA repair capacity, vascular abnormality, and immune suppression make recurrence the rule rather than the exception. Maximal safe resection is followed by focal radiotherapy and temozolomide. Treatment options for recurrence include re-operation, re-irradiation, lomustine, temozolomide rechallenge in some MGMT-methylated diseases, bevacizumab for edema management, clinical studies, and optimum supportive care. However, there is currently no widely recognized salvage standard.[7-11]

Over the past ten years, GBM has been reframed as a delivery, heterogeneity, and immune-ecology issue rather than just a cytotoxic chemotherapy issue. As demonstrated by the phase 3 CheckMate 143 comparison of nivolumab and bevacizumab, immune checkpoint inhibition has altered a number of extracranial malignancies but has not improved survival as monotherapy in unselected recurrent GBM. Although antigen loss, corticosteroid exposure, lymphopenia, low mutational burden in many cancers, and a myeloid-enriched microenvironment limit response durability, adoptive cellular treatment, dendritic-cell vaccines, and antigen vaccines have generated biologically significant signals.[12]

In actuality, gene therapy is a different kind of treatment. To put it another way, gene therapy entails the transfer of healthy genes to replace damaged genes or groups of genes in order to correct genetic defects in diseases for which there is no specific treatment; this procedure can be done directly or indirectly. Gene therapy has been illuminated by various prospective approaches, such as inhibiting or silencing faulty genes (RNAi-miRNA processing), as is widely known. Gene therapy and gene therapy techniques are the exclusive focus of translational medicine. For the therapeutic gene to be transferred, a delivery method is necessary. DNA is a large, electrically charged molecule; that is why human cells do not readily accept or take up DNA in gene therapy methods. Scientists have used genetic engineering techniques to employ viruses as vectors to deliver therapeutic DNA or RNA molecules into human cells. By using viral vectors, they have been able to integrate the relevant therapeutic gene into the cell without infecting or damaging it. In this therapeutic gap, gene therapy plays a crucial role. Payloads may be delivered locally, sustained intratumoral expression can be produced, innate antiviral sensing can be activated, prodrugs can be converted into cytotoxins, resistance pathways can be edited, oncogenic transcripts can be silenced, and immune cells can be armed with synthetic receptors.[13-16] The literature from 2016 to 2026 is the main topic of this narrative review, which assesses GBM gene therapy from the perspectives of vector design, transport method, genetic payload, clinical data, and immune microenvironment reprogramming.

In glioblastoma, the rationale for gene therapy extends well beyond the conventional concept of replacing or supplementing defective genes. Within the framework of modern neuro-oncology, gene therapy has evolved into a multifaceted therapeutic strategy aimed at interfering with tumor-driving molecular pathways, overcoming resistance mechanisms associated with DNA repair, selectively targeting glioma stem-like cell populations, promoting immunogenic tumor cell death, and remodeling the highly immunosuppressive tumor microenvironment. Consequently, the therapeutic efficacy of gene-based interventions in GBM depends not only on the identification of appropriate therapeutic genes but also on the optimization of vector tropism, efficient intratumoral distribution, successful traversal of the blood–brain barrier, precise regulation of transgene expression, controlled activation of local immune responses, and their rational integration with conventional treatment modalities and emerging immunotherapeutic approaches.

Viral Vectors for Gene Therapy

Retroviral Vectors

Retroviral vectors were among the first viral delivery systems to be extensively employed in gene therapy applications. Owing to their ability to achieve stable integration into the host genome, retroviruses have long been considered attractive vehicles for the long-term expression of therapeutic genes. Retroviruses possess a single-stranded RNA genome. During vector construction, viral genes responsible for replication and pathogenicity are removed and replaced with the therapeutic gene of interest. Following administration, the engineered viral vector infects target cells but is incapable of autonomous replication due to the deletion of essential replication-associated genes. After cellular entry, the viral RNA genome is reverse-transcribed into complementary DNA

(cDNA) by the viral reverse transcriptase enzyme. The resulting DNA is subsequently transported into the nucleus, where it integrates into the host chromosomal DNA, thereby enabling long-term expression of the therapeutic transgene. The principal advantage of retroviral vectors lies in their ability to provide stable and sustained transgene expression through genomic integration. However, this integration also represents a major safety concern, as random insertion into the host genome may disrupt endogenous genes or activate proto-oncogenes, leading to insertional mutagenesis and potentially severe toxic effects.[17-20]

