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Herpes Simplex Oncolytic Viral Therapy for Malignant Glioma and Mechanisms of Delivery

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Abstract

The authors present a comprehensive review on the history and development of oncolytic herpes simplex viral therapies for malignant glioma with a focus on mechanisms of delivery in prior and ongoing clinical trials. This review highlights the advancements made with regard to delivering these therapies to a highly complex immunologic environment in the setting of the blood-brain and blood-tumor barrier in a safe and effective manner.

Keywords

Magnetic resonance-guided focused ultrasound; Malignant glioma; Oncolytic viral therapy

HISTORY OF VIRAL THERAPY

The concept of viral therapy for cancer dates back over a century when DePace described the regression of cervical cancer in a patient after receiving Pasteur's attenuated rabies vaccine following a dog bite.[1] Years later, the same vaccine was used to treat 30 patients with melanomatosis; 8 of these patients showed evidence of tumor regression.[2] In addition, reports surfaced of patients with Burkitt and Hodgkin lymphoma showing regression after a naturally occurring infection with the measles virus.[3] Progress was eventually halted in the face of adverse reactions to virotherapy as well as to advancements in chemotherapy.[4] Over the past 30 years, advancements in molecular virology have led to the revival of these therapies with diminished side effect profiles.

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CRediT AUTHORSHIP CONTRIBUTION STATEMENT

Nicholas J. Erickson: Data curation, Writing – original draft. **Mihaela Stavarache:** Data curation, Writing – original draft. **Ibrahim Tekedereli:** Writing – original draft, Writing – review & editing. **Michael G. Kaplitt:** Conceptualization, Funding acquisition, Supervision, Writing – original draft, Writing – review & editing. **James M. Markert:** Conceptualization, Funding acquisition, Resources, Supervision, Writing – original draft, Writing – review & editing.

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Viral therapy can be separated into 2 major and distinct groups: 1) replication-competent oncolytic viruses (OVs) and 2) replication-deficient viral vectors used solely as a delivery mechanism for therapeutic genes.[4] For the purposes of this review, we will focus on OVs, specifically on oncolytic herpes simplex virus type 1 (oHSV).

The ability of OVs to specifically infect and destroy tumor cells is at the cornerstone of their efficacy. The nature of the cancer cell, which allows aberrant genetic material to replicate, can also allow unchecked replication of the OVs since normal host cell mechanisms for antiviral defense are often lost as well. Viral proteins produced by the infected cell can serve as antigens which stimulate a systemic antiviral immune response. Genetically "armed" viruses expressing exogenous (so-called foreign) genes coding for immunogenic molecules —such as cytokines and immunostimulatory cell surface proteins—can further enhance this response. After completion of the viral replication cycle, the infected tumor cell lyses, releasing viral progeny locally, which then can infect susceptible neighboring tumor cells and the process repeats.[5] Even unarmed OVs are also capable of inducing antitumor immune responses, as a normal immunologic response to viral infection. Exposure of tumor-specific antigens to the immune system after cell lysis may play a large role in the antitumor response, allowing so-called epitope spreading to occur. Such epitope spreading can result in the development of an immune response against tumor-specific antigens; however, the exact mechanisms of such responses are still being identified.[6] While the subject of this review is oHSV, it should be noted that other genetically modified viruses that have completed or are in ongoing early phase clinical trials include adenovirus (Ad), reovirus, paramyxovirus, Newcastle disease virus, measles, poliovirus, and parvovirus. Excellent reviews discussing completed and ongoing clinical trials incorporating these viruses are available elsewhere.[7,8]

Herpes simplex virus type 1 (HSV-1), perhaps the best studied of the OVs, is a large (152 kb) double-stranded DNA virus capable of establishing both active and latent infection in humans. Other properties, including a natural neurotropism, high transduction efficiency, and large transgene capacity, combine to make it a particularly useful OV for treating central nervous system (CNS) diseases.[9] In 1991, Martuza et al. described a HSV-1 thymidine kinase (tk)-deleted mutant, *dl*sptk, which did not replicate in quiescent cells such as neurons and demonstrated its ability to shrink gliomas and increase survival in a murine model of glioma.[10] This variant relied on actively dividing cells to supply tk *in trans* for viral DNA replication, and ultimately, viral replication. Despite promising preclinical results, the absence of viral tk (a target of antiviral therapy) rendered it resistant to acyclovir and ganciclovir resulted in the decision not to advance it into clinical trials,[11] although the virus remained sensitive to less commonly used antivirals such as foscarnet and vidarabine. Further advancements in genetic engineering have allowed for the neuroattenuation of HSV-1 mutants while maintaining their propensity for conditional replication in actively dividing cells and sensitivity to the antivirals acyclovir and ganciclovir, as well as foscarnet and vidarabine.[12–15] A notable example is talimogene laherparepvec (Imlygic/T-VEC, Amgen),[16] an HSV-1 mutant armed with granulocyte macrophage colony-stimulating factor and approved by the Food and Drug Administration in 2015 to treat Stage IIIB/IV metastatic melanoma. This approval was a major milestone for the field of oncolytic virotherapy as a whole and highlights the immunotherapeutic anticancer potential of this treatment modality.

