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REVIEW

Exploring the Latest Developments in Natural Killer (NK) Cell-Based Therapies for Diffuse Intrinsic Pontine Glioma (DIPG)

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ABSTRACT: Diffuse intrinsic pontine glioma (DIPG) is a pediatric brainstem tumor with a very poor prognosis, characterized by immunosuppressive tumor microenvironment (TME) that limits immune infiltration, including a significant reduction in circulating natural killer (NK) cells. This drop in NK cell levels and activity may promote tumor growth and immune evasion, making NK cells a promising target for immunotherapy. NK cells can attack and eliminate DIPG tumor cells, including glioma stem cells, while counteracting certain immune evasion strategies. Although the DIPG microenvironment and blood-brain barrier present challenges, NK cell-based therapies have shown encouraging tumor control and survival benefits in animal models with promising safety results. Current clinical trials for DIPG mostly focus on chimeric antigen receptor (CAR)-T cells targeting disialoganglioside (GD2) and cluster of differentiation 276 (CD276 or B7-H3) antigens with early signs of success, while NK cell therapies, such as CAR-NK cells, are still in preclinical or early stages, requiring further development. The tumor's immunosuppressive nature poses challenges that may need combination strategies or immune priming. Despite these obstacles, NK cell-based immunotherapy is an exciting and growing field. Upcoming clinical trials emphasize the potential for NK cell therapies to play a critical role in treating this aggressive pediatric brain cancer.

KEYWORDS: Diffuse intrinsic pontine glioma; tumor microenvironment; natural killer (NK) cells; chimeric antigen receptor (CAR)-NK cells

1 Introduction and Background: Diffuse Intrinsic Pontine Glioma

Diffuse intrinsic pontine glioma (DIPG) is an aggressive pediatric brain tumor that develops in the pons, a part of the brainstem responsible for essential functions like breathing, heart rate, and blood pressure, as well as controlling vision, hearing, and movement [1–4]. DIPG originates from monopotent stem cells derived from oligodendrocyte precursor cells, which can self-renew and produce myelinating oligodendrocytes [5]. The glioma growth factor neuroligin-3 (NLGN3) in the DIPG tumor microenvironment significantly promotes tumor growth [1,2]. These tumors often impact deep midline brain structures in young children, likely tied to epigenetic regulation in early brain development [3]. Over 85% of DIPG tumors involve a lysine-to-methionine mutation at position 27 in the histone (H3K27M), which drives cancer growth [3–5]. The H3K27M mutation in histone H3 disrupts the polycomb repressive complex 2 (PRC2), hindering proper trimethylation at lysine 27 (H3K27me3) [6–8]. This causes a global decrease in transcriptional repression marks and an increase in activating acetylation marks (H3K27ac), enhancing chromatin accessibility and triggering oncogenic pathways [6,9,10]. Tumors with this mutation are classified as grade 4 under the World Health Organization's H3 K27-altered diffuse midline glioma category [8]. DIPG's

challenging location and interaction with the blood-brain barrier (BBB) make it particularly severe, with 150–400 new cases diagnosed annually in the United States [11,12]. Tragically, only 10% of children diagnosed survive beyond two years, with most cases occurring in children under age 9 [3,13–15]. Symptoms, which can appear suddenly, include vision issues, difficulty talking or swallowing, nausea, weakness, balance problems, trouble walking, and behavioral changes [16]. Several treatments for DIPG have been explored, but success rates remain minimal [16]. Due to its delicate location, treatment options like surgery, chemotherapy, and radiation are limited, underscoring the urgent need for advancements in immune cell-based therapies to address the tumor's immunosuppressive microenvironment and poor prognosis [3,4,12,17,18].

Research indicates that natural killer (NK) cells, derived from the peripheral blood of healthy individuals, can effectively target and differentiate glioma tumors [19–21]. These cytotoxic lymphocytes, which make up 5%–15% of the immune cells in healthy people, serve as a crucial first line of defense against tumor growth. NK cells hold therapeutic potential due to their innate ability to attack cells lacking or mutating major histocompatibility complex (MHC) class I [22]. They recognize and kill tumor cells without prior activation, relying on a balance of stimulatory and inhibitory receptors. NK cells play a significant role in cancer inhibition through direct cytotoxicity, antibody-dependent cellular cytotoxicity (ADCC), and by regulating other immune effectors with their cytokines and chemokines [23–25]. They act swiftly to eliminate threats, both directly and by modulating immune responses. Preclinical studies have shown that NK cells are effective against brain tumors, with glioblastoma multiforme (GBM) responding well to IL-2 or IL-15-activated NK cells [26,27]. NK cells combined with monoclonal antibodies have also shown success in treating GBM [28]. However, cancer patients' NK cells often face challenges like tumor-induced inactivation and short lifespans, which reduce their therapeutic viability without enhancements [29,30].