Lentiviral Vectors

Lentiviral vectors represent a highly valuable gene delivery platform in glioblastoma (GBM) research, largely because they are capable of producing sustained transgene expression in diverse cellular populations, including cells with limited or absent proliferative activity. This property makes lentiviral systems suitable not only for direct genetic manipulation of glioma cells but also for the ex vivo modification of immune effector cells used in advanced cellular therapies. Within this framework, lentiviral vectors are widely employed in the generation of engineered immune products such as CAR-T cells, CAR-NK cells, and TCR-modified T cells targeting GBM-associated antigens, including EGFRvIII, IL13Rα2, HER2, and EphA2. By enabling persistent expression of synthetic receptors, immune-regulatory molecules, or cytokine-modulating constructs, lentiviral platforms function as a practical bridge between gene transfer technologies and adoptive cellular immunotherapy. Nevertheless, their integrating nature requires rigorous biosafety assessment. Insertional mutagenesis, vector-driven clonal expansion, altered cellular fitness, and possible long-term genomic instability remain critical considerations, especially during the manufacture of clinical-grade immune cell products intended for patients with intracranial malignancies.[60-61-62]

Adenoviral Vectors

Adenoviral vectors represent another widely utilized viral platform for gene delivery and have been employed in numerous pioneering gene therapy studies. One of their major advantages is their relatively large packaging capacity, allowing the delivery of large or complex therapeutic genes that exceed the carrying capacity of several other viral vector systems. Unlike retroviral vectors, adenoviral vectors generally remain episomal and do not integrate into the host genome, thereby minimizing the risk of insertional mutagenesis. Nevertheless, their clinical application is limited by their strong immunogenicity. Following vector administration, the host immune system rapidly recognizes adenoviral capsid proteins and generates both innate and adaptive immune responses. Consequently, neutralizing antibodies and cytotoxic immune cells may eliminate the vector before sufficient therapeutic gene expression is achieved. In addition, excessive immune activation may induce inflammatory responses and, in some cases, severe systemic adverse events.[21-24]

Adeno-Associated Viral (AAV) Vectors

Adeno-associated viruses (AAVs) are currently among the most extensively utilized viral vectors in clinical gene therapy owing to their favorable safety profile and relatively low immunogenicity. Wild-type AAV is considered non-pathogenic in humans and generally elicits only a limited immune response compared with other viral vectors. Various engineered AAV serotypes have been developed to improve tissue specificity and transduction efficiency, enabling targeted delivery of therapeutic genes to specific cell types and organs. Following infection, recombinant AAV vectors predominantly persist within the nucleus as episomal circular DNA molecules rather than integrating into the host genome. These episomal DNA structures support long-term transgene expression in non-dividing or slowly dividing cells while substantially reducing the risk of insertional mutagenesis. Despite these advantages, AAV vectors possess several limitations. Their packaging capacity is relatively small (approximately 4.7 kb), restricting the size of therapeutic genes that can be delivered. Furthermore, because recombinant AAV genomes generally remain episomal, transgene expression may gradually decline in rapidly proliferating tissues as episomal DNA molecules become diluted during successive cell divisions.[25-29]

AAV Capsid Engineering

Adeno-associated virus vectors provide a different therapeutic framework for GBM gene delivery because they are generally associated with a favorable safety profile and can be engineered to improve tissue tropism. Recent AAV development has increasingly focused on capsid modification strategies that enhance central nervous system targeting, promote glioma cell entry, and reduce undesired vector uptake in peripheral or non-tumoral tissues. These modifications may be achieved through rational capsid engineering, directed evolution, peptide insertion, receptor-guided retargeting, or hybrid design approaches. In the context of GBM, potential targeting axes include glioma-enriched surface receptors, integrin-mediated entry pathways, transferrin receptor-associated transport systems, EGFR-related uptake mechanisms, and other membrane signatures that are preferentially represented within the tumor microenvironment. However, capsid engineering should not be considered sufficient on its own. Vector biodistribution, tumor penetration, and off-target exposure are strongly shaped by the route of administration. Intratumoral injection or delivery into the postoperative resection cavity can provide high local exposure and partly circumvent the blood–brain barrier, whereas intraventricular or systemic delivery may extend CNS distribution but also increases the likelihood of peripheral sequestration, neutralization by pre-existing antibodies, hepatic accumulation, and reduced tumor specificity. Therefore, AAV-based GBM gene therapy should be approached as an integrated design problem in which capsid structure, promoter activity, therapeutic cargo, dose, and administration route are optimized together. [60-62-63]

Herpes simplex virus (HSV)-based vectors

Herpes simplex virus-derived vectors are particularly relevant for GBM gene therapy because HSV has natural neurotropic properties, allows insertion of relatively large genetic payloads, and can be redesigned for oncolytic purposes. Through deletion or functional modification of viral genes involved in neurovirulence, HSV-based systems can be attenuated while retaining a capacity for preferential activity in malignant cells. Tumor selectivity may be further strengthened by incorporating tumor-responsive promoters, restricting viral replication to cancer-associated cellular states, or inserting immunostimulatory transgenes. In GBM, oncolytic HSV platforms can produce therapeutic effects through complementary mechanisms. One mechanism is direct tumor injury mediated by viral replication and cancer cell lysis. A second mechanism involves immune activation following tumor cell destruction, during which tumor-associated antigens and danger-associated molecular signals are released into the local microenvironment. These events may stimulate innate immune recognition, promote dendritic cell maturation, and facilitate adaptive antitumor responses. Thus, HSV-based oncolytic therapy has the potential not only to kill GBM cells directly but also to convert an immunologically suppressed tumor niche into a more inflamed and immune-responsive environment. [58-60-64]

