Molecular Therapy
Oncology
Commentary

# Gene expression signatures predict the sensitivity of pediatric brain tumors to different oncolytic viruses

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https://doi.org/10.1016/j.omton.2024.200860

As the most frequent solid tumor in children, pediatric central nervous system (CNS) malignancies are the leading cause of cancer-related mortality in the age range of 0–18.[1] To improve survival rates and alleviate long-term negative effects of treatment, effective and less invasive therapeutic approaches are desperately needed. Oncolytic viruses (OVs) represent an emerging modality that may be able to address such unmet medical need in patients with pediatric brain tumors (PBTs). OVs preferentially infect tumor cells and multiply and disseminate to nearby uninfected tumor cells, destroying them. Furthermore, the oncolytic activity of OVs can elicit local inflammation and initiate a systemic anti-tumor immune response. A recent clinical trial testing intratumoral administration of Delta24-RGD (also known as DNX-2401) in 12 patients with diffuse midline glioma produced promising results, with a median overall survival of 17.8 months as compared to the historical control of 11 months.[2] Medulloblastoma and high-grade gliomas (HGGs) are the only PBT types for which OVs have been tested in clinical trials.[3] In this issue of *Molecular Therapy Oncology*, Vazaios and colleagues evaluated the oncolytic efficacy of four OVs, Delta24-RGD, rQNestin34.5v1, R124, and rNDV-F0-GFP, derived from adenovirus, herpes simplex virus type 1, reovirus, and Newcastle disease virus, respectively, in 14 patient-derived tumor sphere cultures established from three types of PBTs: HGGs, atypical teratoid/rhabdoid tumors (AT/RTs), and ependymomas (EPNs).[4] Overall, all four OVs exhibited significant oncolytic activity against the PBT cells, consistent with their expression of viral entry-related molecules. However, the oncolytic potency of each OV significantly varied among the cultures, revealing the pronounced heterogeneity of the response. An interesting trend was that Delta24-RGD had a strong oncolytic potential in different AT/RT and EPN cells with MOIs <10, while rQNestin34.5v1 was consistently potent against HGGs with MOIs <1. Overall, however, no preferential effectiveness of particular OVs was observed for each tumor type.

Traditionally, decisions for pediatric cancer treatment have been based on broad tumor classification and clinical staging, a "one-size-fits-all" approach that often fails to account for the unique molecular makeup of each child's tumor. To challenge this paradigm, the authors asked whether gene expression profiles of PBT cells could be biomarkers to predict a tumor's susceptibility to the four OVs. Using RNA sequencing, the authors acquired the transcriptional profiles of the 14 diverse PBT sphere cultures of HGG ($n$ = 7), AT/RT ($n$ = 4), and EPN ($n$ = 3) in their uninfected state, with the goal of determining genes linked to susceptibility and resistance to the OVs. Genes linked to virus entry did not significantly correlate with OV sensitivity except for *syndecan 1* (SDC1) and rQNestin34.5v1. The analyses did reveal genes that were significantly associated with the sensitivity and resistance of each OV. Interestingly, those genes linked to response to each OV did not overlap, indicating that different biological pathways underlie the cytotoxicity of the OVs tested.[4]

The authors used Gene Ontology (GO) term enrichment analysis to identify distinct biological processes linked to sensitivity and resistance to each OV and uncovered a unique landscape of biological processes that may underlie OV efficacy. For example, Delta24-RGD sensitivity was linked to viral transcription and gene expression, highlighting the importance of the virus's ability to hijack the cellular machinery for replication. Conversely, resistance to Delta24-RGD was associated with nuclear factor κB (NF-κB) signaling and hypoxia together with increased expression of *HIF-1A*. On the other hand, cellular proliferation, WNT/β-catenin signaling, glycoprotein synthesis, and exosomal secretion were linked to rQNestin34.5v1 sensitivity, whereas the development of the cerebellar vasculature and ribosomal protein import into the nucleus were linked to resistance. To validate the ability of the identified gene signatures to predict the cytotoxic activity of each OV, they incorporated four additional PBT sphere cultures as a validation cohort in the transcriptomics and *in vitro* efficacy studies. Gene expression signatures of the cells were able to predict the relative sensitivity or resistance of PBT cultures to OV-induced oncolysis, and did so particularly accurately in the case of Delta24-RGD and rQnestin34.5v1,[4] corroborating the utility of the identified transcriptomic profiles as biomarkers predictive of efficacy.

The authors employed a rigorous methodology that integrated a panel of patient-derived tumor sphere cultures with statistically robust gene expression analysis. This work is translationally significant, as it provides a framework for customizing OV therapy to individual pediatric patients. The research outcome holds promise for the development of personalized treatment strategies, potentially improving outcomes for patients. However, future studies should incorporate the tumor microenvironment and immune components into more complex models (*in vitro*, *ex vivo*, and *in vivo*) to gain a holistic understanding of OV therapy. This would

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allow for the investigation of the interplay between OV-induced oncolysis and the immune response, a crucial aspect of OV therapy that was not captured in the current model. Ultimately, however, the true translational value of this work will have to be assessed clinically by determining whether the transcriptional biomarkers of the tumor correlate with patient outcome after OV treatment. Beyond its translational potential, the current study also provides valuable insights into the molecular mechanisms underlying the heterogeneous response of PBTs to different OVs. These findings not only deepen our understanding of the complex interactions between OVs and tumor cells but also open up new avenues for research. Targeting the molecular pathways identified in this study offers opportunities to develop strategies to enhance OV efficacy or overcome resistance mechanisms, which may ultimately improve patient outcomes.

While the focus of this study is on PBTs, its implications extend beyond this specific context. The concept of using gene expression profiles to predict OV sensitivity could be applied to other types of pediatric cancers and even adult malignancies. This could lead to a paradigm shift in cancer treatment, where OVs are not just a tool in the arsenal but a personalized weapon tailored to each patient's unique tumor biology. Furthermore, the study's findings could pave the way for the development of novel combination therapies. Better understanding of the molecular mechanisms of OV sensitivity and resistance could lead us to identify synergistic combinations of OVs with other treatment modalities, such as chemotherapy or immunotherapy, to maximize therapeutic efficacy.

In conclusion, Vazaios and colleagues have made a significant contribution to the field of oncolytic virotherapy by paving the way for a cancer treatment paradigm that is not just effective but also personalized. Given the unique vulnerabilities of the developing brain, understanding the potential risks of acute and late complications subsequent to OV treatment will continue to be critical. As we move forward, it is essential to build upon this foundation with further research and clinical trials to ensure that this promising therapeutic approach reaches its full potential.

## DECLARATION OF INTERESTS

The authors declare no competing interests.

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