TITLE

Deciphering the tumor microenvironment and role of immunotherapy in Diffuse Midline Glioma: a scoping review

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ABBREVIATION TABLE

ACT	Adoptive cell transfer
AMPA	α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid
APC	Antigen presenting cells
B7-H3	B7-homolog 3 protein
B7-H3	B7-Homolog 3 protein
B-ALL	B cell lineage acute lymphoblastic leukemia
BBB	Blood brain barrier
BDNF	Brain-derived neurotrophic factor
BMDM	Bone marrow-derived macrophages, CCR2+ populations
CAR-T	T-cells genetically modified to express chimeric antigen receptor
CED	Convection enhanced delivery
CNS	Central Nervous System
CRS	Cytokine release syndrome
CSF	Cerebrospinal fluid
CTLA-4	Cytotoxic T lymphocyte-associated antigen-4
DAM	Disease-associated myeloid
DC	Dendritic cell
DIPG/DMG	Diffuse Midline Glioma
EGFR	Epidermal growth factor receptor
EPSCs	Synaptic excitatory postsynaptic currents
FAK	Focal adhesion kinase
FUS	Focused ultrasound

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G/TAM	Glioma/tumor-associated microphage
GAM	Glioma-associated myeloid
GBM	Adult glioblastoma
GD2	Disialoganglioside GD2
GEMM	Genetically engineered mouse model
GRP78	Glioma mitogen 78 kDa glucose-regulated protein
H3.1K27M	Mutation in HIST1H3B/C
H3.3K27M	Mutation in H3F3A
HER2	ERBB2 receptor tyrosine kinase
HybISS	Hybridization-based in situ sequencing
ICANS	Immune effector cell-associated neurotoxicity syndrome
ICI	Immune checkpoint inhibitor
ICB	Immune checkpoint blockade
ICP	Intracranial pressure
ICV	Intracerebroventricular
IHC	Immunohistochemistry
IL	Interleukin
IMC	Imaging mass cytometry
IUE	In utero electroporation
IV	Intravenous
LDT	Laterodorsal tegmentum nucleus
MDM	Monocyte-derived macrophage
MMRD	Mismatch repair deficient
MSC	Mesenchymal stem cell
NLGN3	Neuroligin 3
oHSV	Oncolytic herpes virus
OPC	Oligodendrocyte precursor cells
OS	Overall Survival
OSM	Oncostatin M
OT/OT	On-tumor/off-target
OV	Oncolytic virus
PD-1	Programmed cell death protein-1
PD-L1	Programmed cell death ligand protein-1
PDX	Patient-derived xenograft
pHGG	Pediatric High Grade Glioma
PPN	Pedunculopontine nucleus
PRC2	Polycomb repressive complex 2
R/R	Relapsed/Refractory
Rb	Retinoblastoma tumor suppressor
RMAT	Regenerative Medicine Advanced Therapy
scRNA-seq	single-cell RNA sequencing
SIRPa	CD47-Signal retention protein alpha
TAA	Tumor-associated antigen
TCR	T cell receptor
TGF-β	Transforming growth factor β
TIAN	Tumor inflammation-related neurotoxicity

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TIM-3	T cell immunoglobulin and mucin domain-containing protein 3
TIS	Tumor inflammation signature
TMB	Tumor mutational burden
TME/TIME	Tumor (Immune) microenvironment
TSA	Tumor-specific antigen
TSP1/Thbs1	Tumor-derived thrombospondin 1
WHO	World Health Organization
WT	Wild type

ABSTRACT

Diffuse midline glioma, H3 K27-altered, formerly known as diffuse intrinsic pontine glioma, (DIPG/DMG) is the most aggressive form of pediatric brain malignancy, with <10% 2-year overall survival after standard of care. The limited success of traditional immune checkpoint inhibitors in pediatric high-grade gliomas, including DMG, has highlighted the urgent need to re-examine the tumor's intrinsic and microenvironmental barriers to immunotherapy. Advances in molecular and spatial profiling have revealed the profound intratumoral heterogeneity, lineage plasticity, and complex immunosuppressive tumor microenvironment characteristic of DMG, which are shaped by diverse myeloid populations, neuronal integration, and spatially distinct tumor niches. These insights are informing the development of non-traditional immunotherapeutic approaches, including alternative checkpoint blockade, chimeric antigen receptor T cells, and viro-immunotherapy strategies, which aim to overcome DMG's unique immune escape mechanisms. We also outline key translational challenges and future directions necessary to accelerate progress, including the refinement of preclinical models, optimization of CNS-specific immunotherapy delivery, and the integration of patient-derived data into streamlined, collaborative clinical trial platforms.

KEYWORDS

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Diffuse midline glioma (DMG), Pediatric brain tumors, Immunotherapy, Tumor microenvironment, Cancer neuroscience

BRIEF KEY POINTS

- Immunotherapy for diffuse midline glioma faces unique challenges rooted in epigenetic dysregulation, immune evasion, and neural–tumor crosstalk.
- Novel immunotherapeutic strategies leverage spatial, molecular, and neural insights to overcome immune evasion.

INTRODUCTION

Tumors of the central nervous system (CNS) are the most common solid malignancy in children as well as the predominant pediatric cause of cancer-associated mortality. The most aggressive CNS neoplasms in children are classified as pediatric high-grade gliomas (pHGGs), which initiate from oligodendroglial lineage precursors in the developing CNS. Diffuse midline glioma (DMG) is a subclassification of pHGGs that originates along the midline structures of the brain

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(**Figure 1A**).[1, 2] When located in the pons, it is also known as diffuse intrinsic pontine glioma (DIPG). Clinicians may also still refer to a tumor as DIPG if it does not meet the molecular requirements to be DMG, or if they are not biopsied. DMGs account for approximately 20% of all pediatric CNS cancer diagnoses, which is nearly 350 new cases each year in the United States alone. The overall survival (OS) of patients diagnosed with DMG is extremely poor at 9-11 months with a two-year survival of less than 10%.[3, 4]

CLASSIFICATION, EPIGENETICS, AND MOLECULAR SUBTYPES OF DMG

The World Health Organization (WHO) classification and overall approach towards DMG has been revised several times in recent years as summarized in **Figure 2**. As of the 5th edition of the WHO's Classification of CNS Tumors in 2021, DMG is designated as "H3 K27-altered," (alteration at lysine 27 of histone 3) emphasizing that global hypomethylation of H3K27 is a hallmark feature of DMG.[5, 6] Mechanistically, this is driven by either recurring somatic mutations in H3 genes, including *HIST1H3B/C (*H3.1K27M) or H3F3A (H3.3K27M) in which the lysine 27 residue is substituted with methionine, or overexpression of the Enhancer of Zest Homologs Inhibitory Protein (EZHIP) in patients with H3 wildtype. These alterations drive oncogenic transcriptional programs for which few effective treatments currently exist. It is important to note that the H3.1 and H3.3 histone variants are mutually exclusive. H3.1K27M accounts for approximately 12–19% of DMG cases, with a median OS rate of 15 months, while 65% of cases are characterized as H3.3K27M, with a median OS of 9 months.[7] Wildtype H3 is identified in 10–15% of cases, with a median OS of 15 months.[8] **Table 1** summarizes the implications for immunotherapy response by subtype, with a more in-depth discussion later in this review.

