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Oncolytic viral therapy for gliomas: Advances in the mechanisms and approaches to delivery

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Highlights

- Current GBM therapeutic approaches have limited potential on the prognosis and show 15 months of median survival.
- Oncolytic viral therapy is a cutting-edge therapeutic approach that has shown significant promise due to their ability selectively targeting and inducing apoptosis in cancer cells.
- Recent findings suggest that OV therapy may enhance the effectiveness of the “gold standard” treatment, potentially alleviating side effects and improving patient outcomes.

Abstract

Glioma is a diverse category of tumors originating from glial cells encompasses various subtypes, based on the specific type of glial cells involved. The most aggressive is glioblastoma multiforme (GBM), which stands as the predominant primary malignant tumor within the central nervous system in adults. Despite the application of treatment strategy, the median survival rate for GBM patients still hovers around 15 months. Oncolytic viruses (OVs) are artificially engineered viruses designed to selectively target and induce apoptosis in cancer cells. While clinical trials have demonstrated encouraging results with intratumoral OV injections for some cancers, applying this approach to GBM presents unique challenges. Here we elaborate on current trends in oncolytic viral therapy and their delivery methods. We delve into the various methods of delivering OVs for therapy, exploring their respective advantages and disadvantages and discussing how selecting the optimal delivery method can enhance the efficacy of this innovative treatment approach.

Introduction

Contemporary glioma therapy comprises maximal surgical resection followed by concomitant radiotherapy and chemotherapy employing temozolomide (TMZ) (Norden and Wen, 2006). Nevertheless, despite these treatments, the median overall survival (OS) for glioblastoma (GBM) patients remains relatively short, ranging from 12 to 15 months post-diagnosis, with a mere 11% surviving beyond 2 years. In 2016, David-Louis and colleagues introduced the first classification of gliomas, taking into account not only their histological features but also key mutations, such as IDH1/2, TP53, ATRX loss, and 1p/19q co-deletion, as these mutations significantly influence carcinogenesis (Louis et al., 2016). Notably, the *IDH1* variant R132H serves as a prognostic marker, as its presence, in combination with TMZ treatment, correlates with more favorable outcomes compared to the wild-type IDH1. Conversely, poor patient outcomes are associated with mutations in *PTEN*, the *TERT* promoter, PI3-kinase, and *EGFR*, each of which plays a distinct role in abnormal cellular metabolism. Epigenetic alterations, such as CpG island methylation (particularly common in IDH-mutant gliomas and GBM) and H3K27 modifications, can also reduce OS. Developing an effective therapy that considers all these diverse alterations and their combinations presents a formidable challenge, especially given that much of the therapy still relies on FDA-approved agents like TMZ, Lomustine, Carmustine, and Bevacizumab, the first of which was introduced in 2005, indicating limited therapeutic progress (Fisher and Adamson, 2021).

With the advent of CRISPR/Cas9 and its initial application in lung cancer therapy in 2016, extensive discussions emerged surrounding the potential of gene therapy for gliomas. Researchers have since been actively investigating the utility of CRISPR in knocking out specific genes to assess their impact on glioma metabolism and immune system evasion. Numerous genes have been identified as promising molecular targets for anticancer therapy, including *PD-1*, *PD-L1*, and *TIMM3*, both in *in vivo* and *in vitro* studies. However, these advantages are tempered by notable drawbacks, such as off-target effects, delivery challenges, and immune responses to the Cas9 protein (Kang et al., 2023).

Meanwhile, oncolytic viruses (OVs) present an elegant and effective solution, offering a variety of mechanisms and packaging possibilities. However, a different set of challenges arises when it comes to OV delivery. OVs have to bypass several checkpoints, including the blood-brain barrier (BBB), potential toxicity, and immune reactions, to reach the tumor site. Many of these obstacles stem from the chosen delivery method, whether viral or non-viral, and the point of entry. According to the majority of *in vivo* experiments, the most suitable delivery types are intravenous or intra-arterial, whereas intratumoral administration, catheters, or convection-enhanced delivery methods are often overlooked due to the complexities of implementing them in clinical practice. Nevertheless, these overlooked methods have been extensively researched and proven to be more efficient, especially for GBM therapy, due to unique physiological characteristics of the brain.

Glioma, the second most prevalent brain tumor, underwent a significant reclassification by WHO in 2016 and further refinement in 2021 (Louis et al., 2007, 2016, 2021). Among the various treatment protocols, the Stupp regimen stands out as the most renowned. This regimen involves surgical resection followed by a combination of chemotherapy using TMZ and radiotherapy (Hegi et al., 2005; Stupp et al., 2005; Choi et al., 2018). However, despite its established clinical effectiveness, this therapy exhibits significant shortcomings. First, TMZ-based treatment demonstrates its efficacy primarily in IDH-mutant gliomas due to their hypermethylated status—a characteristic absent in other glioma types (Choi et al., 2018; Gulaia et al., 2022; Fernandes et al., 2017). Second, TMZ treatment is associated with side effects, as it induces mutations linked to methylations in O6- and N7-guanine residues (Singh et al., 2020). These epigenetic mutations predominantly persist in IDH-mutant gliomas, rendering them more susceptible to TMZ treatment but complicating future strategies for recurrence treatment (Choi et al., 2018; Pavlova et al., 2022). Most critically, this therapeutic approach exerts a profound impact on the brain microenvironment. Unfortunately, only a limited number of studies have explored the adverse effects of glioma therapy on the microenvironment. For instance, some studies have reported changes in the epigenetic landscape, giving rise to new subpopulations of tumor cells (Malta et al., 2021). Of note, research by Tamura et al. demonstrated that TMZ and radiation had divergent effects depending on the duration of patient exposure. One such effect was the increased expression of FOXP3, a specific marker for T-cell lineage differentiation and, more importantly, regulatory T cell (Treg) production (Tamura et al., 2020); (McMurchy et al., 2013). In contrast, another study by Tamura et al. revealed upregulated PD-1 in recurrent gliomas following post-surgery and TMZ-radiotherapy treatment, a factor also responsible for Treg development (Tamura et al., 2020).