Non-Viral Gene Delivery Methods

To overcome the limitations associated with viral vectors, considerable effort has been devoted to the development of non-viral gene delivery systems for both **in vivo** and **ex vivo** applications. These approaches are generally associated with improved safety profiles, reduced immunogenicity, lower manufacturing costs, and greater flexibility in cargo design. Non-viral delivery strategies include chemical transfection methods, polymer- and lipid-based nanoparticles, electroporation, and liposome-mediated gene transfer. Liposomes are artificial phospholipid vesicles capable of encapsulating nucleic acids and facilitating their fusion with cellular membranes, thereby enabling intracellular delivery of therapeutic DNA or RNA molecules. Similarly, nanoparticle-based systems protect nucleic acids from enzymatic degradation while enhancing cellular uptake and improving biodistribution. An emerging concept currently under investigation is the development of oral gene delivery systems, commonly referred to as **gene pills**. In this experimental strategy, therapeutic DNA is encapsulated within protective delivery vehicles capable of surviving the gastrointestinal environment. Following absorption by intestinal epithelial cells, the therapeutic nucleic acid is released, allowing transgene expression and subsequent systemic distribution of the encoded therapeutic product. Although this approach remains in the preclinical and early translational stages, it represents a promising non-invasive alternative for future gene therapy applications.[30-34]

Lipid Nanoparticles and RNA-Based Therapeutic Delivery

Lipid nanoparticles are among the most flexible non-viral systems for nucleic acid delivery. Their relevance to GBM arises from their ability to carry RNA-based and genome-editing cargos, including siRNA, mRNA, microRNA mimics, antisense oligonucleotides, and CRISPR/Cas components, without requiring stable integration into the host genome. This transient activity is advantageous when the therapeutic goal is reversible gene silencing, temporary protein production, or time-limited genome-editing exposure. Properly formulated LNPs can protect nucleic acid cargos from degradation, improve cellular uptake, enhance endosomal escape, and support repeated administration when this is clinically justified. In GBM models, LNP-based approaches have been investigated for modulating molecular pathways linked to therapeutic resistance, immune escape, glioma stem-like cell persistence, and DNA repair capacity. Targets of interest include MGMT, STAT3, PD-L1, TGF-β signaling, and other oncogenic or immunosuppressive regulators. Despite this promise, several delivery barriers remain unresolved. Efficient transport across the blood–brain barrier, selective accumulation within infiltrative tumor areas, adequate endosomal release, and uniform distribution across heterogeneous tumor tissue continue to restrict the translational impact of LNP-mediated GBM gene therapy. [65]

Lipid–Polymer Hybrid Nanoparticles and Focused Ultrasound

Lipid–polymer hybrid nanoparticles offer another promising delivery strategy by combining the structural stability and controlled-release properties of polymeric systems with the biocompatibility and membrane-interactive behavior of lipid-based carriers. This hybrid design is especially relevant for GBM because successful delivery must overcome multiple biological barriers, including the blood–brain barrier, the blood–brain tumor barrier, irregular vascular permeability, elevated interstitial pressure, extracellular matrix complexity, and diffuse tumor infiltration. Surface functionalization with targeting ligands can further improve tumor recognition and cellular internalization. Examples include cRGD peptides that exploit integrin-associated uptake and transferrin-inspired motifs that may engage receptor-mediated transport routes. In addition to biochemical targeting, physical delivery enhancement has gained increasing attention. Focused ultrasound combined with circulating microbubbles can temporarily increase cerebrovascular permeability in a spatially controlled manner. When this approach is paired with targeted nanoparticles, it may improve regional delivery of genetic cargos such as plasmid DNA, siRNA, mRNA, or CRISPR/Cas9 components. Accordingly, GBM vector design should not be limited to carrier composition alone; it should also integrate delivery-enhancing technologies that improve tissue penetration, spatial control, and tumor selectivity. [66]

Blood–Brain Barrier and Delivery Route Engineering

The blood–brain barrier and blood–brain tumor barrier remain among the most important obstacles to effective GBM gene therapy. Although contrast-enhancing tumor regions often reflect vascular disruption and partial barrier breakdown, GBM cells frequently extend beyond these areas into infiltrative margins where barrier function may remain relatively preserved. As a result, systemically

administered viral or non-viral vectors may inadequately reach the tumor borders that often contribute to recurrence. Several delivery strategies have been explored to address these anatomical and physiological limitations, including direct intratumoral administration, delivery into the surgical resection cavity, convection-enhanced delivery, intraventricular infusion, focused ultrasound-mediated barrier modulation, and implantable local-release platforms. Each strategy has specific advantages and limitations. Local administration can generate high regional vector exposure but may fail to cover widely disseminated infiltrative cells. Systemic delivery is less invasive and may be more compatible with repeated dosing, but it is constrained by immune neutralization, peripheral clearance, non-specific organ uptake, and limited CNS entry. Focused ultrasound-based approaches are attractive because they offer temporary and regionally controlled barrier modulation; however, their reproducibility, long-term safety, and compatibility with different vector systems still require further clinical validation. Therefore, successful GBM gene therapy will depend on aligning the vector platform with the most appropriate delivery route and with the spatial biology of the tumor. [58-60-63-64-66]

