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Evolution of Malignant Glioma Treatment: From Chemotherapy to Vaccines to Viruses

Richard Lee Price, Ph.D.¹ and Ennio Antonio Chiocca, M.D., Ph.D.²

¹Dardinger Neuro-oncology Center, Department of Neurological Surgery, The Ohio State University Wexner Medical Center, Columbus, Ohio, USA

²Harvey Cushing Neuro-oncology Laboratories, Harvard Institutes of Medicine, Department of Neurosurgery and Institute for the Neurosciences at the Brigham, Brigham and Women's/ Faulkner Hospital and Center for Neuro-oncology, Dana-Farber Cancer Institute, Boston, Massachusetts, USA

Introduction

Malignant glioma is the most common type and most severe form of primary brain cancer. Each year, around 17,000 new patients are diagnosed in the United States, or about 5 cases in 100,000 people ¹. The disease most commonly occurs in the sixth through eighth decades of life. The prevalence of the disease will increase with an aging populace. According to the World Health Organization classification system, grade III and IV gliomas are collectively termed malignant gliomas ². Grade III gliomas are anaplastic high-grade gliomas and grade IV tumors are glioblastomas. Glioblastoma accounts for about 80% of malignant gliomas ^{1, 3}. Glioblastoma carries a worse prognosis and is characterized histologically by vascular proliferation and necrosis. The median survival for a patient with glioblastoma is only about 14 months with a 5-year survival rate near zero ⁴. Median survival for grade III gliomas is two to five years ⁵.

Glioblastomas are classified as either primary or secondary glioblastomas ⁶. Primary tumors (>90%) arise *de novo* without previous medical evidence of lower tumor grade formation, whereas secondary glioblastomas develop from a lower grade (II or III) glioma. Primary and secondary classification only refers to the progression pathway to glioblastoma development; the histopathology for the tumors is the same. Regardless of being a primary or secondary glioblastoma, the prognosis and current treatment is the same. Recent research has suggested differences in the tumors ⁶. These tumors develop at different ages and are comprised of different genetic mutations ^{7, 8}. Each tumor type has different molecular signatures as well. Primary glioblastomas are characterized by the EGFR/PTEN/Akt/mTOR pathway ⁹. Amplification of EGFR occurs in 60% of these tumors, but is seldom seen in

Corresponding author: Ennio Antonio Chiocca, M.D., Ph.D., PBB-301, Department of Neurosurgery, 75 Francis Street, Brigham and Women's Hospital, Boston, MA 02115, Phone: 614-293-9312, EACHIOCCA@PARTNERS.ORG.

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secondary tumors^{10, 11}. Secondary glioblastomas are primarily characterized by point mutations in the *TP53* tumor suppressor pathway¹¹. Recently, isocitrate dehydrogenase 1 (*IDH1*) mutations have been discovered in secondary glioblastomas¹². Interestingly, presence of this mutation signifies a more favorable prognosis¹³. The genetic signatures between primary and secondary tumors suggest underlying distinctions that may potentially guide targeted therapy in the future.

Recent research has focused on further defining the genetics of malignant gliomas¹⁴. No unequivocal causal mutations of malignant gliomas have been discovered. Instead, the tumor appears to be induced by an accumulation of multiple mutations over time. As opposed to identifying specific mutations, identification of altered molecular pathways and how the pathways interact is much more informative. Integrated pathway analyses have determined important genetic pathways implicated in glioblastoma^{15, 16}. Genetic alterations in the RTK/RAS/PI(3)K pathway are most common, affecting 88% of glioblastomas. Signaling alterations in the p53 and RB pathways are second and third most frequent, altered in 87% and 78% of cases, respectively. Besides determining genes promoting gliomagenesis, other studies have shown how genes can serve as prognostic indicators. Several prognostic markers have been shown to correlate with glioblastoma survival. For instance, glioblastoma patients with *IDH1* mutations survive longer compared to patients without mutations in this gene¹².