Apart from TMZ, the impact of radiation therapy on the microenvironment has garnered attention. Several studies have identified potential mechanisms by which glioma cells can restore their state post-radiation therapy, including increased CXCL12 expression to promote angiogenesis through interaction with VEGF. Radiation may also trigger a new lineage of undifferentiated oligodendrocyte precursor cells, thereby creating a new niche of radioresistant cancer cell population (Teicher and Fricker, 2010; Giordano et al., 2019). Additionally, radiation promotes the formation of an invasive phenotype in glioma cells through the production of hyaluronic acid, which binds to CD44 receptors—a specific marker for the epithelial-to-mesenchymal transition (EMT) phenotype and glioma stem-like cells (Gulaia et al., 2022; Yoo et al., 2018). Furthermore, radiotherapy induces an increased expression of endoplasmic reticulum stress-responsive genes, such as *ATF4* and *ATF6* in glioblastoma, leading to radioresistance by activating *VEGF*, *FGF2*, and other genes encoding growth and angiogenic factors (Kim et al., 2019). Notably, IDH1/2 mutations mitigate the impact of radiotherapy by stabilizing the kinetochore, reducing the number of micronuclei (MN), which are a crucial hallmark of genomic instability. Since mutations in these genes enhance genomic stability by inducing hypermethylation, the rationale for radiation therapy becomes questionable.

Ever since the discovery and validation of CRISPR/Cas9 technology for precise gene editing, its application has extended beyond *in vitro* experimental models to clinical approaches and the identification of novel therapeutic targets (Uddin et al., 2020). For instance, in the realm of glioma research, CRISPR/Cas9 has played a pivotal role in uncovering promising candidate genes. Through pooled CRISPR/Cas9 knock-out experiments targeting the H3K27M mutant pediatric high-grade glioma (HGG), researchers have identified genes like *EED*, *KANSL3*, *LSM11*, and *CHD4* as potential therapeutic targets (Wenger et al., 2023). Likewise, this innovative technology has been instrumental in the discovery of long-noncoding RNA radiation sensitizers (lncGRS), which are associated with cell growth and resistance to radiotherapy (Liu et al., 2020). Notably, the knock-out of these lncGRS molecules has led to a reduction in glioma stem cells (GSCs), suggesting their role in sustaining stemness in glioblastoma, a phenomenon previously observed in other types of stem cells (Liu et al., 2023; Nio et al., 2015; Ferreyra Solari et al., 2016).

Furthermore, CRISPR/Cas9 has been employed to achieve efficient knock-out of programmed death receptor ligand (PD-L1) through dual guide RNAs (gRNAs). This approach resulted in reduced proliferation and

migration of glioma cells, while the surrounding macrophages adopted the M1 phenotype, known for its capacity to inhibit tissue repair (Fierro et al., 2022; Mills, 2012). Additionally, CRISPR/Cas9 has demonstrated its potential in correcting TERT mutations *in vivo*, offering therapeutic possibility for TERT-mutant gliomas. However, it's worth noting that this correction was performed on specific single mutations, such as −124C>T, requiring a prior tumor genotyping for the therapy to be optimally effective (Li et al., 2020). Furthermore, CRISPR-pooled screening has identified CASHRP1 as a contributor to improved radioresistance *in vitro*, underscoring the significance of CRISPR as a tool for *in vitro* modeling and target exploration (Zhu et al., 2021). These advancements highlight both the promise and the complexities associated with CRISPR-based therapy in the context of glioma research and treatment.

While CRISPR-based therapy has gained significant popularity, numerous research papers have tackled the associated challenges. One notable concern is the potential for the CRISPR/Cas9 system to function off-target, leading to unintended mutations in undesired genomic locations. Many efforts have been made to address this issue, resulting in the development of bioinformatic tools that enhance specificity and the utilization of Cas9. Long-read sequencing and adapted algorithms like Nano-OTS and DISCOVER-Seq have made it easier to identify off-target sites (Guo et al., 2023). However, these advancements merely underscore the potential for off-target variants, emphasizing the need for ongoing optimization (Zhang et al., 2015).

Furthermore, despite attempts to improve gRNA selection and Cas9 delivery, it has been found that both on- and off-target mutations can be inherited by future generations, as demonstrated in a *Zebrafish* model (Höijer et al., 2022). Another concern is related to the fact that Cas9 is not native to eukaryotes, potentially triggering immune reactions. This concern has been substantiated in an *in vivo* canine model and extensively reviewed (Hakim et al., 2021). Notably, two of the most commonly used Cas9 coding sequences are derived from Streptococcus aureus and Streptococcus pyogenes, leading to the recognition of Cas9 by human T-cells in a significant proportion of cases (Mehta and Merkel, 2020; Charlesworth et al., 2019). While it has been suggested to use CRISPR therapy in young organisms with undeveloped immune systems, ethical dilemmas arise from such an approach.

In general, overcoming immune response obstacles can be achieved by targeting tissues with lower immunogenicity (Yip, 2020; Rasul et al., 2022). For instance, recently published research has shown the correction of small eye syndrome in a murine model through CRISPR/Cas9 editing of PAX6, highlighting the immune privilege of certain organs like the eye, brain, placenta, and testicles (Crudele and Chamberlain, 2018). It is worth noting that there are currently 72 clinical trials in progress, none of which are recruiting patients with brain tumors.

Initially, viral vectors were developed and have found extensive use in *in vitro* experiments, primarily for the purpose of overexpressing specific genetic constructs. Among these vectors, a few stand out for their utility in both dividing and non-dividing cells: Lentiviral (LV), Adeno- and Adeno-associated viruses (AV and AAV, respectively), and virus-like particles (VLP) (Muratori et al., 2010). VLP are of particular interest due to their potential to offer a completely safe approach, free from the risks associated with viral replication, while efficiently delivering specific proteins. While the existing data is insufficient to support full-fledged clinical trials, there are presently 153 registered clinical trials employing VLPs as a delivery method. Notably, none of these trials employ VLPs for the delivery of Cas9 (Taha et al., 2022). Hence, while VLPs hold promise, it is prudent to also consider traditional viral vectors.

To begin with, unlike lentiviral vectors that integrate randomly into the genome, AV and AAV vectors transiently transduce cells, making them well-suited for *in vivo* experiments. For example, AAV-delivered Cas9 has been utilized in the treatment of conditions such as atherosclerosis, cancer cell telomere shortening, and metabolic liver disease (Yang et al., 2016; Dai et al., 2020; Zhao et al., 2020). Conversely, the application of LV-based CRISPR/Cas9 therapy *in vivo* remains limited due to potential complications associated with LV delivery (Dong and Kantor, 2021). LVs are seldom used for Cas9 delivery due to associated risks, including uncontrolled

Cas9 expression that may result in multiple double-strand breaks (DSB). However, one approach that has been explored involves co-transfecting plasmids into human adipose mesenchymal stem cells to introduce Thymidine kinase 2 (hTK2) through a knock-in mechanism. Subsequently, these modified cells are employed in both *in vitro* and *in vivo* studies to assess the feasibility of this delivery method for targeting glioblastoma with Ganciclovir administration (Meca-Cortés et al., 2017).