Additionally, DELYTACT, or oncolytic HSV known as G47Δ, has been conditionally approved in Japan for malignant glioma based on the results of a Phase 2 study.[17]

MALIGNANT GLIOMA

The National Cancer Institute estimates the annual incidence of CNS malignancies to be 25,000, with almost 19,000 resulting deaths in 2023 (https://seer.cancer.gov/statfacts/html/brain.html). Glioblastoma is the most common primary CNS malignancy and carries a poor prognosis despite the current standard of care which includes maximal surgical resection followed by radiation with concomitant temozolomide (TMZ).[18] The tumor's infiltrative nature and persistent growth in an immune-privileged organ, along with increased resistance to current therapies that develop over time, contribute heavily to its grim prognosis. Median survival is just more than 14 months and is characterized by progressive neurologic deterioration and resulting decline in health-related quality of life.[19] The desperate need for more effective therapies for this disease is well established and cannot be overstated.

Advancements in the fields of cancer and immunology highlight the importance of effectively arming the patient's immune system when treating this disease. Although this review focuses on oHSV, other immunotherapy modalities including other OV, peptide vaccines, dendritic cell vaccines, chimeric T-cell therapy, and immune checkpoint inhibitors also aim to achieve this goal. Despite these ongoing efforts, the intricate interplay between cancer cells and the host immune response has remained an overwhelming challenge.

Clinical Trials of OV for Malignant Glioma

Since *dls*ptk was described back in 1991,[10] several phase I clinical trials using genetically engineered HSV-1 mutant G207 have demonstrated safety in patients with recurrent glioblastoma multiforme (GBM). The first trial, published in 2000, was performed by stereotactically injecting the modified virus, with 5 separate enhancing foci chosen by the surgeon injected at the highest dose level. The patients were observed for 4 days in the intensive care unit and a magnetic resonance imaging (MRI) was obtained prior to discharge. There were no clinical or radiographic signs of encephalitis at doses up to 3×10^9 plaque-forming units (pfu).[20] The second trial enrolled 6 patients and used a 2-dose regimen with interval resection of the GBM. Patients received 2 doses totaling 1.15×10^9 pfu as follows: 13% of the total was initially injected stereotactically via a catheter placed within the tumor. Two or 5 days later, the tumor was resected e*n bloc* with the catheter in place and the remaining G207 dose was injected into the tumor-infiltrated brain surrounding the resection bed. No patient developed HSV encephalitis; however, 1 patient developed transient fever with hemiparesis that resolved with high-dose steroids. The Principal Investigator attributed this adverse event to inadvertent transit of the virus and its associated vehicle into the ventricular system. High-dose steroids were administered but no antiviral agent and the patient quickly (<36 hours) recovered. The reported findings noted both radiographic and neuropathologic evidence of antitumor activity.[21] The third trial of G207 consisted of injecting a single dose of G207 at 1×10^9 pfu into the enhancing portion of the tumor followed by the administration of 5 Gy of radiation within 24 hours. This was the first clinical trial to demonstrate the safety of adjuvant radiation following oncolytic

therapy using an HSV-1 mutant.[22] Three additional trials using a different HSV-1 mutant HSV1716 have been successfully conducted in the United Kingdom.[23–25]

Friedman et al. very recently reported the results of a phase I trial at the University of Alabama at Birmingham using a continuous infusion of G207 in pediatric patients with biopsy confirmed recurrent or progressive supratentorial gliomas. The group reported that intratumoral delivery of G207 either alone or in conjunction with radiation was associated with minimal low-grade toxicity along with neuropathological antitumor responses in many of the children.[26] Compared to historical controls, the trial demonstrated an approximate doubling of survival of this population, although the sample size was small. A phase 2 clinical trial is forthcoming (ClinicalTrials.gov number, NCT04482933).

Newer HSV mutants, M032 and G47Δ,have been designed to enhance the immunogenicity of the tumor, and thus increase the antitumor immune response. M032 combines the oncolytic effects of the oHSV with immune-mediated mechanisms by also serving as a gene therapy vector. It is an HSV mutant expressing a foreign gene which encodes for both subunits of human interleukin-12, a cytokine crucial for T cell–mediated immunity. The success of preclinical studies has led to a phase I clinical trial at the University of Alabama Birmingham (ClinicalTrials.gov number, NCT02062827) seeking to determine the maximum tolerated dose in patients with recurrent or progressive GBM, anaplastic astrocytoma, and gliosarcoma.[27]