Standard of care for malignant glioma

Surgery is the first line of therapy for malignant glioma, allowing for debulking, cytoreduction, and tissue for analysis. Extent of resection correlates with overall survival (OS), with gross total resection (GTR) being the goal¹⁷. Resection >78% of total tumor volume provides some survival benefit. Resection for tumors located in eloquent areas in the brain can be aided by using intra-operative MRI and intra-operative neuromonitoring. Tumor cell invasion into normal brain precludes complete resection of the whole tumor. Historically, glioblastoma has been treated with post-operative radiotherapy to kill remaining tumor cells. Addition of radiotherapy extends survival from 3–4 months to about 12 months^{18, 19}. In the 1990s, the DNA alkylating agent temozolomide was tested and approved by the FDA as a chemotherapeutic agent for the treatment of malignant glioma²⁰. Addition of temozolomide to surgical resection and radiotherapy extends median survival to 14.6 months and the 2-year survival rate to 27% compared to 10%⁴. Additional studies have shown that patients with DNA methylation in the promoter region of the DNA repair enzyme O6-methylguanine-DNA methyltransferase (MGMT) are more likely to respond to temozolomide therapy²¹. The current standard of care for glioblastoma is GTR with concomitant temozolomide and radiotherapy followed by adjuvant temozolomide.

Carmustine is the only other FDA-approved first-line chemotherapeutic agent approved for glioblastoma. Like temozolomide, carmustine is a DNA-alkylating agent. BCNU (carmustine)-polymer wafers are positioned in the tumor bed after tumor resection. A Phase III clinical trial showed evidence of survival benefit²². However, the efficacy of carmustine has never been directly compared to that of temozolomide.

Grade III malignant gliomas are much less common than glioblastoma. There is little evidence to guide specific therapies for these tumors. Most clinicians treat these tumors based on guidelines for glioblastomas using a combination of surgical resection, temozolomide, and radiotherapy²³. Unfortunately, many grade III tumors recur as secondary glioblastomas.

Nearly all patients with malignant glioma will recur. The median progression-free survival interval (PFS) is 7–10 months after initial surgery²⁴. Once glioblastoma recurs, the treatment regimen is not clearly delineated. No salvage therapy has shown to have a clear benefit, but there are several options. Additional surgical resection is first line if the mass is resectable. If GTR is achieved during re-operation, then OS is maximized despite initial extent of resection²⁵. Furthermore, additional surgery permits new tissue for histology and molecular analysis. Temozolomide and BCNU (carmustine)-polymer wafers are approved for recurrent glioblastoma, but results in median survival of less than six months^{26, 27}. Bevacuzimab is a humanized monoclonal antibody that targets VEGF, thereby blocking angiogenesis. It is approved for use in recurrent glioblastoma and is currently being investigated in Phase III clinical trials as a first line therapy for glioblastoma²⁸. Several different types of therapy for malignant glioma are in various phases of clinical trials for use in the clinic. The remainder of this review will discuss how therapy for malignant glioma is evolving.

Future of chemotherapies

The current standard of care for malignant glioma has limited efficacy, only extending life expectancy about a year longer than the natural course of the disease. One limitation of radiotherapy and temozolomide chemotherapy is that the therapy is non-specific. The therapy does not exploit specific weakness of individual tumors. As we enter a time of greater understanding of the genetic landscape and gene expression of malignant gliomas, we will have a better idea of the targets to attack²⁹. The Cancer Genome Atlas (TCGA) is a project sponsored by the National Institutes of Health (NIH) to better elucidate the genetics and gene expression of multiple cancer types, including glioblastoma. The TCGA has analyzed over 500 untreated glioblastoma samples for DNA sequence and epigenetic modification, gene expression, and microRNA expression¹⁶. This project has led to a deeper understanding of glioblastoma enabling high-throughput pathway analysis and massive data synthesis. One of the major findings of the project was that glioblastoma is divided into four distinct subtypes: mesenchymal, proneural, classical, and neuronal³⁰. Each subtype has novel mutations and expression patterns. Some of these novel pathways and targets will hopefully prove to be exploitable for effective treatments in the future.

Utilizing TCGA data and other genome-wide studies, new molecular targets for malignant gliomas have been detected. Molecular targets are common in pathways central to malignant glioma survival such as proliferation, evasion of apoptosis, invasiveness, and angiogenesis³¹. Aberrant growth factor signaling drives proliferation in many malignant gliomas. Epidermal growth factor (EGFR), platelet-derived growth factor (PDGF), insulin-like growth factor (IGF), and fibroblast growth factor (FGF) are either highly upregulated or mutated in a large percentage of malignant gliomas³². Recent clinical trials have tried to