Though advancements in therapeutics have been made, DIPG remains difficult to treat due to its location, genetic complexity, and the intact BBB [31,32]. Current clinical trials focus on safety, pharmacokinetics, and efficacy, though further evaluation is required. While a definitive cure is lacking, recent trials integrating novel delivery methods, targeted therapies, and immunotherapy show promise. Effective drug delivery techniques like convection-enhanced delivery (CED), intra-arterial infusion, and MR-guided focused ultrasound (MRgFUS) are essential for success [31,33]. Analyses of the tumor microenvironment reveal that DIPG tumors are immunologically "cold", with little infiltration of NK cells and other lymphocytes [34]. Clinical studies show that DIPG patients have much lower peripheral NK cell levels and functions compared to healthy controls [21,35]. Decreased number and activity of NK cells may play a role in DIPG tumor development and immune evasion [35]. To overcome these issues, allogeneic NK cell therapies are being explored in clinical trials as standalone or combination treatments [36–39].

This review focuses on the challenges of DIPG and the obstacles they pose for effective treatments like immunotherapy. NK cell-based therapies, which utilize innate immunity and low toxicity, offer a promising and innovative approach, especially if issues like tumor infiltration and persistence are resolved. Several clinical trials are investigating NK-cell-based CAR constructs and antibody combinations targeting DIPG antigens. Moreover, advancements in crossing the BBB and altering the tumor microenvironment are vital for better outcomes. Progress in NK cell engineering, delivery systems, and combined immunotherapy strategies brings hope for future breakthroughs in DIPG treatment.

2 Current Treatments of DIPG

The relationship between DIPG and its tumor microenvironment (TME) presents challenges and opportunities for treatment. Key targets are genetic alterations, neurotransmitters, immune checkpoints, and ligand-receptor interactions. Identifying and targeting specific molecular pathways and immune interactions within the TME could lead to improved therapies for this aggressive pediatric brain tumor (Fig. 1).

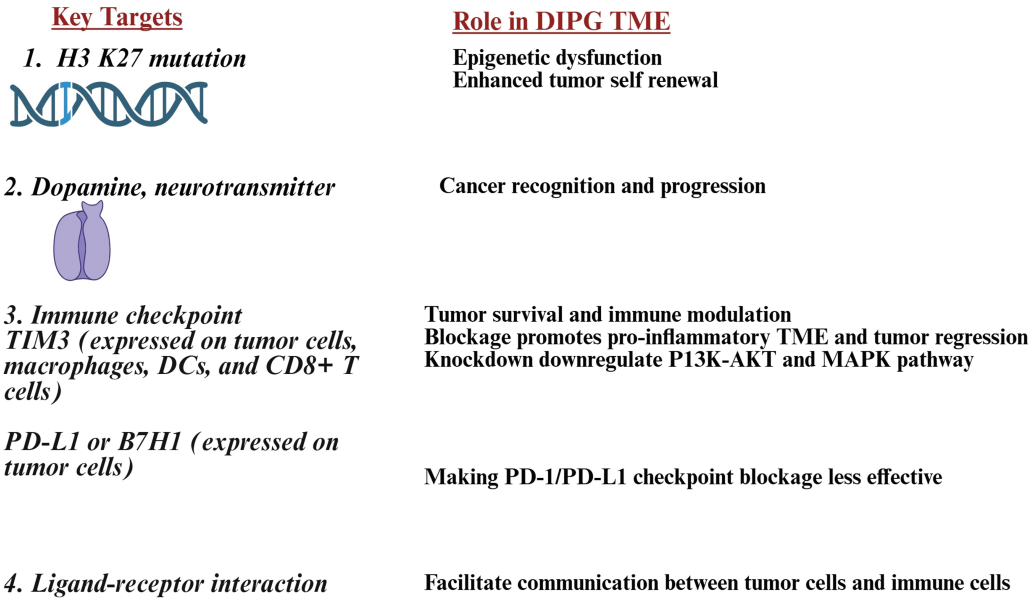

Figure 1: Illustration showing Key targets of diffuse intrinsic pontine glioma (DIPG) and their role in the tumor microenvironment (TME), these are genetic alterations, neurotransmitters, immune checkpoints, and ligand-receptor interactions. Abb: H3K27M: histone 3 K27M; TIM3: T-cell immunoglobulin and mucin-domain containing 3; DCs: dendritic cells; P13K-AKT: phosphatidylinositol 3-kinase Akt; MAPK: mitogen-activated protein kinase; PD-L1: programmed death ligand 1; B7H1: B7 homolog 1; PD-1: programmed cell death protein 1. Created in BioRender. Kaur K https://BioRender.com/1372szq (accessed on 17 November 2025)

