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Review article

Gene therapy in glioblastoma multiforme: Can it be a role changer?

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ABSTRACT

Glioblastoma multiforme (GBM) is one of the most lethal cancers with a poor prognosis. Over the past century since its initial discovery and medical description, the development of effective treatments for this condition has seen limited progress. Despite numerous efforts, only a handful of drugs have gained approval for its treatment. However, these treatments have not yielded substantial improvements in both overall survival and progression-free survival rates. One reason for this is its unique features such as heterogeneity and difficulty of drug delivery because of two formidable barriers, namely the blood-brain barrier and the tumor-blood barrier. Over the past few years, significant developments in therapeutic approaches have given rise to promising novel and advanced therapies. Target-specific therapies, such as monoclonal antibodies (mAbs) and small molecules, stand as two important examples; however, they have not yielded a significant improvement in survival among GBM patients. Gene therapy, a relatively nascent advanced approach, holds promise as a potential treatment for cancer, particularly GBM. It possesses the potential to address the limitations of previous treatments and even newer advanced therapies like mAbs, owing to its distinct properties. This review aims to elucidate the current status and advancements in gene therapy for GBM treatment, while also presenting its future prospects.

1. Introduction

Glioblastoma multiforme (GBM) is a grade IV glioma originating from glial cells in the central nervous system (CNS). It accounts for 14.5% and 48.6% of all central nervous system tumors and malignant central nervous system tumors, respectively. Its incidence is approximately 0.85 per 100,000 in under 18 population and about 4 in 100,000 in adults [1]. It is one of the most complex and treatment-resistant cancers, with an estimated annual mortality rate of over 10,000 individuals in the United States. The five-year overall survival rate stands at less than 7%, and the mean survival time is estimated to be under one year, approximately 8 months. The situation has remained largely unchanged for decades. The current standard of care (SOC) involves surgical resection with a maximum safe margin, followed by radiation therapy (RT) and temozolomide (TMZ). Despite its description in scientific literature over a century ago, only five drugs (TMZ, lomustine, intravenous carmustine, carmustine wafer implants, and bevacizumab (BVZ)) and one device (tumor treatment fields (TTFields)) have received FDA approval. Remarkably, none of these interventions have achieved significant success in extending patient survival beyond a few months [2,3].

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2. Why GBM treatment is too difficult: treatment challenges

GBM's pronounced resistance to conventional radiotherapy and chemotherapy makes it the most lethal intracranial tumor [4]. Despite surgical advances, it almost invariably recurs with a poor prognosis. This can be attributed to the complex genetic heterogeneity, highly invasive behavior, and infiltrative characteristics of GBM cells, which rapidly engage neighboring healthy brain tissues even before symptoms manifest [5,6].

An additional challenge in brain tumors lies in the delivery of therapeutic agents, a predicament restricted by two barriers: the blood–brain barrier (BBB), an intricate neurovascular construct governing the passage of substances into healthy brain tissue [7], and the secretion of substances like protease enzymes, vascular endothelial growth factor (VEGF), and scatter factor/hepatocyte growth factor (SF/HGF) from GBM cells. These factors contribute to the development of an aberrant vascular structure with heterogeneous permeability known as the brain-tumor barrier (BTB). This heterogeneous penetration forms hypoxic regions, where drug delivery is restricted, accelerating the development of drug resistance. In addition, both of these structures can express P-glycoprotein (P-gp), which enhances the efflux of drugs into the extracellular space [8].

Another challenge in treating GBM arises from its location deeply within the brain. This tumor primarily affects the anterior subcortical regions of the brain (86%), with the frontal lobe being most frequently involved (40%), followed by the temporal lobe (29%), parietal lobe (14%), and occipital lobe (3%) [9].

3. Innovative and advanced cancer treatments

The drug delivery system and resistance seem to be the most important challenges in cancer treatment. Numerous therapeutic approaches have been developed to address the aforementioned limitations in GBM patients. Some of these include direct intratumoral drug delivery methods like convection-enhanced delivery (CED), utilizing drug-loaded nanoparticles, and strategies for disrupting the BBB(8). However, these treatment options for GBM are still unable to significantly extend patient survival beyond 14 months, and recurrence typically occurs within 7 months post-diagnosis [10,11]. For many patients, participation in clinical trials remains their sole hope. Therefore, there is an urgent need to understand the tumor environment and enhance therapeutic strategies.

In recent years, a wave of more effective and advanced treatments has emerged to counter the shortcomings of previous therapies. Stem cell therapy, targeted therapy, ablation therapy, and gene therapy stand as some of the most recent advancements [12].

Stem cell treatment strategy is one of the treatment options for cancer, which is considered to be safe and effective. Within this concept, pluripotent stem cells, adult stem cells, and cancer stem cells are three licensed subtypes [12]. Stem cells have been extensively utilized with different strategies in GBM [13]. While stem cell cancer therapy has demonstrated effectiveness and a degree of safety, it is also associated with potential drawbacks including tumorigenesis, side effects in allogeneic haematopoietic stem cell transplantation (HSCT), toxicity and resistance, heightened host immune response, potential autoimmunity, and susceptibility to viral infections [14].

