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REVIEW

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Advances in preclinical models for pediatric diffuse intrinsic pontine glioma

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Abstract

Diffuse intrinsic pontine gliomas (DIPG) are highly aggressive brainstem tumors, representing the leading cause of pediatric cancer-related mortality despite their rarity. DIPGs are predominantly characterized by the H3K27M mutation, which drives tumorigenesis through epigenomic reprogramming and dysregulated gene expression. A major barrier to therapeutic advancement has been the scarcity of representative preclinical models, historically limited by the rarity of tumor tissue samples. Recent advancements in biopsy safety and model development have accelerated progress in understanding DIPG biology and developing novel therapies. While current murine models offer valuable insights, they often fail to replicate the tumor's genetic and microenvironmental complexity fully. Non-murine models offer cost-effective platforms but are limited by anatomical and immunological differences that reduce their relevance to human DIPG. This review highlights advances and limitations in DIPG models, emphasizing the need for integrative approaches using multiple systems to validate therapies, as no single model can fully capture the disease's complexity. Addressing these gaps could lead to the development of novel treatments for DIPG.

KEYWORDS
diffuse intrinsic pontine glioma, pediatric brain tumor, preclinical models, tumor immune microenvironment

Abbreviations: BBB, blood–brain barrier; BBTB, blood–brain tumor barrier; CAM, chick-embryo chorioallantoic membrane assay; CAR, chimeric antigen receptor; DIPG, diffuse intrinsic pontine glioma; DMG, diffuse midline glioma; DNp53, dominant negative p53 mutant; FUS, focused ultrasound; GBM, glioblastoma multiforme; GEMM, genetically engineered mouse model; hES-NPCs, human embryonic stem cell-derived neural progenitor cells; Hu-BLT, human bone marrow–liver–thymus; Hu-HSC, human hematopoietic stem cells; Hu-PBMC, human peripheral blood mononuclear cells; iPSC, induced pluripotent stem cell; LOC, lab-on-a-chip; MRI, magnetic resonance imaging; NK cells, natural killer cells; NSG mice, NOD scid gamma mice; OPC, oligodendrocyte progenitor cell; PBMC, peripheral blood mononuclear cells; PDGFB, platelet-derived growth factor B; PDGFRA, platelet-derived growth factor receptor alpha; PDOX, patient-derived orthotopic xenograft; PDX, patient-derived xenograft; RCAS, replication-competent avian sarcoma-leukosis virus long-terminal repeat with splice acceptor; TMZ, temozolomide; TP53, tumor protein p53; TVA, tumor virus A receptor; WHO, World Health Organization.

An Coosemans and Matteo Riva are considered as last authors.

1 | INTRODUCTION

Diffuse intrinsic pontine gliomas (DIPG), a subset of diffuse midline gliomas (DMG) located in the brainstem, pose a significant challenge in pediatric oncology. Encompassing approximately 75%–80% of pediatric brainstem tumors,[1,2] DIPG is associated with a median survival time of no more than 12 months.[3] Surgical resection is deemed unfeasible due to the location and diffuse, infiltrative growth characteristics of DIPG, leaving fractionated radiation therapy as the primary and only treatment proven to provide a survival benefit.[4] Despite decades of research, conventional chemotherapy and targeted therapies have

not improved survival rates.[5,6] Very recently (August 2025), dordaviprone (ONC201, Modeyso™) received FDA accelerated approval as a post-radiotherapy treatment for H3K27M-mutant diffuse glioma, with continued approval dependent on results from the ongoing Phase III ACTION trial assessing its safety and clinical benefit.[7–9] In addition, treatments such as GD2- and B7-H3-directed CAR T cells,[10,11] and convection-enhanced delivery of panobinostat show promise,[12] but these approaches remain in early clinical trials.

Multiple studies have revealed that 80% of DIPGs harbor the histone H3K27M mutation, encoded by HIST1H3B, HIST1H3C, and H3F3A genes, with this mutation exerting broad effects on gene expression and being considered a tumor driver.[13–15]

Since then, further understanding of the H3K27M mutation's role in DIPG pathogenesis has emerged. This mutation reprograms the epigenome, causing a global reduction in H3K27 methylation and altering chromatin accessibility.[16,17] H3K27M contributes to DIPG pathogenesis by promoting stem cell properties, increasing proliferation, and interfering with normal differentiation.[18] However, the H3 K27M mutation alone is insufficient for full DIPG development. Aberrations in DNA damage control, including TP53 mutations, are found in 75% of cases,[19] while also overactive kinase receptors, such as PDGFRA amplifications (30%),[20] and ACVR1 mutations occur in 20%–32% of DIPGs.[21–24] These genetic alterations often co-occur, indicating possible cooperative effects in tumor development. For instance, ACVR1 mutations frequently coincide with activating PIK3CA or PIK3R1 mutations.[24] Such molecular heterogeneity has important implications for preclinical research: models need to capture these diverse and co-occurring alterations to be clinically meaningful.

Over the past decades, no progress has been made in treating DIPG, largely due to the lack of representative models. Preclinical research has faced challenges, particularly due to the rarity of DIPG and difficulty in obtaining tumor tissue. Historically, biopsies were not performed because of the tumor's critical location, and since surgical resection is not part of the standard treatment, acquiring human DIPG samples has been difficult. As a result, glioblastoma (GBM) cell lines were initially used to establish preclinical models. However, these were later shown to be unrepresentative, as therapies that appeared effective in preclinical studies failed in DIPG patients, highlighting the models' inability to reflect the disease's unique biology.

While biopsies historically considered to carry unacceptable risks of morbidity and mortality, multiple studies have demonstrated that stereotactic biopsies for DIPG are safe.[25,26] The rise of targeted therapies has further encouraged neurosurgeons to perform biopsies to identify druggable mutations. This has increased tissue availability, enabling research to progress and new models to be developed. Despite these advancements, many existing models still have significant limitations, for example, lacking an intact immune system, not fully capturing patient heterogeneity, or being based on adult animal models for a pediatric disease. While further improvement is needed, this review will highlight recent advances in DIPG modeling and summarize ongoing research on both animal and non-animal models, along with their applications and limitations.

2 | MURINE DIPG MODELS

A range of murine models for DIPG has been developed, each contributing unique insights into the disease. In this review, we will focus on four key types: genetically engineered mouse models (GEMMs), patient-derived orthotopic xenograft (PDOX) models, syngeneic models, and humanized mouse models (Figure 1).

2.1 | Patient-derived orthotopic xenograft models

PDOX models have become increasingly important in cancer research, including for DIPG. These models involve implanting human tumor cells (Table 1) into immunocompromised mice, allowing researchers to study the tumor in a living organism while retaining the key characteristics of the original donor tumors. This ability to maintain the histological and genetic features of the patient's cancer makes PDX models particularly valuable for drug screening and personalized medicine strategies.[27,28] Over time, PDX models for DIPG have evolved significantly, driven by technological advancements and our understanding of the disease.