2.1 Targeting H3K27 Mutation

Treatments for H3K27M-mutant diffuse midline gliomas include histone deacetylase and demethylase inhibitors, bromodomain inhibitors to block oncogenic transcription, and vaccines targeting the H3K27M neoepitope to activate the immune system [10,40,41]. Several epigenetic compounds have shown effectiveness and specificity in preclinical studies [42]. H3K27M peptide vaccines have strong preclinical evidence for immunogenicity and anti-tumor effects, and are in early-stage development [40,43]. Epigenetic therapies work by restoring balance, either by increasing H3K27me3 levels with demethylase inhibitors, reducing acetylation-driven transcription with histone deacetylase (HDAC) and bromodomain and extraterminal (BET) inhibitors, or targeting mutant epitopes through immunotherapy. Inhibiting Jumonji domain-containing protein-3 (JMJD3) demethylase with β-Alanine, N-[2-(2-pyridinyl)-6-(1,2,4,5-tetrahydro-3H-3-benzazepin-3-yl)-4-pyrimidinyl] ethylester (GSK-J4) restores K27 methylation, delays tumor progression, and extends survival in DIPG models, though its clinical development is limited due to conversion to N-[2-(2-Pyridinyl)-6-(1,2,4,5-tetrahydro-3H-3-benzazepin-3-yl)-4-pyrimidinyl]-beta-alanine (GSK-J1), which has poor permeability. A stable GSK-J1 analog developed by Suri et al. showed better anti-tumor activity and survival benefits in DIPG models [28]. Panobinostat, a pan-HDAC inhibitor, caused significant cytotoxicity in DIPG *in vitro* [44]. BET inhibitors have also improved survival in mouse models of H3K27-altered gliomas, with clinical trials ongoing [45,46]. Additionally, the indoleamine-2,3-dioxygenase 1 IDO1 inhibitor indoximod has shown early clinical promise against H3K27M-mutant gliomas [6,47]. This multifaceted approach offers hope for improving outcomes in this fatal pediatric brain cancer, with preclinical studies supporting these treatments individually and in combination, while clinical trials assess their safety, dosing, and efficacy [10].

2.2 Targeting Neurotransmitter

Dopamine, a neurotransmitter, plays a key role in cancer initiation and progression [48]. Dopamine receptor antagonists ONC201 and ONC206 have shown cytotoxic effects against DIPG and other diffuse midline gliomas (DMGs) with histone H3.3K27M mutations [13,49,50]. Both pediatric and adult brain tumor patients have experienced significant clinical responses to these drugs [50]. ONC201 and ONC206, part of the imipramine family of anti-cancer drugs, were discovered as a selective antagonist of the GPCR dopamine receptor D2 (DRD2) [51]. ONC201, initially identified as TIC10, induces TNF-related apoptosis and shares a similar anti-cancer mechanism as ONC206 [51,52]. These drugs have demonstrated cytotoxicity against various cancer cell lines, can cross the BBB via oral administration to target high-grade glioma, and are in clinical trials for treating central nervous system cancers [53,54]. ONC201 also activates the APO-2 pathway in cancer cells, with increased levels of APO2-L (TRAIL)-secreting NK cells observed in the peripheral blood of treated mice [55] and human patients [56].

2.3 Radiation, Chemotherapy, and Combination Therapeutics

Radiation therapy, typically fractionated external beam RT at doses of 54–59 Gy, remains the standard treatment for DIPG [57]. Re-irradiation in progressive cases may slightly extend survival, but radiation is primarily palliative and doesn't provide long-term tumor control [57,58]. Epidermal growth factor receptor (EGFR) inhibitors like nimotuzumab, when used with radiation, can reduce side effects but haven't significantly improved survival rates [59]. Most systemic chemotherapy regimens have been ineffective, likely due to challenges with drug delivery and tumor heterogeneity [31,32]. Additionally, DIPG shows resistance to chemotherapeutics like temozolomide [60]. Trials with kinase inhibitors like ribociclib (a CDK4/6 inhibitor) and everolimus (a mammalian target of rapamycin (mTOR) inhibitor) demonstrate safety but unclear effectiveness [61,62]. Similarly, studies on poly ADP ribose polymerase (PARP) inhibitors (e.g., veliparib), alkylating agents (temozolomide, capecitabine), topoisomerase inhibitors (irinotecan), and microtubule inhibitors (cabazitaxel) combined with radiation or chemotherapy or as adjuvants have not shown notable survival improvements [63,64].

