A paucity of effective treatments exists for glioblastoma, the most malignant form of brain cancer. However, novel therapies are in active development, including therapies based on the use of viruses. At least 20 different clinical trials using more than seven different viruses have been studied as a treatment for brain tumors; however, although these studies have managed to confirm the proof-of-concept and in a few individual cases treatment responses have been seen, they have yet to bring forth durable clinical benefit to the bedside. In this article, we highlight the development of viral therapy for brain tumors and some important questions that remain to be answered as this therapy is implemented.

A perspective on viral replication: what virus type to use?

The challenges of viral therapy in the brain are highlighted by the disease itself. After the initial surgical resection, there may be bulky residual tumor near the resection side, or there may be microscopic foci of disease some centimeters away. An important question is then how to deliver an effective virus capable of killing these residual tumor cells either directly through viral lysis or by delivering a gene product that would render it susceptible to killing via an external agent (e.g., chemotherapy) or innate mechanism (e.g., immune mediated)? Existing studies may help determine some of the best features of viruses used to attack brain tumors.

A replication-defective virus is likely to produce only a minimal short-term effect in terms of gene expression. For example, when Oldfield et al. injected human glioblastoma multiforme (GBM) patients with residual tumor with a replication-defective retrovirus that encoded the HSV-TK gene, only a small amount of gene expression was noted near the tumor [1]. Using this construct, only a few small clusters of tk-positive cells were found within several cell diameter of the needle track with little dissemination [2]. Later, the first randomized Phase III clinical trial in GBM viral therapy involved the injection of this construct with subsequent administration of ganciclovir, which revealed no significant difference in survival when comparing patients treated with viral therapy versus control patients [3].

We also know that attenuated replication-competent viruses may also suffer from a similar limitation of viral persistence and spread, albeit by a different mechanism. For example, the doubly mutated G207 herpes virus designed by Martuza and colleagues similarly shows minimal propagation of virus through brain tissue [4]. Moreover, Chiocca and colleagues showed that similarly constructed herpes viruses were often limited by the host immune response [5]. The results of clinical trials using this construct have been equivocal. Markert et al. showed that eight of 21 participant patients treated with G207 had a positive response with one patient...
showing long-term survival of more than 5.5 years; however, the mean survival remained modest at 15.9 months [6]. Later, in a follow-up Phase Ib trial of G207 with six participants, no significant difference in survival was noted comparing control with treated group [7]. It was discovered that viral replication was present in only half of the patients [7]. By the year 2000, it was evident that the genetic payload approach by itself using genes such as HSV-TK, delivered by replication-defective or attenuated viruses, was not sufficient to produce favorable clinical results in GBM patients. This immune-based limitation may also inhibit other viruses that act by target cell lysis such as adenovirus. However, it may be possible to augment viral anti-tumor effects by combining viral treatment with an immune-response-limiting drug or a chemotherapeutic approach.

What about a replication-competent virus that is not directly pathogenic to host cells? The work with replication-competent retrovirus is of interest in this regard. For example, one of the newly developed viruses utilizes the genetic payload approach combined with a non-oncolytic variation. Toca-511 is a nonlytic replication-competent retrovirus, genetically engineered from murine leukemia virus, that is capable of stable tumor cell DNA integration and propagation while delivering a prodrug-activating gene, cytosine deaminase [8]. Without initially causing cell lysis, this virus is able to achieve substantial viral transduction with impressive tumor selectivity [9] and minimal host immune cell activation [10]. Subsequently, once enough time has been given for replication and propagation of the virus through the tumor tissue, prodrug 5-fluorocytosine is administered intravenously, which is then locally converted to 5-fluorouracil in the infected tumor cells. In this method, the nonlytic genetic payload approach may be an effective way of suppressing GBM, as suggested by Toca-511 preclinical studies.