Early models for studying DIPG involved the use of non-DIPG glioma cells in rodents.[29] In 2010, Hashizume et al. developed GBM xenograft models by injecting adult GBM cell lines into the brainstems of mice and rats.[30,31] Treatments like temozolomide (TMZ) and PD-0332991, a CDK4/6 inhibitor, showed tumor growth delay in these models, suggesting potential effectiveness.[15,30,31] However, clinical trials in children with DIPG did not show meaningful survival benefits.[15,32,33] This highlights that adult glioma cells, even when transplanted into the brainstem, do not fully capture the distinct biology or treatment responses of pediatric DIPG.[15]

Significant progress came with PDOX models derived from DIPG autopsy specimens, allowing for a more accurate representation of the disease's genetic landscape.[29] Monje et al. pioneered the development of human DIPG cell lines in 2011, utilizing a post-mortem cell culture technique to generate DIPG neurospheres in vitro, followed by successful stereotactic transplantation of these cell lines into immunodeficient neonatal mice.[15,34] A key consideration when using these models is their prior exposure to radiation and various therapies, which may alter the tumor biology. As a result, post-mortem models may not reflect tumor characteristics at diagnosis or during progression, as shown by differing mutation rates between autopsy and biopsy samples.[35,36]

The increased adoption of stereotactic biopsies at diagnosis in recent years has facilitated the development of more representative DIPG models by providing access to treatment-naïve patient tumor tissue. These models better mimic the molecular diversity and invasive nature of treatment-naïve DIPG tumors. In 2012, Hashizume et al. pioneered the development of a DIPG model from biopsy specimens, paving the way for the subsequent establishment of several primary cultured DIPG cell lines and PDOX models.[15,30,37] Plessier et al. (2017) successfully created eight patient-derived orthotopic xenograft models and six cell-derived xenograft models using stereotactic

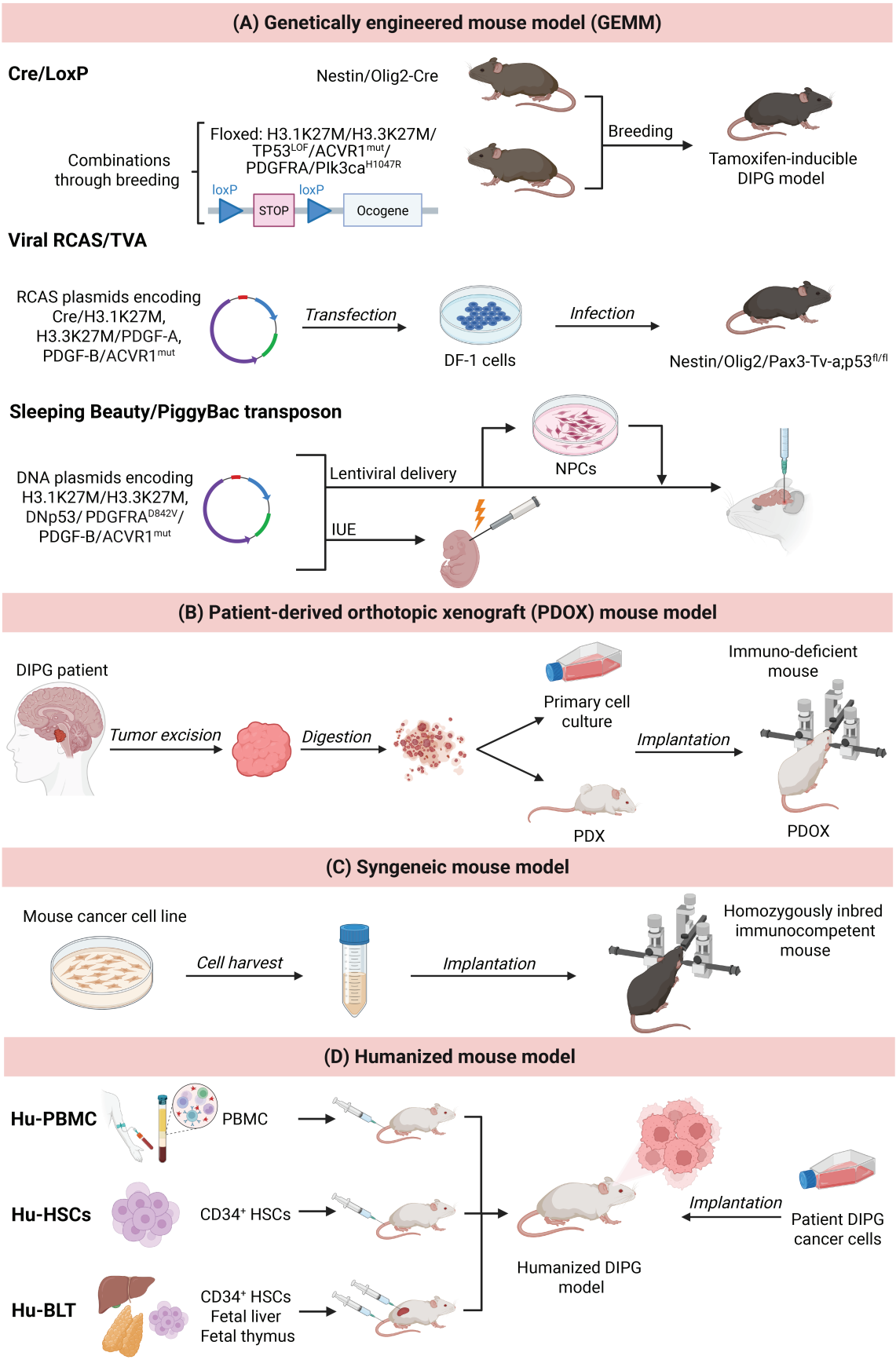

FIGURE 1 Legend on next page.

biopsies. These models accurately replicate DIPG's invasive nature, H3K27 trimethylation loss, and tumor-type diversity observed in patients. Both direct (patient-derived) and indirect (cell-derived) orthotopic xenografts exhibited histological and MRI features similar to treatment-naïve human DIPG.[38]

Building on these foundational models, researchers began to tackle key challenges such as the intact BBB, a major obstacle for drug delivery in DIPG. In 2023, Martinez P. advanced the field by integrating MRI-guided focused ultrasound (FUS) into PDOX models to transiently open the BBB. By combining FUS with microbubbles, they enabled the delivery of larger chemotherapeutic agents, such as panobinostat, into the brain parenchyma. This approach increased drug concentration within tumors by threefold, reduced tumor volume by 71%, and extended survival from 21 to 31 days. Martinez's work demonstrated how new strategies could augment existing PDOX models to address therapeutic challenges, advancing the refinement of these models.[39]

Importantly, PDOX models have played a critical role in enabling translational advances in DIPG research. In 2018, the laboratory of Monje et al. identified uniformly high expression of GD2, a disialoganglioside, on H3K27M-mutant DIPG tumor cells. Leveraging these PDOX models, they demonstrated that systemic administration of GD2-targeting chimeric antigen receptor (CAR) T cells resulted in complete tumor regression in vivo.[40] This preclinical success laid the foundation for a first-in-human phase I clinical trial (NCT04196413) of GD2-CAR T cells in patients with DIPG and spinal diffuse midline glioma, with results published in 2024 showing promising outcomes.[11]