2.4 Immunotherapies

Immunotherapy, including immune checkpoint inhibitors, CAR-T, T-cell receptor (TCR-T), vaccines, oncolytic virus, and combination therapies, has become a growing focus due to the poor outcomes and molecular features of DIPG. Understanding the tumor-immune microenvironment opens new doors for immunotherapy strategies [65] (Table 1). However, the highly immunosuppressive and low-inflammatory tumor microenvironment in DIPG limits immunotherapy effectiveness. Immune checkpoint inhibitor therapies like programmed death protein-1 (PD-1) and cytotoxic T-lymphocyte associated protein 4 (CTLA-4) have shown good tolerance in DIPG patients [58,66,67]. However, immune checkpoint blockade therapies, such as anti-PD1, have largely failed to improve survival, likely due to the "immune-cold" tumor microenvironment with low PD-1 expression [58]. DIPG tumors typically show minimal immune cell infiltration, and infiltrated immune cells often lose PD-1 or PD-L1 expression, limiting the efficacy of immune checkpoint inhibitors [16,34]. Targeting alternative checkpoints like T cell immunoglobulin and mucin domain-containing protein 3 (TIM3) has shown promising preclinical results [68,69]. Peptide vaccines have demonstrated efficacy in inducing cytotoxic T-cell and helper-1-cell mediated responses in K27M-mutated MHC-humanized mice [70,71]. CAR-T cells, particularly anti-GD2 CAR-T cells, have shown safety and initial clinical benefits [4,72–74]. Other CAR-T approaches targeting B7-H3, HER2, and H3K27M mutant peptides are in early clinical trials, showing some encouraging tumor responses [75,76]. Autologous dendritic cell vaccines have demonstrated safety, feasibility, and anti-tumor immune responses in DIPG patients [77,78].

Cancer vaccines targeting WT1, survivin, and H3K27M antigens are under investigation to trigger tumor-specific immune reactions [79,80]. Immunomodulatory drugs like pomalidomide and pelareorep (an oncolytic virus) are being studied alone or with radiation. The efficacy of H3.3K27M-specific T-cell receptors and CAR-T was shown against DIPG in xenograft mouse models [81,82]. Positive outcomes were observed in DIPG patients treated with the immune-modulating antibody MDV9300 (pidilizumab) [83].

Table 1: Ongoing immunotherapy-based clinical trials for diffuse intrinsic pontine glioma (DIPG)

Immunotherapy	Trial phase	ClinicalTrials.Gov identifier	Patients enrolled
B7-H3-CAR T	Phase 1	NCT04897321	32
C7R-GD2.CART cells	Phase 1	NCT04099797	37
IV cyclophosphamide (200 mg/m^2) or oral (cytoxan) followed by dendritic cells (DC) vaccine and IV bevacizumab (15 mg/kg) in 24 h	Phase 1	NCT03914768	10
Agonistic CD40 monoclonal antibody	Phase 1	NCT03389802	NA
IL-12 with oral veledimex	Terminated	NCT03330197	6
ONC201	Phase 3	NCT05580562	450
T Cell Therapy, DC Vaccines, and Hematopoietic Stem Cells Combined with Immune Checkpoint Blockade	Phase 1	NCT06514898	12
GD2 CAR T	Phase 1	NCT04196413	54
B7H3-CAR T	Phase 1	NCT04185038	NA
H3.3-K27M Neoantigen Vaccine Therapy	Completed	NCT04749641	16

Note: Abb: CAR: chimeric antigen receptor; B7H3: B7 homolog 3; C7R-GD2: constitutively active interleukin-7 receptor against antigen GD2; CD: cluster of differentiation; IL-12: interleukin-12; ONC201: dordaviprone; H3K27M: histone 3 K27M; NA: not applicable.