G47Δ is an HSV mutant constructed based upon G207 by an additional gene deletion resulting in enhanced major histocompatibility complex I presentation and viral replication.[28] Recently, Todo's group published the results of a single-arm phase II study of patients with recurrent GBM as well as a phase I/II study of the same virus, administered under different protocols. The 12-month survival in recurrent patients was nearly 85% when administered under a 6-dose protocol, but only 34% when dosed in a 2-dose protocol. Thus, the therapy showed exciting potential, but required multiple surgeries, each with a risk of hemorrhage and infection, to achieve optimal results.[29]

The impact of the immune response after the administration of OVs remains controversial, as clearly the virus produces an immune response that can limit viral spread but also that can produce antitumor effects. The initial response to OV administration may be significantly impacted by the route of administration.[5]

Additional trials of oHSV for glioma that are still in progress include C134, a chimeric oncolytic HSV that is deleted for $\gamma_1$34·5, the HSV-1 gene responsible for neurovirulence, but includes in its stead, the human cytomegalovirus gene homolog, IRS-1 gene (ClinicalTrials.gov NCT03657576) which serves as a PKR evasion gene but does not produce neurovirulence as does the native ICP 34·5 protein, and M032 in combination with immune checkpoint inhibitor Pembrolizumab (clinicaltrials.gov NCT05084430) and rQNestin34.5v.2, which, like C134, increases the replication ability of the oHSV by including the $\gamma_1$34·5, but under the control of the nestin promoter. Each of these trials uses direct inoculation of the virus under study into the tumor or the tumor bed adjacent to the tumor. The group developing rQNestin34·5v2 (renamed CAN-3110) and showed

the preclinical safety of this oHSV1 without any significant adverse events in a phase I clinical trial with recurrent high-grade glioma or recurrent glioblastoma (clinicaltrials.gov NCT03152318).[30] The study concluded that CAN-3110 was safe and had no dose limiting toxicity. Additionally, it led to an increase in T cells in tumor microenvironment which was linked to improved survival along with the pretreatment HSV-1 serologic status. Survival was related to T cell clonotype metrics such as T cell fraction and T Cell Receptor β diversity as well as tumor immune signature.[30]

Host Immune Response and Route of Delivery

Depending on the route of administration, OVs face various immunologic obstacles. If administered intravenously, virus may adhere to endothelium, become neutralized by the complement cascade,[31] or engulfed by circulating antibodies and leukocytes.[32] In addition, the reticuloendothelial system of the liver and spleen act to filter out circulating virus.[33] Even if a small proportion of selectively replicating virions reach the tumor-feeding vessel, it must transit the vessel wall more challenged in glioma with its blood-tumor barrier (BTB) and traverse the extracellular space to reach the tumor cells. Although intravenous delivery method is inexpensive and a familiar approach, the above challenges, in addition to the systemic response from the large doses required to provide an effective inoculum, make this delivery route particularly challenging for an oHSV approach to treating GBM.[5]

Intra-arterial administration eliminates some of the systemic immunologic challenges faced with intravenous delivery, although studies in rodents have suggested that circulating complement and NK cells can still be a factor.[34] Using this technique, OV can be delivered selectively to a particular organ or tumor. In addition, dwell time can be optimized with the use of occlusive balloons and embolic devices. The nature of the virus to selectively replicate within tumor cells combined with selective vessel injection largely eliminates the risk of off-target delivery. Angiography and selective arterial access for this application is more expensive than intravenous administration, riskier, and not currently reimbursed by insurance which has limited its use in clinical trials to date.[5]

There remains significant uncertainty regarding the optimal method of delivery for OVs in the treatment of glioma. To date, in all clinical trials assessing HSV-1 in glioma as a selectively replicating vector, administration has been performed by direct intratumoral or intraparenchymal injection or infusion (Figure 1). Early trials targeted vector injections at the enhancing tumor mass on MRI.[20,24,25] Despite demonstrating safety, there is currently no conclusive evidence of significant vector distribution or treatment efficacy with this route of administration.[35] The uneven response to treatment seen in these trials, as well as the increased efficacy seen in G47Δ when administered at 6 different locations over time, has sparked significant interest into the role of the host immune response, and the impact of methods of delivery and their implications on viral replication and spread through tumor.

The discrepancy between the success of viral replication in glioma cells *in vitro* compared to *in vivo* has been attributed to interference by the host immune system. The adaptive immune response following viral lysis of glioma cells is thought to be beneficial, serving as an anticancer vaccination effect. The initial innate immune response after vector delivery, however, could impede the efficacy of treatment. Intravenous HSV administration is subject