A significant drawback is that PDOX models do not allow researchers to isolate and examine the specific contributions of single mutations to the disease's progression and pathology. This limits the ability to understand how various genetic alterations interact and influence tumor behavior. To address this, a new model was developed in 2021 using human induced pluripotent stem cells (iPSCs) edited via CRISPR to express the H3.3K27M mutation. Edited iPSCs were chemically differentiated into neural progenitor cells, which upon implantation into the brainstems of immunodeficient mice formed diffusely invasive tumors that closely mimicked DIPG transcriptionally and histologically, offering a new tool for investigating pathogenesis and drug responses.[41,42]

In 2024, Bhatia et al. identified a significant problem in preclinical DIPG research: many patient-derived DIPG cell lines and PDOX models lacked full characterization. To address this, efforts were made to create, characterize, and catalogue these models, resulting in an expanded set of resources for researchers. As a result, 21 growth-verified models are now accessible, with 14 of them genomically validated. This data will be made publicly available via cBioPortal, providing researchers with well-characterized models for drug discovery, testing, and more consistent study comparisons.[43]

Despite these advancements, patient material remains scarce and can undergo genetic and phenotypic changes during serial passaging. Moreover, biopsy samples typically represent only a small portion of the tumor, which may not reflect its entire complexity. PDX models lack immune components, limiting the study of non-cell-autonomous mechanisms, tumor immunology, and immunotherapy.[44] While PDX models can be used for patient-specific drug screening and personalized medicine strategies,[27] they may not fully capture the tumor microenvironment's role in treatment outcomes.[44] Incorporating a human immune system through "humanized models" would provide a more accurate and optimal approach.

2.2 | Genetically engineered mouse models (GEMMs)

GEMMs are powerful tools for identifying tumor drivers by introducing specific genetic mutations in mice to replicate the development of human DIPG. Until recently, the creation of these models was limited by a lack of understanding of genetic tumor drivers in DIPG. However, several GEMMs have now been developed using three primary systems: Nestin-Cre/LoxP recombination breeding-based, Viral RCAS/TVA-based, and Sleeping Beauty/PiggyBac transposon-based.

2.2.1 | Cre/LoxP recombination breeding

This system uses the Cre-LoxP recombination technique to achieve targeted gene modifications. Mice are engineered to express Cre recombinase in Nestin or Olig2-expressing cells. LoxP sites are strategically inserted into the genes of interest within the mouse genome, allowing for precise manipulation of gene expression.

Using Cre/LoxP recombination in genetically engineered mice, researchers discovered that H3.3K27M mutations enhance neural stem cell self-renewal while preserving regional identity. When combined with PDGFRA activation and Trp53 loss, these mutations accelerated brainstem glioma development, recapitulating human DIPG gene expression signatures. This process was driven by

FIGURE 1 Murine DIPG mouse models. (A) Genetically Engineered Mouse Models (GEMMs): Mice are genetically modified to harbor human DIPG mutations, using Cre/LoxP (floxed alleles with Nestin- or Olig2-Cre mice), RCAS/TVA (RCAS-transfected DF-1 cells infecting Nestin/Olig2/Pax3-Tv-a;p53^fl/fl mice), or Sleeping Beauty/PiggyBac transposons (plasmids delivered via in utero electroporation (IUE), directly into the brainstem via lentivirus, or into neural progenitor cells (NPCs) followed by brainstem implantation). (B) Patient-Derived Orthotopic Xenograft (PDOX) Models: Human DIPG cells are implanted into the brainstem of immunodeficient mice. (C) Syngeneic Models: Murine DIPG cells are transplanted into genetically identical mice. (D) Humanized Mouse Models: Immunodeficient mice are engrafted with human immune components including peripheral blood mononuclear cells (Hu-PBMC), $CD34^+$ hematopoietic stem cells (Hu-HSC), or fetal liver and thymus tissue with $CD34^+$ HSCs (Hu-BLT), followed by DIPG xenograft implantation. Created in BioRender. Van den Ende, B. (2025) https://BioRender.com/s76f593. [Color figure can be viewed at wileyonlinelibrary.com]

TABLE 1 Key characteristics of patient-derived DIPG cell lines used in in vitro and in vivo research.

Cell name	H3-mutation	Other defined mutations	Tissue origin	Sex	Age
CCHMC-DIPG-1[110–112]	H3WT		Autopsy	ND	ND
CCHMC-DIPG-2[112]	H3.3K27M		Autopsy	ND	ND
CHRU-TC68[36]	H3.3K27M		Biopsy	F	9
DIPGM[34]	H3WT		Autopsy	M	5
DIPG-PBTR3[111]	H3.3K27M		Autopsy	M	5
HSJD-DIPG-007[102,111,113–119]	H3.3K27M	ACVR1 R206H, PPM1D P428fs	Autopsy	M	6
HSJD-DIPG-007-TP53 KO[118]	H3.3K27M	ACVR1 R206H, PPM1D P428fs, P53 KO	Autopsy	M	6
HSJD-DIPG-008[113–116]	H3.3K27M	PPM1D R429fs	Autopsy	ND	ND
HSJD-DIPG-011[117]	H3.3K27M		Autopsy	ND	ND
HSJD-DIPG-012[113–115,118]	H3.3K27M	P53 mutated	Autopsy	M	6
HSJD-DIPG-013[102,116,118]	H3.3K27M	P53 mutated	Autopsy	F	6
HSJD-DIPG-014[102]	H3.3K27M	PPM1D L484fs	Biopsy	F	8
HSJD-DIPG-018[119]	H3.1K27M	ACVR1 R258G	Biopsy	ND	ND
JHH-DIPG1[111,113,114,120,121]	H3.3K27M	P53 mutated	Autopsy	M	8
Li-A[121]	H3.1K27M		Autopsy	ND	5
Li-C[121]	H3.3K27M		ND	ND	13
Li-D[121]	H3.3K27M		ND	ND	12
Li-E[121]	H3.3K27M		ND	F	12
Li-F[121]	WT		ND	ND	8
NEM 157[121]	H3.3K27M		Biopsy	F	5
NEM 163[121]	H3.3K27M		Biopsy	M	5
NEM 165[121]	H3.3K27M		Biopsy	F	3
NEM 168[121]	H3.3K27M		Biopsy	F	9
NEM 175[121]	H3.3K27M		Biopsy	ND	9
NEM-186[121]	H3.1K27M		Biopsy	ND	3
NEM-215[121]	H3.3K27M		Biopsy	ND	6
NEM273[111]	H3.1K27M	ACVR1 G328E	Biopsy	M	4
NEM285[111]	H3.3K27M	P53 mutated	Biopsy	M	7
NEM289[111]	H3.1K27M	P53 mutated	Biopsy	M	4
NEM290[111]	H3.3K27M	P53 mutated	Biopsy	F	11
NEM292[111]	H3.1K27M	P53 mutated	Biopsy	F	5
NEM325[111]	H3.3K27M		Biopsy	F	5
NEM328[111]	H3.1K27M	ACVR1 G328V	Biopsy	F	3
NEM335[111]	H3.3K27M	P53 mutated	Biopsy	M	6
NEM347[111]	H3.1K27M	P53 mutated	Biopsy	M	9
NEM353[111]	H3.3K27M		Biopsy	F	6
OHSU-DIPG#1[121]	H3.3K27M		Autopsy	F	4
OHSU-DIPG#2[121]	H3.3K27M		Autopsy	M	6
RA-055[74,116]	H3.3K27M	P53 mutated, PDGFRa amplified, MYCN amplified	Biopsy	F	ND
SF7761[111,113,114,118,120,122,123]	H3.3K27M	Exogenous hTERT	Biopsy	F	6
SF8628[111,114,122,123]	H3.3K27M	P53 mutated	Biopsy	F	3
SK-DIPG27[121]	H3.3K27M		ND	ND	6
SU-DIPG-I[111,121]	H3 WT	P53 mutated	Autopsy	M	5
SU-DIPG-II[121]	H3.3K27M		Autopsy	M	7
SU-DIPG-III[121]	H3.1K27M		Autopsy	M	12