Several innovative approaches have been explored to facilitate the delivery of the CRISPR/Cas9 system, including the use of lipid nanoparticles (Taha et al., 2022; Kazemian et al., 2022; Han et al., 2022). Lipid nanoparticles have a long history of use in transferring plasmids into cells, with examples like Lipofectamine and similar reagents. As far back as 2006, Lipofectamine was demonstrated to effectively deliver transgenes *in vivo* to chick and mouse embryos. More recently, in 2015, similar success was achieved within the inner ear of adult mice, resulting in significant changes in hair cells (Decastro et al., 2006; Zuris et al., 2015). The only limitation at that time was the inability to perform intravascular injections. However, a lipid-based approach has been devised for inhalation, enabling the subsequent delivery of AAV for the CRISPR/Cas9 system to lung cells. This approach circumvents immune cell interference and penetrates the mucosa layer in the lungs (Li et al., 2023). Furthermore, the utilization of lipid-polymer nanoparticles to target the 06-Methylguanine DNA methyltransferase (MGMT) gene through the CRISPR/Cas9 system, coupled with focused ultrasound, has yielded promising outcomes in enhancing TMZ treatment efficacy in primary glioma cell cultures and mouse xenograft models (Yang et al., 2021a).

The obstacles posed by natural barriers and the body's innate responses have long intrigued researchers in the field of therapy development, particularly in the context of glioma. Among these challenges, the blood-brain barrier (BBB) stands out as one of the most formidable (Mitusova et al., 2022). Its primary role is to safeguard the brain against pathogens and toxic substances, while also maintaining the proper flow of ions and nutrient supply—a function deeply ingrained in its structural composition.

Despite the existence of potential mechanisms to circumvent the BBB, a pivotal rule was established by Lipinski et al., in 2001, known as “The Rule of 5.” This rule outlines criteria for complex molecules that have the capability to penetrate the BBB, including having fewer than 5 hydrogen bonds as donors, no more than 10 hydrogen bonds as acceptors, a molecular weight less than 500Da, and high lipophilicity (Lipinski et al., 2001). It's worth noting, however, that this rule primarily applies to drugs delivered via injection into the bloodstream. Many drugs, including those intended for oral administration, do not meet these criteria (Chowdhury et al., 2021). A notable example is Temozolomide (TMZ), which is administered orally due to its toxicity to blood cells.

Of note, tumor growth tends to disrupt the blood-brain barrier (BBB) (Sarkaria et al., 2018). Several mechanisms may account for BBB disruption, including the invasion of blood vessels, angiogenesis, development of a new vascular network due to hypoxia inside the tumor core mass, activation of the vascular-endothelial growth factor family, or recruitment of endothelial progenitor cells into the tumor (Hardee and Zagzag, 2012; Plate et al., 2012). These processes collectively give rise to a distinct barrier known in the literature as the blood-tumor barrier (Arvanitis et al., 2020). This barrier is considered leaky, but its permeability varies depending on the underlying mechanisms. For example, hypoxia-induced angiogenesis primarily affects the hypoxic core, making it more permeable to drugs, while the tumor's edge and peripheral areas remain relatively intact and unaffected (Hardee and Zagzag, 2012). Assessing the permeability of the BBB can provide valuable insights into differentiating between grade II, III, and IV gliomas, especially when dynamic contrast-enhanced MRI is employed (Li et al., 2015). Furthermore, increased permeability can enhance drug delivery and improve imaging techniques, such as focused ultrasound (FUS) and convection-enhanced delivery (CED) discussed below.

In the realm of GBM immunotherapy, significant progress has been made with numerous potential targets, including CD47 and its ligand SIRPa, PD-1 (programmed cell death) and its ligand PD-L1, CTLA4 (cytotoxic T-

lymphocyte-associated antigen 4), TIM3 (T-cell immunoglobulin and mucin-domain containing 3), and others (Hu et al., 2020; Zhulai and Oleinik, 2022; Scheffel et al., 2020; Togashi et al., 2019; Topalian et al., 2015). These targets represent opportunities to inhibit immune checkpoints. However, for GBM, the clinical outcome has been less promising. For example, Nivolumab (Anti-PD-1) showed minimal to no effect across three Phase 3 clinical trials involving various patient groups (Wang et al., 2019; Yang et al., 2021b). Additionally, CD47 is highly expressed not only on cancer cells but also on healthy red blood cells and platelets, potentially leading to anemia and thrombocytopenia (Oldenborg et al., 2000). Ipilimumab (anti-CTLA4) has shown promise as a checkpoint inhibitor in melanoma therapy, but in the case of GBM, only two Phase 2 trials with published results reported a median overall survival of no longer than 7.7 months when combining anti-CTLA4 and anti-PD-1 therapies, although they displayed few side effects (NCT02794883 and NCT03367715).

This brings us to the issue of toxicity, a critical consideration in therapy development. Toxicity can manifest in various ways, including nausea, vomiting, diarrhea, headache, fever, fatigue, rash, and, importantly, hematologic and neurologic side effects (Lawrence et al., 2011). Hematologic side effects are meticulously examined and taken into account when conducting clinical trials. A review of several Phase 1 clinical trials for GBM therapy reveals a spectrum of adverse effects in patients, ranging from high mortality rates and gastrointestinal or neurological disorders in some cases to minimal or no side effects in others. The latter can often be easily corrected with standard medications (NCT03661723, NCT01280552, NCT01310868, NCT02336165, NCT01846871, NCT02342379, NCT02586857, NCT02315534, NCT00323115).

Furthermore, radiotherapy plays a significant role in GBM therapy by suppressing cancer cell proliferation and metabolism. However, it also poses certain risks. First, it induces double-strand breaks (DSB) in DNA, potentially leading to cell death (Marková et al., 2015). This effect occurs in both normal and cancer cells, particularly when the DNA damage repair system fails to function properly (Kim et al., 2019; Kesari et al., 2011). Second, despite the apparent efficacy of radiotherapy, the tumor microenvironment (TME) is organized in a way that allows some cells to escape the therapy effects, with most of the radiation being absorbed by recruited cells such as cancer-associated fibroblasts and T-regulatory lymphocytes (Wu et al., 2023; Awada et al., 2023).

Given these challenges, the glioma treatment warrants a more direct and precise approach with minimal side effects, extended overall survival, and specific targeting. This is where oncolytic viruses (OVs) come into focus.