Clinical and Research Consequences

Integrated GBM types and therapeutic implications

The phrase "GBM type" needs to be used with caution. Glioblastoma, IDH-wildtype, CNS WHO grade 4, is the most defendable primary disease entity in modern practice. Newly diagnosed versus recurrent GBM, molecularly defined versus histologically overt GBM, MGMT promoter methylated versus unmethylated tumors, EGFR-amplified or EGFRvIII-positive tumors, mesenchymal-enriched tumors, multifocal disease, elderly/frail-patient GBM, and tumors with gliosarcomatous or giant-cell histological patterns are among the clinically significant subdivisions within this entity. These categories are important because they affect immunotherapeutic vulnerability, gene delivery feasibility, and trial stratification. Despite not being official WHO diagnostic entities, expression-based subtypes are nevertheless valuable as biological frameworks. Mesenchymal programs are linked to inflammatory and myeloid-rich microenvironments, proneural/PDGFRA-associated states may represent distinct developmental lineage dependencies, and classical/EGFR-driven tumors may be logical targets for EGFRvIII- or EGFR-directed cellular and viral strategies. A single antigen or single pathway is unlikely to regulate the entire disease, as single-cell and spatial investigations have further demonstrated that GBM cells can change states within the same tumor. This heterogeneity has two implications for gene therapy. Initially, vectors and payloads should be chosen based on molecular eligibility. For instance, HSV-based oncolytic viruses for tumors that can be injected intralesionally, EGFRvIII CAR-T cells for EGFRvIII-positive illness, and immune-stimulatory payloads for tumors with low lymphocyte counts. Second, in order to differentiate biological effect from pseudo-progression, clinical studies should include baseline and on-treatment tissue, cerebrospinal fluid (CSF), circulating tumor DNA, immunological phenotyping, and sophisticated imaging.[14-17]

Therapeutic landscape beyond gene therapy

Instead than treating gene therapy in isolation, a thorough study of the area must compare it to other treatment approaches. One of the few non-pharmacological additions with phase 3 evidence in newly diagnosed GBM is tumor-treating fields, which increased survival when combined with maintenance temozolomide in the EF-14 randomized trial. Bevacizumab-based anti-VEGF therapy can lessen edema and steroid reliance, but it hasn't improved recurrence survival. Because GBM signaling is redundant and spatial medication distribution is irregular, EGFR-targeted small molecules, integrin inhibitors, and many pathway inhibitors have generally worked poorly. The most direct route to gene therapy is through immunotherapies. Despite solid biological justification, the EGFRvIII peptide vaccine rindopepimut did not increase survival in the phase 3 ACT IV trial. Personalized neoantigen vaccines, on the other hand, have demonstrated that GBM can produce vaccine-induced T-cell responses; nevertheless, the results of these investigations were limited and not conclusive regarding survival. Randomized confirmation and biomarker refinement are still crucial because of the atypical design and crossover history of the externally controlled DCVax-L study, which revealed survival extension in newly diagnosed and recurrent GBM. Cellular gene therapy is known as CAR-T cell therapy. Antigen loss and adaptive immune resistance accompanied the viability and safety of EGFRvIII-directed CAR-T cells. In subgroups of recurrent high-grade glioma patients, IL13Rα2-directed CAR-T studies have demonstrated locoregional delivery capability, inflammatory CSF cytokine production, and objective responses. In very small early cohorts, bivalent EGFR/IL13Rα2 constructions and CARv3-TEAM-E cells secreting T-cell engagers have produced quick radiographic and molecular signals, indicating both promise and the necessity of immune-suppressive checkpoint management, multi-antigen coverage, and lasting persistence.[1-14]