(Continues)

TABLE 1 (Continued)

Cell name	H3-mutation	Other defined mutations	Tissue origin	Sex	Age
SU-DIPG-IV[114,119–121,123–126]	H3.1K27M	P53 WT, MDM4 amplified, ACVR1 G328V	Autopsy	F	2
SU-DIPG-IX[121]	WT		Autopsy	M	13
SU-DIPG-VI[111,116,119–121,123]	H3.3K27M	P53 mutated	Autopsy	F	7
SU-DIPG-VII[121]	WT		Autopsy	M	8
SU-DIPG-VIII[121]	WT		Autopsy	M	9
SU-DIPG-X[121]	H3.1K27M		Autopsy	M	11
SU-DIPG-XI[121]	H3.3K27M		Autopsy	M	5
SU-DIPG-XII[121]	H3.3K27M		Autopsy	F	4
SU-DIPG-XIII[114,120,121,123,124]	H3.3K27M	P53 mutated	Autopsy	F	6
SU-DIPG-XVII[116,120,123]	H3.3K27M	P53 mutated	Autopsy	M	8
SU-DIPG-XIX[111,123,126]	H3.3K27M		Autopsy	M	2
SU-DIPG-XXI[114,123,124]	H3.1K27M		Autopsy	M	7
SU-DIPG-XXIV[123,126]	H3.3K27M		Autopsy	F	6
SU-DIPG-XXV[120,121,123]	H3.3K27M		Autopsy	F	4
SU-DIPG-XXVII[123,127]	H3.3K27M		Autopsy	M	5
SU-DIPG-XXIX[123]	H3.3K27M		Autopsy	F	6
SU-DIPG-XXXIII[123]	H3.1K27M		Autopsy	M	8
SU-DIPG-XXXV[123,127]	H3.3K27M	PPM1D S432fs	Autopsy	F	8
SU-DIPG-XXXVI[123]	H3.1K27M		Autopsy	F	3
SU-DIPG-XXXVIII[123,127]	H3.1K27M		Autopsy	F	12
SU-DIPG-XXXXIII[127]	H3.1K27M		Autopsy	ND	ND
SUPSCG1[123]	H3.3K27M			M	12
TP54[125]	H3.3K27M		Biopsy	M	9
TP80[125]	H3.3K27M		Biopsy	F	5
TP83[125]	H3.3K27M		Biopsy	F	5
TP84[125]	H3.3K27M		Biopsy	M	9
TT10603[111,128]	H3.3K27M	P53 mutated	Surgery	M	7
TT10630[111,118,128]	H3.3K27M	PPM1D S516Ter	Biopsy	F	4
TT10714[111,118,128]	H3.3K27M	P53 mutated	Surgery	F	6
TT10728[118,128]	H3.3K27M	P53 mutated	Biopsy	F	7
TT10902[128]	H3.3K27M	P53 mutated	Biopsy	F	8
TT11111[128]	H3.3K27M		Surgery	F	5
TT11118[128]	H3.3K27M	P53 mutated	Surgery	M	6
TT11201[128]	H3.3K27M	P53 mutated	Surgery	F	7
UON-VIBE5[74]	H3.3K27M	PDGRFA amplified, PPM1D mutated	Autopsy	F	ND
UON-JUMP4[74,123]	H3.3K27M	P53 mutated, PIK3CA E545K, ACVR1 G328V, HIST1H3B K28M			
VUMC-DIPG-08[113,114,123]	H3.3K27M		Autopsy	ND	ND
VUMC-DIPG-10[113,114,116,120,123]	H3WT	NF1 Q209*; MYCN amplified	Autopsy	F	12
VUMC-DIPG-11[123]	H3.3K27M		ND	M	15
VUMC-DIPG-A[113,114,121]	H3.3K27M		Biopsy	F	3
VUMC-DIPG-B[121]	H3.1K27M		Biopsy	F	4
VUMC-DIPG-F[111,113]	H3.3K27M		Biopsy	M	7
QCTB-R059[111,119]	H3.3K27M		Surgery	F	10

tamoxifen-inducible Cre recombinase in neonatal nestin-positive cells (neuroepithelial stem cells) across the developing brain.[15,45] More recent studies have focused on Olig2-positive oligodendrocyte progenitor cells (OPC), as these are considered the most likely cell of origin for DIPG.[46] It was shown that the Acvr1^{G328V} mutation halts the differentiation of OPC, and, when combined with Hist1h3b^{K27M} and Pik3ca^{H1047R}, leads to high-grade diffuse gliomas.[15,47] Another study found that deleting Trp53 specifically in Olig2-positive progenitors was sufficient to induce diffuse gliomas with long latency and mild brainstem preference. An inducible H3f3a^{K27M} mutation was also included in these models, and its impact on tumor initiation and progression is currently being evaluated.[48] These models offer crucial insights into the mutation combinations that drive DIPG development.

However, the unpredictable nature of tumor development in Cre/LoxP recombination models makes them complex and costly for therapy assessment studies. As a result, Cre-lox breeding has become less common, replaced by more advanced genetic tools that eliminate the need for time-consuming breeding strategies.

2.2.2 | Viral RCAS/TVA system

The RCAS system uses a retroviral vector to deliver genes into specific cells. It targets cells that express the TVA receptor, and genetically engineered mice are created to express this receptor under specific promoters, usually the Nestin promoter (Nestin-Tv-a mice) for DIPG modeling. This system delivers genetic alterations commonly seen in DIPG, such as PDGFB overexpression, TP53 silencing, and DIPG-specific mutations like H3.1^{K27M}/H3.3^{K27M} and Acvr1^{R206H}, leading to tumor development.[23,49] Becher et al. were the first to use the RCAS/TVA system GEMMs to generate high-grade brainstem gliomas, showing biological similarity to DIPG.[29,50]

Since Nestin labels a broad neural stem and progenitor population across the CNS, spatial specificity is limited. To address this, Pax3—a marker with enriched expression in the brainstem—has been used as an alternative promoter. Using Pax3-Tv-a mice, Misuraca et al. induced *PDGF-B* and *H3.3K27M* overexpression along with *p53* loss, resulting in gliomas throughout the brainstem, including the ventral and dorsal pons, midbrain, and fourth ventricular space.[15,51]