capitalize on blocking these pathways. EGFR is the most widely studied growth factor in malignant glioma. Upregulated EGFR is found in approximately 50% of malignant gliomas, making it an attractive therapeutic target³³. EGFRvIII is a truncated variant of the normal EGFR protein that is commonly found in malignant glioma^{34, 35}. It is expressed in 27%–67% of tumors and rarely seen on normal tissue, making it an attractive target for malignant glioma therapy^{36, 37}. Several small molecular inhibitors are currently in Phase II clinical trials for use in newly diagnosed and recurrent glioblastoma. Gefitinib, erlotinib, and lapatinib are the best-studied EGFR small molecule inhibitors in clinical trials. Additionally, Cetuximab is a chimeric monoclonal antibody against EGFR that recently was studied in a Phase II clinical trial³⁸. Unfortunately, these drugs have only shown modest efficacy for treating malignant glioma. Stratifying patients based on molecular profile of tumors enhanced the efficacy of erlotinib, underscoring the importance of personalizing treatment based on individual tumors³⁹. More detailed discussion on developing small molecule inhibitors for malignant gliomas can be found in a review by Polivka et. al⁴⁰.

Cheaper and more efficient technology is ushering a new era of medicine. Personalized medicine will play a better role of identifying certain exploitable pathways or targets in an individual tumor^{41, 42}. The discovery of temozolomide response being dependent on MGMT promoter status is an example of applying personalized medicine to malignant glioma⁴³. In the near future, genetic tests will determine if a patient will respond to temozolomide. Deep sequencing of tumor DNA and gene expression analysis of fresh tumor samples will eventually direct therapy for patients suffering from malignant glioma. By synthesizing ascertainable data from the tumor, therapy can be tailored and combined to select the appropriate combination of therapies to best target the tumor. As technology evolves to make medicine more personalized, new methods will be utilized to choose the proper combinatorial therapy to treat each malignant glioma.

Modeling malignant glioma in mouse avatars model could become a powerful tool for drug discovery⁴⁴. Developing mouse avatars for malignant glioma involves implanting freshly resected human tumor into a humanized mouse model. This allows for a patient to have their exact tumor growing in a physiologically-relevant *in vivo* model, which can be used to screen and select for the best combinatorial therapy to kill the tumor⁴⁵. Any number and combination of therapeutic options can be tested before being applied to the patient to minimize toxicity and maximize efficacy. Thus far, two groups have published articles using mouse avatars to evaluate efficacy for cancer treatments^{46, 47}. Hopefully, more widespread use of mouse avatars will occur as personalized medicine expands and technology improves.

Another method to discover new therapeutics to treat malignant glioma is to re-examine drugs that have FDA approval to treat other diseases. Drug repositioning, or drug repurposing, is in the process of employing already approved drugs to new indications⁴⁸. The use of chemotherapy already approved for other cancers may intuitively be applied to malignant glioma, as similar pathways can be altered in both cancers. Repositioning FDA-approved drugs is a tremendous advantage in that it reduces drug development cost as well as reduces regulatory hurdles since the drugs is already approved for human use. By using publically available glioblastoma microarray data and drug databases, one group identified the breast cancer drug Fulvestrant as a potential malignant glioma therapy⁴⁹. Another

strategy to reposition drugs is outlined by the International Initiative for Accelerated Improvement of Glioblastoma Care. The group has selected nine drugs that have evidence of blocking well-known glioblastoma pathways⁵⁰. They hypothesize that using these drugs as an adjuvant with temozolomide in recurrent glioblastoma will extend survival. Mounting evidence suggests that cytomegalovirus (CMV) may promote glioblastoma progression⁵¹. An article recently published in the NEJM showed that treating glioblastoma with at least six months of valgancyclovir in addition to standard of care extends median overall survival to 56.4 months⁵². This is additional evidence that drugs can be repurposed to treat malignant glioma based off of increasing knowledge of tumor biology.

Tumor vaccines and immunotherapy

The interaction between host immunity and cancer is complex. Malignant gliomas are no exception. Harnessing host immunity to attack cancer is a formidable option for therapy, but is becoming a reality. With the recent approval of Ipilimumab, a humanized antibody against certain T cells to treat malignant melanoma, and Provenge, an autologous dendritic cell vaccine for hormone-resistant prostate cancer, expectations for immunotherapy of malignant glioma is high. In fact, nearly 20% of all therapies currently being developed are anti-cancer vaccines⁵³. There are currently 22 actively-recruiting immunotherapy trials for malignant glioma (Table 1). Immunotherapy is an attractive option in that it is relatively well-tolerated since the patient uses autologous cells to attack the cancer. Additionally, the cellular specificity afforded by immune responses is of particular importance when dealing with an infiltrating malignant glioma in an eloquent brain region. The goals of designing immunotherapy for malignant glioma are two-fold. First, the vaccine approach must produce a durable and safe immune response that selectively kills tumor cells. Secondly, the response must be robust enough to cause a lasting immune reaction in a patient that is usually immunocompromised⁵⁴. Multiple approaches are currently being evaluated in clinical trials for immunotherapy. They include: dendritic cell (DC) vaccines, peptide vaccines, gene-transfer mediated vaccines, autologous tumor cell vaccines, and T cell immunotherapy.