2.5 Therapeutic Challenges and Advancements

Treating DIPG is challenging due to its difficult location, complex molecular and epigenetic features, suppressive tumor environment, and the obstacle of getting drugs through the BBB. While innovative methods and promising models offer hope, translating them into actual survival improvements remains a big hurdle. Future strategies should prioritize personalized, multimodal treatments that penetrate the BBB, combined with targeted immunomodulation and epigenetic approaches to overcome DIPG's resistance.

Oncolytic viral therapy, such as DNX-2401 delivered via CED and combined with radiation, has shown potential survival benefits [31,84,85]. Super selective intra-arterial cerebral infusion (SIACI) uses BBB disruption agents like mannitol with targeted antibodies such as bevacizumab (anti-VEGF) or cetuximab (anti-EGFR), showing a median overall survival (OS) of about 17.3 months in heavily pretreated patients, demonstrating safety and potential effectiveness [86]. The growing use of stereotactic biopsy aids molecular diagnosis and supports precision medicine [87]. Combination therapies involving radiation, targeted agents, immunotherapy, and BBB bypass are under active investigation [88]. Continued multidisciplinary research on genetics, drug delivery, and immune modulation is crucial to improving outcomes for this devastating pediatric cancer.

3 Natural Killer Cells in DIPG TME

BBB restricts naïve immune cells, including NK cells, to the brain tissue. DIPG creates a complex and challenging tumor immune environment. NK cells, which are vital for innate immunity and have strong tumor-killing abilities, face numerous obstacles within the DIPG TME. Studies using human data, patient-derived models, and translational work highlight these mechanisms. H3K27M-driven chromatin changes in DIPG alter cell surface ligand expression and cytokine secretion, limiting NK cell recognition and activation [89]. miRNAs in both NK and tumor cells regulate receptor expression, signaling pathways, and effector functions, enhancing NK suppression [90].

NK cell activation depends on NK activating receptors, including NKG2D, NKp30, NKp44, NKp46, CD16, and CD94, etc. [91]. These receptors detect stress ligands on tumor cells. DIPG cells and TME components express ligands for inhibitory NK receptors, such as classical major histocompatibility complex (MHC) class I interacting with KIRs, or HLA-E engaging NKG2A [91]. This engagement activates intracellular immunoreceptor tyrosine-based inhibitory motifs (ITIMs), leading to suppression of activation cascades [35]. A high inhibitory-to-activating signal ratio reduces NK cell cytotoxicity, even if tumor cells are recognized [91]. DIPG stem-like cells evade NK attacks through differences in stress ligand expression (e.g., MICA/B, RAET1) and by metabolically impairing NK cytotoxicity. Furthermore, hypoxia and nutrient competition in the TME weaken NK cell metabolic fitness and the ability to mobilize cytotoxic granules [92]. DIPG TME exhibits a low level of cytokines, including IL-15; these cytokines are crucial for NK maturation and cytotoxic activity, lack of these functions impairs NK cell-mediated tumor lysis and differentiation. Inhibitory cytokines in DIPG TME may dampen NK cell function by reducing granule polarization and degranulation efficiency [34]. Elevated B cells may inhibit NK activation indirectly by secreting IL-10 and reducing dendritic cell priming [35]. Low levels of NK-stimulating myeloid cells contribute to NK cell hyporesponsiveness [34]. Low levels of NK cell recruiting chemokines (e.g., CXCL9, CXCL10) limit the homing of peripheral NK cells to the tumor site, ultimately reducing tumor NK cell infiltration [35]. Surviving NK cells in DIPG TME present decreased cytotoxic granule release (perforin, granzyme B) and diminished cytokine secretion capability (IFN-γ, TNF-α), impairing both direct tumor lysis and immune modulation [34].

In short, NK cells are inhibited in DIPG immunosuppression TME, and contributing factors are poor immune cell recruitment, dominant inhibitory receptors, insufficient cytokine activation, and microenvironmental or metabolic challenges (Fig. 2). Effective immunotherapy strategies need to address the recruitment, activation, and sustained presence of NK cells in this immune-resistant TME.