Oncolytic virotherapy as gene-based immunotherapy

The most developed GBM gene-therapy area is oncolytic virotherapy. These vectors are designed to specifically infect, multiply, or lyse tumor cells without harming healthy brain tissue. Through the release of tumor antigens, pathogen-associated molecular patterns, type I interferon signaling, and antigen-presenting cell recruitment, they have a dual therapeutic effect: direct cytolysis and in situ vaccination. With a small percentage of long-lasting radiographic responses, the phase 1 DNX-2401 research showed safety, intratumoral replication, and immunological effects in recurrent malignant glioma. In a phase 1 research, PVSRIPO, a recombinant nonpathogenic poliovirus-rhinovirus chimera, demonstrated a survival tail in recurrent GBM; nonetheless, controlled validation of its effectiveness is necessary. G47Δ/teserpaturev produced encouraging survival in a Japanese phase 2 study of residual or recurrent GBM and became the first oncolytic virus approved for malignant glioma in Japan under a conditional and time-limited pathway. In a first-in-human phase 1 trial, CAN-3110, an HSV-1-based oncolytic virus with nestin promoter-controlled ICP34.5 expression,

was tested in recurrent high-grade glioma/GBM; positive HSV1 serology, tumor viral clearance, and immune activation signatures correlated with survival, supporting the idea that oncolytic viruses can transform GBM into a more inflammatory tumor. Oncolysis alone is giving way to oncolysis with immune engineering in this discipline. Even though the primary effectiveness endpoint was not reached, DNX-2401 plus pembrolizumab was safe and generated responses in a minority of patients with recurrent GBM. This trend reflects the larger issue with GBM immunotherapy: viral stimulation of antigen release and interferon-chemokine circuits may generate a more logical window for PD-1/PD-L1 blockade, CTLA-4 blockade, DC vaccination, or CAR-T infusion, but single-agent checkpoint blockade is typically insufficient.[35-38]

Therapeutic Genetic Cargo

The therapeutic outcome of GBM gene therapy is determined not only by the delivery platform but also by the biological function of the delivered cargo. Candidate payloads include tumor suppressor genes, suicide gene/prodrug-converting enzymes, anti-angiogenic factors, RNA interference molecules, genome-editing systems, cytokines, chemokines, immune checkpoint modulators, and synthetic receptor constructs used in engineered immune cells. Tumor suppressor replacement is intended to restore impaired growth-control or pro-apoptotic signaling, whereas suicide gene strategies create selective cytotoxicity by converting an inactive prodrug into a toxic metabolite within the tumor region. RNAi- and CRISPR/Cas-based approaches provide opportunities to inhibit oncogenic pathways, interfere with DNA repair mechanisms, reduce immune evasion, target glioma stem-like programs, and reverse selected forms of therapy resistance. Immune-oriented gene delivery, including cytokine and chemokine expression, may enhance antigen presentation, recruit cytotoxic lymphocytes, activate NK cells, and strengthen T cell-mediated tumor elimination. For this reason, payload selection should be guided by the molecular and immunological profile of each tumor, including molecular subtype, MGMT promoter methylation status, antigen expression pattern, immune microenvironment phenotype, and the intended combination treatment strategy. [58]

Suicide, prodrug-converting and cytokine-regulated gene systems

Suicide gene therapy introduces an enzyme that converts an otherwise less toxic prodrug into a cytotoxic metabolite inside or near tumor cells. HSV1-thymidine kinase (HSV1-TK) followed by ganciclovir or valacyclovir and cytosine deaminase followed by 5-fluorocytosine are the best-known examples. The bystander effect is important because not every GBM cell is transduced; toxic metabolites and immune-mediated injury can damage neighboring tumor cells. Clinical experience has been mixed. The retroviral replicating vector vocimagene amiretrorepvec (Toca 511) with flucytosine did not improve survival over standard of care in the randomized TOCA 5 trial, despite earlier durable responses in phase 1 cohorts. This failure is scientifically valuable because it shows that promising single-arm results in recurrent GBM can be overturned by randomized testing, and that vector distribution, patient selection, immune contexture and trial endpoints must be optimized before phase 3 escalation. More recent work has revived the suicide-gene concept by combining cytotoxic and immune-stimulatory payloads. A first-in-human phase 1 trial delivered two adenoviral vectors expressing HSV1-TK and Flt3L into the resection cavity of newly diagnosed high-grade glioma patients, followed by valacyclovir and standard chemoradiotherapy; no dose-limiting toxicity was observed and median overall survival was encouraging, although the sample was small and nonrandomized. Mechanistically, TK generates local tumor cell death, while Flt3L recruits and expands dendritic cells, thereby linking cytotoxic gene therapy to antigen presentation and adaptive immunity.[39-41]

