Macrothink Institute™

Journal of Biology and Life Science
ISSN 2157-6076
2026, Vol. 17, No. 1

Novel Insights into Using CRISPR-Mediated Modulation of Dopaminergic Signaling Pathways to Enhance the Therapeutic Efficacy of ONC201 in the Treatment of Diffuse Intrinsic Pontine Glioma (DIPG)

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Received: September 10, 2025 Accepted: November 4, 2025 Published: November 20, 2025
doi:10.5296/jbls.v17i1.23126 URL: https://doi.org/10.5296/jbls.v17i1.23126

Abstract

Diffuse intrinsic pontine glioma (DIPG) is one of the most devastating childhood brain cancers, with a median survival of less than one year and almost no long-term survivors. Classified as a subtype of diffuse midline glioma (DMG) and characterized by the H3K27M mutation, DIPG remains resistant to surgery and conventional chemotherapy due to its location in the brainstem and its highly adaptive biology. ONC201, a first-in-class imipridone, has shown positive early clinical results in children with H3K27M-mutant DIPG by blocking dopamine receptor D2 (DRD2), overactivating the mitochondrial protease ClpP, and triggering stress and apoptosis pathways in tumor cells. While newer imipridones like ONC206 and ONC212 appear even more potent in laboratory models, ONC201 has the strongest clinical track record to date, making it the most practical candidate for exploring combination strategies.

CRISPR-Cas9 gene editing could be used to boost ONC201's effects by targeting the survival mechanisms DIPG cells rely on. Potential strategies include knocking out dopamine receptors like DRD2/3, shutting down integrated stress response regulators such as PERK and GCN2, and removing anti-apoptotic genes like BCL2L1, MCL1, and XIAP that block cell death. Other promising approaches include editing PDGFRA or AKT to weaken compensatory growth pathways, or targeting CLPX to intensify ONC201-induced mitochondrial collapse. These genetic edits could work together to prevent resistance, amplify stress signals, and push DIPG cells past the point of recovery.

Still, major challenges remain. Delivering CRISPR safely across the blood–brain barrier is a significant hurdle, and most studies so far are limited to cell culture or glioblastoma models rather than DIPG itself. This highlights the urgent need for DIPG-specific in vivo research

before clinical translation is possible. This review brings together what is currently known about ONC201, CRISPR, and their potential synergy, outlining both the opportunities and obstacles in moving toward a more effective treatment strategy for this lethal pediatric cancer.

Keywords: ONC201 AND DIPG, dopaminergic signaling in DIPG, CRISPR AND dopamine receptor, CRISPR gene editing in glioma, ClpP activation in DIPG

1. Introduction

Diffuse intrinsic pontine glioma (DIPG) is a childhood brainstem tumor with a median survival of <1 year and a 5-year survival rate <1%. DIPG is now classified as a subtype of diffuse midline glioma (DMG), specifically including the H3K27M mutation (Weisbrod et al., 2024). DIPG, while rare, contributes significantly to overall pediatric brain tumor mortality. Large-scale epidemiological analyses show that survival varies considerably across pediatric brain tumor types, grades, and demographic factors, underscoring the urgent need for targeted therapies (Hossain et al., 2021). DIPG presents with cranial nerve deficits, long tract signs, and ataxia. Despite molecular advances, survival outcomes have not improved, and due to its location in the pons, surgical resection is typically infeasible (Weisbrod et al., 2024). The current standard of treatment remains radiotherapy as the main option for patients over three, and temozolomide is often co-administered but shows limited benefit due to MGMT-mediated resistance (Weisbrod et al., 2024). No current standard treatment has shown prognosis improvement (Stock et al., 2022).