Cancer targeted therapies, alternatively known as molecularly targeted drugs, molecularly targeted therapies, and precision medicine, encompass substances designed to disrupt growth molecules. Their function is to impede the growth and spreading of cancer cells [15]. Within the tumor microenvironment, diverse signaling pathways exist between cells and cancerous cells, offering promising avenues for effective cancer targeting [16]. Monoclonal antibodies (mAbs) and small molecule inhibitors constitute the two primary categories of targeted therapies.

mAbs have heralded a significant revolution in medicine. As previously mentioned, BVZ, an approved drug for GBM, falls within the category of mAbs. While mAbs offer target-specificity and reduced harm to normal tissues, they are not without their limitations. Antibodies and other protein-based drugs hold promise for treating CNS diseases, including malignancies. However, the approval of these products remains exceedingly limited in number [17]. As for GBM, a primary factor contributing to these challenges is the low rate of entry into the brain, largely due to the presence of the BBB [18]. Additionally, general and specific adverse effects, along with concerns about toxicity and cost-effectiveness, pose significant challenges. On the other hand, the production process demands advanced technologies and comes with substantial expenses [19,20]. The majority of small molecules tested in clinical trials for GBM therapy have not demonstrated substantial benefits, often due to the emergence of new mutations. Furthermore, the heterogeneity of GBM cells presents a significant obstacle to targeted therapy. Nevertheless, combining these molecules in a therapy approach holds promise as a strategy for GBM treatment [21]. As we have outlined, GBM treatment faces significant challenges. Many efforts have been dedicated to overcoming these challenges through the utilization of gene therapy.

4. Gene therapy, a young and promising evolution in medicine

Gene therapy is characterized by the introduction of foreign genetic materials (DNA or RNA) into target cells to treat or prevent diseases arising from defective or abnormal genetic conditions. This approach holds promise across a spectrum of diseases, encompassing cancer as well as neurodegenerative and cardiovascular diseases [22]. The first gene therapy clinical trial was conducted in the 1990s. Remarkably, a staggering total of over 1900 clinical trials focusing on gene therapy were carried out solely between 2010 and 2020. Notably, around 57% of these trials were focused on cancer, while the nervous system ranked third [23]. This review is intended to elucidate the role of gene therapy in GBM and provide insights into its limits and future prospects.

Gene therapy can specifically change the behavior of oncogenes and tumor suppressor genes in GBM. Furthermore, it has the potential to surmount challenges like drug resistance through mechanisms such as reducing the expression of resistance genes or implementing strategies involving suicide genes [24]. An advantage of gene therapy, particularly when compared to protein-based

treatments, is its capacity for sustained release of preferred therapeutic agents. This characteristic can obviate the necessity for frequent or repetitive injections [25]. Another significant advantage of this novel strategy is the development of vectors with a strong affinity for the target tissue. Specifically in the context of GBM, the capability to traverse the BBB (as will be elaborated upon later) enhances the potential of this approach.

Our objective was to comprehensively delineate the present status of gene therapy for GBM, encompassing diverse aspects such as strategies, vectors, and their advancements over recent years. Clinical trials serve as informative indicators, capable of illustrating current trends and their changes over time. With this in mind, clinical trials pertinent to gene therapy for GBM were explored in this study. A comprehensive search was conducted on *clinicaltrials.gov*, employing a wide array of pertinent keywords. These included basic terms ("Gene," "Gene Therapy," "Engineered"), key protocols in gene therapy ("CAR T cell," "chimeric antigen receptor," "CRISPR," "DC," "T cell," "TCR," "TALEN," "Zinc finger," "virotherapy"), as well as critical terms for vectors ("Lenti," "Retro," "Adeno," "AAV," "HSV," "Micro RNA," "miR," "miRNA," "siRNA," "virus," "vector"). Additionally, the conditions of interest were indicated by using "glioma" and "glioblastoma." All of the results were reviewed. None of the relevant trials were excluded. Nevertheless, trials that were suspended, terminated, marked as not applicable, withdrawn, or were non-interventional in nature were excluded from our analysis.

The included 137 trials were analyzed based on different aspects of gene therapy, including strategy, most popular genes of interest, and vectors used for gene delivery. The results will be discussed in the following sections.

A summary of trials based on their start year is shown in Fig. 1. As evident, gene therapy trials for GBM commenced as early as 1992, yet 2005 marks a significant turning point with observable advancements in these trials post that year. The emergence of Phase III trials was observed in 2015, reaching its zenith in 2018. Subsequently, a decline became apparent, likely attributed to the impact of the COVID-19 pandemic. However, statistics for the period up until June 2023 are promising, showcasing positive developments.