Mechanisms of Action of Oncolytic Viruses (OVs)

In stark contrast to standard therapies, one of the most significant advantages of oncolytic viruses (OVs) lies in their ability, either naturally or through genetic modification, to target cancer cells while sparing healthy tissue (Islam et al., 2020; Singh et al., 2012).

An OV is essentially a virus, either wild type or genetically engineered, that can be harnessed to selectively target and eliminate cancer cells or serve as a carrier for gene therapy. The history of OVs dates back to 1922 when Levaditi and Nicolau first demonstrated the impact of the vaccinia virus on various animal tumor models (Levaditi and Nicolau). In the context of brain cancers, the initial attempt to employ OVs for GBM treatment occurred in 1991 when Martuza et al. utilized a genetically engineered TK-negative HSV-1 strain called DLSPTK in both *in vitro* and *in vivo* research (Martuza et al., 1991). Subsequently, a phase I clinical trial involving an oncolytic Herpes simplex virus (oHSV) in human subjects was conducted between February 1998 and May 1999 (Markert et al., 2000). However, as of now, Teserpaturev/G47Δ (marketed as Delytact®) remains the first and only OV approved by the Japanese Ministry of Health, Labour and Welfare (MHLW) for GBM treatment (Frampton, 2022). Teserpaturev represents an enhanced version of the second-generation G207 virus, incorporating a third mutation (α47 deletion) (Frampton, 2022). Numerous OVs are currently undergoing clinical trials, spanning a range of viruses, including herpesvirus, adenovirus, poliovirus, reovirus, among others. Extensive data on these OVs and their utilization in clinical trials is available in various

published sources (Hamad et al., 2023).

The fundamental mechanisms by which OVs act on cancer cells are briefly described below. Broadly, OVs can be categorized into two groups:

1. Replication-Competent OVs (lytic viruses): These OVs can actively replicate within cancer cells, leading to their destruction and the release of viral progeny that can infect neighboring cancer cells, thereby amplifying the antitumor effect.

2. Replication-Deficient or Non-Lytic OVs: These serve as vectors for transporting target genes. While they are engineered to lack the replicative ability, they can carry and deliver therapeutic genes specifically to cancer cells, facilitating targeted gene therapy. It's worth noting that replication-competent OV HSV is also used for delivering the HSV-tk gene (Luo et al., 2007).

The mechanisms of OV action in cancer treatment encompass the following:

1. **Oncolytic Virus Therapy**: OVs can selectively infect and replicate within cancer cells, resulting in their lysis and destruction. This targeted oncolysis is a primary mechanism of tumor cell death (Kaufman et al., 2015). OVs are characterized by their infection and subsequent replication within cancer cells, leading to tumor lysis through various mechanisms, including apoptosis, pyroptosis, and necroptosis (Lin et al., 2023). Once OVs enter a cancer cell and replicate, they induce cell lysis and activate the host immune response. This activation attracts antigen-presenting cells (APCs) to the site of the cancer cell due to release of various signaling molecules and cellular components:
    1. Tumor-Associated Antigens (TAAs) are specific molecules present on the surface of cancer cells that act as signals to alert the immune system (Kaufman et al., 2015).
    2. Cell-Derived Damage-Associated Molecular Patterns (DAMPs) are released from damaged cancer cells serving as danger signals to immune system. Examples include calreticulin, ATP, and uric acid (Kaufman et al., 2015).
    3. Viral Pathogen-Associated Molecular Patterns (PAMPs) originate from OVs themselves, including the viral capsid, DNA, single-stranded RNA (ssRNA), self-amplifying RNA (saRNA), and viral proteins. PAMPs function as additional danger signals that trigger the immune response (Bommareddy et al., 2018).
    4. Inflammatory Factors and Chemokines: OVs can stimulate the production of inflammatory factors and chemokines during cancer cells lysis, further contributing to immune response (Lin et al., 2023).

2. **Gene Therapy:** OVs serve as vectors for delivering specific transgenes.

OVs serve as vectors to deliver specific transgene sequences into cancer cells. These transgenes comprise suicidal genes, genes encoding cytokines and their receptors, tumor suppressors, among others. Their functions range from promoting cell death and inhibiting tumor growth to stimulating the immune response against cancer cells or sensitizing tumors to other therapeutic interventions.

a) Delivery of Immunomodulatory Genes

OVs not only exert direct effects on tumor cells but also can significantly modify the tumor microenvironment. By expressing particular immunogenic proteins, OVs have the capacity to convert a “cold tumor” into a “hot tumor” (Melcher et al., 2021). A “cold tumor” typically exhibits limited immune responsiveness, posing challenges for the immune system in recognizing and effectively targeting cancer cells. In contrast, a “hot tumor” has undergone successful immune cell infiltration, resulting in a robust immune reaction against cancer cells. This transformation instigates the immune response, drawing immune cells to the tumor site and ultimately leading to tumor cell demise. To achieve this, cytokine-armed OVs have been developed, with numerous cytokines employed for this purpose, including CCL2, CCL5, CCL19, CXCL11, FGF2, FLT3L, GM-CSF, and more. A comprehensive list of cytokine-armed OVs and their applications in clinical trials

are largely available (Pol et al., 2020).

Towards that end, an intriguing approach for brain tumor treatment was recently proposed by Tian et al. (2022). In their study, the research team engineered an oncolytic herpes simplex virus (oHSV) capable of expressing a secreted single-chain variable fragment of the epidermal growth factor receptor (EGFR) antibody (cetuximab), fused with the chemokine C–C motif ligand 5 (CCL5). This construct specificity was achieved by binding the cetuximab antibody to the EGFR receptor, which is frequently overexpressed in GBM cells. Specifically, cetuximab antibody was tethered to EGFR receptor on GBM cells. It was demonstrated that the administration of the oHSV-Cmab-CCL5 construct enhanced EGFR signaling while continuously expressing CCL5. Furthermore, the construct stimulated the migration and activation of natural killer (NK) cells, macrophages, and T cells. Overall, the administration of oHSV-Cmab-CCL5 extend the survival of tumor-bearing mice. These findings indicate that the combination of OVs with the cetuximab-linked CCL5 construct has a positive impact on the immune response, leading to improved survival outcomes in mice with tumors (Tian et al., 2022).

b) Suicide Gene Therapy

Both replication-competent and replication-deficient OVs can serve as vehicles for delivering genes that encode proteins capable of converting a drug into a cytotoxic prodrug, a technique known as “Suicide Gene Therapy.” In this approach, target genes are selected based on their ability to produce an enzyme, when expressed in cancer cells, that can transform a non-cytotoxic prodrug into a toxic agent. One notable advantage of this approach is the bystander effect, whereby the toxic metabolite can diffuse into neighboring cells through gap junctions, even if these cells are not directly subjected to gene therapy (Mesnil and Yamasaki, 2000).