Beyond spatial targeting, recent research has focused on the cellular origin of DIPG. Tomita et al. used the RCAS/TVA system to generate tumors from Nestin$^+$ progenitors and Olig2$^+$ OPCs in the neonatal mouse brainstem. They found that H3.3K27M had a stronger oncogenic effect in Nestin$^+$ cells than in Olig2$^+$ cells, with Olig2-initiated tumors showing reduced dependence on H3.3K27M and distinct transcriptional programs, indicating that the tumorigenic impact of H3.3K27M is cell-of-origin dependent.[52]

Recent efforts have also combined the RCAS/tv-a system with CRISPR/Cas9 gene editing to streamline DIPG model generation, allowing for rapid perturbation of genes of interest and enabling sophisticated in vivo genetic experiments.[53]

The RCAS/TVA system enables tumor development throughout the whole brainstem, rather than just in the pons, since neither Nestin, Olig2, nor Pax3 expression is specific to that region, which limits the model's spatial accuracy. Despite limitations, RCAS/TVA-based models are an improvement over traditional GEMMs. Viral delivery bypasses the labor-intensive and costly crossbreeding process, and the low penetrance of the virus into receptor-expressing cells more accurately simulates tumor initiation, where only a small number of transforming cells are critical.[54]

2.2.3 | Sleeping beauty/PiggyBac transposon systems

This system uses transposable elements (Sleeping Beauty and PiggyBac) to stably integrate genetic material into the host genome, enabling flexible study of mutations and their roles in tumor development. Delivery to target cells is typically achieved via lentivirus.

Using this approach, it was shown that human embryonic stem cell-derived neural progenitor cells (hES-NPCs) expressing H3.3K27M, modified through lentiviral delivery, do not develop tumors in the mouse pons. However, when combined with TP53 loss and PDGFRA activation, tumors resembling DIPG were successfully formed, showing that H3K27M alone is insufficient to drive DIPG development.[15,55]

Additionally, in utero electroporation has been used to introduce the transposon system directly into the developing brains of mouse embryos.[56] This method involves injecting transposon plasmids and transposase into the brain and applying electrical pulses to facilitate DNA uptake. This system was employed to generate DIPG models by introducing oncogenic combinations, including PDGFB, PdgfraD842V, or PdgfraWT, along with DNp53 and H3.3K27M mutations.[57,58] The combination of PDGFB, DNp53, and H3.3K27M resulted in rapid development of grade IV gliomas, while PdgfraD842V, DNp53, and H3.3K27M led to slower-forming grade III gliomas. Additionally, the combination of PdgfraWT, DNp53, and H3.3K27M produced both high and low-grade gliomas with longer latency periods. Each model exhibited distinct histopathological and molecular characteristics that mirror those found in human DIPGs.[57] These models have enabled the characterization of molecular pathways and histopathological features associated with DIPG. Furthermore, combining in utero electroporation with piggyBac transposon and CRISPR technology has facilitated the development of subtype-specific pediatric high-grade glioma (pHGG) models, allowing for the identification of driver-specific vulnerabilities and potential precision therapies.[59] One significant advantage of in utero electroporation over other GEMMs is its ability to provide precise spatial control over genetic modifications in the developing brainstem. However, it is more technically demanding and requires expertise.

Crucially, these IUE models have been instrumental in evaluating potential therapies. In PDGFRα-driven H3K27M pHGG models, dasatinib combined with everolimus improved CNS drug retention and extended survival in mice, with early clinical use suggesting feasibility in patients.[60] Similarly, ID1 inhibition using cannabidiol (CBD) in IUE

TABLE 2 Genetic profiles of murine diffuse midline glioma models.

Histon 3	Cell line ID	Co-mutations
$H3^{WT}$	UC-BL6-D1[58] UC-BL6-D3[58]	$p53^{LOF}$ $Pdgfra^{D842V}$
$H3.3^{K27M}$	KP[62]	$p53^{LOF}$
	H3K27MPP[82,129]	$p53^{LOF}$ $Pdgfra^{OE}$
	K27M-APP[56,82]	$p53^{LOF}$ $ATRX^{LOF}$ $Pdgfra^{OE}$
	UC-BL6-B1[58] UC-BL6-B7[58] PPK (IUE-24)[74]	$p53^{LOF}$ $Pdgfra^{D842V}$
	KPPMPIK[62]	$PPM1D^{\Delta C}$ $PIK3CA^{E545K}$
	KPF[62]	$p53^{LOF}$ $FGFR1^{N457K}$
	KN[62]	$NF1^{LOF}$
	KF[62]	$FGFR1^{N457K}$
	KPC[62]	$p53^{LOF}$ $CCND2^{WT}$
	KNF[62]	$NF1^{LOF}$ $FGFR1^{N457K}$
$H3.1^{K27M}$	H3.1KP[62]	$p53^{LOF}$
	UC-BL6-C1[58] UC-BL6-C2[58] UC-BL6-C7[58]	$p53^{LOF}$ $Pdgfra^{D842V}$ $Acvr1^{G328V}$
	H3.1KACVPIK[62]	$ACVR1^{G328V}$ $PIK3CA^{E545K}$

Abbreviations: LOF, loss-of-function; OE, overexpression.

models reduced tumor growth and invasion, revealing a migratory program that can be therapeutically targeted.[61] Broad drug screening across multiple IUE-generated pHGG subtypes further identified selective vulnerabilities tied to specific oncogenic alterations, showing how these models recapitulate aspects of tumor heterogeneity relevant for patient-tailored therapy.[62] In summary, GEMMs have proven to be valuable for studying the genetic drivers of DIPG in an immunocompetent setting, offering critical insights. However, these models are based on a limited number of mutations or gene alterations, failing to fully capture the genetic and epigenetic complexity of DIPG tumors. They often lack the spatial precision to confine tumor growth to the pons, leading to discrepancies in tumor behavior and treatment response. Furthermore, it is important to recognize that mouse tumors are not identical to human tumors, and findings from GEMMs may not always accurately predict how human tumors will respond to treatment.

2.3 | Syngeneic models

Syngeneic models are created by implanting murine tumor cells that originate from the same genetic strain as the host mice, ensuring compatibility with the host's immune system. There are few syngeneic models specifically designed for pediatric DIPG. Many models (rat and mice) have been established that do not accurately mimic pediatric DIPG, as they were based on tumor cells derived from other types of cerebral tumors, which lack the unique features of DIPG.[63–69] Recently, syngeneic DIPG mouse models were developed by implanting murine DIPG cell lines (NP53 and XFM) into transgenic mice (C57BL/6 background) and BALB/c mice, respectively.[70,71] These cell lines were established in Dr. Oren Becher's laboratory and were derived from DIPG tumors in genetically modified mice (C57BL/6 and BALB/c backgrounds) using the RCAS-TVA system. The NP53 cell line was derived from tumors in a DIPG model driven by PDGF-B signaling, p53 loss, and ectopic H3.3-K27M expression. The XFM cell line came from tumors in a model driven by PDGF-B signaling and the loss of Ink4a and ARF.[72,73]

Additionally, murine DMG cell lines have been developed from primary tumors in mice using brainstem-targeted in utero electroporation. These cell lines, featuring distinct genetic profiles (Table 2) were used to establish syngeneic C57BL/6 mouse models.[58,62,74] Of note, these models are designed for DMG, of which DIPG is a subset. Consequently, the selection of co-occurring mutations in these models reflects the broader genetic landscape of DMG rather than being specifically tailored to DIPG.