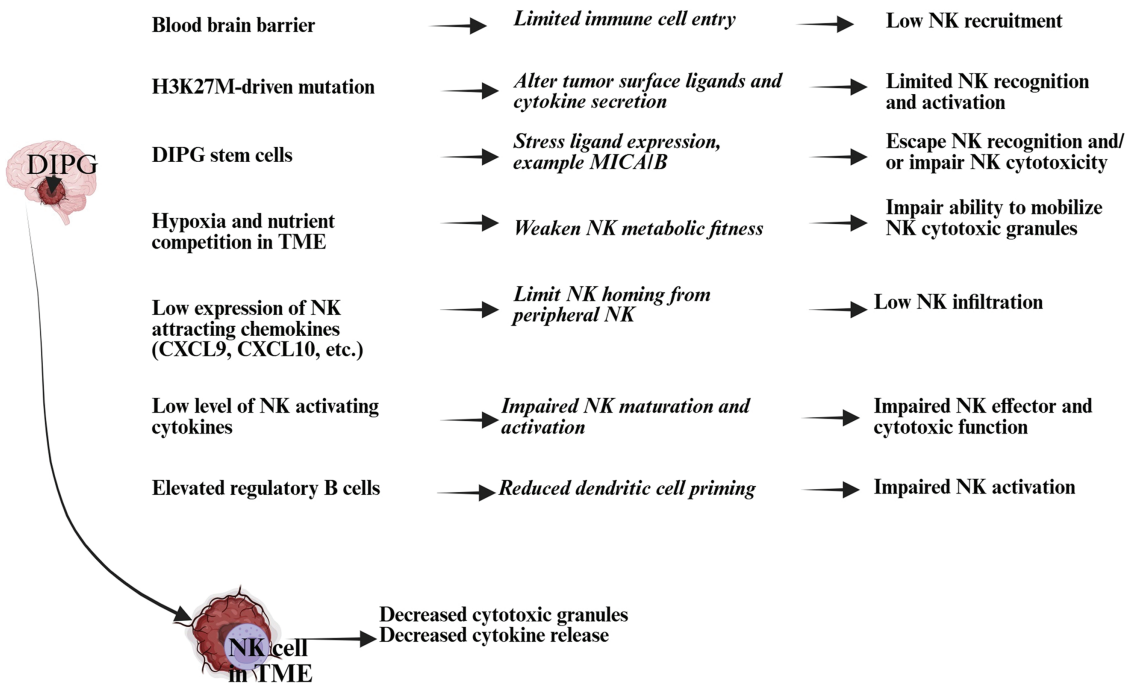

Figure 2: An illustration showing how natural killer (NK) cell activity is suppressed in the DIPG TME. NK cell activity plays a vital role in tumor destruction and growth control; reduced NK cell numbers and functionality lead to tumor progression and spread. Created in BioRender. Kaur K https://BioRender.com/9g38tjj (accessed on 17 November 2025). Abb: CXCL: Chemokine (C-X-X motif) ligand; MICA/B: major histocompatibility complex (MHC) class I chain-related protein A/B

4 NK Cell-Based Immunotherapies for DIPG: Preclinical and Clinical Studies

NK cells have shown the ability to destroy DIPG tumor cells *in vitro* [93,94] (Table 2 and Fig. 3). They can selectively recognize and kill DIPG tumor cells, including glioma stem cells, overcoming some tumor immune evasion mechanisms, suggesting their therapeutic promise [95]. Preclinical studies convincingly demonstrate that NK cells, especially when engineered as CAR-NK cells or engaged via bispecific molecules, offer promising targeted immunotherapeutic approaches for DIPG [96–99]. CAR-NK cells exhibit transient viability and cytotoxicity following irradiation (a safety step before infusion), highlighting their translational potential [100]. CAR-engineered NK (CAR-NK) cells targeting tumor-associated antigens like GD2 have shown strong, antigen-specific cytotoxicity against DIPG cells with high GD2 expression *in vitro* and in orthotopic xenograft models, resulting in tumor growth inhibition and improved survival in preclinical mouse studies [100,101]. In mouse models, GD2-CAR NK-92 cells slowed tumor growth and extended survival in GD2-positive DIPG xenografts with an acceptable safety profile. GD2-CAR NK-92 cell lines specifically kill GD2-expressing DIPG cells with minimal toxicity to normal neural progenitor cells and show favorable safety profiles *in vivo*, avoiding cytokine release syndrome in treated mice [102]. Despite challenges posed by the DIPG tumor microenvironment and blood-brain barrier, these strategies have shown effective tumor control and survival benefits in animal models, with favorable safety profiles [16,17]. Future trials will need to confirm the preclinical efficacy and safety of CAR-NK cell therapies, such as GD2-CAR NK cells, before they reach clinical stages. The adoptive transfer of irradiated CAR-NK-92 cells in preclinical models shows a gradual loss of viability and cytotoxicity over time, raising challenges for *in vivo* persistence [103].