A defining characteristic of DMG is its global hypomethylation. Driven by recurring somatic mutations in H3 genes or EZHIP overexpression, these alterations inhibit the catalysis of

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repressive epigenetic marks on K27 of histone H3 by the Polycomb repressive complex 2 (PRC2).[9-12] In normal tissues, a myriad of marks decorate K27 on histone H3. These include either acetyl (H3K27ac), monomethyl (H3K27me1), dimethyl (H3K27me2), or trimethyl (H3K27me3). While H3K27ac and H3K27me1 are associated with active transcription, the deposition of H3K27me2 and H3K27me3 are catalyzed by PRC2 and associated with transcriptional repression. It is important to highlight that H3K27M has been shown to lead to global disruptions of not only H3K27me3[9, 10, 13-16] but also DNA methylation[13, 16] and H3K9me3[17] as well as increased levels of marks associated with active transcription such as H3K4me3[18], H3K36me2[14, 19], and H3K27ac[2, 20] (**Figure 1B**). Interestingly, the H3K27M substitution impacts the methylation of not only the mutant allele but all H3 alleles.[9, 10, 12] Thus, even though only a fraction of total histone H3.1 and H3.3 harbor the K27M mutation, a global reduction of H3K27me2/H3K27me3 persists in each DMG cell.[10] This dominant negative phenotype of H3K27M makes it analogous to EZHIP overexpression where the global loss of transcriptional silencing is strongly associated with DMG gliomagenesis.

CHALLENGES FROM TARGETING GENOMIC ABERRATIONS AND AFFECTED PATHWAYS IN DMG

DMG has a low mutational rate compared to other cancers, but is frequently associated with mutations in genes involved in cell cycle regulation (*TP53*, *PPMID*), chromatin remodeling (*ATRX*), and growth factors (*ACVR1*).[21] Profiling of the common somatic mutations between the H3K27M mutant subtypes are summarized in **Figure 3**.[22] See **Supplemental Section 1** for a detailed discussion of subtype-specific co-mutations and evolutionary drivers (including H3 variant partnerships).

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DMG tumors exhibit high levels of both inter- and intra-tumoral clonal heterogeneity, with multiple somatic subclones co-existing in the spatial and temporal dimensions.[23, 24] High-throughput drug screening campaigns with molecular analysis have been employed to assess drug sensitivities in a plethora of DMG models, which aim to correlate genomic influences with potential treatment strategies.[25, 26] Subclones are crucial to tumorigenesis, enhancing proliferation, invasion, and oncogenic signaling, particularly in response to CNS-active therapies.[22] Thus, given the complexity of the clonal cancer genome, a one-size-fits-all targeted approach is likely destined for failure as tumors typically harbor multiple mutations with unclear significance, which may complicate the identification of oncogenic drivers.

Transplanting or co-culturing DMG subclones with less mobile colonies has been shown to augment invasiveness, which suggests a cooperative role in tumor cell dissemination.[24] While targeting specific subclone mutations may provide temporary benefits, the genomic diversity of subclones will ultimately limit the long-term efficacy of targeted approaches.[27] Furthermore, the tumor microenvironment (TME) and non-genomic factors such as corticosteroid therapy, growth factors, and stress hormones have been found to contribute to increased invasiveness and intertumoral heterogeneity, complicating the treatment efforts. Moving forward, it is critical that therapeutic regimens for DMG consider not only the characteristics of the tumor itself but also the microenvironment that encapsulates and protects it.

THE TUMOR (IMMUNE) MICROENVIRONMENT (TME/TIME)

While the heterogeneous nature of HGGs like DMGs has stagnated improvements in targeted therapies for decades, landmark studies on the TME of DMGs over the past couple of years (secondary to increased access to biopsy samples) have revolutionized our view and reinvigorated the field's interest in the potential for immunotherapies (**Figure 4**).

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Historically, the CNS has been described as immunologically privileged tissue containing little to no immune cell infiltration. However, this has been disproven by extensive evidence demonstrating immune cell transfer between the CNS and the periphery via the glymphatic system, a glia-based lymphatic drainage system, the choroid plexus, meninges, and perivascular spaces.[28-33] Studies have shown adaptive immune trafficking as well as peripheral immune cell-mediated inflammatory responses in the CNS that have been shown to even regulate social behaviors such as stress.[34-37] Amongst the many specialized immune cell populations in the CNS, microglia, specialized tissue-resident macrophages, are well-characterized to be the primary resident immune cell of the brain with critical functions such as supporting brain development,[38] neural circuit refinement,[39, 40] and immune surveillance[38, 41].

The midline structures of the brain and brainstem, as eloquent areas, are vital for coordinating motor functions like swallowing and essential bodily functions such as cardiac and respiratory control. Thus, it is critical to strike a balance between protection against pathogens and immune-mediated neuroinflammation.[42] For this reason, control of peritumoral inflammation with dexamethasone (corticosteroid) is commonly prescribed to children with DMG. However, dexamethasone has been shown to induce T-cell exhaustion (upregulation of Immune Checkpoint ligand CTLA-4), and thus has been suggested to exacerbate the immunologically cold-DMG TME.[42, 43]

Studies that have analyzed the TME of DMG have found a paucity of lymphocytes and chemokines compared to normal brain tissue.[44] Moreover, the expression of CD3 (T-cell specific marker) as well as expression of immune activating cytokines and chemokines in DMG is substantially lower than both adult glioblastoma and other pediatric gliomas.[45] Corroborating this finding, transcriptomic and immunohistochemistry analysis of autopsy samples from DMG

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patients have found that the majority of DMG tumors have little to no infiltrating CD8+ cells.[44, 46] Remarkably, IBA1 (a marker for macrophages/microglia) has been shown to be markedly upregulated in primary DMG tissue.[45] This has led many to speculate that these myeloid-derived cells and microglia may have a critical role in DMG immunosuppression, but have not been characterized until recently in the studies discussed below.

Recent Advancements from Single-cell, Spatial Analyses, and Preclinical Models

Single-cell and spatial analyses have been paramount in the characterization of the DMG TME. However, it is important to note these technologies were first employed to determine the tumor cell populations themselves. Though debated,[47] many studies support an early oligodendroglial precursor as the predominant cell of origin for DMG,[2, 48-55] including histologic analysis of the developing human brainstem identify pre-OPCs in the pons at the time and place where DIPG typically arises.[48] Super-enhancer profiling also indicates that OPC/pre-OPC chromatin states closely mirror DMG, compared to neural precursors,[2, 53] and single-cell RNA sequencing shows malignant DMG populations are dominated by proliferative OPC-like cells where H3K27M impedes terminal glial differentiation.[56, 57] A landmark multi-omic study of DMG throughout various age groups found a striking absence of neuronal-lineage tumor cells in DMG and proposed that K27M enforces a glial/OPC-like program irrespective of age or site.[55] Of note, previous work has suggested that these “glioma stem cells” play a fundamental role in the suppression of both the innate and adaptive immune system by immunosuppressive cytokine secretion.[58, 59]

The Filbin group observed that glioma-associated myeloid cells (GAMs) comprise the largest population of nonmalignant cells within DMG tumors[55], consistent with previous histological studies demonstrating GAMs as the predominant immune cell present in DMGs.[45] Though

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overall GAM proportions were consistent between tumors from adult and children, pediatric DMGs displayed higher rates for brain-resident microglia while adult DMGs were enriched with monocyte-derived macrophages. The authors highlight evidence that GAMs secrete ligands (such as OSM), contribute to the mesenchymal cell state in adult glioblastoma, and were also found to promote MES-like tumor cell states in H3-K27M DMG-associated GAMs as age increases.[60-62] This marked one of the first instances where microenvironmental factors were found to distinctly shape tumor cellular states in DMG, which has propelled further investigation into the role of the myeloid population in H3K27M tumors.