H3K27M mutation is present in 70–85% of DIPGs. Dopamine receptors, particularly dopamine receptor D2 (DRD2), play a significant role in the biology and treatment responsiveness of diffuse intrinsic pontine glioma (DIPG). DIPG tumors often exhibit increased DRD2 expression alongside reduced DRD5 expression, which enhances tumor sensitivity to DRD2 antagonism as a therapeutic strategy (Hall et al., 2019). ONC201 is a first-in-class imipridone with demonstrated preclinical and early clinical efficacy in H3K27M-mutant DIPG (Weisbrod et al., 2024). The small molecule ONC201, a selective DRD2/3 antagonist, has demonstrated the ability to cross the blood-brain barrier and induce apoptosis in DIPG cells by activating the integrated stress response (ISR) and upregulating TRAIL and DR5, independent of p53 status (Borsuk et al., 2021; Hall et al., 2019). Mechanistically, DRD2 antagonism disrupts survival signaling in glioma stem-like cells, contributing to mitochondrial dysfunction and apoptosis (Kline et al., 2018). Notably, while DRD2 modulation enhances ONC201 sensitivity, ONC201's anticancer effects are not entirely dependent on DRD2 blockade, as it also activates mitochondrial ClpP protease, driving mitochondrial collapse and bioenergetic failure in DIPG cells. Additionally, preclinical findings indicate that dopamine pre-treatment does not protect DIPG cells from ONC201-induced apoptosis, in contrast to other tumor types, suggesting that DIPG cells are already adapted to high dopamine levels within the brainstem microenvironment and remain sensitive to DRD2 antagonism (Zhang et al., 2024). In clinical settings, ONC201 has demonstrated sustained tumor control and neurological improvement in H3K27M-mutant DIPG patients, with a favorable safety profile and minimal adverse effects (Hall et al., 2019). Response to ONC201 correlates with high ClpP and PDGFRA expression, while resistance is associated with high EGFR and FOXG1 expression, suggesting biomarkers for patient stratification and combination therapy strategies (Koschmann et al., 2020). Combination therapy strategies are under investigation, as ONC201 synergizes with HDAC inhibitors (panobinostat, romidepsin) and proteasome inhibitors (marizomib), it enhances ISR activation and apoptotic signaling in DIPG cells, while bifunctional LSD1/HDAC inhibitors have also been shown to reprogram chromatin and induce differentiation in DIPG models (Anastas et al., 2019). Second-generation imipridones ONC206 and ONC212 exhibit even greater potency at lower doses (Borsuk et al., 2021). These insights position dopamine receptor

signaling as both a biological vulnerability in DIPG and a rational therapeutic target that can be leveraged through ONC201 to induce apoptosis in H3K27M-mutant DIPG, supporting its ongoing evaluation in clinical trials. ONC201's ability to target dopaminergic signaling, induce mitochondrial stress, and activate the ISR positions it as a promising therapeutic candidate for DIPG, supporting further investigation in preclinical combination strategies and biomarker-guided clinical trials to improve outcomes in this highly lethal pediatric cancer.

Second-generation imipridones, including ONC206 and ONC212, show significantly enhanced potency compared to ONC201 in preclinical DIPG models. In studies using H3K27M-mutant DIPG cell lines, the half-maximal inhibitory concentrations (IC_{50}s) were 1.46 μM for ONC201, compared with just 0.11 μM for ONC206 and 0.03 μM for ONC212, indicating over an order of magnitude greater cytotoxic strength (Borsuk et al., 2021). Moreover, these second-generation imipridones replicate ONC201's mechanism, such as engaging ClpP/CLPX, activating ATF4, and upregulating TRAIL-DR5. They can also synergize robustly with histone deacetylase inhibitors (panobinostat, romidepsin) and proteasome inhibitor marizomib (Borsuk et al., 2021). Despite these compelling preclinical results, ONC201 remains the most thoroughly characterized imipridone in clinical contexts: it has demonstrated reliable blood–brain barrier penetration, established safety in early-phase pediatric trials, and sustained neurological and radiographic responses in H3K27M-mutant DIPG patients (Hall et al., 2019; Weisbrod et al., 2024). Thus, focusing on ONC201 allows the present study to leverage a solid translational foundation, while recognizing that ONC206 and ONC212 may inform future iterations or combination strategies once clinical validation is achieved.