Enhanced viral therapy efficacy by combinatorial therapy & strategies to manipulate tumor microenvironment

Currently, two standard therapies for glioma exist: radiation therapy and chemotherapy. In addition, immunotherapy and antiangiogenic therapy are also being tested in glioma. Do these therapies facilitate or disrupt viral therapy and what is the best way to combine viral therapy with a regimen that includes these agents? An interesting study in 2006 demonstrated that temozolomide, a widely used chemotherapy drug, exhibited strong synergy with G207 in both O6-methylguanine-DNA methyltransferase-negative and O6-methylguanine-DNA methyltransferase-inhibited gliomas [11]. In vivo, all athymic mice with U87 intracranial xenograft tumors that were treated with combination G207 and temozolomide exhibited a 100% 90-day survival. This was much improved compared with either G207 or temozolomide alone treated control mice with 10 and 0% 90-day survival, respectively [11].

Concerning the combination of viral therapy with radiation, there is less data available. Hadijianayis et al. showed in brain tumor models that a similar improvement in herpes viral efficacy could also be observed with combination radiation therapy [12], paving the way for safety testing of this approach in humans. This approach may apply to other viruses as well, considering that oncolytic parvovirus has been shown to synergize with radiation in glioma cell killing in vivo [13]. In non-brain-tumor models, it has been shown that the combination of G207 with low-dose radiation results in increased cytotoxicity of in vivo mouse flank colon cancer model, and that this is dependent on the upregulation of cellular ribonucleotide reductase activity [14].

In addition, the combination of viral therapy with other therapies that alter the tumor microenvironment can have the capacity to alter the efficacy of viral therapy. For example, Thaci et al. showed improved oncolytic adenovirus distribution when combined with antiangiogenic therapy [15]. While it has been shown that oncolytic viruses have an immediate direct antiangiogenic effect, other studies have demonstrated that the resultant changes in the tumor microenvironment may nonetheless facilitate the regrowth of vasculature in the remaining tumor after viral clearance. For instance, Aghi et al. found that there was a decrease in antiangiogenic peptide expression and a delayed tumor regrowth mediated in part by a resultant increased local vascularity after the injection of an oncolytic herpes virus in a glioma xenograft model [16]. However, this effect could be mitigated by combining G207 with 3TSR, a peptide containing thrombospondin-1 region, which reduced the virus-associated increase in vascularity.

There may be other strategies that can be applied to brain tumors that are currently undergoing testing in other tumor types. For example, the combination of virus injection with collagenases has been shown to be effective in promoting virus spread by overcoming physical barriers that may exist within the tumor tissue. It has been observed that compared with injection of HSV virion into subcutaneous melanoma tumors grown in mice, co-injection of these viruses into tumor with bacterial collagenase resulted in increased tumor distribution and anti-tumor efficacy of the oncolytic virus [17]. On the basis of this principle, an adenovirus has been constructed with relaxin (matrix metalloproteinase-inducing protein) expression, which has been shown to be more effective in disseminating within tumor spheroids compared with control vectors lacking relaxin [18]. Given the limitations observed with viral distribution in the brain, such techniques are ripe for testing in brain tumor models as well.

The innate immune system, which typically plays an important beneficial role in clearing natural viral infections, actually appears to provide potent obstacles for achieving therapeutic virus replication and tumor destruction [19]. However, the stimulation of adaptive immunity may also have a positive impact on tumor therapy through its promotion of antibodies against virally infected tumor cells and priming of the cytotoxic T-cell response. It has been described that both intratumoral viral titers and anti-tumor efficacy is improved by pretreatment of tumor-bearing animals with cyclophosphamide [20]. The enhanced efficacy is thought to be due to elimination of antiviral cytokines and depletion of mononuclear cells. In addition, understanding the interplay between host immunity and viral vector is important, as it can have significant bearing on safety.
Viral vectors: promising new therapeutics in the battle against glioblastoma

Conclusion
While viral therapy for brain tumors remains an as yet unfulfilled promise, it is apparent that the work of the past 20 years has revealed necessary hurdles to overcome before making this a therapeutic reality. The pioneers who developed viral therapy for use in the brain have laid a foundation for success for the next generation of clinician scientists who work to bring this therapy to effective use in the clinic.

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References
16 Aghi M, Rabkin SD, Martuza RL. Angiogenic response caused by oncolytic herpes simplex virus-induced reduced thrombospondin expression can be prevented by specific viral mutations or by administering a thrombospondin-derived peptide. Cancer Res. 66(2), 440–444 (2007).