In 2024, a series of breakthrough papers shed light on the TME of DMG, specifically the myeloid-derived subpopulations, which has critical clinical implications for future immunotherapies. While we provide a summary below, please refer to our expanded discussion in **Supplemental Section 2** for a more thorough analysis.

Using scRNA-seq plus imaging mass cytometry, Andrade et al. reported DMGs contain myeloid-dominant infiltrates with modest T-cell presence, and K27M-derived microglia have immunosuppressive signatures and high immune checkpoint expression.[63] Thus, highlighting the potential role of myeloid cells in promoting the immunosuppressive TME. Serial engraftments in a syngeneic K27M model accelerated growth and progressively skewed the TME toward myeloid dominance. Moreover, while monotherapy targeting microglia (CSF1R) or anti-PD-1 was ineffective, combinatorial approaches increased $CD3^+$ infiltration and extended survival, implicating myeloid modulation as a prerequisite for T-cell recruitment.[63] Complementary work from Ross and colleagues show that H3.3K27M DMGs are enriched for disease-associated microglia/monocyte-derived macrophage (MDMs) that downregulate inflammatory signaling and facilitate immune escape. Similarly, they found that targeting this microglia state (dual CCR1/CCR5 inhibition) reduced myeloid recruitment, increased $CD8^+$

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infiltration, and prolonged survival to an extent comparable with radiotherapy, without altering circulating myeloid counts.[64] Collectively, these studies highlight the importance of co-targeting microglia/myeloid populations with traditional immunotherapeutic approaches (like anti-PD-1) to overcome the immunosuppressive DMG TME.

Despite the DMG TME being described as immunosuppressive, a large-scale immune-oncology profiling in a cohort of 1,382 total pediatric brain tumors demonstrated an unexpectedly high tumor inflammation signature (TIS) score in DMG relative to non-tumor brain and hemispheric HGG. Immunohistochemistry confirmed high expression of several immune checkpoints on a subset of DMG samples including those with currently available FDA-approved drugs (PD-1, PD-L1) and drugs under development (LAG3, TIGIT).[65] They also assessed the utility of TIS compared to tumor mutational burden (TMB) for its use as a biomarker for predicting immunotherapy treatment response. The best outcomes occurred in recurrent HGG patients with both high TIS and TMB, several achieving over three years of progression-free survival. This data suggests that a subset of DMG patients may be candidates for radiation- and chemotherapy-free regimens given their robust immunotherapy response.[65, 66] Stratification in the neoadjuvant setting, utilizing biomarkers such as TIS/TMB, holds tremendous promise to change how we view the DMG TME as an monolith entity.

Although large-scale transcriptomic datasets have transformed the field and are increasingly clinically relevant, recent spatial multi-omic analyses by Damodharan et al. caution against relying solely on RNA reads for TME profiling and target selection. Digital spatial profiling revealed limited RNA–protein concordance in DMG, with notably poor alignment for clinically relevant immune checkpoints (PD-L1, CTLA-4) and EGFR. These findings underscore the importance of incorporating proteomics into discovery pipelines and early-phase trial design.[67, 68]

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Collectively, these advances delineate a DMG ecosystem dominated by OPC-like tumor states and immunosuppressive myeloid programs, identify biomarker frameworks (TIS/TMB) that may enrich for immunotherapy benefit, and point to combination strategies that pair myeloid-targeting agents with checkpoint blockade to overcome resistance.[63-66]

DIPG/DMG INTEGRATION WITH NEURAL NETWORKS

Although the majority of studies in DMG focus on immune-resident population, few studies have suggested the critical role of neurons in DMG immunomodulation. Collectively, cancer neuroscience reframes DMG as a malignancy that is embedded within and sculpted by neural circuits, rather than a mass merely residing in the CNS. Building on developmental neurobiology, recent work from multiple groups shows that DMG co-opts activity-regulated programs to drive tumor intrinsic proliferation, invasion, and therapeutic resistance via secreted factors, bona fide synapses, neuromodulatory inputs, and coordinated immune crosstalk.[69, 70] This triangular interaction among neurons, glioma cells, and immune cells provides a mechanistic framework for why conventional therapies underperform and how circuit-level interventions might potentiate immunotherapies.[71, 72] See our expanded discussion in **Supplemental Section 3**.

During development, oligodendrocyte precursor cells (OPCs), a cell state closely linked to DMG, are regulated by neuronal activity,[73] and the landmark study by Venkatash et al. illuminated that tumors exploit this relationship.[74] They identified neuroligin-3 (NLGN3) as an activity-regulated, secreted factor that accelerates glioma growth. NLGN3 initiates a feed-forward loop in tumor cells, engaging PI3K–mTOR and elevating oncogenic programs (PDGFA, TTYH1) across multiple glioma subtypes, including DMG. Follow-up work discerned key mechanistic sources

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and consequences of NLGN3 in the glioma-context.[75] In particular, the sheddase ADAM10 was identified as the mediator of NLGN3 release. Further, ADAM10 inhibition was shown to reduce tumor burden in DIPG models, highlighting a tractable extracellular target and demonstrating neuronal paracrine signaling as a major mitogenic driver in DMG.

Beyond secreted signals, DMG cells were also found to electrically integrate into neural networks. Venkatesh and colleagues pioneered this discovery that tumor cells form excitatory neuron-to-glioma synapses mediated by calcium-permeable AMPA receptors, while also exhibiting prolonged, non-synaptic depolarizations driven by activity-dependent extracellular potassium that propagate through gap-junction-coupled glioma networks.[76] These inputs increase proliferation, and pharmacologic blockade of AMPA currents or gap-junction signaling significantly reduced tumor growth in vivo.[74, 76] Importantly, independent work in glioblastoma corroborated glutamatergic synapses to glioma cells, underscoring the generality of neuron–glioma electrochemical communication.[77]

As CNS regulation of cancer became increasingly appreciated, more complex questions, including adaptive plasticity, arose. In pediatric glioma models, Taylor and colleagues revealed neuronal firing induces an adaptive response in malignant synapses via a brain-derived neurotrophic factor (BDNF)–TrkB–CAMKII axis. DMG upregulates the BDNF receptor, TrkB, which allows for augmentation of excitatory currents, synapse number expansion, and growth acceleration. Genetic deletion or pharmacologic inhibition of TrkB weakens these malignant synapses and prolongs survival.[70] These results reveal that gliomas not only passively receive neuronal input but actively reinforce and expand malignant synapses through activity-regulated plasticity mechanisms. Analogous to learning and memory processes in healthy brain circuits, gliomas hijack the BDNF–TrkB–AMPAR axis to sustain excitatory signaling that fuels their proliferation.[70] The work highlights TrkB inhibition as a promising therapeutic strategy,

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particularly as clinically approved TRK inhibitors could be repurposed for non–NTRK fusion gliomas.