DC vaccines are the most common modality being evaluated. They have proven to be safe and have shown some promise in Phase I and II clinical trials, with one Phase III clinical trial underway. DCs are professional antigen presenting cells (APCs) that survey the host looking for pathogens-associated molecular patterns. Immature DCs endocytose foreign pathogens and subsequently activate CD4 and CD8 T cells to attack the pathogen⁵⁵. DC vaccines exploit this immunological process. Autologous DCs are removed from the patient and cultured *in vitro*. While in culture, DCs are pulsed with either synthetic tumor-associated antigens (TAAs) or autologous tumor lysate. The exposure to tumor antigen primes the DCs, at which point the cells are re-introduced into the host which activates T lymphocytes to attack the tumor. The dose, antigen, adjuvant, and location of administration vary between trials⁵⁶.

The VICTOR I study used DCs loaded with EGFRvIII peptide conjugated to keyhole limpet hemocyanin (KLH)⁵⁷. Twelve patients participated in this phase I trial for newly diagnosed glioblastoma. A total of five doses were administered, three within the first two weeks of resection and then two additional doses at four and six weeks post-surgery. Not only did this

study prove that DC vaccination was safe, but it suggested a modest survival benefit. The average time to progression was 10.2 months and median survival was 18.7 months⁵⁷. The ICT-107 vaccine utilizes autologous DCs loaded with multiple synthetic TAAs (HER2, TRP-2, gp100, MAGE-1, IL13Ra2 and AIM-2)⁵⁸. The study enrolled 17 newly diagnosed glioblastoma patients. They reported a PFS of 16.9 months and median survival of 38.4 months. A randomized, double-blind, placebo-controlled Phase II clinical trial has been completed and its results were recently publicized online to show a benefit in progression-free, but not overall survival⁵⁹.

As mentioned earlier, there is mounting evidence that CMV is present in malignant gliomas^{60, 61}. CMV is only present intratumorally and not in normal surrounding brain parenchyma. Viral epitopes can be exploited as a tumor-specific target. John Sampson and Duane Mitchell's groups have engineered a unique DC vaccine to capitalize on intratumoral CMV epitopes. Instead of being primed with protein, autologous dendritic cells are pulsed with CMV RNA. The RNA elicits a DC immune response against CMV epitopes. Once re-injected into the host, the primed DCs activate T cells to attack tumor-associated CMV epitopes. Phase I and II clinical trial data are forthcoming (personal communication).

Another approach to DC vaccination utilizes tumor lysate from the autologous patient tumor. In one Phase II clinical trial, 32 newly diagnosed or recurrent glioblastoma patients received DC vaccination starting 15 weeks after surgery. Autologous DCs were pulsed *in vitro* with autologous tumor lysate before being re-introduced into the patient⁶². In this study, immunologic responders (53% of patients) were defined as having an elevated interferon- γ response. Importantly, immunologic responders demonstrated a time to progression (TTP) and OS significantly longer than non-responders to the vaccine. In a similar Phase I clinical trial of 23 patients with newly diagnosed or recurrent glioblastoma, Prins et al. employed DC vaccination with autologous tumor lysate⁶³. The trial included a booster phase in which the virus was re-administered every 3 months with an immunoadjuvant (imiquimod or poly-ICLC) to enhance immunoreactivity. Serum TNF- α and IL-6 levels increased after each DC vaccination, increasing to a greater extent after booster doses, indicating a sustained immune response to the vaccine. The median survival for patients receiving the vaccine was 31.4 months⁶³. Gene expression analysis showed that tumors with a mesenchymal subtype phenotype mounted a stronger immune response to the vaccine compared to other patients in the trial, which resulted in a significantly longer survival when compared to a randomly selected mesenchymal control group.