CRISPR-based strategies hold promise for enhancing ONC201 efficacy in DIPG by targeting resistance pathways and stress adaptation mechanisms that enable tumor survival. DIPG tumors frequently activate compensatory oncogenic signaling pathways, including PI3K/AKT/mTOR, PDGFRA, and ACVR1, contributing to therapeutic resistance and necessitating combination strategies to overcome pathway redundancy (Duchatel et al., 2019). Additionally, DIPG's adaptation to *integrated stress response (ISR) signaling under hypoxic and nutrient-deprived microenvironments enables resistance to ISR-activating therapies like ONC201 (Lines et al., 2023). CRISPR-Cas9 can be utilized to knock out key resistance-associated genes such as DRD2, XIAP, and components of the ISR pathway (e.g., ATF4, PERK, GCN2) to sensitize DIPG cells to ONC201-induced apoptosis while blocking compensatory survival pathways (Panganiban et al., 2019). Additionally, CRISPR can be leveraged for functional genomic screens to identify synthetic lethal targets in combination with ONC201, guiding precision therapy strategies in DIPG (Shivram et al., 2021). By integrating CRISPR-mediated pathway disruption with ONC201's activation of mitochondrial stress and TRAIL-mediated apoptosis, it may be possible to develop effective, biomarker-driven treatment approaches for this highly resistant pediatric brain tumor. Given all these current findings, there are still many gaps on the implications CRISPR could have in mediating dopaminergic signaling to improve the efficacy of ONC201 in the treatment of DIPG, which this paper aims to address.

2. Methods

This paper was conducted as a literature review, drawing on recent peer-reviewed publications to evaluate the therapeutic potential of combining CRISPR-Cas9 gene editing with ONC201 in the treatment of diffuse intrinsic pontine glioma (DIPG). To ensure a comprehensive and up-to-date analysis, major scientific databases, including PubMed, Google Scholar, and ScienceDirect, all of which provide access to high-quality and peer-reviewed biomedical research literature will be used. Additionally, I will examine the reference lists of key primary studies and review articles to identify relevant supporting or foundational research. The search

will be limited to publications from 2017 to 2025, prioritizing the most current findings on DIPG biology, ONC201 (and related imipridone compounds such as ONC206 and ONC212), dopamine receptor signaling in gliomas, and CRISPR-Cas9–based gene editing technologies. Emphasis will be placed on studies that explore mechanisms of treatment resistance, dopaminergic vulnerabilities, mitochondrial dysfunction, and integrated stress response (ISR) pathways in DIPG.

Inclusion criteria will consist of:

- Peer-reviewed research articles and reviews
- Studies specifically focused on DIPG or H3K27M-mutant gliomas
- Research directly related to ONC201, DRD2/DRD3 signaling, CRISPR-Cas9, and associated resistance or apoptotic mechanisms
- Experimental data from in vitro, in vivo, or early-phase clinical studies where applicable

Articles not available in English, not peer-reviewed, or not specific to DIPG/Gliomas or the relevant therapeutic pathways will be excluded. The goal of this review is to synthesize existing knowledge and identify both the current scientific consensus and the key gaps in research that may guide future therapeutic development.