Interestingly, tumor-intrinsic epigenetic dysregulation further shapes how DMG integrates into neural circuits.[78] In H3.1K27M DMG, Zhang and colleagues elucidate that dysregulated epigenetic mechanisms increase neuron-to-glioma synapses in DMG, specifically the CDH2-FOSL1 axis. Genetic depletion of this axis reduces active chromatin marks, downregulates synaptic programs, and diminishes neuron-induced proliferation, independent of canonical regulators (NLGN3, BDNF, and TRKB).[78] Despite this epigenetic axis being highly targetable, different subtypes likely exhibit distinct epigenetic mechanisms to mediate these interactions, and thus future work is needed.

Most recently, studies have addressed a longstanding gap in the field by showing that DMG also exploits non-glutamatergic neurotransmission to its advantage. Secondary to NKCC1-mediated chloride accumulation, GABAergic inputs become depolarizing (growth-promoting) in DMG.[79] In preclinical models, enhancing GABA signaling with benzodiazepines increases tumor growth, whereas IDH-wild-type hemispheric HGGs show minimal depolarizing GABA currents, highlighting an important clinical consideration for DMG patients.[79, 80] In parallel, neuromodulatory brainstem circuits have been found to exert region-specific control. Cholinergic projections from the pedunculopontine nucleus promote pontine DMG growth, and laterodorsal tegmentum inputs stimulate thalamic disease via muscarinic receptors (CHRM1/CHRM3). In DMG xenografts, targeted antagonism abolished this proliferative response.[81]

In summary, DMG integration into synaptic networks is pervasive and robust. Increased expression of synaptic proteins and enhanced neuronal signaling cooperate to drive glioma proliferation in a durable positive feedback loop. Ongoing work seeks to uncover the role of

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immune cells in this crosstalk, and if immunotherapy can combine with circuit-level blockade (e.g., ADAM10, AMPA, gap junctions, NKCC1, muscarinic or TrkB inhibition) to dismantle the neuronal drive while remodeling the tumor immune milieu.[70, 75, 76, 79, 81, 82]

OPPORTUNITIES AND CLINICAL CHALLENGES WITH IMMUNOTHERAPIES IN DMG TO DATE

Immune Checkpoint Blockade

Immune Checkpoint Inhibitors (ICIs) function by enhancing anti-tumoral adaptive immunity by restoring cytotoxic T cell activity and reversing T-Cell exhaustion. Currently, the most common ICIs used clinically are antibodies against cytotoxic T lymphocyte-associated antigen-4 (CTLA-4), programmed cell death protein-1 (PD-1), or programmed cell death ligand protein-1 (PD-L1). These ICIs have had tremendous success in hematological cancers and other solid tumors, namely metastatic melanoma. Unfortunately, the preliminary use of ICIs in DMG has lacked efficacy.[83] A list of the current ICI clinical trials for the treatment of DMG is summarized in **Table 2**.

In a retrospective cohort analysis, Kline and colleagues reported no significant difference in outcomes between patients treated with PD-1 blockade combined with re-radiation versus re-radiation alone.[84] Nevertheless, new phase I and I/II clinical trials have been launched to evaluate the therapeutic potential of immune checkpoint inhibition in DMG. These trials include anti-PD1 therapies such as pembrolizumab (NCT02359565), pidilizumab (NCT01952769), and cemiplimab (NCT03690869), anti-PDL1 therapy with durvalumab (NCT02793466) and, most recently, a combination of anti-CTLA4 and anti-PD1 therapies with nivolumab and ipilimumab (NCT03130959), which did not improve survival in patients with newly diagnosed diffuse intrinsic pontine glioma following upfront irradiation relative to historical data.[42, 85] Though more

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recent evidence may imply a more complex relationship,[65] it was previously postulated that this lack of response was due to the lack of PD-L1 expression on the DMG cell surface as well as the absence of T cells in the tumor microenvironment.[86] As discussed in the previous section, combinatorial strategies to enhance immune checkpoint expression such as PD-L1, stimulate activating cytokines/chemokines, and target immunosuppressive myeloid populations in the DMG may be helpful in sensitizing tumors to ICI. In addition, recent attention has turned to less studied immune checkpoint pathways such as T cell immunoglobulin and mucin domain-containing protein 3 (TIM-3) and the CD47-signal retention protein alpha (SIRPa) pathways. These approaches may be more attractive in DMG since they also target (immunosuppressive) myeloid cells, which comprise the majority of the DMG TME.

In a recently published study, Ausejo-Mauleon and colleagues found that TIM-3 was highly expressed in both tumor cells and immune cells, namely microglia and macrophages, in DMG patients.[87] Further, they demonstrated TIM-3 blockade in immunocompetent DMG orthotopic models prolonged survival, with 50% of long-term survivors being disease-free and acquiring immunological memory. This pre-clinical data offers strong support for future clinical trials with an anti-TIM-3 antibody against DMG as monotherapy or combined with other proven immunologic treatments, such as oncolytic viruses or adoptive cell transfer.

Similarly, CD47, a well-studied anti-phagocytic ligand that is overexpressed in a variety of tumors,[88] binds SIRPa from macrophage/dendritic cells to decrease their phagocytic capacity and promote tumor-mediated immune evasion.[88] To counter this, Gholamin et al. developed a humanized antibody against CD47 that blocks this "don't eat me" signal. Using patient-derived xenograft models of pediatric brain tumors, the antibody effectively inhibited CD47, killed tumor cells, and extended the animals' survival without causing toxicity to normal tissues.[89] These promising preclinical results prompted a clinical trial (NCT05169944), which seeks to employ the

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anti-CD47 antibody, magrolimab, against recurrent or progressive malignant brain tumors in both adults and children. Although this study excluded DMG patients, we suggest future studies consider including DMG patients, potentially as an exploratory arm, given the disproportionally high expression of CD47 in the DMG TME.

Adoptive Cell Transfer

Adoptive cell transfer (ACT) is an immunotherapy treatment wherein immune cells are isolated from a patient, modified, expanded *ex vivo*, and transferred back into the patient. Currently, T-cells genetically modified to express a chimeric antigen receptor (CAR-T) are the most popular form of ACT due to their remarkable clinical response rates in the context of hematologic malignancies, and have become the first FDA-approved ACT for refractory/relapsed B cell lineage acute lymphoblastic leukemia (B-ALL).[90] Now, less than a decade later, six CAR-T therapies are commercially available for the treatment of various B cell lineage malignancies.[91] This work has paved the way for their application in solid tumors, though achieving meaningful survival benefits continues to be a challenge. Given the “cold” immunological features of DMG, CAR-T cell therapy is touted by many as being the most promising immunotherapeutic approach with at least 10 CAR-T cell clinical trials for DMG, active as of December 2025. An exhaustive list of the current ACT clinical trials for the treatment of DMG are summarized in **Table 3.**

A CAR, or chimeric antigen receptor, is a fusion protein that incorporates five main components: an antigen-binding domain (typically composed of an antibody-derived single-chain variable fragment, or ScFv), a flexible hinge domain, a transmembrane domain, costimulatory domains (such as 4-1BB or CD28), and an intracellular signaling domain derived from the CD3ζ subunit of the T cell receptor (TCR) complex.[92, 93] Variations to CAR designs to include auxiliary genes,

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such as cytokine receptors, are also typical for enhancing their antigen recognition capabilities as well as signaling efficacy.

