Neuro-Oncology Advances

5(1), 1–7, 2023 | https://doi.org/10.1093/noajnl/vdad081 | Advance Access date 7 July 2023

Recent oncolytic virotherapy clinical trials outline a roadmap for the treatment of high-grade glioma

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Abstract

Adult and pediatric high-grade gliomas (HGGs) are aggressive cancers of the central nervous system that confer dismal clinical prognoses. Standard radiation and chemotherapy have demonstrated only limited efficacy in HGGs, motivating the accelerated investigation of novel modalities such as oncolytic virus (OV) therapies. OV centered therapies work through a mixed mechanism centered on oncolysis and the stimulation of an antitumor immune response. Three recent clinical trials utilizing herpes simplex virus-1 and adenovirus-based oncolytic virotherapy demonstrated not only the safety and efficacy of OVs but also novel dosing strategies that augment OV response potential. Considering these recent trials, herein we present a roadmap for future clinical trials of oncolytic immunovirotherapy in both adult and pediatric HGG, as well as persistent roadblocks related to the assessment of OV efficacy within and between trials.

Keywords:

brain tumors | high-grade glioma | immunotherapy | neuro-oncology | neurosurgery | oncolytic virotherapy | pediatrics

High-grade gliomas (HGGs) are the most frequently diagnosed primary central nervous system (CNS) tumor in adults and are the leading cause of cancer-related mortality in children.[1] They bear a poor prognosis due to a lack of efficacious treatments and the high frequency of tumor recurrence.[1] The median overall survival of newly diagnosed HGG in studies since the initiation of temozolomide/radiation is 15.6 months[2,3]; recurrent HGG confers an even more dismal outlook, with a median survival of only 6–9 months.[4,5] Current standard-of-care treatment regimens for newly diagnosed HGG includes maximal surgical resection, radiation, systemic therapy with the DNA alkylating agent temozolomide, and regional therapy with alternating electrical fields.[6] However, development of standardized protocols for management of progressive disease remain elusive.

Oncolytic viruses (OVs) are emerging as promising treatment strategies to meet the clinical challenge of HGG, as they confer several advantages over traditional therapies. OVs are capable of selectively infecting tumor cells and replicating within them,[7] generating antitumoral effects through both induction of direct oncolysis and stimulation of antitumor immune activity.[7] Intratumoral injection of OVs also bypasses

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the blood–brain barrier and does not necessitate a baseline immunogenic tumor microenvironment, thereby avoiding major obstacles hindering other immunotherapeutic efforts in HGG.[8] Finally, OVs can be modified with genetic alterations that increase the safety/efficacy of the virus.

Among the ~15 different virus species currently being studied in adult and pediatric glioma patients, herpes simplex virus-based OV therapies (oHSV) and adenovirus-based OV therapies have been most frequently employed.[9] Many OVs have demonstrated promising results and provide clinical support that such an approach has potential to make strides in treating intractable CNS cancers.[10–13] We have examined these in a prior review,[9] and we have herein elected to highlight/discuss two recent pediatric trials and one recent adult phase II trial given rather intriguing clinical results/future implications (Table 1).

In the first clinical trial of OVs in pediatric HGG, Friedman et al. reported on a phase 1 trial of children and adolescents with progressive supratentorial HGG with intratumoral administration of the herpesvirus (HSV-1) G207.[14] Next, Gállego Pérez-Larraya et al. completed a phase 1 trial of children and adolescents with newly diagnosed diffuse intrinsic pontine glioma (DIPG) using intratumoral administration of the adenovirus DNX-2401.[15] In the most recently completed OV trial, Todo et al. completed a phase 2 trial of adults with supratentorial glioblastoma with intratumoral administration of the herpesvirus G47Δ.[16] Beyond the promising clinical data, these three trials model several key innovations and highlight persistent challenges in the implementation of OV therapy for both pediatric and adult HGG.

