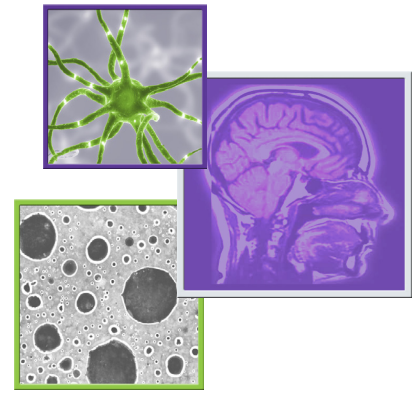
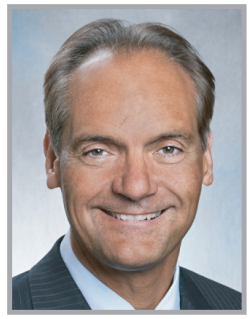
EDITORIAL



Oncolytic virotherapy for gliomas: steps toward the future



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"Several oncolytic agents have recently progressed to advanced efficacy trials for solid non-CNS tumors..."

Oncolytic virotherapy is a relatively novel and increasingly examined strategy to combat various tumor types including those of the CNS [1]. In this approach, replication-competent viruses are engineered to specifically infect and replicate in cancer cells, while normal cells are spared. In the process of progeny particle release, tumor cells are killed and tumor-associated antigens are released. Exploiting their self-perpetuating and replicative nature, oncolytic viruses (OVs) can spread throughout the tumor, ideally leading to complete tumor destruction by anti-tumor immune activation, disruption of tumor blood supply, and virally encoded therapeutic transgenes in addition to direct oncolysis [2]. Several oncolytic agents have recently progressed to advanced efficacy trials for solid non-CNS tumors, including the pioneering herpes simplex virus (HSV) talimogene laherparepvec, which may be the closest to receiving US FDA approval for intratumoral treatment of malignant melanoma [101]. Local administration is also applicable for oncolytic virotherapy of gliomas, for which viral infusions into the tumor or the resection cavity are performed either by free-hand [3] or convection-enhanced delivery [4].

Phase I/II trials have been carried out using OVs for the treatment of glioblastoma (GBM), and different viral species and strains have been utilized. Some have been chosen on the basis of their natural oncotropism, as in the case of Newcastle disease virus administered intravenously [5] or the Dearing strain of reovirus (named Reolysin®; Oncolytics Biotech, Inc., Calgary, Canada) administered intratumorally [6]. Other OVs are genetically modified to ensure tumor-specific replication: examples include the adenovirus ONYX-015 with deletions in the early genes *E1B* and *E3* [3], or HSVs G207 [7], G47Delta [8], HSV1716 [9] or rQNestin34.5 [10] with mutations that attenuate the virus so that it replicates primarily in tumor cells. All these viruses have been administered into the tumor or peritumorally.

All trials of OV therapy for GBM so far have shown relative safety even at the highest administered doses. In fact, evidence for a maximum tolerated dose has not been currently shown in any of the published trials. These safety data are especially

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encouraging in the context of intratumoral injection of gliomas, where no signs of focal or diffuse viral encephalitis have been observed. Furthermore, OV transmission to others in contact with treated subjects has not been observed [11], despite the occasional detection of viral shedding in saliva, urine and feces [5–7]. Regardless, monitoring for serious adverse events remains paramount as manufacturing techniques are allowing for administration of even higher doses of OVs and new, more potent, OVs are being engineered and tested.

Since the power for detection of efficacy is nonexistent in early-stage clinical trials, reports of benefit remain anecdotal. For instance, one out of 11 patients treated intravenously with multiple doses of Newcastle disease virus was reported to exhibit a complete remission accompanied by an improved neurological status leading to discontinuation of corticosteroid therapy. However, within 3 months, histologically confirmed GBM regrew [5]. Similarly, one out of 12 patients participating in a trial establishing safety of a reovirus experienced stable disease for 39 weeks after a single dose of Reolysin® (Oncolytics Biotech, AB, Canada) until the astrocytoma eventually progressed [6]. As expected, subjects with grade III astrocytomas survived longer than those with GBM after virotherapy, but this is more likely owing to the more favorable behavior of the lower grade tumor [6,9,12]. Overall, all trials using an OV as a single agent provide hints of a therapeutic effect, but further improvements in virus design and/or delivery are required.

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Currently, a series of new OVs are being tested. In addition to a Phase I/II trial using the rat parvovirus H-1 [13], a dose-escalation study investigating the genetically engineered poliovirus PVS-RIPO for intratumoral infusion into recurrent GBM was initiated in January 2012. This virus relies on entering cells via the nectin-5 receptor, overexpressed in GBM and other malignancies. It has also been engineered to be non-neuropathogenic by exchange of the translation-regulating sequences of poliovirus with those of human rhinovirus 2 [14]. Another OV consists of Delta24-RGD, an engineered adenovirus that selectively replicates in pRB-deficient tumor cells and also enters via surface integrins [15]. This OV is currently under investigation in two early clinical trials in humans with GBM [102]. Clinical trials for GBM with a measles virus are currently ongoing [103].