Effective T cell engagement and resultant tumor destruction relies upon the recognition of tumor-associated antigens (TAA) that are highly expressed on cancer cell surfaces in an MHC-independent manner. This triggers effector T cell signaling pathways, leading to the release of cytotoxic molecules such as perforin and granzyme B, along with proinflammatory cytokines like IL-2, IL-6, IFN-γ, and TNF-α.[92] Thus, the selection of the TAA is critical to minimize on-tumor/off-target (OT/OT) toxicities. For example, an ideal TAA is homogeneously expressed on tumor cells but absent or minimally expressed in healthy tissues.[93] Beyond CAR-T cell design, factors such as T cell subset phenotypes, macrophage populations, and circulating cytokines and chemokines also influence therapeutic efficacy. While early results from CAR-T cell therapies in DMG patients have shown promise for longer-term survival, intratumoral heterogeneity and the TME remain the primary therapeutic challenges.

Epidermal growth factor receptor (EGFR) is a well-studied therapeutic target linked with tumor progression and upregulation of a tumor-specific variant (EGFRvIII) in DMG.[94, 95] Numerous trials evaluating EGFR CAR-T therapy against HGGs have displayed safety and feasibility with some clinical efficacy. Two Phase 1 trials (NCT02209376, NCT01454596) utilizing EGFRvIII CAR-T cells against adults with HGGs have demonstrated tolerable responses with IV and intracerebroventricular (ICV) delivery, respectively.[96, 97] Although there were no evidence of OT/OT toxicities, neither trial demonstrated a clinically meaningful effect in patients. Currently, a Phase I trial (BrainChild-02, NCT03638167) is evaluating a CAR-T targeting EGFR806 in pediatric HGG patients but is excluding pontine gliomas. Thus, future trials are needed to show the efficacy of EGFR-targeting CARs against the classic DMG/DIPG tumors.

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CAR-T cell trials (NCT03500991, NCT02442297) targeting ERBB2 receptor tyrosine kinase (HER2) for CNS tumors at progression in both pediatric and adult settings have demonstrated safety and tolerability.[98, 99] Between these two trials, systemic, tumor cavity, and ventricular system infusion delivery were evaluated. Infusions via a CNS catheter showed evidence of local CNS immune activation, including high concentrations of CXCL10 and CCL2 in the cerebrospinal fluid.[98] Whereas the trial with systemic administration found that of 16 evaluable patients (9 adults and 7 children), 1 had a partial response for more than 9 months, 7 had stable disease for 8 weeks to 29 months, and 8 progressed after T-cell infusion.[99]

Several CNS tumors, including DMG, highly express IL-13Rα2, which is targetable through a IL-13 cytokine-directed CAR.[92, 100] Interestingly, IL-13Rα2 is poorly expressed in healthy tissue, besides the testis, and thus has prompted clinical trials for DMG. Phase I clinical trials (NCT04510051) for pediatric brain tumor patients as well as (NCT02208362) for adults with glioblastoma are currently active. Although preliminary evidence supported the feasibility and tolerability of IL-13Rα2 directed CAR-Ts, disease recurrence occurred due to reduced IL-13Rα2 expression.[100, 101] We anticipate that combination treatment with ICIs may be the next step against DMG to parallel the Phase I trial (NCT04003649) for glioblastoma, which combines IL-13Rα2 CAR-Ts with the ICIs, nivolumab (PD1) and ipilimumab (CTLA4).

Recent Landmark Successes for CAR-Ts in DMG

Screening the cell surface of patient-derived DMG cells, Mount and colleagues revealed that the now popular CAR-T antigen, disialoganglioside GD2, is highly and uniformly expressed on H3K27M cells and GD2-targeting CAR-T therapy has remarkable efficacy in preclinical models.[102] Currently being investigated in four active clinical trials for DMG alone, GD2 has also been found to be expressed on several solid and CNS tumors while being minimally expressed

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in healthy tissue.[103] In 2020, a Phase 1 clinical trial (NCT04196413) began recruiting to evaluate intravenous infusion of autologous GD2 CAR-T cell therapy, with the option for repeated intracerebroventricular dosing, in young adult and pediatric patients with H3K27M-mutant diffuse midline glioma. Impressively, preliminary data demonstrates radiographic and clinical benefit in three out of four patients, including significant tumor reductions and neurological improvements. Of note, the single non-responsive patient still exhibited an immune response of elevated levels of immunosuppressive cytokines, such as transforming growth factor β (TGF-β), in the cerebrospinal fluid (CSF). Importantly, ICV dosing was associated with improved or stabilized responses, reduced cytokine release syndrome (CRS), enhanced proinflammatory cytokine and chemokine levels in the CNS, and a decrease in immunosuppressive myeloid cells in the CSF compared to IV infusions. Tumor inflammation-related neurotoxicity (TIAN), likely due to the tumor's midline location, caused transient neurological worsening in all four patients, with intratumoral edema contributing to temporary CSF obstruction and hydrocephalus, requiring intensive care management.

More recently, the final results of this phase I trial was presented by Monje et al. for NCT04196413, which assessed the feasibility and safety of intravenous and intracranial GD2-CAR-T cells for H3K27M+ diffuse midline gliomas.[104] Reported adverse events included CRS and TIAN, which were effectively managed through intensive monitoring and comprehensive supportive care. Notably, ICV administration was associated with reduced systemic toxicity, emphasizing its potential as a targeted approach for gliomas while minimizing systemic immune activation. Promising clinical outcomes were observed, including substantial tumor volume reductions in multiple patients, with one patient achieving a complete response that has persisted for over 30 months. Clinical neurological improvements were generally consistent with radiographic findings, although some discordance between imaging outcomes and clinical metrics was noted, highlighting the complexity of response assessment in DMGs. Key

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mechanistic insights demonstrated the prolonged persistence of GD2-CAR-T cells in CSF and peripheral blood for over 500 days in select patients. This persistence was coupled with reductions in tumor-specific DNA markers and correlated with elevated cytokine levels in both CSF and blood, further supporting evidence of robust, on-target antitumor activity.

In 2024, results were published from NCT04099797, a Phase 1 trial assessing modified GD2-directed CAR-T cells with a constitutively active interleukin (IL)-7 receptor (C7R-GD2.CARTs).[105] In an effort to improve expansion and cytotoxicity of CAR-T cells while avoiding toxicities associated with recombinant cytokines, this group at Texas Children's Cancer Center engineered a constitutively active C7R to safely augment antitumor activity of CAR-T cells against glioblastoma (GBM) xenografts.[106] Inspired by the early successes of the GD2 CAR-T against DMG, the authors combined their technology to assess the safety and efficacy of the C7R-GD2.CARTs against DMG and other recurrent GD2-expressing pediatric CNS tumors.[105] Among patients treated with C7R-GD2.CART cells, partial responses were reported in 2 out of 7 patients. 7 out of 8 developed grade 1 tumor inflammation–associated neurotoxicity (TIAN) and 6 out of 8 cytokine release syndrome (CRS), but all events were controlled with anti-cytokine agents. Of note, 7 out of 8 patients received multiple cycles and exhibited transient neurological and radiographic improvement with higher levels of granzyme B and interferon-γ expression in peripheral blood compared with patients treated with GD2.CART cells without C7R.

Despite the encouraging advancements of Monje et al., significant challenges remain. The limited sample size of the study and single-institution design constrain the generalizability of its findings, and the exclusion of patients with bulky thalamic or cerebellar disease further narrows its applicability. Moreover, the immunosuppressive tumor microenvironment continues to impede durable responses, as evidenced by elevated TGF-β levels in non-responders. Mechanisms of

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resistance, including limited persistence and immune suppressive mechanisms warrant further investigation to optimize therapeutic durability.