Peptide vaccination utilizes direct administration of synthetic TAAs along with an adjuvant to stimulate an immune response. The goal of this strategy is to select a tumor-specific antigen that is abundantly expressed in malignant cells, but is absent in normal cells. The ACTIVATE phase II trial proved that targeting EGFRvIII was safe and possibly efficacious⁶⁴. This trial was limited to 18 patients with EGFRvIII-expressing tumors. Patients received three doses of a 14-amino acid epitope from the EGFRvIII protein conjugated to a KLH adjuvant, later to be named CDX-110, every two weeks starting two weeks after surgery. After initial load dosing, patients received a maintenance dose once a month until radiographic progression was evident. The median TTP for this trial was 14.2

months and median survival was 26 months. During the ACTIVATE trial the standard of care for glioblastoma changed to include temozolomide. The ACTIVATE II trials included 21 patients with EGFRvIII glioblastoma that received the first dose of vaccine within six weeks of completing concomitant radiotherapy and temozolomide⁶⁵. The results of this trial were similar to ACTIVATE, with a median TTP of 15.2 months and median survival of 23.6 months. ACTIII is an ongoing large Phase II clinical trial comparing standard of care + CDX-110 to standard of care. There is an additional Phase II trial for recurrent glioblastoma and a Phase III trial for newly diagnosed patients (Table 1).

Heat shock proteins (HSPs) are overexpressed in malignant glioma cells and have been shown to interact with drivers of malignant glioma tumorigenesis such as EGFR, PDGFR, and PI3K^{66, 67}. HSP vaccines are created by binding tumor-specific HSPs with TAAs. Data suggest HSPs promote a pro-inflammatory response to the TAAs resulting in a tumor-specific immune response⁶⁸. Oncophage is an HSP vaccine that is composed of a 96kDa HSP, gp96, that is bound to TAAs⁶⁹. Twelve patients with recurrent glioblastoma were enrolled in a Phase I clinical trial. Immunologic responses based on IFN- producing cells on brain biopsy were elicited in 11/12 of patients. The median survival for the eleven responders was 47 weeks. A Phase II clinical trial is currently recruiting patients.

Gene transfer vaccination is another method for creating a local immunologic effect in a tumor. A gene of interest can be locally expressed by injecting the vector directly into the tumor cavity producing tumor-specific expression that stimulates a local immune response⁷⁰. AdV-tk is an Adenoviral vector that contains the herpes simplex virus (HSV) thymidine kinase (tk) gene. The virus is directly inoculated into the tumor bed at the time of resection followed by oral valacyclovir, an antiviral prodrug. Valacyclovir is converted into a toxic metabolite by thymidine kinase in tumor cells. Local cytokine induction and leaky toxic metabolites leads to an additional anti-tumor response in neighboring cells via a “bystander effect”⁷¹. In a Phase I clinical trial, patients were inoculated at the time of resection followed by 14 days of valacyclovir⁷². Radiotherapy was started one week after AdV-tk injection and temozolomide was started after the course of valacyclovir. There was no dose limiting toxicities. Furthermore, analysis of tissue demonstrated significant T cell and macrophage infiltration in the tumor bed after AdV-tk administration. Phase II trials are currently underway.

Adoptive Immunotherapy utilizes the inherent anti-tumor ability of autologous effector immune cells. Autologous peripheral lymphocytes are activated *ex vivo* and expanded before infusion back into the host. Typically, lymphocytes are cultured with IL-2 to generate lymphocyte-activated killer cells (LAKs) and cytotoxic T cells (CTLs). Once reintroduced into the host, APCs activate the cells to attack malignant glioma. Tumor-derived T cells and T cells from draining lymph nodes can be used for adoptive immunotherapy too. Multiple clinical trials using LAKs have shown only modest response in treating malignant glioma (reviewed in⁵⁶). The most recent completed Phase I/II clinical trial was published by Dillman and colleagues⁷³. This trial enrolled 33 patients achieving a median survival of 20.5 months. A follow-up Phase II clinical trial was terminated.

An exciting new immunotherapy concept is using chimeric antigen receptors (CARs) integrated to T cells to generate tumor-specific T cells (CAR-expressing T cell). Tumor-specific CARs are formed by fusing an extracellular domain created from a monoclonal antibody that is reactive to a TAA of interest coupled to the intracellular signaling domain of a T cell ⁷⁴. Recognition of a TAA by the extracellular domain of a CAR signal initiates an intracellular reaction in the CAR-expressing T cell, ultimately resulting in T cell activation and killing. A large advantage of this technology is tumor specificity and MHC-independent cytotoxicity ⁷⁵. EGFRvIII as well as HER2 CAR-expressing T cells have shown efficacy in preclinical models ^{76,77}. These platforms are currently being evaluated in several clinical trials (Table 1).