4.1. Gene therapy strategies for GBM

Cancer gene therapy encompasses various strategies that can be categorized into two main approaches: directly altering the behavior of tumor cells and assisting the immune system in recognizing and eradicating these cells. These strategies have led to the classification of main gene therapy approaches for cancer as follows: 1) Immunogene therapy, 2) Oncolytic virotherapy (OV), 3) Suicide gene therapy, 4) Gene suppression, and 5) Gene correction and editing [26]. All of these strategies have been used in GBM trials. These strategies are summarized in Fig. 2.

4.1.1. Immunogene therapy

Immunogene therapy constituted the majority of the employed methods (n = 78). GBM utilizes several approaches to alter the tumor microenvironment (TME) in favor of suppressing the immune system to evade anti-GBM immune responses. Cytokines and chemokines secreted from and available in the TME lead to the recruitment of immunosuppressive cells. T-cell migration and activation blockage ensue subsequently [27,28]. Among cytokines, TGF-β and IL-10 have a key role. These cytokines are secreted by both GBM-infiltrating Tregs and GBM cells themselves. IL-10 inactivates T cells, dendritic cells (DCs), macrophages, and major histocompatibility complex II (MHC-II) expression in monocytes [29,30]. In contrast, it increases Tregs, myeloid-derived suppressor cells

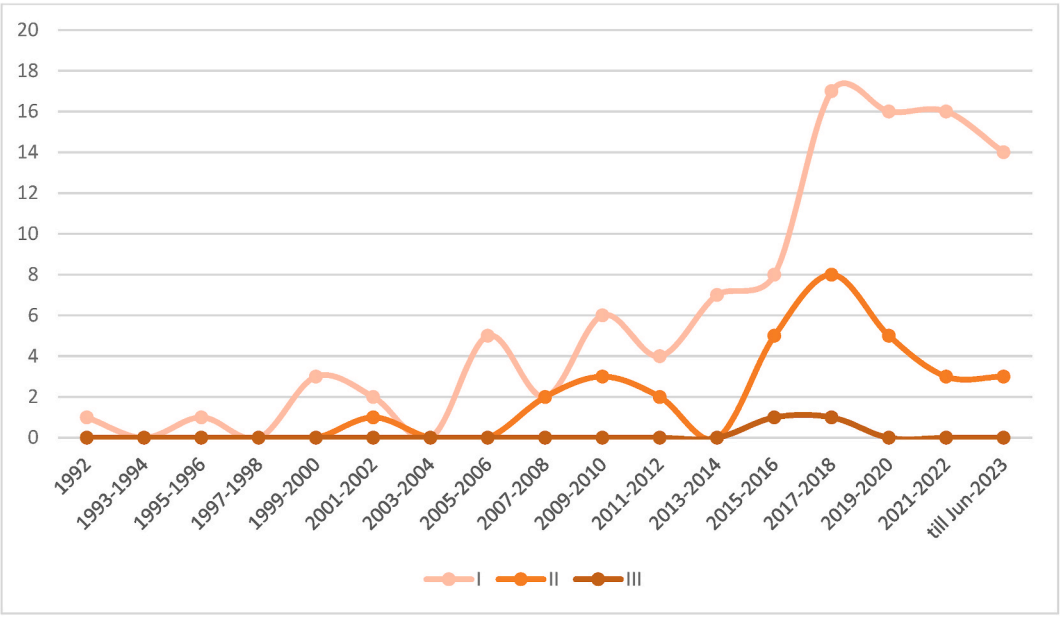

Fig. 1. GBM gene therapy clinical trials until January 2023 sorted by their start date and phase. Phases I/II and II/III were merged to II and III, respectively, to avoid unnecessary complexity and simplify the presentation.