In late 2024, the FDA designated GD2-CAR-T-cell therapy for DMG as a *Regenerative Medicine Advanced Therapy (RMAT)*, underscoring its potential transformative impact that reflects the agency's assessment that early clinical evidence demonstrates substantial improvement over existing therapies for DMG and its development should be accelerated. Future directions should refine delivery strategies, such as ICV-only administration, while addressing the logistical complexities of repeated infusions. Combinatorial approaches incorporating immune checkpoint inhibitors or agents targeting the tumor microenvironment may offer synergistic enhancements to CAR-T-cell efficacy. These early results suggest promise for continued targeting of optimized GD2 CAR-Ts in DMG and other cancers as we await more data from multiple ongoing clinical trials (i.e. NCT05298995, NCT05544526).

B7-homolog 3 protein (B7-H3), or CD276, has also been found to be upregulated in multiple cancers, including DMG.[93, 107] An open, single-site, Phase 1 trial (NCT04185038) is evaluating B7-H3 CAR-T cells in pediatric CNS tumors including DMGs. ICV administration was repeated as outpatients with an alternate week dosing regimen for a total of 40 doses for the first three patients.[108] Preliminary results pointed to an increased immune engagement by cytokine evaluation in serum and CSF, CAR-T persistence in the CNS, and ICV tolerability.

In early 2025, the final results of this first-in-human phase 1 trial were published.[109] Twenty-three pediatric and young adult patients were enrolled, with 21 receiving multiple ICV infusions without prior lymphodepletion. The therapy was well-tolerated overall. Primarily grade 1–2 adverse events were documented, and only one dose-limiting toxicity was observed. Pharmacodynamic analyses demonstrated local CAR-T cell expansion, cytokine induction, and

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evidence of immune activation in the cerebrospinal fluid. Median overall survival from diagnosis was 19.8 months, notably longer than the historical median of ~11 months, with several patients surviving beyond 30–44 months. Given these encouraging results, a multisite phase 2 trial is planned to more rigorously assess clinical efficacy.

In recognition of these promising outcomes, the FDA granted the B7-H3-targeted CAR-T cell product (SCRI-CARB7-H3v1) both *Breakthrough Therapy Designation* in April 2025 and *RMAT Designation* later that month. Collectively, they mark a major regulatory milestone and underscore the therapeutic potential of ICV-delivered CAR-T cells in DMG, as further preclinical efforts continue to refine CAR design, enhance CNS trafficking, and explore combinatorial immunomodulatory approaches.

Although B7-H3 CAR-T therapy has demonstrated remarkable early phase success, therapeutic resistance and incomplete responses remain major challenges, which include tumor heterogeneity and antigen escape. These limitations underscore the need for multi-antigen approaches that can sustain antitumor efficacy across diverse tumor subclones. Thus, this Seattle-based research group has recently initiated a first-in-human Phase I CAR-Trial (BrainChild-04, NCT05768880) for children and young adults with DMG as well as R/R CNS tumors, targeting four TAAs: B7-H3, HER2, EGFR806, and IL-13-Rα2. Patients will receive a heterogenous CAR-T population expressing four distinct CARs by different subsets to address challenges associated with tumor heterogeneity. This “Quad CAR-T” approach holds tremendous promise to reduce the likelihood of immune evasion and broaden tumor coverage within heterogeneous DMG lesions.

Overall, CAR-T cell therapy for DMG is widely regarded as one of the most encouraging avenues for a cure to DMG. Rapid progress is noticeable as clinical trials are refining

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approaches from a single bolus IV infusion to repeated, fractionated locoregional infusions of multi-antigen targeting CAR-Ts, which have demonstrable positive tolerability and anti-tumor profiles with effectiveness over antigen escape.[93] Challenges that lie ahead include the limited accessibility of living tumor samples for TAA phenotyping and treatment stratification and the financially demanding production costs.[93]

Vaccines

Tumor vaccines are a category of immunotherapy that induces a T-cell response to tumor-specific antigens (TSAs). Vaccine epitopes are typically conjugated to immunostimulatory biological adjuvants, which enhance the potency of the adaptive immune response. A list of the current vaccines in clinical trial for the treatment of DMG are summarized in **Table 4.**

Tumor vaccine design is multi-faceted and still evolving. It must address challenges such as inter- and intra-tumor heterogeneity, the classification of epitopes that influence the immune response, and key considerations for antigen selection, including their role in cellular survival and immunogenicity. Among the many promising antigen candidates for DMG, the H3K27M mutation is most notable given its inherent specificity. Preclinical studies of an H3.3K27M peptide vaccine demonstrated a robust T-cell-mediated immune response, leading to the initiation of a phase I clinical trial in combination with polyIC ± nivolumab (NCT02960230).[110] Preliminary evidence shows that the treatment is well tolerated, and 6 out of 18 patients developed an immune response.[111] Two other H3.3K27M peptide vaccine trials are currently ongoing in combination with IR (NCT04749641) and IR with the anti-PD-L1 antibody atezolizumab (NCT04808245). Results have yet to be posted.

Aside from peptide vaccines, novel vaccination modalities are increasingly receiving attention for high grade gliomas. Professional antigen presenting cells (APCs) are a critical player in

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tumor-specific vaccination with several current trials seeking to evaluate the efficacy of APCs that have been transfected with tumor antigens (i.e. whole tumor cells, peptide extract, or tumor-derived RNA). Since the discovery of H3.3K27M, a multitude of studies have investigated the use of H3.3K27M in APC vaccines.[8, 112-114] Moreover, RNA lipid particle-based immunotherapies are becoming heavily studied in glioblastoma, with the goal of developing a "universal" vaccine. That is, the vaccine is not meant to selectively target mutated cells of cancer but instead simply prompt a strong immune response.[115, 116]

Despite the clinical benefit of tumor vaccines remaining elusive to date, novel strategies remain an important pillar of immunotherapeutic development for DMG, especially as an adjuvant.

Oncolytic Viruses

Oncolytic viruses (OVs) are an emerging class of immunotherapies that promote selective tumor lysis and induction of anti-tumor immunity.[117] Some OVs that are derived from nature (Seneca Valley virus, Newcastle virus, and reovirus) have immediate anti-tumor effects, while most (oncolytic herpes virus, oncolytic adenovirus, and oncolytic measles virus) have been modified for specificity, immunogenicity, and safety.[118] Fundamentally, cancer cells are thought to be more sensitized to OV infection and viral replication, secondary to intrinsic abnormalities of cell signaling and anti-viral machinery.[119] OV-mediated cytolysis is a coordinated series of events that depend on viral entry, viral replication, and the secondary immune response to the virus.[118, 120] A list of the current OV clinical trials for the treatment of DMG are summarized in **Table 5.**

The exploitation of specific surface proteins is critical to enhancing OV entry and subsequent oncolysis. Nectin-1 (CD111) is significantly higher in pediatric brain tumor xenografts compared to adult glioblastoma, which prompted increased investigation into oncolytic herpes virus (oHSV) virotherapy.[121] CD111 is a receptor molecule vital to promoting oHSV entry and is used

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to predict sensitivity to herpes oncolytic therapy.[122] HSV-G207 is a genetically engineered HSV-1 mutant designed for cancer treatment.[123] It originated from HSV mutants such as HSV-1716 and HSV-R3616, which have both copies of the RL1 gene deleted. This gene encodes ICP34.5, a protein critical for the virus's neurovirulence and immune evasion by counteracting host defenses like PKR-mediated protein synthesis shutdown and autophagy. Without ICP34.5, these mutants are unable to replicate in most normal cells but retain selective replication in tumor cells, where antiviral responses are often impaired, triggering an oncolytic effect. To further enhance safety and tumor specificity, HSV-G207 was created by inserting the Escherichia coli lacZ gene into the virus's ICP6 gene, which encodes ribonucleotide reductase, thereby weakening the virus further while retaining its ability to target and destroy cancer cells. A phase I study with intratumorally delivered G207 as a treatment for pHGG (NCT02457845) reported tolerable safety and promising efficacy results with radiographic, neuropathological, and/or clinical responses in 11 out of 12 patients.[124] The median overall survival (OS) was extended from 5.6 months in historical controls to 12.2 receiving G207. In addition, there is currently a clinical trial utilizing oHSV G207 in recurrent pediatric cerebellar brain tumors.[125] Of note, in any clinical trial, no patient, pediatric or adult, treated with G207 has suffered from virus-associated neurotoxicity such as encephalitis.