Suicidal genes like Herpes simplex virus thymidine kinase (HSV-TK), cytosine deaminase (CD), and others exemplify genes whose products facilitate the conversion of a harmless chemical compound, known as a prodrug, into toxic agents that specifically target cancer cells (Karjoo et al., 2016).

Presently, there are 12 different enzymes utilized in gene therapy applications (Denny, 2003). In the subsequent section, we provide a succinct overview of the most extensively studied enzymes employed in suicide gene therapy experiments, employing both *in vivo* and *in vitro* models.

Thymidine kinases (TK) (EC. 2.7.1.21) derived from various viruses are widely employed in suicide gene therapy. Presently, the most extensively studied enzyme for suicide gene therapy is HSV-tk, sourced from herpes simplex viruses. The first application of the HSV-tk/GCV system dates back to 1991, and since that time, it has remained the most thoroughly investigated enzyme-prodrug system (Ezzeddine et al., 1991). HSV-tk facilitates the conversion of the non-cytotoxic prodrug ganciclovir (GCV) (NCT01913106 (Hasenburg et al., 1999),) into the toxic metabolite GCV-phosphate. This metabolite is further converted to GCV triphosphate by intracellular kinases. During the replication cycle in cancer cells, GCV triphosphate is integrated into their DNA. Of note, the alternative prodrugs for HSV-tk include acyclovir, valacyclovir, penciclovir, ganciclovir elaidic acid ester, (E)-5-(2-bromovinyl)-2′-deoxyuridine, zidovudine, and 2′-Exo-mrthanocarbathymidine. An interesting finding in a study by Hossain et al. demonstrated a significant survival advantage when valganciclovir (a ganciclovir prodrug) was administered over an extended period compared to long-term ganciclovir administration in an orthotopic xenograft model (Hossain et al., 2019). To mitigate the latent toxicity associated with wild-type TKs, a mutated A168H TK (TKA168H) has been introduced into SHED cells (Stem cells from human exfoliated deciduous teeth) to establish the therapeutic cells (Oishi et al., 2022). Other types of TK used in suicide gene therapy comprise the varicella-zoster virus (VZV) – TK (prodrug GCV, 6-methoxypurine arabinoside (ara-M) (Duarte et al., 2012)), Tomato-TK (Stedt et al., 2015), (Christiansen et al., 2015), and EHV4-TK (McSorley et al., 2014). In a study by Stedt et al., Tomato-TK (prodrug azidothymidine) exerted no statistically significant effect in a cell culture compared to the HSV-tk/GCV system. Nonetheless,

Christiansen et al. attempted to enhance the sensitivity of Tomato-TK to the prodrug Azidothymidine through mutagenesis (Stedt et al., 2015).

Cytosine deaminase (CD; EC 3.5.4.1) is another extensively studied enzyme for suicide gene therapy. This enzyme is found exclusively in bacteria and fungi and possesses the capability to convert the precursor 5-FC into the toxic agent 5-FU. It's worth noting that 5-FU received FDA approval as a drug in 1962 and has since been widely employed in various cancer treatments, both as a standalone therapy and in combination with other drugs for treating different types of cancer, including adenocarcinoma (Argilés et al., 2020), neck squamous cell carcinoma (Vermorken et al., 2007), colorectal cancer (Vodenkova et al., 2020), and breast cancer (Cameron et al., 1994).

CD facilitates the conversion of 5-FC into 5-FU, which is subsequently transformed into intracellular metabolites, including fluorodeoxyuridine monophosphate (FdUMP), fluorodeoxyuridine triphosphate (FdUTP), and fluorouridine triphosphate (FUTP). These active metabolites impact cancer cells through two distinct pathways. First, they downregulate thymidylate synthase activity, a critical component in DNA repair and replication. Second, they interfere with pre-mRNA splicing and pseudouridylation of U2 snRNA by incorporating metabolites of 5-FU into U2 snRNA. These processes collectively lead to the inhibition or termination of cancer cell division (Longley et al., 2003).

Various types of viruses have been employed in both *in vivo* and *in vitro* settings to deliver Yeast cytosine deaminase (yCD) into cancer cells, including oHSV (Yamada et al., 2012), Vaccinia Virus (Chalikonda et al., 2008), and adenoviral vectors (Liu and Deisseroth, 2006), all of which have demonstrated promising results. However, in clinical trials, only Toca511 has been evaluated. Toca511 (Vocimagene amiretrorepvec/flucytosine) is a non-lytic retroviral replication-competent murine leukemia virus encoding yCD. It showed promising results in early-phase (I-II) clinical trials for the treatment of high-grade glioma. Nevertheless, a randomized, open-label phase 2/3 trial in patients with recurrent high-grade glioma revealed no advantage over standard treatment, with the Toca 511/FC group exhibiting a median survival of 11.1 months compared to 12.22 months for patients receiving single approved therapies (SOC) (Cloughesy et al., 2020).

Other enzymes used in suicide gene therapy include Rabbit carboxylesterase (rCE)/irinotecan system, deoxycytidine kinase (dCK)/cytosine arabinoside (AraC) system, Purine nucleoside Phosphorylase (PNP), cytochrome P450(CYP), Thymidylate monophosphate kinase (TMPK), and Carboxypeptidase G2 (CPG2).

3. Combination of Oncolytic Virotherapy and Gene Therapy

Certain OVs can be genetically engineered to merge oncolytic virotherapy with gene therapy, generating a synergistic impact that enhances the overall treatment's effectiveness.

In the context of GBM therapy, numerous OVs have undergone genetic modifications to increase their capacity for selective interaction with and elimination of cancer cells. These alterations are designed to refine tumor targeting, amplify viral replication within cancer cells, and bolster the immune response against tumors, ultimately resulting in more potent treatment outcomes.

The combined effect of oncolysis and the release of the signaling molecules fosters a conducive environment for immune activation against cancer cells, culminating in a systemic antitumor immune response. This process can elevate the body's ability to identify and combat not only the infected cancer cells but also other cancer cells throughout the body, thereby augmenting the overall therapeutic effectiveness of oncolytic virotherapy.