3. Results

CRISPR-mediated gene editing offers a precise and programmable strategy to enhance the therapeutic efficacy of ONC201 in diffuse intrinsic pontine glioma (DIPG) by targeting specific resistance pathways and stress-adaptive mechanisms that limit ONC201-induced apoptosis. One of the most compelling targets is dopamine receptor D2 (DRD2), which is frequently overexpressed in DIPG and drives tumor cell survival through proliferative signaling pathways. While ONC201 functions as a selective DRD2 antagonist, residual dopaminergic activity via DRD3 and other dopamine receptors may still support cell survival. Using CRISPR-Cas9 to knock out DRD2, and possibly DRD3, could help abolish dopamine-driven oncogenic signaling altogether, thus impairing tumor proliferation and increasing sensitivity to ONC201-induced mitochondrial stress (Panganiban et al., 2019). Similarly, dopamine has been reported to induce mitochondrial dysfunction, cytochrome c release, and caspase-dependent apoptosis in glioma models, suggesting a shared vulnerability of gliomas to dopaminergic disruption of mitochondrial pathways (Liu et al., 2017). ONC201 is known to activate the integrated stress response (ISR), primarily through the upregulation of ATF4, which subsequently increases pro-apoptotic downstream targets such as CHOP and DR5. However, prolonged or chronic ISR activation can paradoxically lead to tumor adaptation rather than cell death, allowing cancer cells to survive under persistent stress. This adaptation often involves phosphorylation of eIF2α (encoded by EIF2S1), which reduces global protein translation and allows the cell to conserve resources during stress. Targeted CRISPR knockout of upstream ISR regulators such as EIF2AK3 (encoding PERK) and GCN2 could prevent this adaptive translational arrest, forcing the cell into apoptosis rather than survival (Shivram et al., 2021). Furthermore, editing EIF2S1 itself may block the phosphorylation of eIF2α, thereby sustaining translation and exacerbating proteotoxic stress when combined with ONC201, pushing tumor cells past the tipping point.

In addition to modulating stress responses, targeting intrinsic apoptosis resistance is another promising application of CRISPR in this context. DIPG tumors often overexpress anti-apoptotic genes like BCL2L1 (which encodes Bcl-xL), MCL1, and XIAP, all of which work to inhibit downstream caspases and block programmed cell death. Knocking out these genes via CRISPR could sensitize cells to ONC201 and its downstream activation of the TRAIL (TNF-

related apoptosis-inducing ligand) pathway. ONC201 already upregulates DR5, a TRAIL death receptor, and by simultaneously suppressing apoptosis inhibitors, CRISPR-mediated editing would tip the balance in favor of apoptosis (Guo et al., 2023). This could be particularly effective in overcoming intrinsic or acquired resistance to ONC201 observed in some DIPG subtypes. Moreover, ONC201's therapeutic efficacy can be amplified by disrupting compensatory survival pathways such as PI3K/AKT/mTOR signaling, which is frequently activated in DIPG, especially in tumors harboring the H3K27M mutation. Activation of this pathway has been associated with treatment resistance and enhanced proliferation in high-grade gliomas. CRISPR knockout of key pathway components like AKT1, MTOR, or PDGFRA (commonly amplified in H3K27M DIPG) could block this axis and eliminate the tumor's ability to bypass ONC201-induced apoptosis (Duchatel et al., 2019). Targeting PDGFRA is especially relevant, as its amplification has been linked to poor prognosis in DIPG, and its downstream signaling supports metabolic adaptation and angiogenesis, which are traits ONC201 alone may not fully suppress. Another novel approach involves exploiting ONC201's mechanism of mitochondrial disruption. ONC201 activates ClpP, a serine protease in the mitochondrial matrix responsible for degrading damaged or misfolded proteins. Hyperactivation of ClpP leads to the breakdown of mitochondrial protein homeostasis and induces bioenergetic collapse. In healthy individuals, ClpP activity is tightly regulated by CLPX, a mitochondrial chaperone that delivers specific substrates to ClpP. Knocking out CLPX using CRISPR removes this regulatory gatekeeper, allowing ClpP to function unchecked and accelerating mitochondrial stress to a lethal level. In preclinical models, this synergy between ONC201 and ClpP deregulation has been shown to selectively impair tumor cells while sparing normal tissue (Borsuk et al., 2021). Given the central role of mitochondrial metabolism in DIPG, especially in treatment-resistant phenotypes, this strategy holds strong translational potential. In combination, these CRISPR strategies, such as targeting DRD2, stress-adaptive ISR genes, anti-apoptotic regulators, survival pathways, and mitochondrial proteostasis, create a multi-pronged framework for amplifying ONC201 efficacy in DIPG. Importantly, many of these CRISPR targets converge on shared molecular endpoints like apoptosis induction, energy failure, or ISR dysregulation, allowing for rational design of combinatorial approaches. For example, simultaneous knockout of DRD2 and BCL2L1 could both remove proliferative signaling and enhance TRAIL sensitivity, while editing EIF2AK3 and CLPX might combine translational overload with mitochondrial collapse for maximal cytotoxicity.