Oncolytic adenoviruses have been genetically altered to selectively infect tumor cells by targeting oncogenic mutations such as those in the retinoblastoma tumor suppressor (Rb) signaling pathway, which is present in most gliomas.[126, 127] For example, Fueyo and colleagues engineered a tumor-selective adenovirus by modifying the virus's early region 1A (E1A) protein, which in wild-type (WT) adenovirus binds to the Rb protein and release E2F-family transcription factors, thereby promoting cell cycle progression and transcriptional activation.[127] By deleting the Rb binding domain in the viral E1A protein, the virus now selectively infects and kills cancer cells, demonstrating *in vitro* and *in vivo* cytolytic activity. Of note, 59% of pHGG cell lines contain

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Rb mutations, and thus this strategy has garnered attention for use in DMG.[8] DNX-2401 is a modified oncolytic adenovirus that contains an integrin-binding RGD-4C motif to enhance glioma tropism. A phase I clinical trial for recurrent malignant adult glioma using DNX-2401 (NCT00805376) found a 3-year survival of 20%.[128] In addition to radiographic signs of inflammation, histopathologic examination of immune markers in post-treatment specimens showed intratumoral T cell infiltration and TIM3 downregulation after treatment. These promising results, in addition to preclinical data specific to DMG, have motivated a phase I/II clinical trial (NCT03178032).[129, 130] Intratumoral infusion of DNX-2401, followed by radiotherapy in pediatric patients with DMG, led to tumor reduction in 9 out of 12 patients as well as increased infiltrative CD8+/CD4+ T-cells and absent regulatory T cells in the one patient with available tissue from repeat biopsy/autopsy. Impressively, the median overall survival was 17.8 months compared to the 8-12 months of historical DIPG patients treated with radiation therapy alone.[4, 131] Nevertheless, the small sample size (n=12) and the lack of a randomized design preclude any conclusion regarding survival, but do provide a rationale for a larger trial even in light of some serious adverse events (n=3).

The efficacy of OV-mediated immunotherapy for brain tumors are heavily dependent on selective delivery. Mesenchymal stem cells (MSCs) can cross the BBB and localize to areas of brain tumor even with systemic administration.[132] This homing capacity makes them excellent vectors of anticancer therapeutics. Promising preclinical data from GBM models showed that MSCs carrying a virus delayed its elimination by the host immune system and provided neuroprotection to normal peritumoral tissue[133] and thus, led to a phase I clinical trial investigating the safety of OV-carrying MSCs for GBM (NCT03072134). Since then, encouraging preclinical findings have also emerged using DMG patient-derived xenografts (PDX) and intranasal delivery.[134] Carceller and colleagues administered Celyvir, an autologous mesenchymal stem cell infected with ICOVIR-5 (an oncolytic adenovirus that selectively

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replicates in cancer cells) by means of superselective intra-arterial delivery, in a 9 year old patient diagnosed with DMG.[135] They reported impressive tolerance to the 2 administrations with no acute or delayed adverse effect, which underscores the feasibility of this technique for the delivery of virotherapies and/or cellular therapies in midline regions. This experimental approach is particularly inspiring for the development of less invasive methods of CNS delivery.

Controlling Toxicities

A vital aspect of achieving success with immunotherapies in pHGGs is inducing a localized inflammatory response.[136] That said, inflammatory responses and toxicities from acute therapy can be harmful and potentially life-threatening especially in compact, eloquent areas such as the brainstem where immune-related edema is not well-tolerated. Toxicities are an important consideration with all immunotherapy, but have rarely been reported for DMG tumors.[93] Cytokine release syndrome (CRS) is a potentially lethal toxicity from immunotherapies such as CAR-T treatments, and describes a systemic inflammatory episode resulting in fever, rigors, and even organ failure.[93, 137] It is typically myeloid-mediated through the production of pro-inflammatory cytokines or nitric oxide.[137] Of note, almost 90% of patients administered IV CAR-T therapy experience some degree of CRS, and nearly 30-50% of patients may develop more severe symptoms.[93, 137] Immune effector cell-associated neurotoxicity syndrome (ICANS) is another toxicity documented in several immunotherapy approaches, and is characterized by an array of neurological symptoms such as headache, tremors, and aphasia as well as increased intracranial pressure, hemorrhage, and coma.[93, 137, 138] Notably, ICANS has not been reported frequently in DMG CAR-T cell trials.

Lastly, tumor inflammation-associated neurotoxicity (TIAN) is a newly termed syndrome experienced by patients with CNS malignancy, primarily after immunotherapy treatment. Importantly, TIAN is relevant not only to cellular therapies but to all therapies that seek to

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stimulate the immune system of the central nervous system (CNS).[139] As opposed to the generalized and diffuse cerebral edema of severe ICANS, TIAN involves localized inflammation at the tumor site but extends beyond the concept of pseudoprogression, and can lead to significant edema that poses risks such as neuronal dysfunction, increased intracranial pressure (ICP), tissue herniation, and obstruction of cerebrospinal fluid (CSF) flow.[93, 139] While Type 1 TIAN is used to describe neurological symptoms secondary to mechanical factors, Type 2 is due to primary local neural dysfunction and can even occur in the absence of edema.

Given the increasing application of immunotherapies in DMG, the establishment of distinct subtypes as well as a standardized clinical grading scale for TIAN has become crucial. Unlike CRS and ICANS, the TIAN grading framework accounts for localized, on-target, and anatomy-dependent manifestations such as peritumoral edema, elevated intracranial pressure, and region-specific neural dysfunction. This targeted classification provides a more practical and clinically relevant approach for evaluating CNS-directed immunotherapy toxicities, which facilitates early recognition, guides intervention, and enables consistent comparison across studies.