Beyond CAR-NK cells, innovative therapies like NK cell Engagers (NKCEs) are being developed to bind NK cells and tumor antigens simultaneously, and have the potential to boost NK cell recruitment

and activation against DIPG cells [104,105]. Investigational NK cell-derived exosomes and other NK-based approaches are also being explored to target glioma stem cells (GSCs), which contribute to DIPG aggressiveness and treatment resistance [106,107]. *Ex vivo* osteoclasts-induced expanded supercharged NK cells were found to be effective in targeting DIPG tumors alone or in combination with the dopamine receptor antagonists (ONC201 and ONC206) [93,94]. Treating NK cells with epigenetic modifiers like HDAC inhibitors (e.g., MS-275) boosts their cytotoxicity against solid cancers by enhancing genes linked to movement and cell killing [108,109].

Table 2: Preclinical studies showing efficacy of NK cells against DIPG

NK cell approach	Experimental model	Outcome	References
NK cells isolated from PBMCs were activated overnight with IL-2 (1000 U/mL).	DIPG cell lines (SU-DIPG-IV, SU-DIPG-XIII, and SU-DIPG-XVII)	*In vitro*: NK cells lysed all DIPG cultures	[89]
NK cells from PBMCs were activated overnight with IL-2 (1000 U/mL) and anti-CD16 mAbs before being co-cultured with osteoclasts and probiotics.	K27M DIPG cell lines	*In vitro*: NK cells lysed K27M DIPG cell lines	[93,94]
NK-92 cells were genetically modified to express a CAR targeting GD2.	Patient-derived DIPG cell lines and DIPG xenografts in immunodeficient mice	*In vitro*: CAR-NK cells exhibited cytotoxic activity against DIPG cells. *In vivo*: Anti-GD2 CAR NK-92 cells reduced tumor burden and prolonged survival in xenograft models.	[101]
Human pluripotent stem cells (hPSCs) were differentiated into NK cells.	DIPG cell lines (SF8628)	*In vitro*: NK cells lysed DIPG cell lines	[110]

Note: Abb: NK: natural killer; PBMC: peripheral blood mononuclear cell; CAR: chimeric antigen receptor; GD: beta-1,4-N-acetyl-galactosaminyltransferase 1; DIPG: diffuse intrinsic pontine glioma.

Translation into clinical trials is underway or imminent, underscoring the potential for NK cell-based therapies to become a vital component in treating this devastating pediatric brain cancer [17,97]. Currently, no large-scale clinical trials have been published deploying NK cell therapies (unmodified or CAR-engineered) in DIPG patients. Most ongoing clinical research focuses on CAR-T cell therapies targeting antigens like GD2 and B7-H3, which have shown promising results in early to mid-phase trials (ClinicalTrials.gov. ID NCT04196413). Preliminary data suggest that CAR-NK therapies might have more favorable safety profiles and lower cytokine release syndrome (CRS) risks compared to CAR-T therapies [111,112]. CAR-NK cells offer improved tumor specificity and cytotoxicity with lower risks of graft-versus-host disease or cytokine release syndrome compared to CAR-T cells [37]. Understanding NK cell trafficking, persistence, and activation in the DIPG microenvironment is critical. The potential of combining NK cell therapy with epigenetic-modifying agents has yet to be clinically tested. There is a pressing need for clinical trials focusing on NK-cell therapies, including CAR-NK cells, in DIPG.