These genome editing interventions also show promise in overcoming known resistance mechanisms to ONC201. In some DIPG models, resistance arises through adaptation of the ISR or upregulation of alternative survival pathways. By targeting these compensatory circuits directly at the genetic level, CRISPR provides a durable solution that is less prone to reversal than small-molecule inhibition. For instance, targeting eIF2α phosphorylation with CRISPR, rather than ISR inhibitors like ISRIB, may yield longer-lasting inhibition of the stress adaptation machinery, thereby preserving ONC201's apoptotic potential even in chronically stressed tumor cells (Guo et al., 2023). Additionally, the use of CRISPR in this context is supported by a growing arsenal of high-fidelity Cas9 variants and delivery systems that minimize off-target effects. For example, using enhanced SpCas9 (eSpCas9) or SpCas9-HF1 combined with lipid nanoparticle (LNP)-based delivery could achieve efficient gene editing with reduced risk of genomic damage to healthy brain tissue, which is a critical concern in pediatric brain tumor therapy (Guo et al., 2023). RNP (ribonucleoprotein) electroporation of Cas9-sgRNA complexes also ensures transient activity, minimizing prolonged exposure and reducing the likelihood of undesired mutations.

Another potential use for CRISPR-ONC201 synergy involves gene editing to enhance ONC201

delivery or expression in target cells. For example, editing genes that affect blood-brain barrier permeability or tumor microenvironment stiffness could improve drug penetration. Although this approach is still in early exploration, initial research suggests that modifying extracellular matrix components or endothelial regulators could significantly enhance drug distribution in infiltrative tumors like DIPG. Furthermore, the concept of synthetic lethality could be explored by using CRISPR screens to identify genes whose loss synergizes with ONC201-induced mitochondrial dysfunction. Genome-wide CRISPR-Cas9 libraries can be used in DIPG cell lines treated with ONC201 to find new sensitizer genes, which can inform future therapeutic targets or combination regimens. Finally, while CRISPR holds significant promise in this context, practical limitations remain. Off-target effects, although increasingly minimized with next-gen Cas variants and detection tools like GUIDE-seq and BLISS, still pose a risk. Immunogenicity of CRISPR components, delivery challenges across the blood-brain barrier, and ethical considerations in pediatric patients also warrant careful consideration. Additionally, the rapid evolution of resistance mechanisms within DIPG means that static gene edits may eventually be circumvented. To overcome this, future approaches may incorporate inducible CRISPR systems or base editors that allow for reversible or tunable modulation of gene expression without permanent double-strand breaks (Guo et al., 2023). In conclusion, CRISPR-Cas9 technology represents a powerful complementary tool to ONC201 therapy in DIPG. Through precise genetic manipulation of resistance and stress pathways, including DRD2 signaling, ISR regulators, anti-apoptotic genes, mitochondrial chaperones, and survival kinases, CRISPR provides a robust platform for enhancing the anti-tumor effects of ONC201. As ONC201 continues to progress through clinical trials, integrating gene editing strategies may yield more durable, personalized, and effective treatments for this devastating pediatric cancer.