Non-viral RNA delivery and genome editing

Because they may be produced without infectious viral particles, repeat-dosed, and modularly tailored to siRNA, microRNA, mRNA, antisense oligonucleotides, plasmids, or CRISPR components, non-viral methods are appealing. GBM delivery has been investigated using lipid nanoparticles, polymeric nanoparticles, inorganic carriers, modified exosomes, and cell-membrane-coated particles, frequently with ligands intended to take advantage of transferrin, integrin, low-density lipoprotein receptor-related, or tumor-homing pathways. Achieving enough brain and tumor distribution without systemic toxicity is their main difficulty, not payload design. Oncogenic or resistant pathways such STAT3, MGMT, PD-L1, TGF-β signaling, miR-21, SOX2-linked stemness programs, and DNA repair networks can be silenced by RNA therapies. Studies using biomimetic and exosome-based nanoparticles that deliver STAT3 siRNA have demonstrated preclinical potential to alter immunosuppressive signaling and overcome temozolomide resistance. A controlled substitute for integrating vectors, mRNA delivery could temporarily encode cytokines, chemokines, tumor antigens, or genome editors. The idea is extended from gene insertion to genome editing by CRISPR/Cas systems.Genes related to proliferation, invasion, stemness, DNA repair, immune evasion, and therapeutic resistance have been the focus of preclinical GBM research. However, because intracranial editing necessitates strict regulation of off-target effects, p53-related selection, immunological responses to Cas proteins, mosaic editing, and long-term surveillance, direct clinical translation is still in its early stages. Clinical editing will probably start with local or ex vivo approaches rather than widespread systemic brain editing, and CRISPR may be most helpful in the immediate future as a discovery platform to find synthetic fatal dependencies and immunotherapy-resistant nodes.[42-47]

Combination Strategies with Standard and Immunotherapy-Based Treatments

Because GBM is highly heterogeneous and therapeutically resistant, gene therapy is unlikely to achieve durable disease control as a stand-alone intervention. Its greatest potential may emerge when it is rationally combined with established and emerging modalities such as maximal safe surgical resection, radiotherapy, temozolomide, tumor treating fields, immune checkpoint blockade,

dendritic cell vaccination, and adoptive cellular immunotherapy. For instance, gene-silencing or genome-editing strategies targeting MGMT may enhance temozolomide responsiveness in selected molecular contexts. Suicide gene systems may provide localized cytotoxic amplification after surgical debulking or in combination with radiotherapy. Oncolytic viral vectors may increase immunogenic tumor cell death, antigen release, and inflammatory signaling, thereby improving the biological setting for checkpoint inhibition or dendritic cell-based vaccination. Similarly, cytokine- or chemokine-encoding constructs may complement CAR-based immune cell therapies by improving immune cell trafficking, persistence, and effector activity within the GBM microenvironment. The effectiveness of such combinations will depend not only on mechanistic compatibility but also on treatment timing, therapeutic sequence, tumor burden, antigen expression, corticosteroid exposure, immune activation status, and therapy-induced inflammation.

Reprogramming the immune microenvironment

This review's main translational hypothesis is that GBM gene therapy should be evaluated based on its capacity to alter the immune milieu rather than just radiographic reduction. Because tumor-associated microglia/macrophages and myeloid-derived suppressor cells dominate the immune compartment, effector T cells are scarce or exhausted, antigen-presenting cells are limited, and suppressive mediators like TGF-β, IL-10, adenosine, PD-L1, and hypoxia-linked metabolic pathways blunt cytotoxic immunity, GBM is often referred to as "immune cold. This biology can be addressed in a number of ways by gene therapy. Viral pattern recognition and immunogenic cell death are caused by oncolytic viruses. Flt3L attracts precursors of dendritic cells. Although IL-12 platforms can increase NK-cell activity, cytotoxic T-cell activation, and interferon-γ, their expression must be controlled to prevent neuroinflammation. In preclinical GBM models, AAV-CXCL9 enhanced cytotoxic lymphocyte infiltration and made tumors more susceptible to anti-PD-1 therapy, demonstrating how chemokine gene transfer can address a trafficking deficiency instead of destroying tumor cells. Theoretically, PD-L1, CD47, TGF-β signaling, STAT3, or other inhibitory nodes can be reduced by CRISPR or RNA interference, preparing the tumor for CAR-T cells or vaccinations. The necessity of combination therapy is also explained by this immunological logic. Although it may result in lymphopenia, radiotherapy can boost MHC expression and antigen release.Temozolomide may produce lymphodepleted niches for adoptive T cells while also suppressing the immune system. Pre-existing or induced T-cell infiltration is necessary for checkpoint blocking. Antigen expression, transport, and persistence are necessary for CAR-T cells. Thus, cytoreduction and tissue acquisition surgery, local gene therapy into the resection cavity, short-interval immune monitoring, and subsequent vaccination, checkpoint inhibitor, or CAR-T consolidation in patients exhibiting inflammatory conversion may all be part of rational sequencing.[48-54]

Future Perspectives: Toward Multilayered Gene-Based Platforms for Neuro-Oncology

Future GBM gene therapy is likely to move beyond single-vector and single-cargo concepts toward more coordinated therapeutic systems. Next-generation strategies may combine tumor-selective promoters, inducible expression circuits, engineered AAV capsids, RNA-loaded lipid nanoparticles, exosome-inspired carriers, dual-targeted hybrid nanoparticles, focused ultrasound-assisted delivery, and genetically modified immune cells. The therapeutic aim should not be limited to shrinking the contrast-enhancing tumor mass. More durable approaches will need to address glioma stem-like populations, infiltrative tumor borders, vascular and perivascular niches, and immunosuppressive myeloid compartments that support recurrence and treatment resistance. In this sense, gene therapy may be most effective when it is used not simply as a method for introducing a therapeutic gene, but as a platform for coordinating tumor cell killing, immune activation, microenvironmental remodeling, and improved drug delivery within a personalized treatment strategy. Biomarker-based patient selection, scalable manufacturing, vector safety monitoring, and clinically compliant quality-control systems will be essential for translating these complex platforms into meaningful therapeutic benefit.