Syngeneic models offer significant advantages for cancer research, particularly in studying tumor biology and the immune response. Their immune system allows for a precise representation of tumor-immune interactions, making them particularly valuable for assessing the effectiveness of immunotherapies and understanding the complexities of tumor progression. Additionally, syngeneic models are typically more cost-effective and easier to establish than GEMMs, which necessitate extensive genetic modifications and complex breeding strategies.

However, the number of available syngeneic models is still limited, capturing only a fraction of the diverse genetic alterations found in patient tumors and leaving many co-occurring mutations underrepresented.[75] Additionally, their reliance on mouse biology may influence tumor behavior differently than in humans. Furthermore, with only a few laboratories working with these models, progress is hindered, as research advancement depends on reproducibility across multiple research groups.

2.4 | Humanized mouse models

Humanized mouse models are immunodeficient mice engrafted with human immune components, designed to mimic a functional human immune system. These models have been a step forward in studying human diseases and immune responses, as they offer a more accurate representation of the human tumor microenvironment, bridging the gap between traditional mouse xenografts and human clinical trials.[76] Three main types of humanized mouse models have been developed: Hu-PBMCs (peripheral blood mononuclear cells), Hu-HSCs (hematopoietic stem cells), and Hu-BLT (Bone marrow–Liver–Thymus).[77] These models are created using immunodeficient mice, which lack functional T, B, and NK cells.[78] The Hu-PBMC model involves injecting mature human immune cells, while the Hu-HSC model uses

TABLE 3 Comparative analysis of murine models for diffuse intrinsic pontine glioma.

Model type	PDOX	GEMM	Syngeneic model	Humanized mouse model
Source of tumor cells	Patient-derived DIPG tissue, directly implanted	Mice genetically engineered to develop DIPG-like brainstem tumors	Mouse DIPG-like tumor cells from same genetic background	Patient-derived DIPG tissue implanted after partial immune humanization of mice
Genetic fidelity	High – preserves patient-specific DIPG mutations and heterogeneity	Moderate – these models incorporate only a limited set of mutations relevant to DIPG.	Moderate – tumor cell lines may represent DIPG but lack the heterogeneity seen in patients	High – maintains patient-specific mutations and heterogeneity
Immune system	Immunocompromised host (lacks immune system)	Fully intact, mouse immune-competent	Fully intact, mouse immune-competent	Humanized – partial human immune system in immunocompromised mouse
Use in immunotherapy development	Limited – does not allow for human immune-tumor interactions	Useful – allows for testing of immunotherapies in a mouse context	Useful – allows for testing of immunotherapies in a mouse context	High – suitable for testing human-targeted immunotherapies
Tumor location	Orthotopic – implanted in brainstem	Spontaneous – tumors arise in brainstem through genetic induction	Typically orthotopic, implanted in brainstem	Orthotopic – implanted in brainstem
Advantages	High patient specificity; reflects DIPG heterogeneity	Reflects DIPG-like development and mouse immune interactions	Cost-effective, allows immune studies	High patient specificity; reflects DIPG heterogeneity; closest approximation to human immune response in DIPG models
Limitations	Lacks immune system; expensive; DIPG tissue difficult to obtain	Time-consuming, costly; requires precision in gene editing; lacks patient-specific genetic heterogeneity; tumor development not restricted to the pons	Lacks patient-specific genetic heterogeneity	Complex, costly; human immune reconstitution is partial; ethical concerns
Typical applications	Precision medicine, patient-specific drug screening	Genetic studies, immune interaction testing, drug efficacy	Immunotherapy studies, testing standard DIPG treatments	Testing immunotherapies

hematopoietic stem cells to reconstitute the murine immune system. The Hu-BLT model integrates human hematopoietic stem cells along with fetal liver and thymus tissue to support the development of human T cells through thymopoiesis.[79]

Humanized models are increasingly used in brain cancer research to better mimic human immune–tumor interactions. In GBM, several humanized mouse models have been developed. For example, Srivastava et al. created a Hu-HSCs mouse model, which successfully engrafted patient-derived GBM tumorsphere cells, closely replicating human GBM characteristics.[80] Similarly, Liu et al. introduced three distinct human GBM models in Hu-BLT mice, revealing unique immune landscapes within the tumors.[81]

In DIPG, the use of humanized models is still emerging, but early studies have begun to reveal key aspects of the tumor immune microenvironment. For instance, a Hu-HSC model was created using $CD34^{+}$ stem cell-engrafted NSG mice, in which a PDOX was established using the H3.3K27M SU-DIPG-XIII cell line.[82] This model recapitulated several immunological features of human DIPG, including high infiltration of myeloid cells and scarce presence of T cells. These findings were consistent with immune profiling from patient samples and further confirmed that the immune infiltrate in H3.3K27M tumors is predominantly of myeloid origin.

While humanized mice offer valuable insights, they are costly, labor-intensive, and raise ethical concerns, particularly regarding the use of human fetal tissue in Hu-BLT models. Additionally, most models fail to include key innate immune components, such as microglia. Without a fully representative tumor microenvironment, it is challenging to address critical questions about the stroma's role in tumor vulnerability and the impact of immunotherapy.[75]

To conclude, there are multiple types of murine DIPG models available, each with distinct advantages and limitations. These models vary in their ability to replicate the tumor's microenvironment, genetic landscape, and response to therapy. A comprehensive overview of these murine models and their respective strengths and weaknesses is summarized in Table 3.

3 | NON-MURINE DIPG MODELS

3.1 | Chick-embryo chorioallantoic membrane assay

Recently, there has been growing interest in non-murine models for studying DIPG (Figure 2). In 2022, Erica A. Power and colleagues