4. Discussion

The integration of CRISPR gene editing in combination with ONC201 presents a promising and innovative approach to overcoming therapeutic resistance in DIPG, which has previously had a very poor prognosis (Weisbrod et al., 2024). ONC201's mechanisms of selectively antagonizing DRD2/3, activating ClpP, and inducing the integrated stress response has shown early clinical success in the treatment of H3K27M-mutant DIPG (Hall et al., 2019; Borsuk et al., 2021). However, resistance mechanisms and cellular adaptations to mitochondrial stress limit its full therapeutic potential (Lines et al., 2023; Zhang et al., 2024). The use of CRISPR-Cas9 to disrupt key resistance and survival pathways may significantly enhance ONC201 efficacy by sensitizing tumor cells to apoptosis and disabling the protective mechanisms that allow DIPG cells to persist under metabolic stress (Panganiban et al., 2019; Shivram et al., 2021). Targeting dopaminergic signaling components such as DRD2 and DRD3 with CRISPR could eliminate residual dopamine-mediated survival signaling, thereby enhancing ONC201-induced stress (Hall et al., 2019; Panganiban et al., 2019). Furthermore, disrupting ISR regulators (such as PERK, GCN2, or eIF2α) could prevent protective translational arrest, a known resistance mechanism to ISR-based therapies. CRISPR editing of anti-apoptotic genes like XIAP, BCL2L1, or MCL1 may also reduce the apoptotic threshold, further enabling ONC201's pro-death signals, particularly through TRAIL/DR5 activation. In addition, targeting pathways commonly activated in DIPG, such as PI3K/AKT/mTOR and PDGFRA, may suppress compensatory oncogenic signaling and limit tumor adaptation. Finally, CRISPR-based disruption of mitochondrial regulators like CLPX may enhance ClpP-mediated mitochondrial collapse, strengthening ONC201's effects.

While the combination of CRISPR-Cas9 gene editing and ONC201 represents a promising therapeutic strategy for DIPG, several significant translational challenges must be addressed.

The most important among these are the difficulty of delivering CRISPR components across the blood–brain barrier (BBB) and the current lack of in vivo validation supporting the safety and efficacy of this approach. Another major limitation lies in the scarcity and variability of animal models for DIPG, as these tumors are difficult to replicate in vivo, limiting the accuracy of CRISPR delivery and therapeutic testing. The BBB is a highly selective physiological barrier that limits the passage of large biomolecules, including the viral vectors, ribonucleoprotein complexes, or nanoparticles typically used to deliver CRISPR systems, making efficient targeting of tumor cells within the brainstem particularly challenging. The BBB is a major obstacle to the delivery of CRISPR-based therapies for DIPG, as Cas9/gRNA complexes are too large and unstable to cross unaided. The BBB is composed of tightly connected endothelial cells supported by pericytes and astrocytic end-feet, which together maintain strict regulation of ionic and molecular transport. This structure effectively excludes macromolecules larger than 400–500 Da, preventing the passive diffusion of most CRISPR-associated components. Even viral vectors like AAV and lentivirus, which have been used in CNS gene therapy, face barriers related to size, receptor specificity, and the risk of triggering neuroinflammation. Additionally, efflux transporters such as P-glycoprotein actively pump many foreign molecules back into the bloodstream, further reducing intracellular accumulation of therapeutic agents within the brain parenchyma. Recent studies in glioblastoma have demonstrated the feasibility of engineering nanoparticles functionalized with angiopep-2 to achieve BBB penetration and efficient in vivo gene editing (Ruan et al., 2022). A 2022 study even showed that angiopep-2 nanocapsules could cross the BBB and drive strong CRISPR edits in brain tumors (Zou et al., 2022). While these findings are not DIPG-specific, they suggest that advanced nanoparticle-based strategies could eventually enable CRISPR delivery in pediatric brainstem tumors. While ONC201 is orally bioavailable and capable of penetrating the BBB, gene-editing systems do not yet have a clinically validated delivery platform that can achieve comparable distribution in deep brain regions without invasive administration (Bentayebi et al., 2023). In addition, the absence of comprehensive in vivo DIPG studies means that key questions regarding long-term gene-editing safety, off-target effects, immune responses, and therapeutic durability remain unanswered, all of which must be resolved before clinical translation is feasible. The BBB remains a major obstacle to the effective delivery of CRISPR-based therapeutics. DIPG arises in the pons, a region of the brainstem that is both anatomically sensitive and highly protected by the BBB. While ONC201 is a small molecule capable of crossing the BBB due to its lipophilic properties and oral bioavailability, CRISPR-Cas9 systems—whether delivered as DNA, mRNA, or ribonucleoprotein complexes—are significantly larger and generally unable to cross the barrier unaided (Tang et al., 2022). Current delivery approaches rely heavily on vectors such as adeno-associated viruses (AAVs), which have demonstrated success in central nervous system applications due to their high transduction efficiency and persistence in non-dividing cells. However, AAVs are constrained by limited cargo capacity and the potential for prolonged Cas9 expression, which can increase the risk of off-target genome editing and immune responses (Guo et al., 2023).