to complement mediated destruction, while intratumoral delivery can lead to neutralization by microglia ($CD68^+$), macrophages ($CD163^+$), and interferon-gamma produced by natural killer cells, as well as type I or antiviral interferons.[36,37] Some of this NK-mediated antiviral activity has been shown in preclinical models to be suppressed by pre-administration of cyclophosphamide[38] which may lead to enhanced viral replication, oncolysis, tumor-specific immune responses, and a reduction in the HSV dose required to achieve these effects.[39–42] More importantly, cyclophosphamide administration did not seem to hamper the adaptive response developed against the tumor at a later timepoint.[37] These results may support transient inhibition of the initial innate immune response as a mechanism of improving the efficacy of OV therapy. Despite these promising results in nonhuman models, concern has been raised regarding intratumoral injection. Directly injecting a viral vector which relies on actively replicating tumor cells into a glioma characterized by areas of necrosis may limit its efficacy. In addition, the primary tumor mass is often surgically resected, limiting the amount of tumor antigens available to incite an antitumor immune response. Interestingly, 80% of malignant gliomas recur within 3 cm of the original tumor site[43] making this target a feasible location for injection. Markert et al.[21] and Harrow et al.[23] demonstrated the safety of peritumoral injections of oHSV in patients undergoing resection of malignant glioma. Irrespective of the route of administration, the optimization of viral distribution remains critical to successful transduction of infiltrating tumor cells and the efficacy of OV therapy. Despite the considerable number of preclinical studies incorporating direct injection of OVs into the brain for various pathologies, there is a paucity of literature on the assessment and optimization of their distribution, likely due to the lack of preclinical large animal models of the disease. In 2003, a phase I trial used peritumoral injections of an adenoviral vector and found a mean distribution of only 4·9 mm from the needle tip[44] highlighting the inefficiency of simple injection on viral distribution, at least of this nonreplicating vector. In addition, concern has been raised regarding the size of the most commonly injected vector, HSV-1. The diameter of HSV-1 vectors ranges from 120 to 300 nm,[45] while the average diameter of the extracellular space within the brain is around 38 to 64 nm.[46]This discrepancy suggests that achieving widespread, interstitial distribution of this vector may prove unattainable based on size alone. A preclinical study in 2011 by E White et al.[47] was the first to systematically evaluate and attempt to optimize the delivery of an HSV-1 vector using a convection-enhanced delivery (CED) approach, and this work did demonstrate increases in delivery but required administration of heparin sulfate. The anticoagulant effects of this agent make it undesirable for human trials.

White et al. used an HSV-1 viral construct delivered by CED and examined the distribution in both gray and white matter. CED appears to address the shortcomings in achieving widespread distribution by simple injection and represents a promising new strategy for the administration of OVs into the peritumoral region of the brain.[48–50] It involves the use of fine catheters to distribute therapeutic agents into the extracellular space at carefully selected infusion rates under a constant pressure head. As opposed to relying on simple diffusion for drug distribution, CED allows for the homogenous distribution of macromolecules to large volumes of brain by bulk flow within a relatively short amount of time.[51] The study found that HSV-1 was too large for successful direct infusion by CED. However, if the tissue was preinfused with an isotonic solution of albumin prior to administering the vector, improved

distribution was achieved. These results suggest that the prior methods of delivery used by preclinical and clinical trials may have been hampered by insufficient viral distribution within the tumor.[47] The downside of CED, however, is the requirement for multiple passes of catheters to various locations within and adjacent to the tumor, which may have increased risk of hemorrhage. Additionally, if optimal treatment strategies involve multiple dosing schemes, administration of virus via multiple catheters becomes even more cumbersome and dangerous.

MAGNETIC RESONANCE-GUIDED FOCUSED ULTRASOUND

The delivery of therapeutic agents to glioblastomas and other primary brain malignancies has generally been hindered by the invasive nature of such tumors, the location of tumors within eloquent portions of the normal brain and the selective permeability of the blood-brain barrier (BBB)/BTB,[52] perhaps the most important limiting factor in the future development of neurotherapeutics. The presence of tight junctions in the BBB structure, while beneficial for the organism through their role in protecting the homeostasis of the CNS, decreases the bioavailability to the tumor site of potentially efficacious therapeutic agents. Although the BTB is “leaky” in the core part of the tumor, an intact BBB with fully functional tight junctions is still present in the invasive regions of most GBM tumors.[53] A novel approach to allow a safe, widespread distribution of therapeutic agents including OVs is sorely needed.

In recent years, a new technology, magnetic resonance-guided focused ultrasound (MRgFUS), has emerged as a viable option for targeted noninvasive treatment to specific areas in the brain.[54] MRgFUS has great potential, by allowing for the safe and widespread delivery of antiglioma agents, for improving quality of life and survival in these patients.