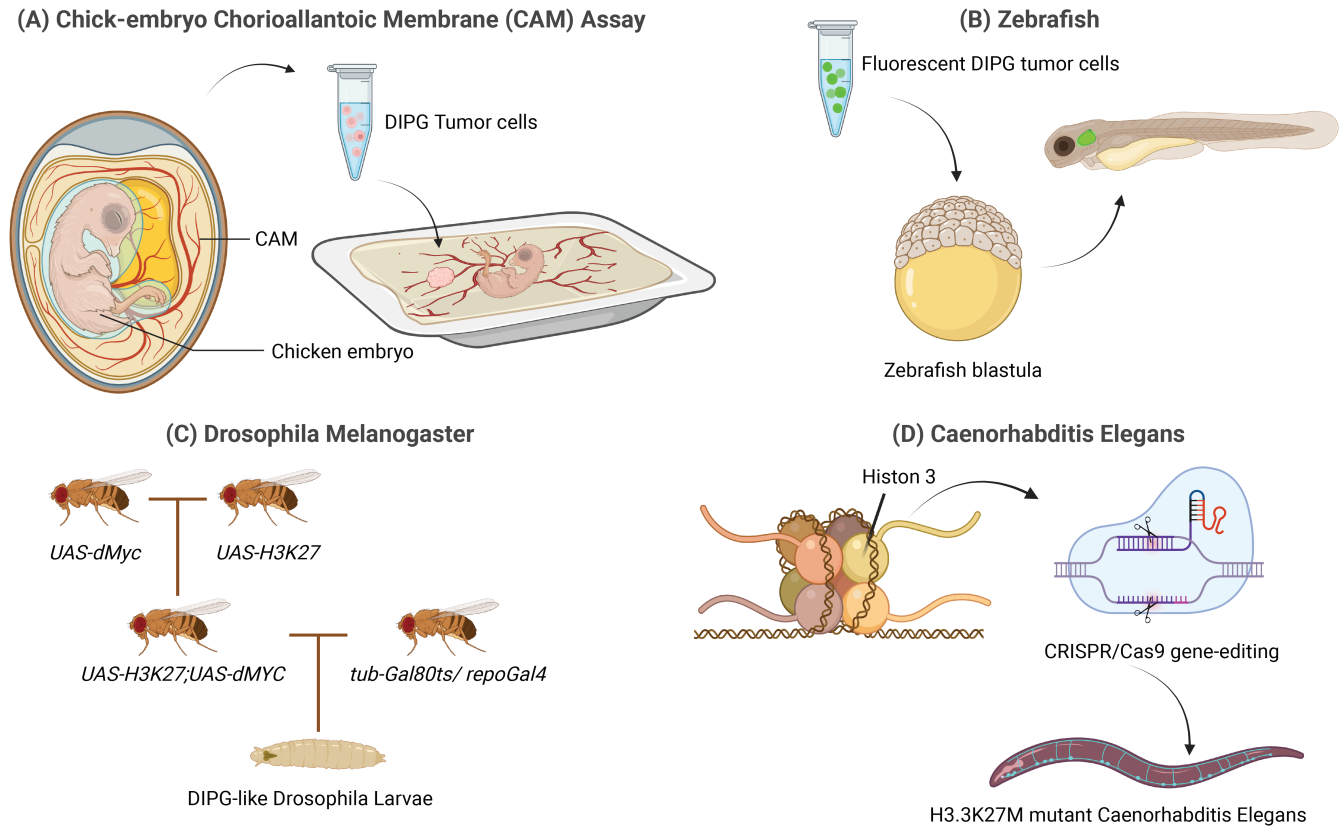

FIGURE 2 Non-murine DIPG Models. (A) Patient-derived DIPG cells implanted on the chorioallantoic membrane of a chicken embryo. (B) Fluorescent DIPG cells injected orthotopically into zebrafish blastula. (C) Drosophila melanogaster bred for glial cell overexpression of H3K27 and Myc. (D) Caenorhabditis elegans edited with CRISPR/Cas9 to express H3.3 K27M. Created in BioRender. Van den Ende, B. (2025) https://BioRender.com/cuob4mk. [Color figure can be viewed at wileyonlinelibrary.com]

introduced the chick-embryo chorioallantoic membrane (CAM) assay as a novel xenograft model for DIPG.[83] While the CAM assay has been used to study tumor biology and treatment response in other cancers, such as renal cell carcinoma[84] and pancreatic cancer,[85] its application to DIPG is novel. This model enables human tissue engraftment due to the embryo's lack of an immune system. In this study, patient-derived DIPG cells were implanted into the chorioallantoic membrane—a highly vascularized structure formed by the fusion of the chorion and allantois. Remarkably, tumor formation was observed within 48 h, enabling rapid testing of potential anti-cancer therapies. CAM tumors retained key histological and molecular features of the original tumors, including H3K27M positivity and H3K27me3 reduction. The model was also responsive to drug treatment and allowed assessment of tumor volume, vascularity, and radiation effects.[83]

The CAM model offers a rapid in vivo platform, producing results in just a few weeks, compared to the months required for tumor engraftment and testing in murine models. It is a cost-effective, time-efficient intermediate model that helps bridge the gap between in vitro and in vivo studies, making it ideal for drug screening. However, the absence of an intact immune system in this model limits its effectiveness for immunotherapy research. It also does not permit the evaluation of BBB penetration—an essential aspect of DIPG treatment, considering that the BBB remains intact in these tumors. Moreover, the tumor microenvironment within the CAM fails to accurately replicate the conditions of the brain.

3.2 | Zebrafish

Zebrafish models have also emerged as valuable tools for studying pediatric brain cancers, offering unique advantages over traditional models. Their genetic similarity to humans, rapid development, and transparent bodies enable precise tumor cell tracking and expedited drug screening.[86]

Zebrafish models have been successfully used to study various pediatric brain tumors, including high-grade gliomas, providing insights into tumor biology and potential therapeutic strategies.[87,88] These models allow for real-time monitoring of tumor development, efficient gene manipulation, and evaluation of genetic interactions in disease pathogenesis.[87] Recent studies have demonstrated the utility of zebrafish in assessing drug toxicity, developing orthotopic xenograft models, and screening potential therapies for pediatric brain cancers.[86] For instance, a novel zebrafish model of DIPG was used to validate potential therapies, such as quinacrine, within just 7 days by orthotopically injecting fluorescent DIPG cells into the developing zebrafish blastula.[89]

Quinacrine reduced DIPG growth by 40% in xenografted larvae, with no toxicity observed in normal neural cells. Building on these advancements, a more recent preclinical model has further optimized cell injection and imaging techniques, enabling large-scale screening and real-time analysis of tumor–immune interactions. By fluorescently labeling both tumor cells and microglia, this model allows for simultaneous visualization of tumor growth and the dynamic interactions between tumor cells and the immune microenvironment.[90]

However, zebrafish face limitations in DIPG research due to significant anatomical differences—they lack a direct equivalent to the pons—as well as differences in their immune system and BBB, making the model less translatable than mouse models. Nonetheless, zebrafish offer a rapid, cost-effective approach, complementing mammalian systems and providing promising avenues for advancing pediatric brain cancer research and treatment.[86,88]

3.3 | Drosophila melanogaster

Drosophila melanogaster has emerged as a valuable model organism for studying human brain diseases, including DIPG and GBM. In 2023, C. de Pablo et al. released a preprint discussing a novel model for DIPG using Drosophila melanogaster. This model incorporates the overexpression of H3K27 and Myc in glial cells, reflecting the epigenetic activation of MYC, a key transcriptional feature of H3K27M tumors.[91] The study demonstrates that the co-expression of H3K27 and MYC in glial cells leads to increased cell numbers and glial membrane volume within the ventral ganglion of larvae, a region analogous to the human brainstem where DIPG originates. The H3K27 + MYC co-expression model successfully mimicked DIPG tumor characteristics, including glial invasion, tissue specificity, and the pediatric nature of the disease.