While CRISPR/Cas9 offers remarkable precision in gene editing, one of its most critical challenges is the occurrence of off-target effects, where Cas9 introduces double-stranded breaks at unintended genomic sites. These inaccuracies may arise from partial mismatches between the guide RNA and non-target DNA sequences or from PAM sequences permissive to near matches (Zhang et al., 2015; Guo et al., 2023). Such off-target modifications can disrupt normal gene expression, induce genomic instability, and complicate downstream therapeutic outcomes—issues that are particularly concerning in neuro-oncologic contexts like DIPG, where even minor genomic perturbations in neuronal tissue may have severe clinical consequences. Modern detection technologies such as Digenome-seq, GUIDE-seq, and

CIRCLE-seq enable more comprehensive identification of these off-target events, improving confidence in the specificity of gene edits (Guo et al., 2023). To mitigate these risks, researchers have engineered higher-fidelity Cas9 variants such as eSpCas9, SpCas9-HF1, and HypaCas9, which demonstrate significantly reduced off-target cleavage without compromising on-target efficiency (Guo et al., 2023). Additionally, strategies such as sgRNA truncation, chemical modification, and transient delivery of Cas9–sgRNA ribonucleoprotein complexes have been shown to further enhance specificity (Zhang et al., 2015). These improvements are essential when considering CRISPR as a tool for modulating dopaminergic signaling in combination with ONC201, as maintaining neuronal genomic integrity is vital for both therapeutic efficacy and safety. Ensuring accurate CRISPR targeting not only minimizes risk but also strengthens the translational potential of gene-editing–based approaches for DIPG and other high-grade pediatric gliomas.

Non-viral alternatives such as lipid nanoparticles (LNPs) offer the advantage of transient expression and reduced immunogenicity. Nonetheless, their inability to efficiently penetrate the BBB under physiological conditions has limited their utility in brain-targeted gene editing applications. While novel approaches, such as exosome-mediated transport, receptor-targeted delivery systems, and focused ultrasound to transiently disrupt the BBB, are being actively explored, these methods remain largely experimental and have not yet been optimized for DIPG or other high-grade gliomas (Tang et al., 2022). A recent review of brain-targeted gene therapy strategies highlights non-viral vectors, which include lipid nanoparticles, polymer-based systems, and exosomes, as promising alternatives to viral approaches, given their transient expression and reduced immunogenicity (Xie et al., 2023). These systems can be engineered with ligands such as transferrin or angiopep-2 to exploit receptor-mediated transcytosis and have achieved measurable gene delivery in preclinical brain models (Non-viral delivery systems for gene therapy across the BBB, 2023). However, while these advances demonstrate proof-of-concept for overcoming BBB limitations, their translation to DIPG remains untested, and efficiency in the brainstem microenvironment is still a major challenge. Until an efficient, tumor-specific, and clinically viable delivery system is developed, the implementation of CRISPR-based therapeutics in DIPG will remain limited to preclinical contexts.

Another essential consideration involves the ethical and developmental implications of applying CRISPR-based therapies in pediatric contexts. Because children cannot fully consent, parental authorization and medical oversight must ensure that interventions serve only clear medical necessity rather than enhancement purposes. Germline edits that could affect future generations present additional moral challenges and are currently discouraged in clinical settings (Brokowski & Adli, 2018). Safety concerns, including off-target effects, immune reactions, and unknown developmental consequences, require rigorous preclinical data and long-term monitoring (Foulkes et al., 2019). Furthermore, equity issues arise, as advanced CRISPR treatments may only be available to certain populations, widening global health disparities (Vigliotti & Martinez, 2018). To prevent misuse and ensure fairness, transparent regulation, strong ethical review, and child-centered governance frameworks must accompany any future application of CRISPR-based treatments in pediatric neuro-oncology (Devi, 2025).