Several challenges remain before OV therapy shows meaningful efficacy. For instance, OV replication within tumors appears to be relatively low [7]. A possible explanation for low levels of viral replication may be the effects of the innate immune system, which attempts to limit the initial phases of OV infection and replication within tumors [16–18]. OV biodistribution and spread throughout the tumor may also be an issue. Spread throughout the tumor could be improved using slow and continuous infusions with a microcatheter [19]. To increase spread through the tumor stroma, OVs have been engineered with transgenes that can dissolve components of the extracellular matrix, such as hyaluronidase [20] or chondroitinase ABC [21]. Alternatively, virus species with intrinsic properties beneficial for movement through the tumor may be chosen, such as measles viruses spreading via cell fusion or vaccinia viruses via drilling themselves into neighboring cells with an actin tail [1]. Finally, tumor infection selectivity could be increased by redirecting OV tropism towards molecules expressed on the tumor surface [1].

Clearly, the immune system influences virotherapy. It could pose a barrier to viral delivery and replication, but it can also support tumor clearance when directed toward tumor-associated antigens rather than viral epitopes. In fact, the two OVs that have progressed the furthest in clinical trials, talimogene laherparepvec and the vaccinia virus JX-549, actively express granulocyte–macrophage colony-stimulating factor to provoke anti-tumor immunity after infection. Malignant melanoma, known to be highly susceptible to immunotherapies, appears to respond particularly well to these OVs. However, other tumor types, such as GBM, could also benefit from oncolysis-mediated cross-priming of tumor antigens and immunostimulation by virally encoded cytokines [22]. The temporal sequence of modulating the innate immune system to initially allow OV replication and lysis, followed by restimulation of the innate and adoptive immune responses with a lysed tumor antigen 'debris' field is thought to be the appropriate strategy to achieve OV efficacy.

Another approach consists of combination therapies in which the OV is one component of a multimodal regimen. This can include arming of viruses with prodrug convertases able to activate innocuous prodrugs into active chemotherapeutic metabolites that can not only kill infected tumor cells, but also uninfected

neighboring tumor cells [23,24]. The most popular systems consist of HSV thymidine kinase plus gancyclovir (or derivatives) and bacterial or yeast cytosine deaminase plus 5-fluorocytosine. In fact, a nonlytic replicating retrovirus Toca-511 expressing an optimized cytosine deaminase is currently under clinical investigation for glioma therapy in combination with 5-fluorocytosine [25]. Importantly, OVs are able to synergize with radiation or chemotherapies offering a plethora of combination regimens to be evaluated in the future. Promising results from a scheme uniting glioma surgery, adenoviral oncolysis, thymidine kinase-mediated valacyclovir activation and radiotherapy achieved median overall survival of 12.4 months [12] encourage the development of other complex protocols. Of note, tumor killing may also be augmented by combining two OVs selected to complement each other's effect: in a seminal study, a vaccinia virus and a vesicular stomatitis virus were effectively combined and viral yields in human brain cancer tissue samples could be increased up to 1000-fold [26]. Additional synergies between these and other virotherapeutics are certainly worth investigating. Combination therapies not only offer a way to enhance efficacy, but could also solve the heterogeneous responses of tumors to single agents.

Ultimately, the field will benefit considerably from developing OV platforms for efficient systemic delivery to allow convenient repeated dosing and treatment of distant metastases. Multiple factors have hampered the success of intravenously administered OVs, including sequestration in the liver and spleen as well as neutralization by complement and antibodies [27–29]. Strategies to overcome this include pharmacologic agents [30], chemical modification of the viral surface typically using polyethylene glycol or clodronate, chimeric viruses displaying neutralizing epitopes of alternative noncrossreactive serotypes [31], or viral strains naturally evolved for blood-borne dissemination. Another promising strategy relies on mesenchymal stem cells or other cells with tumor-homing capacities as 'Trojan horses' to carry OVs to the tumor, thus rendering viral particles invisible to neutralizing serum factors [1]. Importantly, this strategy has been shown to be suitable for CNS applications when neural stem cells were used to deliver a conditionally replicating adenovirus to glioma xenografts in nude mice [32]. All these approaches provide a toolkit to improve the outcome of oncolytic virotherapy of gliomas. Viral engineering, immunomodulation and combination therapies are yet to be fully exploited for progress in this clinically young field.

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EA Chiocca serves as a consultant to DNAtrix, Inc., which is developing Delta24-RGD. For this he receives stock options. The authors have no other relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript apart from those disclosed.

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