The acoustic energy delivered to the tissue by focused ultrasound (FUS) can generate either *thermal energy* that induces coagulative necrosis of the tumor or *mechanical energy* that generates stable or inertial cavitation. During stable cavitation, ultrasound waves, applied as short pulses at high acoustic power to minimize thermal increase in tissue, initiate rapid changes of pressure at the ultrasound focal point, causing the steady oscillation of intravenously injected small inert gas-filled microbubbles, acoustic microstreaming of fluid around the bubbles, and sheer stress. Mechanical energy generated during stable cavitation prompts the transitory local detachment of tightly sealed junctions of the BBB, allowing the passage of particles across the BBB. A high enough acoustic power will cause a violent collapse of the microbubbles, a phenomenon known as inertial cavitation, generating shock waves that can destroy tissue.[55,56]

Origins

The effects of acoustic power on tissue were first reported in 1942 when high-intensity ultrasound produced focal thermal lesioning in liver tissue with minimal effects at the organ surface and no effects in surrounding tissue.[57] In the first preclinical study, the application of high-intensity FUS in nonhuman primates induced local cerebral changes resulting in focal behavioral disabilities.[58] Soon afterward, clinicians sought to use therapeutically the thermal effects of sonication, attempts being made to use the technique in the neuroablative

treatment of psychiatric disorders,[59] and for cancer-related pain[60] but limitations in controlling the effects of sonication on the brain outweighed the benefits of the treatment. In the mid-1950s, Ballantine et al. published the first observations suggesting that the destructive action of ultrasound wave is the result of mechanical strain combined with an increase in temperature at the level of the target.[61] However, decades later, the mechanical effects of ultrasound to temporarily disrupt the integrity of BBB were confirmed and demonstrated under appropriate conditions to be reversible and reproducible.[62–64] A major game changer in the FUS field was the employment of MRI to precisely target and monitor ultrasound effects upon specific target areas in the brain, which led to an increase in the number of studies focused on developing new therapies for disorders of the brain which had previously being hindered by the presence of BBB.

Brain Tumor Treatment Using Focused Ultrasound

Currently there are 2 different approaches to treating brain cancer with ultrasound: direct destruction of the tumor by thermal ablation and transitory opening of the BBB to allow the passage of intravascular therapeutic agents into tissue harboring active tumor cells. The first attempt at using FUS-mediated hyperthermia to treat a brain tumor was made in 1985,[65] and 2 decades later, in 2006, the first clinical trial for recurrent GBM was initiated. Imaging suggested that ultrasound-induced thermocoagulation of the enhancing tumor mass was achieved, which was confirmed histologically as well.[66] In parallel, preclinical studies confirmed that FUS increased the bioavailability of chemotherapeutic agents, such as Doxorubicin,[67–71] TMZ,[72] and cisplatin-loaded nanoparticles[73] while also reducing tumor growth and increasing animal survival. Other studies have investigated FUS enhancement of antitumor immune responses[74,75] as an alternative mechanism for increasing antitumor efficacy of this methodology.

MRgFUS-Mediated Delivery of Oncolytic Viruses

To date, a number of preclinical studies have explored the potential of FUS to improve the delivery of OVs to cells of interest, both *in vitro* and *in vivo*. Several challenges specific to OVs could influence effectiveness of FUS-mediated delivery to the tumor tissue (Figure 2), including the size of particular agents which might limit passage across openings made in the BBB, neutralization by the complement system in the bloodstream which could reduce availability of active viral particles, and first-pass filtering in liver and spleen prior to reaching the brain and target vasculature. Therefore, the effectiveness of OV delivery following FUS cannot be predicted from other studies that used the same method to deliver different agents, such as chemotherapy or even other biologicals, with very different properties than OVs.

The first preclinical studies were performed *in vitro*, in either monolayer or gel matrix systems. Bazan-Peregrino, using a BT-474 breast cancer cell-based tumor-mimicking flow-vessel model, showed that ultrasound-induced inertial cavitation increased the expression of a luciferase transgene encoded by a potent breast-cancer-selective oncolytic Ad, AdEHE2F-Luc.[76] The increase was greatly improved particularly in the direction of the ultrasound beam. FUS alone induced an increase in the number of plaques in both Vero monkey kidney cells and human oral squamous cell carcinoma (SCC) cells infected with the HSV-1 R849

mutant virus, but the addition of microbubbles further increased plaque formation, indicating that ultrasound promotes the entry of oHSV-1 into cells.[77] Ultrasound had similar effects on the oHSV-1 RH2 mutant virus in SCC cells, but also on virus injected directly intratumorally into subcutaneous SCC tumors generated in athymic mice.[78]

In vivo studies have demonstrated that ultrasound was similarly efficient at increasing OV penetration in cells when directly injected into the tumor. Greco showed that replication-incompetent Ad, expressing melanoma differentiation-associated gene-7/interleukin-24 (Ad.mda-7), in combination with microbubbles completely eradicated not only the targeted DU-145/Bcl-XL therapy resistant tumors generated subcutaneously in nude mice but also the nontargeted distant tumors established on the opposite flank.[79] Moreover, a significant increase in ultrasound-mediated tumor transgene expression was reported when AdEHE2F-Luc virus was co-injected with microbubbles and administered either into tumors generated from ZR-75–1 human breast cancer cells or intravenously, the effect being limited to the sonicated side.[80] Promising results were also reported when using the combined effects of ultrasound and oncolytic Ad "stealthing", which was accomplished by the coating of Ad with a biocompatible polymer that removes the natural Ad tropism, thereby preventing binding to blood components, and thus increasing its circulation time.[81] Intravenous injection of Ad in mice bearing ZR-75–1 tumors followed by the application of ultrasound waves to the tumor dramatically increased virus activity, leading to substantial and statistically significant regression of tumor growth and increased survival.[81]