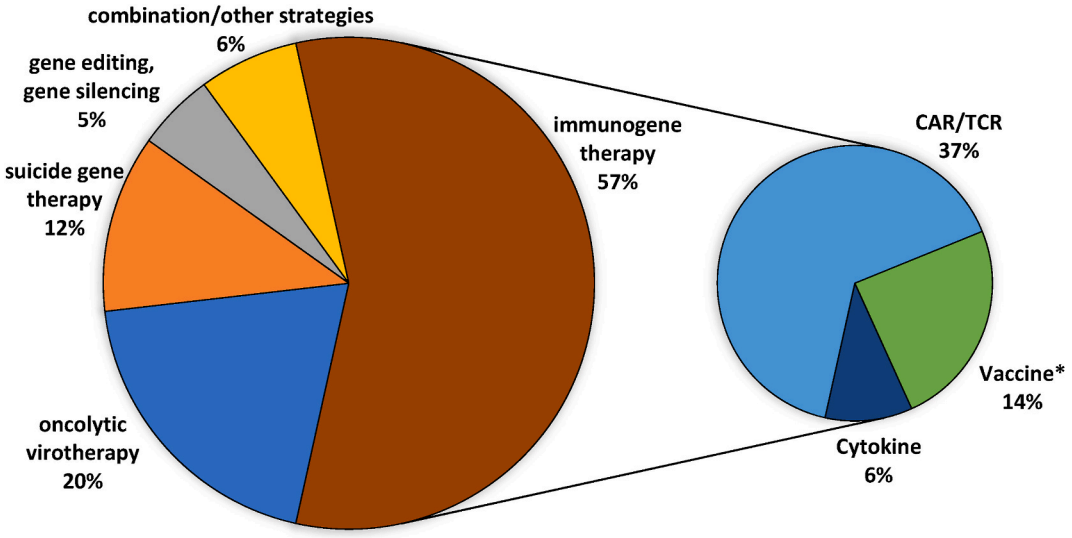

Fig. 2. Strategies used in GBM gene therapy clinical trials until June 2023. As evident, assisting the immune system in overcoming tumor immunosuppressive microenvironment and eliminating tumoral cells have been the trend so far. CAR: chimeric antigen receptor, TCR: T cell receptor *Dendritic cell and DNA vaccine.

(MDSCs), and PD-L1 expression in tumor-associated macrophages (TAMs) and monocytes [31,32]. TGF-β expressed by GBM cells reduces T cell proliferation and activation and has a distinct correlation with prognosis and glioma grade [33,34]. Moreover, Systemic adaptive immunity suppression has been also detected in murine and human GBM cases [31,35].

An immunosuppressive TME plays a pivotal role in tumor growth, and this is precisely where immunotherapy comes into play. The primary immunogene therapy strategies currently under investigation for treating GBM comprise chimeric antigen receptor (CAR) T-cell therapy, T cell receptor (TCR) therapy, immunostimulatory cytokine gene therapy, autologous activated lymphocytes (ALT), and active immunotherapy utilizing tumor cell-based peptides and antigens, DC or DNA vaccines. Among these strategies, TCR and CAR cell therapies have been more extensively employed. Oncolytic viral therapy has been discussed as an independent strategy and categorized beneath immunogene therapy because of its mechanism of action [36,37].

TCR and CAR T cells, as well as peripheral natural killer (pNK) cells, are characterized by possessing modified receptors capable of targeting specific ligands. They accounted for 37.2% and 65.4% of all trials and immunogene therapy trials, respectively. All of these studies started since 2010 except one, which began in 2002. Among these, 84.3% were in phase I, and the others were in phase I/II or II. The most targeted molecule, accounting for 39.2% of TCR and CAR T or pNK therapy trials, is the epidermal growth factor receptor (EGFR). EGFR represents one of the most prevalent oncogenic mutations, detectable in 50% of all GBM cases. Its alterations include amplification (the most common), mutation, rearrangement, splicing site changes, etc. [38,39] EGFR is associated with proliferation, migration and escape from apoptosis [40]. The most common EGFR gene mutation is version III (EGFRvIII) [38], which has been targeted as a tumor associated antigen (TAA) in GBM in several studies.

Interluekin-13 receptor alpha 2 (IL13Rα2) was targeted in 21.6% of TCR and CAR T or pNK therapy trials. IL13Rα2 is absent in the normal brain tissue, while it is overexpressed in more than 75% of GBM cases [41,42]. It drastically contributes to GBM invasion and metastasis and is associated with late stages of the disease and a poor prognosis [43]. Due to its function and exclusive presence in GBM, it serves as an appealing target for cytotoxic therapeutics.

Another common target in TCR and CAR T or pNK therapy trials has been B7 homolog 3 protein (B7–H3), also known as CD276 (17.6%). This molecule functions as a bifunctional immune checkpoint, displaying both costimulatory and coinhibitory immunoregulatory effects [44]. The human B7–H3 protein exists as either as a transmembrane or a soluble isoform. The transmembrane form is composed of an extracellular, a transmembrane, and a short intracellular domain [45]. Soluble B7–H3 (sB7-H3) is either extracted from the surface by a matrix metallopeptidase (MMP) or produced through alternative splicing [46,47]. B7–H3 has different roles. In contrast to other immune checkpoints, B7–H3 also plays a role in cancer cells aggressiveness, simultaneously regulating innate and adaptive immunity [48].

Among GBM immunogene therapy trials, 10.3% employed cytokines. As previously mentioned, cytokines play a pivotal role in immunosuppression. In contrast, there are also stimulatory cytokines. Gene therapy methods can be harnessed to induce tumor-selective production of various cytokines, such as IL2, IL4, IL12, and IFNβ/γ, leading to immune responses against glioma cells [49,50]. IL12 is a very potent anti-tumor cytokine, inducing a Th1 response [51]. The systemic administration of its recombinant form has been limited because of its toxicity in spite of its therapeutic success in animal models of cancer. local IL12 delivery by gene transfer is a solution. Additionally, the ligand-inducible expression switch, RheoSwitch Therapeutic System (RTS) is used for local control of the production of IL12 to minimize systemic toxicity. Administration of RTS-IL12 to glioma patients increases the production of tumor infiltrating lymphocytes (TILs) IFNγ and expression of PD1, resulting in immunological anti-glioma effects [52,53].