The efficacy of OV therapy hinges not only on the inherent qualities of OVs—such as their tumor-targeting abilities, viral stability, potential pathogenicity, immunogenicity, and capacity to encode therapeutic transgenes—but also on the delivery method. The method of delivery plays a crucial role in treatment effectiveness, particularly for brain tumors, where the blood-brain barrier (BBB) and tumor microenvironment

(TME) pose significant challenges to achieving maximum therapeutic impact. Yet, there is currently no standardized protocol for OV delivery, whether intravenously, intratumorally, or by other means.

The ideal OV delivery method should be minimally invasive, patient-friendly, devoid of side effects, capable of circumventing the BBB, and amenable to repeated dosing. Additionally, the immunogenicity of a virus also influences the choice of delivery route. For instance, intratumoral administration benefits from highly immunogenic viruses, as they trigger robust antitumor immunity. In contrast, less immunogenic viruses may be more suitable for intravenous delivery, as they can persist in the body longer without provoking an immune response. However, achieving the perfect delivery method is challenging due to these multifaceted requirements. Selection depends on various factors, including virus type, tumor resectability, glioma malignancy, and customization for each specific case. Moreover, each delivery method presents its own set of advantages and disadvantages.

Table 1 presents an overview of various OV delivery methods for GBM tumor sites, delineating their respective pros and cons. Each method possesses unique strengths and weaknesses, and the choice of the most suitable approach is based on factors such as tumor location and specific OV attributes. Employing multimodal delivery techniques can further enhance treatment efficacy.

Below, we describe existing methods for delivering OVs to tumor sites and delve into their respective advantages and disadvantages. Fig. 1 illustrates the methods used in OV therapy *in vitro*, *in vivo*, and in ongoing clinical trials.

The treatment of glioblastoma employs various methods for delivering oncolytic viruses (OVs), including:

- ***Intratumoral Delivery:***
  a. Intratumoral Stereotactic Injection
  b. Post-operative Injection into the Resection Cavity
  c. Convection Enhanced Delivery (CED)
- ***Systemic Delivery:***
  a. Intravenous Administration
  b. Intra-arterial Administration
  c. Inhalation

a. Direct Intratumoral Injection

The intratumoral delivery method involves the direct injection of oncolytic viruses (OVs) into tumor sites using stereotactic techniques with intraoperative MRI guidance. This method was utilized in the pioneering experiment for GBM treatment *in vivo* with OVs therapy (Martuza et al., 1991). Despite the availability of alternative administration routes for OVs, such as intravenous or intra-arterial delivery, direct intratumoral injection remains the predominant strategy due to its precision in targeting OVs directly to the tumor site, minimizing the risk of affecting non-targeted organs and tissues (Macedo et al., 2020). An important advantage of intratumoral OVs delivery is its ability to bypass the blood-brain barrier (BBB). Therefore, regardless of the type of delivery, it is more reasonable to select OVs with enhanced immunogenicity through genetic manipulation or those with naturally higher immunogenicity. Direct injection into the tumor, compared to intravenous administration, allows for the circumvention of preexisting immunity and helps avoiding an antiviral immune response (Ricca et al., 2018; Groeneveldt et al., 2023; Marelli et al., 2018).

Notably, Teserpaturev (G47Δ), the first OV approved for GBM treatment in Japan, was also administered using intratumoral delivery. Despite the challenges associated with repeated injections, G47Δ was introduced into

the tumor up to six times over a five-month period, along with concurrent tumor biopsies. This approach demonstrated good tolerance and holds promise for future applications (Todo et al., 2022). To streamline the process, many clinical trials perform intratumoral delivery immediately after or simultaneously with a biopsy, often in the same coordinates (NCT03178032, NCT02798406, UMIN000015995). Safety and efficacy of this delivery method have been confirmed in numerous clinical trials involving oHSV, retroviral OVs, adenovirus, and others (a comprehensive list is provided in Table 1).

Stereotactic intratumoral delivery of OVs is particularly suitable for inoperable or recurrent GBM cases. In cases prior to surgical resection, it enables the attainment of the desired virus concentration in the tumor margin. For instance, in a recent phase 1/2 trial, patients with recurrent glioblastoma who did not undergo repeated surgical resection received a combined intratumoral injection of DNX-2401 alongside systemic administration of pembrolizumab. The study yielded an overall survival of 12.5 months, a promising outcome, especially considering that it marks the first study on brain tumors to combine direct oncolytic viral therapy with systemic checkpoint inhibition (Nassiri et al., 2023).

Intratumoral delivery, relying on simple diffusion, has the limitation of covering only a small volume of tissue with oncolytic viruses (OVs) after injection, which is a drawback of this delivery approach. To address this limitation, innovative methods have been developed. For example, in a Phase I trial conducted by Tejada et al., the OVs DNX-2401 were delivered using Alcyone's MEMS Cannula (AMC) Targeted Delivery System®, employing the 'aura-method.' This method is grounded in the use of a dual-channel, MRI-safe cannula. In this technique, a small dose of gadolinium was injected through the first independent channel, while DNX-2401 was administered through the second channel of the cannula. This approach allowed gadolinium to distribute around the periphery of DNX-2401, providing control over distribution and backflow through MRI monitoring (Tejada et al., 2018).

Another limitation of intratumoral delivery is its confinement to the tumor area and its immediate vicinity, as well as the challenge of reintroducing the virus. Additionally, when compared to intravenous delivery, intratumoral delivery is considered an invasive method. Nonetheless, as mentioned previously, it can be performed with minimal observed side effects (Todo et al., 2022).

b. Post-operative Injection in Resection Cavity

Intracavitary injection involves administering the virus into the wall of the resection cavity following the surgical removal of the tumor. This method, employed after direct intratumoral injection, is frequently utilized for delivering oncolytic viruses (OVs) to tumors.

This approach can result in higher concentrations of the virus, thereby enhancing the effectiveness of OV treatment. For instance, a phase I clinical trial assessed the safety of two inoculations of G207, an oncolytic herpes simplex virus (oHSV) with a deletion of γ134.5 and inactivated UL39. In this study, researchers employed a combination of pre-resection stereotactic delivery of G207 (2–5 days prior to surgery), followed by multiple injections of G207 immediately after surgical tumor resection. Notably, only one out of six patients enrolled in the study experienced side effects, and none developed HSV encephalitis. This study demonstrated the feasibility of combining these two methods, at least in the case of G207 (Markert et al., 2009).

c. Convection Enhanced Delivery (CED)

CED, initially developed in 1994 by Edward Oldfield's group at the National Institute of Health as a system for delivering macromolecules into the brain (Griggs et al., 2023), has become a valuable method for delivering oncolytic viruses (OVs) to brain tumors. The CED setup involves the stereotactic placement of one of several catheters within the tumor. These catheters are connected to a delivery pump that establishes a stable positive pressure gradient, enabling them to cover a larger volume of the tumor compared to other delivery methods (Mehta et al., 2017).