Virotherapy

Over the last two decades, viral therapy in the treatment of malignant glioma has garnered great interest. With developments of molecular biology and better understanding of virology, viruses can now be manipulated to combat cancer. The concept of viral therapy is to not only utilize a virus to kill a tumor cell, but also to invoke an immune response against released tumor antigens. The goal is to create a virus that specifically attacks cancer cells without harming normal tissue surrounding the tumor ⁷⁸. A vast array of viruses can be engineered for viral therapy to target different types of cancer. With fifteen years of experience in clinical trials for glioblastoma, viral therapy has proven to be a safe option. Additionally, it is becoming a viable therapeutic option for other cancers ⁷⁹. One oncolytic virus (OV), ONYX-015, is approved in China for head and neck cancer. Additionally, the T-Vec virus is in Phase III trials in the United States for melanoma ⁸⁰. To date, multiple Phase I clinical trials for glioblastoma have been completed or are ongoing.

The first generation of viral therapy for glioblastoma utilized replication-incompetent viruses. The use of replication-competent viruses was avoided so uncontrolled viral infection of the host would not occur. Retroviruses or Adenoviruses were rendered replication-incompetent by deletion of a portion of the viral genome ⁸¹. These viruses were then “armed” with the thymidine kinase gene from HSV (HSV-TK), which converts the pro-drug gancyclovir (GCV) into a cytotoxic metabolite ⁸². There were high expectations for efficacy in glioblastoma since this virus would theoretically selectively kill replicating tumor cells and generate a secondary immune response via the “bystander effect”. The first clinical trial employed a replication-incompetent retrovirus containing HSV-TK. In this Phase I clinical trial, 15 patients were stereotactically injected with vector-producing cells into the tumor site and given oral GCV ⁸³. The results of this trial proved that the gene therapy was safe. The median survival was 8.1 months with four patients displaying modest anti-tumor responses. This virus was abandoned when Phase III trials did not show any statistically significant efficacy when compared to standard therapy ⁸⁴.

As replication-incompetent viruses proved to be safe yet ineffective, researchers forged ahead with bolder replication-competent viruses. The next generation of viral therapy, oncolytic viruses (OVs), utilizes attenuated viruses that are capable of replicating in and destroying tumor cells. OVs are engineered to infect tumor cells, replicate, lyse the tumor cell, and spread to adjacent tumor cells ⁸⁵. Additional tumor killing would theoretically be

achieved by local immune responses created by tumor antigen release after oncolysis. Currently, four different viruses have been used in published clinical trials.

Newcastle disease virus (NDV) is a single-stranded RNA virus. The HDV-HUJ strain showed great efficacy to kill human tumor cells *in vitro* when it was found to selectively target and kill human and rat neuroblastoma cells without affecting normal fibroblasts *in vitro*⁸⁶. Fourteen patients with recurrent malignant glioma participated in a Phase I clinical trial. Patients received eight cycles of virus delivered intravenously⁸⁷. Minimal toxicity was observed among patients and three out of fourteen patients survived greater than 61 weeks. This virus is currently being tested in a Phase I/II clinical trial (Table 2).

Adenovirus (AdV) and Reoviruses have also been used in Phase I clinical trials for glioblastoma patients. The ONYX-015 virus, currently approved in China for head and neck cancer, lacks the E1B gene prohibiting replication in normal cells⁸⁸. A Phase I clinical trial was conducted with 24 recurrent glioblastoma patients. Up to 10^{10} plaque forming units (p.f.u.) of the virus was injected into the resection cavity in these patients⁸⁹. Therapeutic effect of the virus was not observed; however, the maximally tolerated dose (MTD) was never achieved in patients. Reoviruses are double-stranded RNA viruses that are tropic to mammalian cells. In glioblastoma cells, upregulated RAS signaling promotes viral replication in tumor cells⁹⁰. Twelve patients with recurrent glioblastoma participated in a Phase I clinical trial. In the trial, patients received up to a 10^{10} p.f.u. intratumoral dose of virus⁹¹. The virus was tolerated in all the patients and the MTD was not reached. One of the patients exhibited PFS at six years after treatment.