4.1.2. Oncolytic virotherapy

The second most commonly employed method was OV (n = 27). Oncolytic viruses are replication-competent wild-type or engineered viruses, capable of targeting tumor receptors or replicating under oncogene promoters to enhance their specificity for neoplastic cells. Evidence indicates that the immunosuppression within the tumor microenvironment can actually augment the infection capacity and oncolytic efficacy of oncolytic viruses [49,54,55]. Several oncolytic viruses have been evaluated in preclinical studies or clinical trials for the treatment of GBM.

In OV trials, 44.4% employed adenoviruses (Ad), with DNX-2401 (delta 24-RGD) being the more prevalent choice (58.3%). DNX-2401 is a conditionally replicating adenovirus (CRAd) designed to target glioma cells. A 24-nucleotide deletion in the early 1A (E1A) gene results in enhanced replication within cancerous cells as compared to normal cells. Conversely, Ad entry into cells occurs through coxsackie-adenovirus receptors, which are not universally expressed in all cancers. The insertion of RGD-4C peptide into the adenoviral surface fiber amplifies its attachment to cancerous cells, aiding in its attachment to integrins and thereby enhancing its tropism [56].

In the second position, herpes simplex virus (HSV) accounted for 33.3% of OV trials, with HSV G207 being more prevalent than other engineered HSVs (55.5%). HSV G207 is a conditionally replicating virus, with reduced neurovirulence achieved through the deletion of γ34.5 copies. Additionally, the deletion of the UL39 gene results in the cessation of viral DNA synthesis in nondividing cells [57].

PVS-RIPO, oncolytic Measles virus, human wild-type reovirus, vaccinia virus, and wild-type parvovirus were other oncolytic viruses used in clinical trials.

4.1.3. Suicide gene therapy

The first registered trial for GBM was conducted in 1992, involving the delivery of a suicide gene via retrovirus. Suicide gene therapy ranked third (11.7%) among all GBM gene therapy trials.

Suicide genes used in GBM trials were Herpes Simplex Virus Thymidine Kinase (HSV-TK) and Cytosine Deaminase 5-fluorocytosine (CD/5-FC) (56.3% and 43.7%, respectively). The product of HSV-TK, as implied by its name, is a kinase that phosphorylates nucleoside analogues such as ganciclovir (GCV), which exhibit low affinity for mammalian TK. The final product is cytotoxic and possesses the ability to disrupt DNA replication in actively proliferating cells [58]. As predictable, the HSV-TK gene therapy relies on the cell cycle, specifying its activity and toxicity to actively proliferating cells. another advantage is a process known as the bystander effect, in which cytotoxicity is transferred directly from involved cells to adjacent non-infected cells, possibly through gap-junctions, facilitated by cell-to-cell contacts, enhancing the treatment effect. However, this can involve normal cells, resulting in side effects [59].

CD/5-FC has been vastly studied for glioma gene therapy [49]. CD is a bacterial or yeast enzyme that is absent in mammalian cells. It converts an effective antifungal drug, 5-FC, to a potent cytotoxic substance, resulting in DNA synthesis disruption. This method also leads to the bystander effect, even more pronounced compared to TK, as the final product is a small molecule, enabling it to diffuse through transduced and neighboring cells [60,61].

It is important to note that the vector used should possess a highly specific tropism for the target tumor cells to minimize potential side effects. Mesenchymal stem cells (MSCs) and neural stem cells (NSCs) are two novel vectors used in suicide gene therapy. NSCs have a great tropism and invasive behavior toward tumor cells, with continuous proliferation and differentiation to both glial and neuronal cells [62,63]. MSCs are non-hematopoietic multipotent stem cells, capable of migrating to the site of injury and inflammation, such as the tumor environment [64]. They are more readily available than NSCs and can be obtained from various sources including bone marrow, adipose tissue, muscle tissue, and the peripheral bloodstream [65].

4.1.4. Other methods

Gene editing and silencing involve the repair of damaged tumor suppressors or hyperactive oncogenes to regulate tumor growth [26]. This constituted 5% of GBM gene therapy trials and included genes such as P53 and (O)6-methylguanine-DNA-methyltransferase (MGMT). P53 is a tumor suppressor that is inactivated in 25–30% of primary GBMs and 60–70% of recurrent ones [66]. The overexpression of the MGMT gene by removing alkyl adducts at the O-6 position of guanine causes drug resistance in GBM patients treated with alkylating agents such as TMZ [67]. A mutated form of MGMT can be introduced into hematopoietic stem cells or T cells, leading to the protection of bone marrow and the prevention of lymphodepletion caused by TMZ toxic effects. This approach reduces side effects and enables an increase in the therapeutic dose.

Many studies have been carried out to elucidate the role of angiogenesis in solid tumors and its underlying mechanisms, aiming to

Table 1
GBM gene therapy trials with a combination of two strategies of cancer gene therapy.