Clinical Trials

To date, there are still a limited number of preclinical studies addressing the efficiency of ultrasound in mediating delivery of OV to gliomas. However, as multiple types of OV proving to be efficient in vitro and in vivo are developed, there is an increasing demand for a technology that has the potential to repeatedly deliver clinical levels of OV at the level of tumors. Clinical trials focused on optimizing the opening of BBB for chemotherapy and viral therapy are in progress. The safety of opening the BBB along the periphery of the tumor resection cavity in patients undergoing TMZ as part of the standard of care is currently recruiting patients (clinicaltrials.gov NCT03551249). A clinical trial was recently completed that evaluated the safety of BBB disruption using transcranial MRI-guided FUS in conjunction with an intravenous ultrasound contrast agent to increase the accumulation of doxorubicin in brain tumors and the adjacent brain. The procedure was well tolerated with no adverse clinical or radiologic effects and T1-weighted MRI acquired postsonication revealed a 15%–50% increase in contrast enhancement within the target volume. Additional biochemical analysis performed in sonicated and unsonicated tissue suggested that chemotherapy delivery is feasible[74] (clinicaltrials.gov NCT02343991).[82] Other trials focus on the safety and efficiency of BBB disruption on 1 of the first 3 days of TMZ treatment in patients with glioblastoma undergoing standard care (clinicaltrials.gov NCT03712293) and in patients with first GBM following a maximal safe surgical resection and standard TMZ (clinicaltrials.gov NCT03616860). Clinical trials in progress focus on the safety of opening the BBB in patients scheduled to undergo surgical resection of the tumor (clinicaltrials.gov NCT03626896, NCT03322813) and to evaluate the influence of BBB disruption on brain perfusion (clinicaltrials.gov NCT04063514).[83]

Despite the lack of clinical trials to study the efficiency of MRgFUS-mediated delivery of oncolytic viral gene therapy to brain tumors, the current clinical trials will provide valuable data on the safety and efficiency of opening the BBB in patients with new or recurrent tumors, under chemotherapy treatment or preparing for surgical resection of the tumor, as part of the current standard of care for gliomas. These data in combination with the results generated from clinical trials using OV delivered via other routes of administration and clinical trials assessing the efficiency of MRgFUS-mediated ablation of tumor will clear the path for clinical studies that will explore the efficiency of MRgFUS in delivering OV therapy to targeted tumoral or residual tumoral tissue in the brain. Finally, we have recently reported that MRgFUS can efficiently open the BBB in the hippocampus of subjects with Alzheimer disease. Gadolinium enhancement confirmed efficient coverage of the targeted hippocampus with no off-target BBB opening, and subjects were treated in 3 sessions, each separated by 2 weeks, with similar results in each session for each subject without adverse effects.[83] This confirms that current human MRgFUS technology can safely and efficiently target complex brain structures repeatedly in the same subject, which could be a critical capability for treating tumors which may not have all cells capable of responding to OV therapy in a single session. The ability to combine advances in OVs, which have been directly infused into many human subjects, with an MRgFUS BBB disruption technology that has also proven to be safe and feasible in humans provides the realistic possibility of combining these methods for less invasive, more efficient, and conformal delivery of biological agents such as OVs to the human brain.

CONCLUSION

Malignant glioma remains a recalcitrant disease to the standard therapies of surgery, chemotherapy, and radiation. Oncolytic virotherapy has emerged as a potential exciting new therapy for this disorder, with the first clinical approval of an oncolytic herpes simplex virus for malignant glioma occurring recently in Japan for the oncolytic HSV G47Δ. A major obstacle in the widespread adoption of this treatment will undoubtedly be the fact that optimal results were achieved with multiple (6 times in 1 year) viral administration, each being conducted via stereotactic inoculation of the tumor directly via a surgical approach. Approaches which eliminate the need for multiple surgical interventions are critically needed. Unfortunately, the virus is too large for successful administration via an intravascular approach under ordinary conditions due to the presence of the BTB. However, high-frequency ultrasound mediated interruption of the BBB, first used to deliver chemotherapies and then other biologic therapies, has emerged as an instrument to allow such dosing of oncolytic herpes simplex in a less invasive approach. Data with biologics and other viral therapies support the investigation of this approach using oncolytic HSV, particularly considering the success of multiple dosing regimen of this therapy, in malignant glioma.