HSV-1 viruses are the best-studied OV. Two replication-competent viruses have been used in clinical trials for glioblastoma to date, G207 and HSV1716. G207 lacks ribonucleotide reductase, rendering it unable to replicate in normal cells. So far, 30 total patients have received the virus via intratumoral injection in two separate Phase I clinical trials. The first trial demonstrated safety at doses as high as 3×10^9 p.f.u.⁹². Of the 21 patients in the first trial, 8 patients had a reduction in tumor size based off MRI. The second trial included six patients that safely received multiple intratumoral viral injections⁹³. Additionally, the investigators were able to prove that the virus was able to replicate in tumor tissue. HSV1716 is another HSV-1 OV that has been tested in three Phase I clinical trials for glioblastoma. This virus contains an attenuating mutation that selects it for replication in rapidly dividing cells, but not in differentiated cells. A total of 33 patients with either newly diagnosed or recurrent malignant glioma have participated in the clinical trials. The first trial demonstrated that the virus was safe up to doses of 3×10^5 p.f.u.⁹⁴. Three of the nine patients in this study showed a response to the virus, with two patients alive after four years post-treatment. The second Phase I clinical trial enrolled 12 patients with malignant gliomas. They received an intratumoral injection of 10^5 p.f.u. of HSV1716 followed by resection of the tumor. The resected tumor was analyzed and assayed for evidence of viral replication⁹⁵. As seen in the previous study, none of the patients experienced any adverse effects related to virus administration. Infectious virus was recovered from the tumors of two of the patients. A third Phase I clinical trial demonstrated the safety of the virus at a dose of 10^5 p.f.u.⁹⁶. Three of the twelve patients in this study were long-term survivors (15–22 months).

In Phase I clinical trials, OV's have proven to be safe. In fact, the MTD was never established in most trials. Unfortunately, the OV's tested to date have not proven to be as efficacious as hoped. This is disappointing in light of promising preclinical data. New research is being conducted into understanding roadblocks to successful OV therapy as well as developing new viruses. Further development of OV's has produced more complex viruses. New viruses are being created that are "armed" with cytotoxic agents or cytokines to promote tumor killing⁹⁷. Furthermore, more potent viruses are being targeted to specific tumor receptors or stem cell population of glioblastoma^{98, 99}. A significant body of research continues to elucidate how the immune system interacts with the virus. New pre-clinical evidence suggests that NK cells are responsible for impeding virotherapy¹⁰⁰. Future challenges lie in overcoming host immunity to the virus and engineering more potent OV's that are able to replicate and kill glioblastoma cells. Additional clinical trials are still ongoing for Adenovirus and NDV¹⁰¹. Furthermore, new viruses are being tested in Phase I trials. A Measles virus that produces carcinoembryonic antigen (MV-CEA) is now in Phase I trials for recurrent glioblastoma^{102, 103}. Additionally, recombinant Poliovirus and Parvovirus are being tested in Phase I clinical trials (Table 2)^{104–106}. Results from these clinical trials are anxiously awaited.

Conclusion: What should we tell our patients?

Malignant glioma remains a disease with a dismal prognosis. Patients are in desperate need of new therapies to combat the disease and extend life. The advent of high-throughput technology and TCGA allows for a deeper understanding of malignant glioma. Researchers are actively generating vast amounts of data and translating discoveries into therapeutics. There are many pre-clinical studies that are in the pipeline for Phase I clinical trials. Furthermore, there are several Phase III clinical trials underway that appear promising. Unfortunately, these trials are years away from completion and potential FDA-approval.

Malignant glioma is a heterogeneous disease. Observations from clinical trials suggest that certain modalities work best in subsets of patients within a population. Elucidating what patients will respond best to certain therapies is paramount. Implementing personalized medicine practices will help accomplish this. Since individual malignant gliomas are unique, tumor samples from every patient must be analyzed to determine what pathways are dysregulated. Based off this information, the proper therapeutics can be selected to best target the individual tumor. Furthermore, the use of mouse avatars may assist in determining what drugs the tumor responds to *in vivo*. Finally, glioblastoma is a complex disease that overcomes single agent therapy. Single agent therapy will likely never cure the disease. Investigating multi-modal therapy in patients and considering multiple experimental therapies will give patients the best hope of combating malignant glioma.