In addition to these challenges, there is a notable lack of in vivo studies evaluating the combined therapeutic effects of CRISPR-mediated pathway disruption and ONC201 in DIPG models. Most of the current literature supporting this strategy is based on in vitro data using cultured DIPG cell lines, which, while informative, cannot fully recapitulate the tumor microenvironment, cellular heterogeneity, and anatomical constraints present in vivo. DIPG's infiltrative growth pattern within vital brainstem tissue further underscores the need for preclinical studies that utilize patient-derived xenografts (PDXs) or genetically engineered mouse models (GEMMs) that reflect the biology of H3K27M-mutant gliomas. In vivo models

are essential not only for confirming therapeutic efficacy but also for assessing pharmacokinetics, biodistribution, potential neurotoxicity, and immune responses, which are factors that cannot be reliably predicted in vitro (Guo et al., 2023). Without rigorous validation in these systems, the translation of CRISPR–ONC201 therapies into clinical trials, particularly for pediatric patients, remains premature. In summary, although the theoretical framework for combining CRISPR and ONC201 in DIPG is compelling, its advancement is constrained by significant technological and biological limitations. Overcoming these barriers will require innovations in the central nervous system–specific delivery platforms and the expansion of in vivo research to validate both safety and therapeutic benefit.

Another important limitation is that much of the existing literature on CRISPR-based therapeutic strategies is derived from studies in glioblastoma or other high-grade gliomas, rather than DIPG specifically. This reliance on glioblastoma data stems from the extreme rarity of DIPG, which accounts for only 10–15% of pediatric brain tumors, as well as the difficulty of obtaining tumor tissue for research due to its location in the pons. While glioblastoma shares certain molecular features with DIPG, such as activation of PI3K/AKT/mTOR signaling, overexpression of anti-apoptotic proteins, and metabolic adaptations, the two tumors differ in their genetic drivers, microenvironmental context, and treatment responses. For example, the hallmark H3K27M mutation in DIPG is not typically present in glioblastoma, and this epigenetic alteration impacts chromatin regulation and tumor biology. As a result, extrapolating findings from glioblastoma to DIPG may overestimate or misrepresent the efficacy of certain CRISPR targets or ONC201 combination strategies. This underscores the need for DIPG-specific preclinical studies, such as the use of patient-derived xenografts, genetically engineered mouse models, and in vitro cultures from primary DIPG samples, to validate that molecular vulnerabilities identified in glioblastoma are truly relevant to DIPG before advancing toward clinical application.

Acknowledgments

I would like to express my sincere appreciation to Makaila Furderer of the University of Michigan, whose guidance and feedback were invaluable throughout the development of this manuscript. I also thank everyone who contributed their time and expertise to support the conceptual refinement and scientific framing of this review.

Authors contributions

As the sole author, I, Ella Tulloch, was responsible for all components of this work, including the conception of the topic, literature review, data interpretation, drafting of the manuscript, and final revisions. No additional authors contributed to the writing or preparation of the manuscript. Mentorship was provided by Makaila Furderer, who offered general academic guidance but did not participate in authorship or manuscript preparation. There were no special authorship agreements.

Funding

This work received no external funding. All components of the review and manuscript preparation were conducted independently.

Competing interests

I, Ella Tulloch, declare that I have no competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Informed consent

Obtained.

Ethics approval

The Publication Ethics Committee of the Macrothink Institute.

The journal's policies adhere to the Core Practices established by the Committee on Publication Ethics (COPE).

Provenance and peer review

Not commissioned; externally double-blind peer reviewed.

Data availability statement

The data that support the findings of this study are available on request from the corresponding author. The data are not publicly available due to privacy or ethical restrictions.

Data sharing statement

No additional data are available.

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