Novel OV Delivery and Dosing Strategies are Both Safe and Efficacious

There are many benefits to direct intratumoral inoculation, including allowing OVs to bypass the blood–brain barrier which prevents effective delivery of systemic treatments. Intraparenchymal approaches to OV delivery also yield high local concentrations thereby decreasing the incidence of systemic toxicity.[17] Of note, concerns about procedure-associated complications and virotherapy-associated inflammation have precluded direct inoculation of infratentorial tumors, such as those observed in pediatric HGG patients.[18]

All three studies employed intratumoral delivery methods (Table 2). Catheter location for the first two studies was confirmed via postoperative imaging, and no major surgical and/or additional high-grade adverse events were observed. Crucially, Gállego Pérez-Larraya et al. directly inoculated pontine tumors with adenovirus-based OV with no significant sequelae, demonstrating for the first time the safety and efficacy of intratumoral dosing within the brainstem. Intratumoral inoculation is, therefore, a promising/practical approach to OV therapy regardless of location; as such, future trials may employ such methods.

One patient in the DIPG trial and all the patients in the G47 Δ adult trial received additional doses of OV therapy (Table 2), with no recorded limitations on number of doses and/or inoculation levels. Patients in Todo et al. study received a maximum of 6 doses of G47 Δ with the trial design having been based on preclinical data that demonstrated superior efficacy of multiple intratumoral doses as compared with a single, albeit tenfold higher dose.[19] In this trial patients did not suffer from more adverse events in the setting of repeat dosing and increased tumor infiltrating lymphocytes (TILs) were noted to have correlated with additional OV doses; repeat dosing methods may therefore increase the efficacy of OV therapies. One of the primary concerns of combining this strategy with intratumoral inoculation is the need for repeat stereotactic biopsies/injections and associated risks related to possible surgical complication(s). While this did not materialize, advanced systemic delivery methods capable of facilitating multiple rounds of inoculation without the need for repeated surgeries are currently under investigation, which would decrease the physical and financial burden(s) of repeated-dosing strategies.[20–24]

The results of the Todo trial help motivate the incorporation and investigation of multiple dosing strategies in future clinical trials of OV in HGG.[16]

Table 1. Study characteristics for the three clinical trials discussed.

Study	Country	Phase	Patient populations	Virus	Tumor types	Treatment Groups
Friedman et al. 2021[14]	USA	1	Children and adolescents (ages 7–18) with pathologically proven malignant supratentoral brain tumor with diameter of 1.0 cm or more that progressed after surgery, radiotherapy, or chemotherapy	HSV-1 G207	10 GBM 1 AA 1 HGG, not otherwise specified	Four cohorts: 10^7 PFU, 10^8 PFU, 10^7 PFU + 5 Gy radiation, 10^8 PFU + 5 Gy radiation
Gállego Pérez-Larraya et al. 2022[15]	USA	1	Children and adolescents (ages 1–18) with newly diagnosed DIPG (confirmed by clinical and MRI features)	DNX-2401	12 DIPG	Two cohorts: 10^8 viral particles, 5×10^{10} viral particles
Todo et al. 2022[16]	Japan	2	Adult pateints (age > 18) with residual or recurrent supratentorial glioblastoma after surgery, radiation therapy, and TMZ	G47Δ	19 GBM	Single-arm study, 10^9 PFU*

GBM, glioblastoma; AA, anaplastic astrocytoma; HGG, high grade glioma; DIPG, diffuse intrinsic pontine glioma
*In clinical studies for lethal diseases in Japan, setting a non-curable standard-care control arm is considered unethical and not accepted.

Lack of Standardized Response Assessment Criteria and Seropositivity Reporting Hinders Comparison of OV Clinical Trials

Response assessment(s)—Response assessment remains an unsolved challenge for OV treatment of HGGs. False tumor enlargement, or pseudoprogression, has been reported in 10–30% of HGG patients within the first 12 weeks of treatment with radiation and chemotherapy.[25–27] This phenomenon is also commonly observed following OV therapy due to increased immune cell infiltration, which is difficult to distinguish from actual tumor enlargement/progression.[25,28] Although pseudoprogression commonly resolves spontaneously without treatment, its presence complicates interpretation of outcomes such as progression-free survival, confounds clinical decision-making and can lead to inappropriate additional treatments and/or surgical resections.[29] Furthermore, the use of steroids to combat persistent pseudoprogression can also interfere with the antitumor activity of newly recruited immune cells, abrogating the potential goal/success of OV therapy. Finally, published clinical trials implement varying timelines for follow-up imaging assessment of tumor progression following OV treatment. This inconsistency affects comparison of tumor regression vs progression timelines across trials and may delay opportunities for OV re-dosing and/or other therapeutic intervention(s) as appropriate.