Conflict of interest statement:

James M. Markert is a board and equity holding member in Aettis, Inc. and may receive royalties. The company holds frozen oncolytic viral stocks. Mustang Bio Tech is licensing the Intellectual Property of C134 an oncolytic viral therapy. He is blinded to the conditions for the C134 clinical trials. He is a shareholder for a privately held Small Business Innovation Research LLC, Treovir, Inc., concerning G207 oncolytic viral therapy now in clinical

trial. Merck, Inc. provides industry grant support by providing Keytruda (pembrolizumab) for a clinical trial of M032 oncolytic virotherapy and financial support for a clinical trial. He is a listee on Intellectual Property related to a cancer immunotherapy system and to a novel immunovirotherapeutic strategy targeting the glioma secretome. This Intellectual Property has been filed by in8Bio (formerly Incysus, Ltd.) and has royalty earning potential.

DRU-2019-7123. Orphan Drug Designation for Oncolytic Herpes Simplex Virus Expressing IL-12-M032 for “treatment of malignant glioma” Markert JM

U.S. Patent application filed/pending 62/824,685. Oncolytic virus and focused Ultrasound for NonInvasive Focal Gene Delivery to the Mammalian Brain. Markert JM, Kaplitt MG, Stavarache, Mihaela Inventors.

International application published under the patent cooperation treaty (PCTO) filed/pending WO 2020/198680 A1. Appl No. 20723237.2.Oncolytic Virus and Focused Ultrasound for NonInvasive CNS Focal Gene Delivery. Kapplitt, MG, Markert JM, Stavarache Mihaela Inventors

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Abbreviations and Acronyms

Ad	Adenovirus
BBB	Blood-brain barrier
BTB	Blood-tumor barrier
CED	Convection-enhanced delivery
CNS	Central nervous system
FUS	Focused ultrasound
GBM	Glioblastoma multiforme
HSV-1	Herpes simplex virus type 1
MRgFUS	Magnetic resonance-guided focused ultrasound
MRI	Magnetic resonance imaging
oHSV	Oncolytic herpes simplex virus type 1
OV	Oncolytic virus
Pfu	Plaque-forming units
SCC	Squamous cell carcinoma
TMZ	Temozolomide
tk	Thymidine kinase

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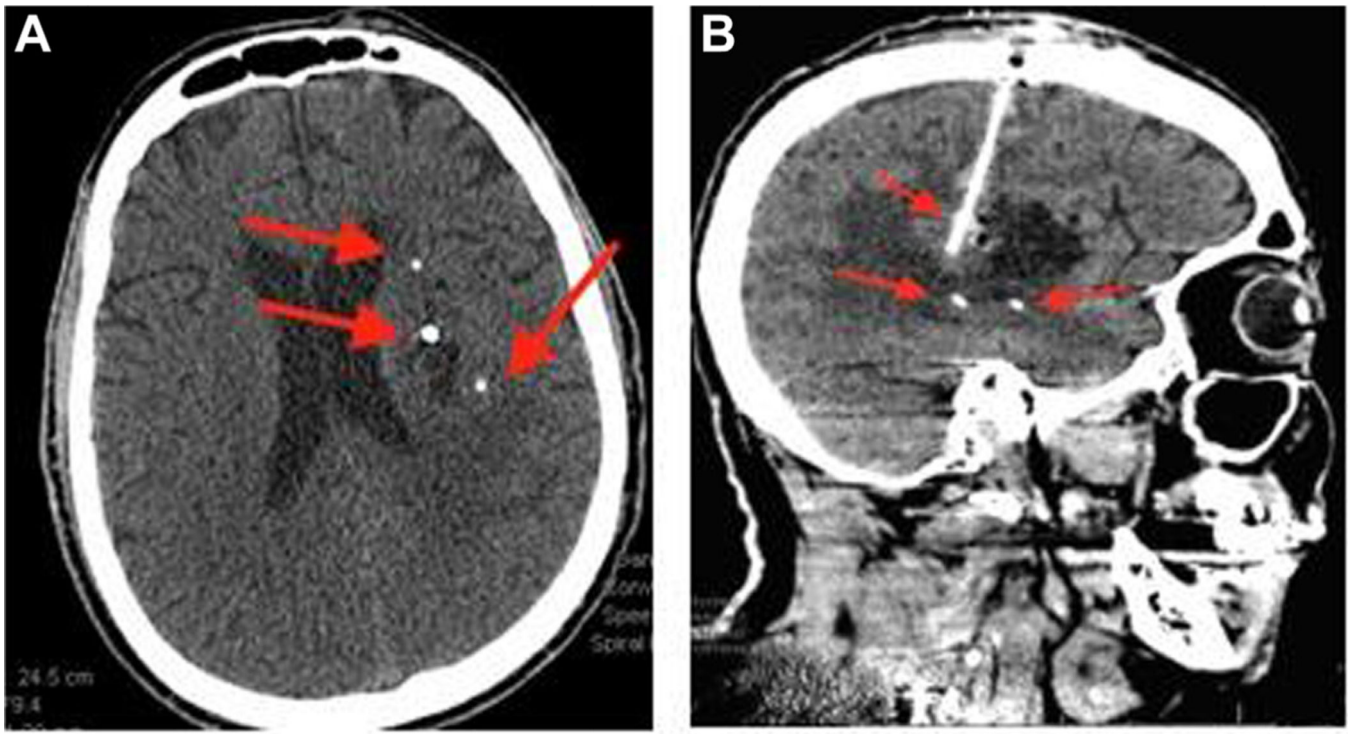

Figure 1.