Drosophila melanogaster is advantageous for research due to its rich array of genetic tools, and genetic homology with humans; approximately 60% of Drosophila genes are conserved in humans, and around 75% of genes associated with human diseases have functional orthologues in fruit flies.[92] Furthermore, their short generation time, low maintenance costs, and ease of handling enhance their suitability for exploring relevant pathways in DIPG pathology. Despite these advantages, notable differences in brain structure and complexity between Drosophila and humans create challenges for accurately modeling DIPG. The absence of a comparable BBB in Drosophila limits its utility in investigating drug penetration and its effects on brain tumors. Furthermore, Drosophila rely exclusively on an innate immune response, lacking the adaptive mechanisms present in mammals. As a result, they are not well suited for studying the complex immune responses crucial for immunotherapy research.

3.4 | Caenorhabditis elegans

Though not traditionally seen as a model organism for cancer research, the nematode Caenorhabditis elegans offers several advantages. In 2019, Delaney et al. developed a C. elegans model featuring the H3.3K27M mutation associated with DIPG. They demonstrated that H3.3K27M-induced chromatin changes lead to ectopic DNA replication and cell cycle progression in germ cells through misregulation of the JNK pathway. Importantly, inhibiting JNK suppressed abnormal replication in both C. elegans and human H3.3K27M tumor cells.[93] This research highlights C. elegans as a valuable model for identifying drug targets for H3.3K27M-driven tumors and underscores its potential for phenotype-based drug screening.

C. elegans features a fully sequenced genome, presents no ethical concerns, enables rapid experimental turnover, and is cost-effective. However, its simplistic biology, lack of complex brain structures, and limited immune system pose significant limitations. While C. elegans is useful for identifying potential drug targets and fundamental biological mechanisms, findings from this model require further validation in more complex organisms, such as mice, before being applicable to humans.

3.5 | Higher-order animals

Higher-order animal models for DIPG have not been developed. However, spontaneous brainstem tumors have been reported in dogs, with tumors located in the pons or medulla oblongata associated with a poor prognosis. Survival following diagnosis with palliative treatment is typically <2 months.[94–96] Molecular analyses of canine high-grade gliomas have revealed shared driver alterations with human pediatric gliomas, including mutations in PDGFRA, PIK3CA, and ACVR1, as well as high levels of aneuploidy, particularly in regions syntenic to human loci carrying HIST1 and PDGFRA.[97]

Similarly, brainstem tumors have also been observed in cats. Among 137 cases of feline brain tumors, one glioma was identified in the pons, mirroring its rarity in humans.[98,99] A more recent pathological study further characterized feline gliomas, identifying two tumors within the brainstem region. One was classified as a Grade II oligoastrocytoma, and the other as a Grade IV GBM, based on WHO 2007 CNS criteria and immunohistochemical staining for markers including GFAP, Olig2, and Ki-67. While the GBM case shares some anatomical and histological features with DIPG, there is no information available on H3K27M mutation status or diffuse growth pattern, precluding any definitive parallels.[100]

4 | NON-ANIMAL DIPG MODELS

Non-animal preclinical models are gaining increasing importance as regulatory guidelines for animal research become more stringent, driving a growing trend to reduce animal experiments. Recent advancements in non-animal models for DIPG research have centered on the development of 3D brain organoids and co-culture systems, offering a more accurate representation of the human brain microenvironment than traditional in vitro models. In 2024, Prior V. et al. developed co-culture systems that combine DIPG spheroids with stem-cell-derived

brain organoids, enhancing the ability to mimic the tumor microenvironment more effectively.[101] These models revealed changes in tumor cell adhesion, DNA synthesis, and dendritic growth signaling when DIPG cells interact with organoids. Another notable in vitro study focused on the BBB in DIPG aimed to develop a human blood–brain tumor barrier (BBTB) model to investigate how DIPG affects brain capillary endothelial cells (ECs). This study adapted a human BBB model, composed of ECs, pericytes, and astrocytes, by replacing the astrocytes with DIPG cells. Results showed that the BBTB's integrity remained intact, and DIPG had minimal impact on drug transport properties and efflux transporter activity.[102] In parallel, major progress has been made in developing immune-competent brain organoids. These organoids incorporate microglia, addressing the absence of an immune component in earlier models.[103] Building on this, a neuroimmune-competent brain organoid model has been developed by fusing microglia-containing brain organoids (MiCBOs) with H3K27M-mutant DIPG spheroids, creating the MiCBO-tumor fusion (MiCBO-TF) model. This system faithfully reproduced the diffuse infiltration pattern of DIPG and revealed dynamic microglia-tumor interactions.[104] By incorporating key elements of the in vivo environment, these models address the limitations of previous preclinical approaches. However, organoid models remain technically challenging, time-consuming, and lack the full stromal component. Despite these limitations, organoids hold great promise for advancing our understanding of DIPG pathogenesis and identifying more effective therapies.[101]

Lab-on-a-chip (LOC) systems have emerged as innovative tools for cancer modeling and therapy testing, offering the ability to replicate complex tumor microenvironments. These platforms have been developed for various cancer types, including GBM, melanoma, breast, lung, prostate, colorectal, pancreatic, and ovarian cancers.[105–107] LOC systems enable the co-culture of cancer cells with non-malignant cells, the incorporation of extracellular matrix components, and the simulation of physiological gradients.[107,108] In brain cancer research, a recent tumor-on-a-chip model incorporated a 3D BBB, allowing for the assessment of drug delivery and selectivity.[109] The integration of artificial intelligence with cancer-on-a-chip platforms may enhance predictive drug screening models in the future,[108] potentially revolutionizing cancer research and personalized medicine approaches. While LOC systems have not yet been applied to DIPG, they hold significant potential as an innovative approach.

5 | CONCLUSION

In recent years, preclinical research on DIPG has made notable strides, progressing from GBM-based models to specialized systems that better capture DIPG's unique biology. Increased access to patient-derived tumor samples and the development of sophisticated modeling techniques have enhanced our capacity to replicate key aspects of DIPG. Additionally, non-traditional models, such as chick-embryo CAM assays, zebrafish, and organoids, offer valuable insights, especially for drug testing.

Despite this progress, current models still struggle to fully mimic DIPG's complex biology, especially the tumor microenvironment, immune interactions, and blood–brain barrier. PDX and GEMMs remain pivotal, yet they lack critical components; PDX models lack a functional immune system, while GEMMs lack a human tumor. Humanized mouse models offer exciting potential by incorporating both human tumor and immune components, bringing us closer to accurately replicating human DIPG. Non-mammalian models offer faster, cost-effective alternatives, but they often lack anatomical and physiological relevance.

To date, no single model can entirely capture the complexity of DIPG. Therefore, we believe that an integrative approach based on the combination of several models represents the best strategy for enhancing our understanding of the disease, and ultimately developing a cure for DIPG patients.

AUTHOR CONTRIBUTIONS

Bieke Van den Ende: Conceptualization; writing – original draft; writing – review and editing; visualization. **Frederik de Smet:** Writing – review and editing. **Sandra Jacobs:** Writing – review and editing. **An Coosemans:** Writing – review and editing; supervision; funding acquisition; conceptualization. **Matteo Riva:** Writing – review and editing; supervision.

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An Coosemans is a contracted researcher for Oncoinvent AS and Novocure and a consultant for Sotio a.s., Epics Therapeutics SA, and Molecular Partners. The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as potential competing interests.

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