Importantly, no accepted criteria yet exist for assessing response to intratumoral OV inoculation. Friedman et al. used the immunotherapy response assessment in neuro-oncology (iRANO) criteria,[25] whereas Gállego Pérez-Larraya et al. used the response assessment in pediatric neuro-oncology (RAPNO) criteria.[26] Both methods are suboptimal for the evaluation of OV therapy response(s).

The iRANO criteria, designed specifically for immunotherapy response assessment, might better identify pseudoprogression than the previous RANO criteria[27,30]; however, it is prudent to note that they were developed prior to the 2016 and 2021 WHO classification schemata which incorporated genetic and epigenetic elements into HGG subclassification.[28,31] These genetic alterations have prognostic implications for survival and response to therapy, and future criteria will require the consideration of such governing biology. The RAPNO criteria for DIPG are newly developed recommendations that are designed specifically for unique characteristics in children/young adults that attempt to overcome the difficulties of reliably measuring response to DIPG therapies.[26] However, their generalizability and ability to guide accurate, reproducible assessments that reflect clinical benefits remain unknown given the limited extent to which they have yet been validated. Moreover, despite the use of RAPNO, Gállego Pérez-Larraya et al. still reported difficulties in determining progression free survival with certainty.[15] Instances of pseudoprogression were also reported by Friedman et al.[14] Although Todo et al. reported that their response criteria functioned well, with 12 patients (63.2%) being able to receive the maximum 6 doses without being considered to have tumor progression[16]; the Todo trial was the only study which conducted repeat biopsies/injections for 6 doses over 5 months. Such a burr hole, injection and biopsy frequency may not be feasible in larger populations including frail patients. Moreover, economic considerations may make such an approach impractical in other settings. It is also noteworthy that Todo et al. report a median progression free survival of 4.7 months compared to a 20.2 median overall survival; a difference clinically indicative of at least a degree of pseudoprogression.

Table 2. Details of drug delivery for the three clinical trials discussed.

Study	Initial inoculation volume (mL)	Infusion rate (mL/hr)	Inoculation method	Radiation	Surgical complications	Additional OV doses	Other post-inoculation treatments (n)
Friedman et al. 2021[14]	2.4	0.6	3–4 catheters	For 2 cohorts, gross tumor volume plus 2-mm margins received 5 Gy radiation within 24 hr of inoculation	small catheter tract hemorrhage CSF leak that required oversewing	None	Steroids (3) Bevacizumab (6) Additional resection (4) Radiation (7) Other (6) None (4)
Gállego Pérez-Larraya et al. 2022[15]	1	0.9	1 cannula	11 patients with median 54 Gy (range 39.0–59.4) started median 17 days after inoculation (range 10–20)	None	1 patient received second dose	Steroids (0) Bevacizumab (2) Additional resection (0) Radiation (3) Other (5) None (6)
Todo et al. 2022[16]	1	0.2	manual injection in 1–3 locations	N/A	None	All received additional doses (range 2–6)	Steroids (0) Bevacizumab (12) Additional resection (3) Radiation (4) Other (1) None (5)

Standardized guidelines for optimal response assessment criteria and follow-up MRI timing will thus be important in determining the efficacy and noninferiority of OV therapies in adult and pediatric HGG. Given the experience of inflammatory related pseudoprogression in these three trials, gadolinium-based MRI alone may not be the optimal modality for response assessment of OV therapy of HGG. An example of a complementary modality was shown in a recent proof-of-concept study which demonstrated in vivo CD8-targeted positron emission tomography (PET) imaging of murine glioma models undergoing OV therapy.[32] Further work addressing translational challenges associated with the widespread use and clinically adoption of PET-based response assessment(s) is therefore encouraged. It is our contention that such modalities may ultimately aid in the determination of OV treatment response.[32]