Clinical development roadmap

Before moving on to definitive randomized investigations, future GBM gene-therapy trials should be more physiologically instrumented, smaller, and smarter. WHO-integrated diagnosis, MGMT promoter status, steroid exposure, tumor volume, number of recurrences, resectability, target antigen expression, and baseline immunological context should all be taken into consideration when determining eligibility. Safety, vector biodistribution, payload expression, immune pharmacodynamics, corticosteroid-sparing effect, neurological function, quality of life, progression-free survival with immune-response criteria, and overall survival should all be included in the endpoint hierarchy. Additionally, pseudo-progression and inflammatory edema must be taken into consideration in trial design. Enhancement on MRI may be temporarily worsened by a gene treatment that effectively stimulates immunity. Therefore, active therapy may be disregarded if early radiographic progression is relied upon. Therapeutic biology may be better captured by an integrated evaluation that includes serial MRI, amino-acid PET when possible, CSF cytokines, tumor DNA, T-cell receptor sequencing, paired biopsies, and steroid-adjusted clinical state. Regulations and manufacturing are equally crucial. Potency tests, replication-competent virus testing, sterility, stability, shedding studies, and long-term monitoring are all necessary for viral platforms. Release requirements for viability, transduction efficiency, sterility, phenotype, potency, and residual activation beads or vector are necessary for cell-based gene treatments. Particle-size distribution, encapsulation effectiveness, endotoxin testing, and repeatable scale-up are necessary for non-viral nanoparticles. The most reasonable conclusion for a journal audience would be that, although GBM gene therapy is no longer hypothetical, it is still not a typical curative technique outside of clinical trials and limited authorization. [55-59]

CONCLUSION

Gene therapy has evolved from an experimental concept into one of the most promising translational strategies for the treatment of glioblastoma. Advances in vector engineering, targeted genetic manipulation, and precision delivery technologies have considerably expanded the therapeutic landscape beyond conventional cytotoxic approaches. Viral vectors, including retroviral, adenoviral, and adeno-associated viral platforms, continue to provide highly efficient gene delivery systems, whereas non-viral technologies such as lipid nanoparticles, engineered exosomes, messenger RNA platforms, and CRISPR/Cas-mediated genome editing are rapidly emerging as safer and increasingly versatile alternatives. These technological developments have enabled the design of more selective therapeutic interventions capable of simultaneously targeting tumor proliferation, therapeutic resistance, and immune escape mechanisms. An important conceptual shift highlighted throughout this review is that the future success of GBM gene therapy will depend not only on efficient gene delivery but also on its capacity to remodel the highly immunosuppressive tumor microenvironment. Oncolytic viruses, immune-stimulatory cytokine gene delivery, RNA interference, genome editing technologies, and genetically engineered immune cells collectively illustrate how gene therapy can transform immunologically "cold" glioblastomas into tumors that are more susceptible to adaptive immune responses. Consequently, rational combination strategies integrating gene therapy with immune checkpoint inhibitors, dendritic cell vaccines, adoptive cellular therapies, radiotherapy, and conventional chemotherapy are likely to represent the next generation of multimodal treatment paradigms. Despite encouraging biological and early clinical findings, several critical challenges remain unresolved, including limited vector distribution within infiltrative tumor tissue, blood–brain barrier penetration, antigenic heterogeneity, adaptive resistance, treatment-related neurotoxicity, manufacturing complexity, and the identification of predictive biomarkers. Addressing these obstacles will require standardized vector production, biomarker-driven patient stratification, advanced molecular imaging, longitudinal immune monitoring, and well-designed randomized clinical trials. Overall, the available evidence indicates that gene therapy is no longer a theoretical therapeutic concept but an increasingly realistic component of precision neuro-oncology. Continued advances in molecular engineering, synthetic biology, genome editing, and systems immunology are expected to accelerate the clinical translation of gene-based therapeutics. Ultimately, personalized gene therapy integrated with comprehensive immune microenvironment reprogramming may redefine the therapeutic management of glioblastoma and contribute to meaningful improvements in long-term patient survival and quality of life.

DECLARATIONS

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Tables and Figures

Table 1. Contemporary GBM categories relevant to gene-therapy design.