A decade after its development, CED was first tested in an animal model using AAV-TK (Cunningham et al., 2000). In addition to OVs, CED serves as a delivery system for various agents, including liposomes (Voges et al., 2003), conjugated toxins, and conventional chemical agents such as Paclitaxel (Lidar et al., 2004), Topotecan (NCT03154996), and TMZ (Enríquez Pérez et al., 2020), for GBM therapy (D'Amico et al., 2021).

The primary advantage of CED delivery in the context of oncolytic virotherapy is its ability to bypass the blood-brain barrier (BBB). Furthermore, the stable positive pressure gradient employed in CED allows for broader virus dispersion over a larger area. The convenience of re-dosing, facilitated by implantable catheters that enable repeated drug delivery at intervals of up to 1 month (Bienemann et al., 2012; Barua et al., 2013), adds to the attractiveness of this delivery method for oncolytic viral therapy.

Recent studies have investigated the application of OVs like Delta24-RGD, demonstrating the safety of prolonged CED techniques using Delta24-RGD in recurrent GBM (van Putten et al., 2022). Clinical trials utilizing CED include those involving PVSRIPO (Desjardins et al., 2018), NCT02986178, NCT03043391, NCT04479241, NCT04599647), Toca 511+Toca FC (Aghi et al., 2014), and a G207 variant of HSV-1 for pediatric glioma (Friedman et al., 2021).

Despite the numerous advantages offered by convection-enhanced delivery (CED), it's important to acknowledge that the complexities involved in the installation process may lead to potential side effects. Furthermore, despite the application of CED in various pre-clinical and clinical studies, a standardized CED protocol has yet to be developed. One significant drawback of CED is backflow, where fluid moves in the opposite direction from the target infusion site back to the source, potentially reducing the effectiveness of drug delivery. However, these challenges can be addressed through the development of new types of catheters and the implementation of reduced catheter insertion speeds (Casanova et al., 2014).

A comprehensive study conducted by Shahar et al. aimed to assess and summarize the potential effects of CED delivery. The study analyzed medical charts and relevant neuroimaging data from 25 patients who received a total of 64 CED catheters in 29 catheter placements, evaluating all adverse effects following catheter placement. The observed side effects included increased edema (31%), infection (6.9%), bleeding (6.9%), and seizures (13.8%). Additionally, 4 patients (13.8%) experienced significant neurological deterioration. Interestingly, edema did not occur in patients who had a single catheter implanted, but its likelihood increased with the number of catheters used (Shahar et al., 2012).

In summary, despite its disadvantages, CED for OVs stands out as one of the most powerful delivery methods, with a notable advantage being the direct delivery of OVs into the brain. The pressure gradients employed enable the even distribution of OV solutions in large volumes. A promising clinical trial involving CED was conducted by Feres and colleagues, demonstrating that CED with NSC-CRAd-S-pk7 enabled the distribution of high drug concentrations without causing systemic toxicity. The trial reported a median progression-free survival of 9.1 months and a median overall survival of 18.4 months (Fares et al., 2021).

a. Intravenous Administration

Intravenous administration involves the injection or infusion of a drug directly into the peripheral vein of a patient using a syringe or a specialized catheter through a needleless port (Kim and De Jesus, 2023). While theoretically allowing the virus to travel throughout the body to reach the target tissue where the tumor has developed, intravenous delivery, despite the convenience and safety, is infrequently used for delivering oncolytic viruses to brain tumors due to several limitations. One significant challenge is the blood-brain barrier (BBB), which limits the ability of oncolytic viruses to penetrate the brain tissue. Additionally, the accumulation of oncolytic viruses in unintended off-target tissues and the potential for virus degradation within circulation or non-tumor tissues along the way can significantly reduce the efficacy of intravenous delivery.

Activation of the innate immune response presents another hurdle for intravenous OVs delivery. Macrophage-mediated phagocytosis of the virus, for example, can impede the spread of OVs (Marelli et al., 2018). Furthermore, pre-existing immunity plays a critical role in determining the effectiveness of intravenous delivery, as viruses have to travel a considerable distance within the bloodstream to reach the tumor, especially in the case of brain tumors. Neutralizing antibodies (NAbs) against well-known oncolytic viruses such as Adenovirus type 5 (Ad5) and HSV-1 are widespread in the human population (Groeneveldt et al., 2023). In summary, intravenous delivery of OVs is likely to be more efficient in patients without pre-existing NAbs. For example, a Phase I study by Russel et al. demonstrated the efficacy of intravenous treatment with oncolytic Measles virus, with the crucial factor being that both patients in the study were without NAbs (Russell et al., 2014).

However, in another study conducted by Adair and colleagues involving oncolytic reovirus in patients with colorectal cancer, it was shown viruses delivered intravenously can reach the tumors and replicate there even in the presence of NAbs (Adair et al., 2012). In the context of GBM treatment, a clinical trial phase Ib (ReoGlio) was conducted, evaluating the effect of intravenous delivery of Reolysin along with subcutaneous GM-CSF on GBM patients (ISRCTN70044565). The results showed a median overall survival of 12.6 months in dose level 1, and 16.1 months in dose level 2, with a median overall survival of 13.1 months for all patients. Importantly, no serious adverse effects were registered.

Of note, intravenous delivery of RNA viruses, in contrast to DNA viruses, may offer greater practicality due to a reduced likelihood of encountering pre-existing immunity, especially for limited treatment durations (Bommareddy et al., 2018). However, viruses with lower immunogenicity, such as adenovirus or HSV, are often preferred because they can persist for longer periods in the body without being rapidly cleared by the immune system (Bommareddy et al., 2018).

To achieve a better tumor penetration via intravenous delivery, where a significant portion of the virus is rapidly cleared by the liver, spleen, and other organs, the repeated administrations become essential for enhancing the effectiveness of OVs treatment. However, it's essential to note that repeated administrations could potentially, on the contrary, reduce the efficacy of OVs therapy. For instance, studies have shown that prior intravenous administration of VSV in BALB/c mice before OVs therapy affected transgene expression, in contrast to naive animals where sustained expression of VSV-gfp was reported. Additionally, the high immunogenicity of the virus and the production of neutralizing antibodies are significant drawbacks associated with intravenous administration (Evgin et al., 2015).