NCT	phase	Treatment Strategy	Vector/gene of interest
NCT01811992	I	suicide gene therapy/immunogene therapy	Ad/hCMV-TK/hCMV-Flt3-L
NCT03294486	I/II	OV/suicide gene therapy	VACV/FCU1
NCT05095441	I	OV/Immunogene therapy	oHSV/IL12
NCT05717712	I	OV/Immunogene therapy	oAd/IL12
NCT05717699	I	OV/Immunogene therapy	oAd/IL12

Abbreviations: OV: oncolytic virotherapy; hCMV: human cytomegalovirus; oHSV: oncolytic herpes simplex virus; oAd: oncolytic adenovirus; VACV: vaccinia virus; IL-12: interleukin-12.

discern its distinctions from normal vascularization [68]. Glioblastoma is no exception to this investigation, as it exhibits characteristics such as a thick basement membrane, large diameter, tortuosity, heterogeneous shape and distribution, as well as excessive branching across all levels of arterioles, venules, and capillaries [69]. Among responsible factors, VEGF and its associated signaling pathway have been extensively studied as the most important proangiogenic factor in tumor angiogenesis. Overall, the activation of VEGFR-2 via VEGF-A is recognized as the cornerstone of tumor angiogenesis [70,71]. Angiogenesis inhibition was purposed in four GBM gene therapy trials, with one of these trials being in phase III.

Five trials utilized a combination strategy. These trials are outlined in Table 1.

4.2. Vector

The genetic material needs to be delivered to the target cells through a carrier known as a vector. Vectors can be categorized into viral and non-viral subgroups [72]. Both types have been used in gliomas (Fig. 3). Every vector has its own advantages and disadvantages, which should be considered prior to selection.

4.2.1. Viral vectors

Viral vectors are purified non-toxic viruses that have been modified to serve as carriers without inducing infection [24]. They can be classified into two types: replication-competent and replication-incompetent. Replication-competent viruses are genetically altered agents that retain the ability to self-replicate while delivering cytotoxic anticancer genes. These fall under the category of OVs. The replication-incompetent vectors are engineered to deliver the desired anticancer gene with minimal or no expression of their own viral genes [73].

In comparison to nonviral vectors, viral vectors exhibit higher efficiency in delivering genes to tumor cells with more prolonged periods of expression. However, they might carry a higher risk of toxicity and immunogenicity, potentially leading to more undesirable side effects [74].

4.2.1.1. Retroviruses and lentiviruses. Retrovirus served as the first vector used for glioma gene therapy. In GBM gene therapy trials, retroviruses and lentiviruses held the leading position, constituting 45.3% of the total. This dominance can be attributed to the extensive application of these vectors in CAR and TCR therapies, which are among the most prevalent immunogene therapy protocols (Fig. 3). Another important use of retroviruses involves the replication of retroviral vectors aiming at delivering a suicide gene into tumor cells [75,76]. Research has demonstrated that due to genome integration, even if tumor cells survive the cytotoxic effects of gene therapy and recur, the effects of the inserted genes will persist [77]. Studies have also highlighted the limitations associated with the transfection of retroviral vectors [78].

TOCA511 is a retroviral vector commonly used to deliver the CD gene into tumor cells [79]. Preclinical studies and molecular analyses have indicated that TOCA511 does not exhibit widespread or uncontrolled replication while possessing radiosensitizing effects [80,81]. Trials have showcased its significant survival benefits (supplementary table).

Lentivirus is a member of the retroviridae family [82]. Its exogenous genome can be integrated into the genomes of both dividing or non-dividing cells [83]. An advantage of lentiviruses, when compared to retroviruses, is their enhanced stability and reduced likelihood of insertion mutation. On the other hand, pre-integration complex is transported to the nucleus actively, which constitutes a distinctive characteristic of lentiviral vectors [84].

4.2.1.2. Adenoviruses. These viruses are widely used for gene therapy. In GBM gene therapy trials, 24% of the vectors employed were Ad vectors. Adenoviruses are classified into seven species, denoted A to G, encompassing at least 57 serotypes, labeled as 1–57 CE. Their cellular entry is contingent on the presence of the coxsackie-adenovirus receptor. The most commonly utilized Ad vectors belong