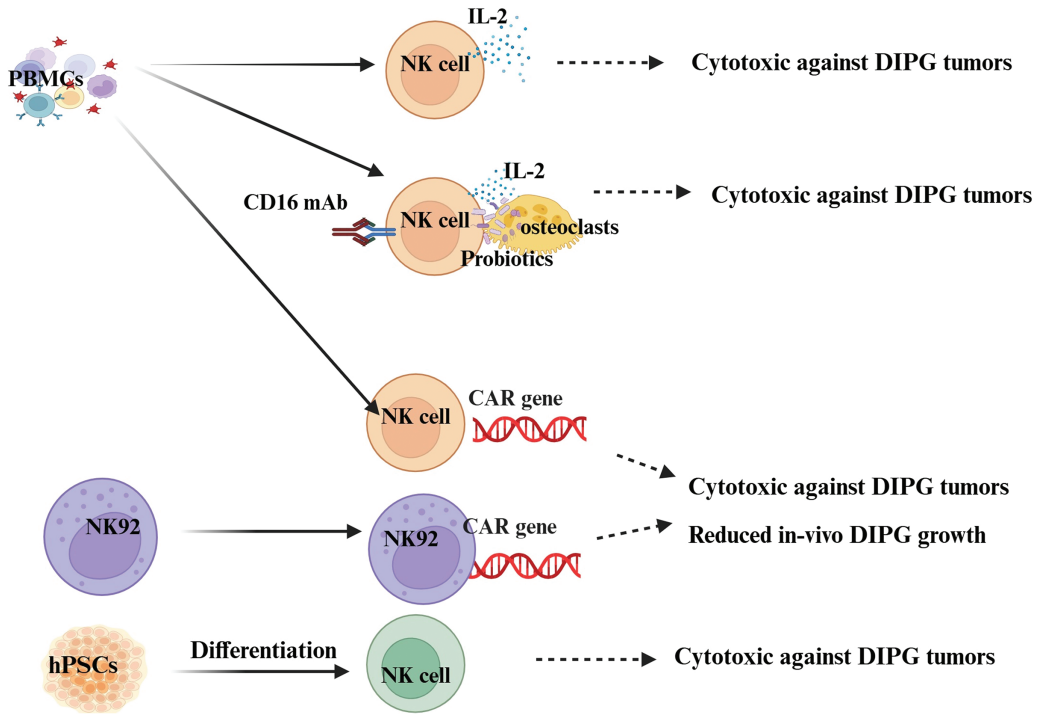

Figure 3: Illustration showcasing NK cell therapy platforms for DIPG. This can be achieved through cytokines, a combination of feeder cells, cytokines, Fc antibodies, and probiotics, or by utilizing CAR technology. Created in BioRender. Kaur et al. https://BioRender.com/rinjnt8 (accessed on 17 November 2025). PBMCs: peripheral blood mononuclear cells; CD16: cluster of differentiation 16; hPSCs: human pluripotent stem cells; CAR: chimeric antigen receptor

5 Challenges and Future Perspectives

Adoptively transferred NK cells face significant challenges with survival and proliferation *in vivo*, especially in the immunosuppressive TME [89]. Physical barriers and low chemokine levels can hinder NK cell recruitment and infiltration into DIPG [89]. Cytokine therapies like IL-2-activated NK cells often cause severe toxicities, such as vascular leak syndrome and cytokine release syndrome [113,114]. Tumors evade NK cell function by downregulating activating ligands or releasing soluble ligands like MICA/B, which interact with inhibitory NK receptors and lead to dysfunction [92]. They may also maintain or increase MHC class I molecule expression, activating inhibitory KIRs on NK cells [35]. Inhibitory checkpoint molecules like PD-1 contribute to NK cell exhaustion and reduced cytotoxicity, while factors like TGF-β, IL-10, and hypoxia further suppress NK cell survival and activation [115–118]. Advanced strategies aim to create memory-like NK cells, such as cytokine-induced memory-like NK cells, to enhance their persistence and antitumor efficacy.

Allogeneic NK cells show great potential for adoptive therapies because they carry a low risk of GVHD, but they face hurdles like immune rejection and limited persistence due to HLA mismatches [119,120]. Functional immune systems in recipients often recognize and reject these foreign cells [39]. Host T cells can attack donor cells with mismatched HLA, B cells may produce alloantibodies tagging NK cells for destruction, and host NK cells might target donor cells lacking self-HLA ligands [121,122]. Additionally, macrophages and complement activation contribute to the elimination of donor NK cells, limiting their effectiveness [123]. Selecting donors based on KIR and HLA compatibility can boost NK cell function and reduce rejection, while repeated doses of off-the-shelf NK products may improve therapeutic outcomes [124].

6 Conclusion

NK cells have shown promise in preclinical studies against DIPG, both naturally and when CAR-engineered. Studies have demonstrated safety and effectiveness in lab and animal models, particularly with GD2-CAR NK-92 cells targeting GD2-positive DIPG cells. However, clinical evidence for NK cell therapies in DIPG remains limited, with no late-phase trials yet, unlike CAR-T therapies. Challenges include the tumor's immunosuppressive environment, limited NK cell persistence after infusion, and determining the best delivery methods—whether intratumoral, intraventricular, or systemic. Enhancing NK cell function through epigenetic priming or combination therapies is a promising research area. Moving forward, priorities include clinical trials on NK cell therapies, identifying biomarkers for treatment response, and ensuring patient safety. The progress of CAR-T cells provides a strong foundation to advance CAR-NK therapies into early-phase trials.

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