To address the challenges related to immune clearance during intravenous OVs delivery, several strategies have been proposed, including combination with immune inhibitors, encapsulation in liposomes and nanopolymers, and the use of cellular delivery systems (Tang et al., 2023). For example, in a recent study, Huang and colleagues utilized erythrocyte-lipid hybrid membrane vesicles (erythroliposomes) to coat OVs, enhancing the efficacy of intravenous delivery. This approach demonstrated that erythroliposome-camouflaged ad11 induced a robust immune response within tumors while minimizing systemic inflammatory responses (Huang et al., 2022).

As previously mentioned, most OVs encounter challenges in bypassing the BBB. Nevertheless, there are exceptions, including reovirus, Seneca valley virus, poliovirus, NDV, and Parvovirus H, which have demonstrated the ability to penetrate the BBB (Kaufman et al., 2015). Based on this capability, there have been successful clinical trials employing OVs treatment and involving intravenous delivery.

For instance, a Phase I/II trial focusing on recurrent GBM demonstrated the well-tolerated intravenous administration of the NDV virus (Freeman et al., 2006). In the initial dose-escalation clinical trial of ParvOryx01, a combination of intratumoral and intravenous delivery methods was applied. This study not only affirmed the safety and tolerability of ParvOryx01, but also demonstrated its capacity to traverse the

bloodstream and reach brain tumors by bypassing the BBB (Geletneky et al., 2017). Another significant example of OVs traversing the BBB was illustrated in a preclinical study led by Samson and colleagues, showcasing the reovirus's ability to overcome this barrier. Additionally, the study revealed that reovirus could stimulate the expression of PD-1 and PD-L1, thereby enhancing the effectiveness of immune checkpoint inhibitor (ICI) therapy through an interferon-based mechanism (Samson et al., 2018).

In light of the mentioned challenges, intravenous delivery may not be the most straightforward method for directly delivering OVs to the tumor site. However, intravenous administration plays a pivotal role in the context of suicide gene therapy, as previously described. Intravenous delivery, along with oral administration, is commonly employed for delivering prodrugs such as acyclovir, ganciclovir, valacyclovir, and others to brain tumors. Detailed information regarding intravenous delivery of prodrugs for suicide gene therapy have been thoroughly reviewed elsewhere (Tamura et al., 2021).

b. Intra-arterial

Intra-arterial (IAA) administration involves the direct delivery of drugs into a patient's artery, typically a carotid or vertebral. This method of delivery can be employed through various techniques, including arterial catheterization, direct injection via a syringe, or application of specialized drug delivery systems. The earliest attempt at intra-arterial delivery for GBM treatment dates back to 1950. IAA has garnered interest as a promising delivery method due to its potential advantages, as demonstrated in a study by Tyler et al., in which a 50-fold increase in substance concentration within tumor tissue compared to intravenous administration has been reported (Tyler et al., 1986).

Endovascular selective intra-arterial (ESIA) and endovascular superselective intra-arterial infusion (ESSIA) represent advancements in IAA delivery techniques. These methods have found success in the retinoblastoma treatment using chemotherapy (Ravindran et al., 2019). ESIA, in particular, has shown impressive results, with a reported 100-fold increase in substance concentration within tumor tissue compared to intravenous administration (Daniels et al., 2018). These approaches employ superselective microcatheterization, enabling the precise introduction of substances into specific arteries, ensuring targeted delivery to tumor cells while minimizing the impact on the surrounding healthy tissues. Notably, ongoing research is currently exploring the potential of perfusion-guided ESIA injections involving mesenchymal stem cells (MSCs) loaded with the oncolytic virus Delta-24 through the left posterior cerebral artery in a phase I trial (NCT03896568) (Chen et al., 2022). In summary, IAA, especially with the advent of ESIA, represents a less common yet promising delivery method.

Its advantages include:

- Precise delivery of virus to tumor site
- Possibility of re-dosing or repeat delivery
- Potential for combination with MSCs or other cell carriers to enhance efficacy

However, it is a more complex procedure compared to intravenous administration, requiring skilled personnel for manipulation. Additionally, the feasibility of repeated delivery remains more challenging than in the case of intravenous administration.

c. Inhalational Delivery Method

The inhalational delivery of oncolytic viruses was initially explored in a phase II/B, a placebo-controlled clinical trial involving 26 patients across multiple centers. In this trial, conducted by Csatry et al., the Newcastle disease virus (NDV) vaccine MTH-68/N was used to treat 33 patients with advanced cancer, with NDV administered via inhalation twice weekly (Csatary et al., 1999). After two years of treatment, all seven survivors belonged to the virus therapy group. However, in a subsequent study focused on high-grade gliomas

utilizing MTH-68/H, authors noted the superiority of intravenous administration of MTH-68/H over the inhalation route (Csatary et al., 2004).

Another example of inhalation-based OV administration was described by Wagner et al., in 2006. This case involved a 12-year-old boy diagnosed with anaplastic astrocytoma, who received inhalation administration of the NDV virus in vaporized form (Wagner et al., 2006). Despite the convenience associated with this delivery method, such as ease of administration, it remains the least studied and least practiced option for delivering OVs.

Section snippets

Conclusion

Current therapies for glioblastoma are beset by limitations, with long-term remission proving elusive due to residual tumor cells following treatment and genetic mutations. Therapies employing anti-PD1 or anti-PD-L1 antibodies hold promise, particularly when used in conjunction with radiotherapy and/or TMZ, rather than as a standalone treatment. However, it's important to note that such a combination can have potentially detrimental effects on the patient's health. Therefore, there is a…

Future perspectives

OVs could be considered as a truly novel and revolutionizing therapy approach. It's landscape of mechanisms encompass many possible ways of targeting GBM starting with regular apoptosis induction and finishing with immune system modulation. To take into consideration, OVs clinical trials are also of great number, what further points out their importance. And despite many arguments on the delivery topic intratumoral delivery could be the best option modern medicine has. While intravenous and…

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A. Romanishin: Writing – review & editing, Writing – original draft, Visualization, Validation, Funding acquisition, Conceptualization. **A. Vasilev:** Writing – review & editing, Writing – original draft, Visualization, Funding acquisition, Conceptualization. **E. Khasanshin:** Formal analysis. **A. Evtekhov:** Formal analysis. **E. Pusynin:** Formal analysis. **K. Rubina:** Writing – review & editing, Validation. **V. Kakotkin:** Validation, Data curation. **M. Agapov:** Validation, Data curation. **E. Semina:** Writing –…

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.…

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