Seroconversion—Most patients in the general population have been exposed to the viruses that form the backbone of OV therapies, but little is known about how previous viral exposure may influence response to OV therapy for HGG. Pretreatment seropositivity, or the presence of antiviral antibodies, for these virus families within control and treatment cohorts is not consistently reported in published trials, frustrating attempts to clarify its potentially confounding role.[14,15,22,33–35] The trials discussed in this commentary may add some additional insights. For example, Gállego Pérez-Larraya et al. reported that all patients were seropositive for adenovirus IgG before treatment and that anti-adenovirus antibody titers increased after treatment. Friedman et al. found that the median survival among patients with baseline HSV-1 seropositivity was shorter than those who seroconverted after oHSV treatment, although the analysis was limited by the small number of patients, while Gállego Pérez-Larraya et al. found that median overall survival was shorter for patients with higher anti-adenovirus titers after infusion. Todo et al., presented patient level data for HSV-1 seropositivity the day before G47Δ initiation. We ran a univariate survival analysis based on these seropositivity data and observed no difference in progression free survival or in overall survival depending on pre-G47Δ seropositivity (data not shown). Finally, beyond these three trials, pre-existing antibodies have been shown to reduce overall survival in solid tumors[36]; whereas oncolytic HSV-1 talimogene laherparepvec efficacy has been shown to not be reduced by seropositivity in the melanoma setting.[37] Together, these often conflicting data suggest a complex relationship between seropositivity and efficacy of OVs; of note, the integrity of the immune system and ability to mount a productive response should be considered in such instances. The impact of active viral infection at the time of therapy or previous infections requiring antiviral therapy on OV treatment for HGG also remains unknown, as patients with these clinical histories are commonly excluded from clinical trial cohorts.

In summary, we encourage documentation of pretreatment and posttreatment seropositivity for patients with HGG treated on OV trials in order to clarify these phenomena and establish guidelines for therapeutic application in previously exposed vs. nonexposed patients.

General versus tumor-specific inflammation—Immune cell evasion is a classic characteristic of HGG. This is especially true in pediatric HGG, likely related in part to low mutational burden, making these tumors immunologically "cold."[38] Preclinical models have demonstrated the effectiveness of OVs in increasing TILs and transitioning the tumor microenvironment to one that is proinflammatory.[39,40] The recent clinical studies found results consistent with preclinical models from posttreatment biopsies/tissue; infiltration was persistent, lasting > 50 months as observed in autopsies in Todo et al. However, it is not clear whether this immune reaction is general inflammation that fails to recognize tumor-specific antigens or if it represents direct recognition and lysis of tumor cells. Moreover, even if it does represent TIL mediated tumor cell lysis, the fact remains that despite persistent CD8 and CD4 infiltration in autopsies, patients died from progressive disease. One explanation could be upregulation of immunosuppressive mechanisms as tumors evolve. PD-1, PD-L1, CTLA-4 and IDO have for example all been shown to be upregulated following G207 OV therapy, revealing a potential role for checkpoint inhibitors.[41] Interrogation of general and peripheral versus tumor-targeted immune responses may distinguish effects specific to the OV. Although Friedman et al. found an increase in TILs from 2 to 9 months post inoculation, it is not yet clear if this phenomenon is tumor-specific. The influx could result from tumor antigen recognition, supported by the correlation between higher T-cell clonality post-treatment and progression-free survival as well as TILs in locations distant from inoculation sites; however, this may represent a generic inflammatory reaction related to viral antigen(s), although no viral antigen was ultimately recovered. Finally, although not a primary focus of these three trials, myeloid cells, such as tumor-associated macrophages and microglia, can act to both potentiate and inhibit OV therapy. Future efforts should be directed towards modulating myeloid responses to maximize antitumor responses while minimizing antiviral responses.[42]

Future studies should work to identify the mechanisms of the immune responses in order to utilize these to improve OV efficacy.

The Distinct Biology of Adult and Pediatric HGGs must Inform OV Clinical Trial Design

Early trials of oHSVs began in the 2000s and many subsequent trials have since investigated OV usage in not only adult but also in pediatric patients (Table 3).[9] OV therapy is an attractive alternative therapeutic strategy for pediatric HGG patients, who are especially susceptible to the adverse effects of standard chemo- and radiotherapeutic treatment regimens such as long-term neurocognitive/neurosensory impairment and endocrine function alteration.[43]

Clinical trials of novel treatments for HGG have historically occurred first in adult populations and significantly

Table 3. Current active pediatric oncolytic virotherapy clinical trials obtained on clinicaltrials.gov.