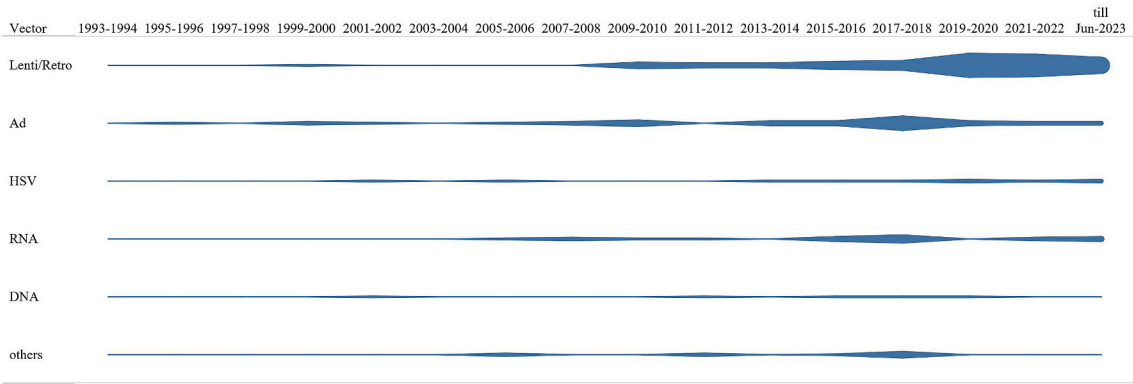

Fig. 3. Trends in utilization of different vectors in GBM gene therapy trials up to June 2023. The most common vectors are separated while the less common ones such as some oncolytic viruses are groups under the category "others". Ad: adenovirus; HSV: herpes simplex virus; Lenti/Retro: Lenti or retrovirus.

to serotype 5 [85]. Both replication-competent and replication-deficient Ads are used in cancer gene therapy. There are three strategies to enhance Ad's target tropism in GBM: employing tumor-specific promoters, deleting crucial viral genes supplied by tumor cells, and modifying the viral capsid to facilitate selective entry into GBM cells [86].

4.2.1.3. Herpes simplex virus. HSV served as the third most common viral vector, accounting for 7.3% of all trials. HSV-1 stands out as an appealing vector for CNS applications. Two distinct applicable forms have been identified. In recombinant (replication-defective) HSV-1, certain or all of the genes essential for viral replication and lytic activity (including ICP0, ICP4, ICP22, ICP27, ICP47, and ICP34.5) are deleted [87,88]. The amplicon type or oncolytic HSV-1 (oHSV-1) maintains its replication capabilities while utilizing tumor-specific promoters to drive gene expression [89,90]. Examples have already been discussed.

4.2.1.4. Adeno-associated viruses (AAV). Long-lasting, stable, efficient, and non-toxic gene delivery, enabling the transduction of various target cells, establish AAV as a robust and successful viral vector [91]. In recent years, targeting GBM via AAV has gained widespread attention and demonstrated success in preclinical studies [92]. The discovery of AAV9 in 2009, followed by the emergence of rAAVrh.8 and rAAVrh.10 in 2014, heralded a revolution in the treatment of CNS diseases through the utilization of AAV vectors. This revolution was underpinned by their capacity to traverse the BBB, rendering systemic injection more efficacious and supplanting the need for invasive local methodologies [93–95]. Up to the present, AAV9, rAAVrh.8, rAAVrh.10, AAVrh.39, and AAVrh.43 have demonstrated glial and neuronal tropism, along with the capability to traverse the BBB following systemic injection [96]. AAV9 variants, namely AAV-PHP.B and AAV-PHP.eB, have exhibited even greater ability to pass the BBB compared to AAV9 [97,98]. An example of this is angiostatin delivery [99]. Another successful experience involved the local injection of an AAV containing the sVEGFR1/R2 gene directly into the tumor site [100].

While no clinical trials involving AAV have been conducted for GBM, its notable success and extensive utilization in preclinical studies suggest that it holds promise as a potential vector for future research endeavors.

4.2.2. Non-viral vectors

The use of non-viral vectors has been limited due to their decreased specificity for target tissues, low transfection rates, and weak stability, [74]. However, recent advances in nanotechnologies have led to the design of nanoparticles (micron-sized molecules) that serve as non-viral vectors with low toxicity and immunogenicity [101].

RNA-based methods encompass a range of approaches, including antisense oligonucleotides (AS-ON), double-stranded RNAs, small interfering RNAs (siRNAs or RNA interference, RNAi), and RNAs extracted from cancerous cells to load DC cells for DC vaccine. These methods collectively accounted for 13% of all vectors used in GBM gene therapy trials. The first three methods consist of 15–30 nucleotide sequences that function by binding to a complementary sequence within a target mRNA, leading to its inactivation [102]. DNA can be also used as single-strand antisense oligodeoxynucleotides (AS-ODN) or double-strand DNA coding for beneficial genes. These approaches accounted for 3% of the trials reviewed. Target cells need to undergo transfection using external forces like electroporation and sonoporation, or through the use of a vector such as a nanoparticle. These methods aid in facilitating the penetration of nucleic acids into the target cells. The combination of RNA or DNA with electroporation is widely utilized in the creation of cancer vaccines for GBM. Clinical trials also investigated the delivery of siRNA using gold nanoparticles to target the oncogene BCL2L12 in GBM.

These methods face the challenge of rapid degradation of nucleic acids by nucleases in the circulation, leading to their short half-lives and the potential need for repeated injections. Additionally, the activation of interferons due to the recognition of atypical nucleic acid structures may hinder their in vivo effectiveness [102].