(**A**) Axial and (**B**) sagittal CT scans. In the classical approach, catheters are implanted into the patients' brains and directly inoculate the virus (*red arrows*).

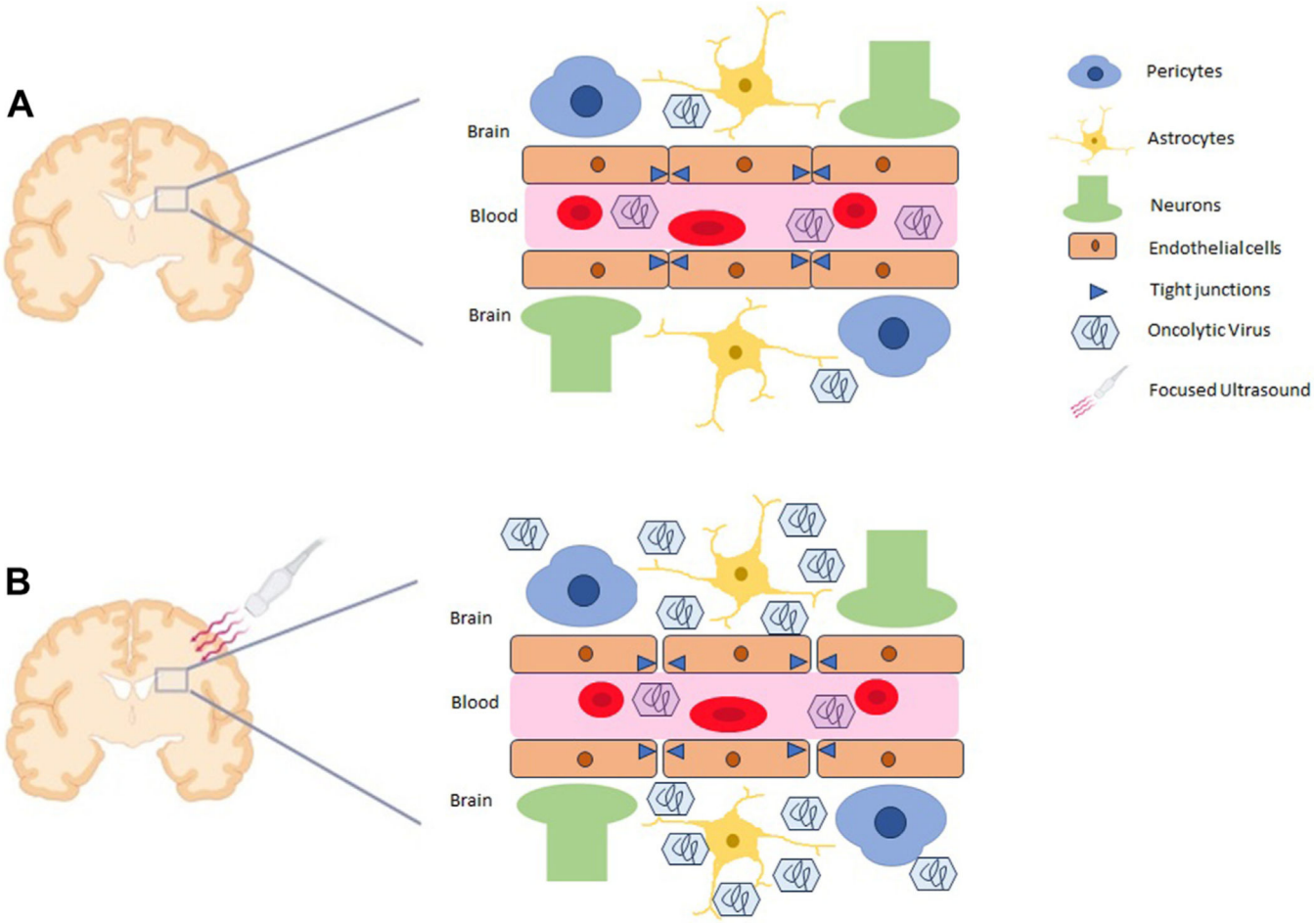

Figure 2.

(**A**) Inadequate delivery due to blood brain barrier. The classical approach accomplishes delivery of the virus to multiple regions of the tumor but has obvious limitations. On the other hand, systemic or intra-arterial approaches provide more feasible ways to deliver the virus to the tumor site; however, the blood-tumor barrier prevents adequate delivery. (**B**) Increased delivery rates with MRgFUS. The use of high-frequency ultrasound will allow delivery of virus to tumor by disruption of the blood-tumor barrier and is a more elegant approach than placing catheters. It will also allow a broader distribution of the virus to the entire tumor. The figure was created in part using biorender.com.