Class	Virus	Phase	NCT
Herpes Simplex Virus	HSV G207	1	NCT02457845
	HSV G207	1	NCT03911388
	HSV G207	2	NCT04482933
Adenovirus	AloCELYVIR	1b/2	NCT04758533
Poliovirus	PVSRIPO	1b	NCT03043391
Reovirus	Pelareorep	1	NCT02444546
Measles Virus	MV-NIS	1	NCT02962167

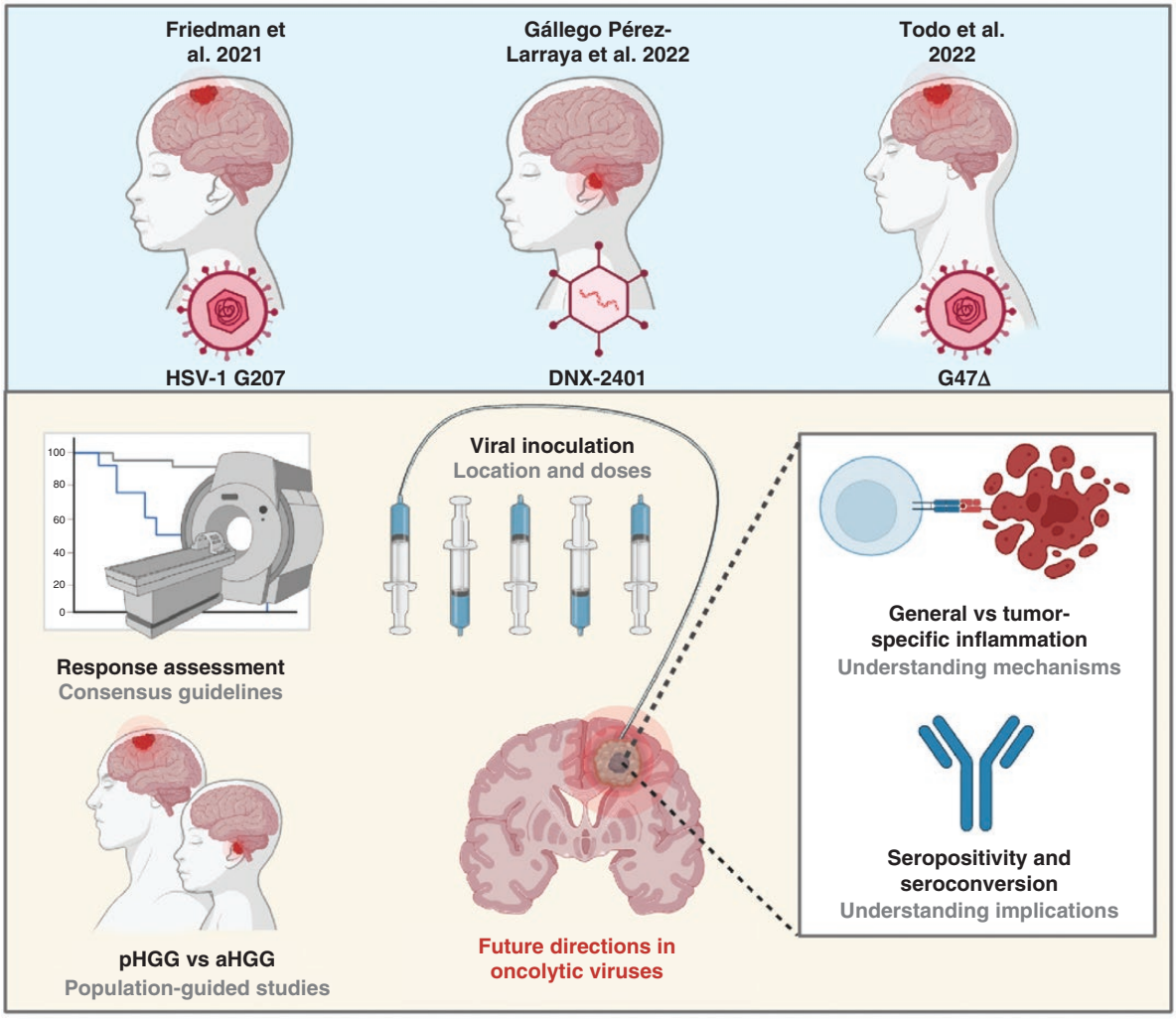

Figure 1. Recent clinical trials reveal path forward for oncolytic virotherapy in adult and pediatric high-grade glioma. High-grade gliomas (HGGs) are aggressive cancers of the adult and pediatric central nervous systems. Limited efficacy of standard chemo- and radiotherapy approaches motivated interest in novel therapeutic directions, including oncolytic virotherapy (OV). Three recently published clinical trials using herpes simplex virus- and adenovirus-based OVs to treat adult and pediatric HGGs demonstrated the strong potential of these therapies to improve patient survival, presented major innovations in OV administration, and highlighted opportunities to optimize assessment of OV efficacy. Intratumoral OV inoculation and repeated dosing strategies were shown to be safe and beneficial to survival and should therefore be included in future clinical trial design. Standardized reporting of pre- and post-OV seropositivity and unification of imaging-based Response Assessment guidelines must be a top priority to facilitate comparison of results across trials and improve patient stratification within future clinical investigations.

inform the design of subsequent trials for the pediatric populations.[44] However, detailed interrogation of the genomics, epigenomics, and tumor microenvironments of pediatric and adult HGG have demonstrated vast biological differences between these two entities as well as heterogeneity within pediatric and adult subsets of HGG, potentially explaining the limited efficacy of adult-based therapies applied to pediatric brain tumor patients.[45,46] For instance, biological predictors of therapy response in adult HGG, such as *MGMT* promoter methylation for TMZ treatment, are not always observed within the pediatric HGG population.[47] In addition, the mutation landscape of adult HGG (eg *IDH1/2, PTEN, EGFR*)[48] and pediatric HGG (eg oncohistone mutations [H3K27M, H3F3A, HIST1H3B], *PDGFRA*, tyrosine kinase fusions)[48] produce different malignant cell lineages that may confer divergent sensitivities to OVs and other therapies. Finally, though much remains unknown about the immune microenvironment of adult and pediatric HGG, the developmental context of each of these diseases contains key biological differences that may confound OV therapy.[49]