Category	Definition/Features	Therapeutic relevance
WHO-integrated GBM	IDH-wildtype, H3-wildtype diffuse astrocytic glioma, CNS WHO grade 4; may be histologically or molecularly defined [1-3].	Defines target indication and avoids mixing with IDH-mutant astrocytoma grade 4.
Newly diagnosed GBM	Disease at first presentation, usually treated with surgery, radiotherapy, temozolomide and selected TTFields [2,4].	Resection cavity enables local vector injection and tissue-rich biomarker design.
Recurrent GBM	Post-standard-therapy progression; no universal salvage standard [2,5].	Most oncolytic and cellular gene-therapy trials are conducted here.
MGMT methylated/unmethylated	Predicts temozolomide sensitivity and prognosis.	Affects combination strategy with TMZ and trial stratification.
EGFR/EGFRvIII-positive GBM	EGFR amplification or EGFRvIII variant in a subset of tumors [6,10,14].	Supports EGFRvIII CAR-T, EGFR-targeted engagers and antigen-specific vaccines.
Mesenchymal-enriched GBM	Inflammatory, invasive and myeloid-rich phenotype [15-17].	May favor immune-reprogramming payloads and TAM-targeting combinations.

Table 2. Non-gene-therapy strategies that frame GBM gene-therapy combinations.

Strategy	Role in GBM care/research	Connection to gene therapy
Surgery	Maximal safe cytoreduction and diagnostic tissue acquisition.	Creates resection cavity for vector delivery and baseline biomarker profiling.
Radiotherapy plus temozolomide	Backbone treatment for fit patients [2].	May release antigen but can produce lymphopenia; sequencing with immune gene therapy matters.
Tumor-treating fields	Improved survival with maintenance temozolomide in EF-14 [4].	Potential non-overlapping modality with local gene therapy.
Bevacizumab/anti-VEGF	Edema control and radiographic response at recurrence; limited survival impact [2,5].	Can reduce steroid need but may alter immune trafficking and imaging interpretation.
Vaccines/DC therapy	Peptide, neoantigen and DC vaccines have shown immunogenicity; survival evidence varies [6-9].	Gene therapy may improve antigen release and dendritic-cell recruitment.
Checkpoint inhibitors	Monotherapy has failed in unselected recurrent GBM [5].	Requires gene-induced inflamed conversion to become more rational.
CAR-T cells	Genetically modified cell therapy targeting EGFRvIII, IL13Ralpha2 or multi-antigen designs [10-14].	Can be combined with oncolytic viruses, chemokine delivery and checkpoint blockade.

Table 3. Gene-therapy platforms in GBM.

Platform	Representative payloads/examples	Strengths	Limitations
Adenoviral vectors	DNX-2401; HSV1-TK/Flt3L; IL-12 systems [18,23,25,36].	High expression; strong immunogenicity; large clinical experience.	Pre-existing immunity; local delivery challenges; inflammatory edema.
HSV/oncolytic HSV	G47Delta/teserpaturev; CAN-3110; G207 [20-22,51].	Neurotropic, large payload capacity, direct oncolysis and immune activation.	Requires careful attenuation; invasive delivery; viral-shedding surveillance.
Retro/lentiviral vectors	Toca 511; CAR-T manufacturing vectors [10-14,24].	Stable gene insertion; useful for ex vivo immune-cell engineering.	Insertional and distribution concerns for direct intracranial use.
AAV vectors	AAV-CXCL9 and other CNS gene-addition strategies [38,43].	Longer expression and neurological delivery experience.	Small payload; neutralizing antibodies; limited tumor spread.
LNP/polymer/exosome systems	siRNA, miRNA, mRNA, ASO, CRISPR cargo [26-29,44].	Repeat dosing, modular payloads, non-infectious manufacturing.	BBB penetration, endosomal escape and tumor specificity remain unresolved.
Genome editing	CRISPR/Cas targeting resistance, immune evasion or stemness genes [30,31,45].	Can disable causal vulnerabilities rather than add genes.	Off-target risk, mosaic editing, delivery and long-term safety.

Table 4. Immune-reprogramming payloads and combination rationale.

Payload/approach	Primary intended effect	Combination opportunity
Oncolytic replication	Tumor lysis, viral sensing, antigen release and local inflammation [18-23].	Checkpoint inhibitors, DC vaccines, CAR-T cells, radiotherapy.
HSV1-TK/prodrug	Local cytotoxicity plus bystander killing [24,25,52].	Flt3L, radiotherapy, temozolomide, dendritic-cell activation.
Flt3L	Recruitment/expansion of dendritic-cell precursors [25].	TK-mediated antigen release and vaccination.

IL-12	IFN-gamma induction, T-cell and NK-cell activation [36,37].	PD-1 blockade; requires regulated expression to manage toxicity.
CXCL9/CXCL10 axis	T-cell trafficking into lymphocyte-poor GBM [38].	Anti-PD-1, CAR-T, neoantigen vaccines.
RNA/CRISPR suppression of PD-L1, STAT3, TGF-beta or CD47	Reduction of local immune-inhibitory signaling [26-31].	Checkpoint blockade, macrophage modulation and adoptive cell therapy.

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