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Table 1

Summary of ongoing immunotherapy clinical trials for malignant glioma

Name of trial/Identifier	Phase	Type	Enrollment	Therapy	Primary Outcome
ATTAC/NCT00639639	I	DC	16	CMV pp65-LAMP mRNA-loaded DCs	Safety
NY-ESO-1 intranodal vaccine/NCT01522820	I	DC	30	DEC-205-NY-ESO-1 fusion protein + sirolimus	Safety
Vaccine Therapy for recurrent GBM/NCT00890032	I	DC	50	BTSC mRNA-loaded DCs	Safety
Phase I study of DC vaccine/NCT02010606	I	DC	40	Allogenic stem cell lysate	Safety
Adjuvant Intra-Nodal Autologous DC vaccine/ NCT00323115	II	DC	11	Intra-nodal autologous DC vaccine	Tumor-specific CTL Response
DC Vaccine for Patients With Brain Tumors/ NCT01204684	II	DC	60	Autologous tumor lysate + adjuvant	Most effective combination of DC vaccine components
ICT-107/NCT01280552	IIb	DC	200	TAA	OS
DCVax-L/NCT0045968	III	DC	300	Autologous tumor cell lysate	PFS
REGULATe/NCT00626483	I	DC/T cell	6	DC + CMV-specific T cell + Basiliximab	Functional capacity of regulatory T cells
ERaDICAte/NCT00693095	I	DC/T cell	12	CMV-autologous lymphocyte transfer + DCs	Safety
alloCTL/NCT01144247	I	Alloreactive CTL	15	Intratumoral alloreactive T cells and IL-2	Safety
Gliatek/NCT00589875	IIa	Gene transfer	52	AdV- β + valacyclovir and RT	Safety
Imiquimod/BTIC vaccine/NCT01400672	I	Tumor lysate vaccine	20	BTIC vaccine + Imiquimod	Dose limiting toxicity
TVI-Brain-1/NCT01290692	II	Tumor lysate vaccine	86	Autologous tumor vaccine + immune expansion adjuvant	PFS
Vaccine Therapy +/- Bevacuzimab/NCT01814813	II	HSP	222	HSPPC-96 + Bevacuzimab	OS
HERT-GBM/NCT01109095	I	CAR CTL	18	Genetically modified HER.CAR CMV-specific CTLs	Dose limiting toxicity
White Blood cells with Anti- EGFRvIII/NCT01454596	I/II	CAR CTL	160	Ant-EGFRvIII engineered CARs, aldeskleukin, fludarabine, CPA	Safety
Vaccine therapy + Sargramostim/NCT01250470	I	Peptide vaccine	9	ISA-51/survivin peptide vaccine + sargramostim	Safety
ZAP IT/NCT00626015	I	Peptide vaccine	20	PEP-3-KLH + daclizumab	Suppression of regulatory T cells
IMA950/NCT01403285	I	Peptide vaccine	25	11 TAAs + GM-CSF and Imiquimod	Safety and immunogenicity
ACT III/NCT00458601	II	Peptide vaccine	82	CDX-110 + TMZ + RT	PFS
ACT IV/NCT01480479	III	Peptide vaccine	440	CDX-110 + TMZ	OS

Data is from clinicaltrials.gov ¹⁰². BTSC: brain tumor stem cell, BTIC: brain tumor initiating cell, CTL: cytotoxic T cell

Table 2

Summary of ongoing OV clinical trials for malignant glioma

Name of trial/Identifier	Phase	Virus type	Viral Modifications	Enrollment	Dose/Route	Primary outcome
MV-CEA for recurrent glioblastoma/NCT00390299	I	Measles	Carcinoembryonic antigen-expressing	40	Up to MTD/IT	Safety, MTD, viral propagation and expression
ParvOryx in patients with glioblastoma/NCT0101430	I/IIa	Parvovirus	H-1PV	18	Up to MTD/IT or IV	Safety and tolerability
NDV-HUJ in Glioblastoma/NCT01174537	I/II	NDV	HUJ strain	30	10 ¹⁰ EID50 5 days/ week IV	PFS
PVS-RIPO for recurrent glioblastoma/NCT01491893	I	Poliovirus	IRES from human rhinovirus 2	18	Up to MTD/IT	MTD
Ad-RTS-hIL-12 + Veldimex in malignant glioma/ NCT02026271	I	Adenovirus	IL-12 secretion	50	10 ¹² vp/ IT	Safety and tolerability
DNX2401 and TMZ in recurrent glioblastoma/ NCT01956734	I	Adenovirus	Mutation in E1A and RGD-related integrin expression	31	3 × 10 ¹⁰ vp/ resection cavity	Number of patients with adverse events
Combined Cytotoxic and immune- stimulatory therapy for glioma/NCT01811992	I	Adenovirus	TK and Flt3L expression	18	10 ⁹ – 10 ¹¹ / peritumoral	Dose-limiting toxicity

Data from clinicaltrials.gov ¹⁰². vp: viral particles, EID50: 50% egg infectious dose, TMZ: Temozolimide, IRES: internal ribosomal entry site, IT: intratumoral, IV; intravenous