Therefore, future study design for OV use in adult and pediatric HGG is recommended to reflect the distinct biological pathophysiologies of these tumors. The unique cellular and molecular determinants of OV therapy response in these disparate patient populations should also be investigated at the preclinical and clinical levels to optimize treatment stratification and clinical trial enrollment criteria. An example of such an investigation was in the repositioning of G207 from the adult to the pediatric setting culminating in the Friedman et al. 2021 trial.[14] Pediatric murine glioblastoma models using patient derived xenografts were shown to express Nectin-1, a cell adhesion molecule, at significantly greater amounts than their adult counterparts. The pediatric models were also shown to be 11 times more sensitive to G207 than the adult models. Finally, Nectin-1 expression was shown to correlate with sensitivity of these brain tumor xenografts to G207.[50] These data helped motivate a trial of G207 in the pediatric setting.[14]

Conclusion

Recently published and ongoing clinical trials continue to show significant promise for OV therapy for the treatment of HGG. The trials discussed herein demonstrate the efficacy and safety of intratumoral inoculation and repeated dosing strategies. Till date, OV therapy has demonstrated a limited side-effect profile, promotion of an antitumoral microenvironment, and an apparent increase in overall survival compared to historical data in both adult and pediatric patients. Continued progress necessitates adoption of these successful practices, unification of therapeutic response criteria, and pre- and posttreatment seropositivity reporting standards (Figure 1).

Supplementary Material

Supplementary material is available online at *Neuro-Oncology Advances* online.

Conflict of Interest:

JDB has an equity position in Treovir Inc., an oHSV clinical stage company and is a member of the POCKiT Diagnostics, Centile Bioscience and NeuroX1 Boards of Scientific Advisors. The remaining authors have no pertinent conflicts to declare.

Funding:

JDB was supported by the Kiki Leptomeningeal Disease Research Fund.

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