4.3. Trials with published results and a new approved drug

Among all 137 GBM gene therapy trials, 48 trials published their results (see Supplementary Table). Among these trials, 79.2% were in phase I. Immunogene therapy, oncolytic virotherapy, and suicide gene therapy accounted for 41.7%, 27.1%, and 18.8% of the trials, respectively. In terms of vectors, 31.2% and 25% of the trials used adenovirus and retrovirus or lentivirus, respectively. Additionally, fewer than 36% of the studies reported serious adverse events (SAEs). It is important to note that SAEs occurred in a minority of the study population in most of these studies, and only a few studies reported deaths due to the treatment. Regarding survival improvement, it is worth noting that a majority of the studies were in phase I, where survival benefit was not the primary focus, and some of these studies lacked a control group. Despite the generally poor prognosis of GBM, there have been about one third of studies that have shown significant survival improvement, clinical benefit, complete or partial response compared to the control group. It is worth noting that durable and multiyear life has been observed repeatedly, which is uncommon among GBM patients.

G47Δ is a triple mutated oncolytic HSV-1 derived from G207. In a phase II trial for malignant glioma, G47Δ demonstrated remarkable outcomes, leading to its conditional and time-limited approval in Japan [103].

5. Challenges of gene therapy in GBM treatment

Gene therapy for GBM is confronted with several distinct challenges. These encompass the limited efficiency of viral vector transduction, the absence of a delivery system capable of bypassing the BBB, the challenge of differentiating between tumor cells and normal cells, and the controlled selective expression of a therapeutic transgene.

The process of determining which genes to alter or substitute is significantly impeded by the inherent genetic diversity of GBMs, as well as the ongoing accumulation of mutations during the progression of the cancer.

A prevalent drawback of all virus-based gene therapies is their potential to instigate immune responses and inflammation. This can be mitigated by adjusting their dosages. The method of drug administration also raises concerns. Given that most viral vehicles are rapidly eliminated from the system, minimally invasive administration methods such as stereotactic or endoscopic have been suggested. These methods have already shown benefits in other diseases [59,104].

The limited distribution of the viral vector within the tumor, attributable to GBM's infiltrative nature, continues to impede optimal clinical effectiveness. However, improvements in vector stability and the extension of therapeutic transgene expression could potentially enhance GBM treatment.

The prospect of gene therapy alone curing GBM in the near future is unlikely. Considering the heterogeneous nature of GBM, blocking one or two pathways may merely activate alternative pathways, leading to continued tumor progression. Consequently, the mere replacement of a single lost gene (such as a tumor suppressor gene) does not result in successful GBM treatment.

The possibility of combining viral gene therapy with other treatments, such as advanced radiation therapy and molecular targeted therapy, could potentially prove more effective than the sole use of viral agents [59,104].

6. Future prospects

As previously detailed, monotherapy has not yielded substantial efficacy in the management of GBM. The potential of combination therapy, particularly the amalgamation of pharmaceutical agents with distinct mechanisms of action, is an area of considerable interest for forthcoming research endeavors. The positive results observed with suicide gene therapy and oncolytic virotherapy underscore their increasing relevance in the field. The recent approval of G47Δ serves as a testament to this trend. Two other therapies within these categories, DNX-2401 and TOCA-511, are currently under review for approval in the treatment of GBM [23].

Previous studies have highlighted considerable progress in intracellular signaling in CAR-expressing T cells over the last decade. Despite the limited advancement in directing these CAR T cells towards glioma and the challenges posed by inconsistent target expression [42,105], it's important to note the promising progress in CAR T cell therapy. Particularly in the treatment of solid tumors, some encouraging results in GBM in recent years (supplementary table) suggest potential advancements in this area of gene therapy.

The CRISPR/Cas system also merits attention. This rapidly advancing tool has demonstrated promising applications in GBM [106,107], thereby positioning it as a strong candidate for further investigation in GBM research.

7. Conclusion

To conclude, gene therapy offers distinct advantages and benefits for GBM when compared to both conventional and innovative strategies, such as mAbs and small molecules. The results from published GBM gene therapy trials demonstrate encouraging clinical benefits with an acceptable level of toxicity. The achievement of durable and even complete responses in such a fatal disease is a significant and promising advancement. However, there remain challenges that need to be addressed. We remain hopeful for the future of gene therapy in the treatment of GBM.

Among the methods employed in GBM gene therapy, immunotherapy, particularly CAR T cell therapy, has undergone significant evolution. Additionally, oncolytic virotherapy and suicide gene therapy stand as other important strategies. The application of lentiviral and retroviral vectors has experienced notable expansion, largely driven by their role in immunotherapy such as CAR T cell therapy. Adenovirus utilization has also seen growth. While AAV has not yet been utilized in GBM trials, results from preclinical studies underscore substantial benefits. Consequently, it holds promise as a potential vector with distinct attributes.

Data availability statement

Data associated with our study is not deposited into a publicly available repository and will be made available on request.

CRediT authorship contribution statement

Mohammad Rayati: Writing – review & editing, Writing – original draft, Data curation. **Vahid Mansouri:** Writing – review & editing, Visualization, Methodology. **Naser Ahmadbeigi:** Writing – review & editing, Supervision, Project administration.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2024.e27087.

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