Improving Delivery

Despite significant progress in preclinical research and clinical trials on targeted, immunotherapeutic agents, biological barriers within the brain and its tumor microenvironment remain a critical challenge to overcome for improving patient outcomes. In neuro-oncology, the BBB is often termed as the blood-brain tumor barrier (BBTB). Typically, brain tumors are described to disrupt the anatomy as well as function of the BBB and provide openings for drug delivery. Several studies to date have characterized the BBTB of DMG to have a relatively intact BBB that renders systemic drug delivery more challenging than other brain cancers.[140-142] Thus, it is critical that novel delivery technologies are explored and optimized to bypass or disrupt the

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DMG BBTB. Over the past five years, these have primarily included Ommaya reservoirs, convection enhanced delivery (CED), and focused ultrasound (FUS).[143]

Ommaya reservoirs are dome-shaped silicone reservoirs that are surgically implanted under the scalp, and connects to an indwelling catheter positioned in the ventricles of the brain. While Ommaya reservoirs have been a valuable neurosurgical device for decades, it has recently emerged as a method to administer immunotherapy agents directly into the ventricular CSF spaces for DMG patients. Promisingly, its ability to bypass the BBTB allows for higher drug concentrations to reach the target site, and has been found to decrease systemic toxicities in addition to improving anti-tumor responses (NCT04185038, NCT04196413, NCT04099797, and NCT04196413).[104, 143] Similar to an Ommaya reservoir, CED is a neurosurgical technique where one or more small catheters are stereotactically placed within the ventricle/CSF spaces or near a tumor to directly administer agents including immunotherapies.[144, 145] While also invasive, CED offers significant advantages over systemic drug delivery and diffusion, where therapeutics passively permeate tissues, CED utilizes convection to create a local hydrostatic pressure gradient that enables more uniform and widespread drug distribution. Though CED has been routinely proven safe for most agents, its efficacy has been impeded by the multitude of parameters that require optimization, including infusion flow rate, distribution area, and number of infusions.[146] For example, a phase I trial with CED of IL13-Pseudomonas toxin in children with DIPG/DMG (NCT00088061) found the treatment to be safe with no radiographic evidence of acute or long-term treatment toxicity, but only 2 out of 5 patients had a transient arrest of disease progression.[147] FUS is a minimally invasive drug delivery technique that uses intravenously administered microbubbles and targeted ultrasound energy to transiently disrupt the BBB. The oscillation of circulating microbubbles under ultrasound induces mechanical stress on the vascular endothelium, leading to temporary tight junction opening, decreased efflux transporter activity, and increased vesicular transcytosis. This results in a localized and

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reversible increase in BBB permeability, with an increase in convective transport within the tumor interstitium. Although not tested yet for immunotherapies in clinical trials of DMG, FUS has shown promise for delivering large molecules such as antibodies and viruses as well as immunomodulatory compounds/proteins.[148-151] For a more detailed discussion on any of these delivery modalities, refer to Arms et al.[143]

FUTURE DIRECTIONS

DMG carries one of the most devastating prognoses among CNS diseases, and although immunotherapy remains the most promising modality for achieving durable responses, numerous obstacles complicate progress toward improved outcomes (Figure 4). We propose a three-tiered strategy to overcome the DMG TME in order to sensitize the tumor to immunotherapy (Figure 5). This framework includes immunogenic priming, bypassing the BBB, and combining therapeutic modalities. Immunogenic priming of the DMG TME may involve a range of approaches, including standard of care IR and TLR agonists. BBB bypass can be achieved through neurosurgical interventions such as an Ommaya reservoir or CED, or noninvasively through FUS. Although combining immunotherapies is conceptually appealing, results to date have been complex.[152] Unreliable TME biomarkers and pronounced tumor heterogeneity further limit the development of well-informed combinations. Despite the large number of new combinatorial trials initiated for DMG each year, serial sampling approaches that generate hypothesis driven biomarkers will facilitate more rational combinations with respect to modality, timing, and scheduling.

Another critical obstacle that DMG research continues to face before clinical translation is the development of robust preclinical models.[153] Although patient derived cell line models have been

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instrumental in elucidating key molecular attributes of H3K27M+ DMG cells, their major limitation lies in the inability to accurately study interactions with other cellular components of the brain tumor microenvironment. Moreover, xenografts derived from these models are typically immunodeficient, require orthotopic engraftment (which may artificially disrupt the BBB), and do not faithfully recapitulate the human TME. Consequently, the field has increasingly adopted genetically engineered mouse models (GEMMs), either traditional systems with modification of oncogenes or in utero electroporation (IUE), that provide opportunities to study immune based therapies. Additional efforts include the establishment of DMG organoid models and co-culture systems that more reliably reproduce the TME, as well as the development of DMG models in larger species.

Recent studies that have characterized these immunocompetent GEMMs, together with analyses of patient samples, indicate that the DMG TME is less uniform than previously appreciated and that some subtypes may not be immunologically cold. Importantly, emerging work is defining how different oncohistones (H3.1 or H3.3 K27M) influence the TME in both models and patient tissue. A deeper understanding of how the mutational profile of DMG shapes its TME is essential. For example, specific mutations may correlate with tumor inflammation signatures and could be leveraged as biomarkers to improve responses to ICI treatment.

With encouraging data from trials and accompanying accelerated approval designations from the FDA, optimism surrounding immunotherapies for DMG is at an all-time high. As additional later phase clinical trial data emerge, they will likely inform refinements in standard of care and help define synergistic combinations among immunotherapy modalities.

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References:

1. Weller M, Wen PY, Chang SM, Dirven L, Lim M, Monje M, Reifenberger G. Glioma. Nature Reviews Disease Primers. 2024;10(1):33. doi: 10.1038/s41572-024-00516-y.

2. Nagaraja S, Vitanza NA, Woo PJ, Taylor KR, Liu F, Zhang L, Li M, Meng W, Ponnuswami A, Sun W, Ma J, Hulleman E, Swigut T, Wysocka J, Tang Y, Monje M. Transcriptional Dependencies in Diffuse Intrinsic Pontine Glioma. Cancer Cell. 2017;31(5):635-52.e6. doi: 10.1016/j.ccell.2017.03.011.

3. Pachocki CJ, Hol EM. Current perspectives on diffuse midline glioma and a different role for the immune microenvironment compared to glioblastoma. J Neuroinflammation. 2022;19(1):276. Epub 20221119. doi: 10.1186/s12974-022-02630-8. PubMed PMID: 36403059; PMCID: PMC9675250.

4. Hoffman LM, Veldhuijzen van Zanten SEM, Colditz N, Baugh J, Chaney B, Hoffmann M, Lane A, Fuller C, Miles L, Hawkins C, Bartels U, Bouffet E, Goldman S, Leary S, Foreman NK, Packer R, Warren KE, Broniscer A, Kieran MW, Minturn J, Comito M, Broxson E, Shih CS, Khatua S, Chintagumpala M, Carret AS, Escorza NY, Hassall T, Ziegler DS, Gottardo N, Dholaria H, Doughman R, Benesch M, Drissi R, Nazarian J, Jabado N, Boddaert N, Varlet P, Giraud G, Castel D, Puget S, Jones C, Hulleman E, Modena P, Giagnacovo M, Antonelli M, Pietsch T, Gielen GH, Jones DTW, Sturm D, Pfister SM, Gerber NU, Grotzer MA, Pfaff E, von Bueren AO, Hargrave D, Solanki GA, Jadrijevic Cvrlje F, Kaspers GJL, Vandertop WP, Grill J, Bailey S, Biassoni V, Massimino M, Calmon R, Sanchez E, Bison B, Warmuth-Metz M, Leach J, Jones B, van Vuurden DG, Kramm CM, Fouladi M. Clinical, Radiologic, Pathologic, and Molecular Characteristics of Long-Term Survivors of Diffuse Intrinsic Pontine Glioma (DIPG): A Collaborative Report From the International and European Society for Pediatric Oncology DIPG Registries. J Clin Oncol. 2018;36(19):1963-72. Epub 20180510. doi: 10.1200/jco.2017.75.9308. PubMed PMID: 29746225; PMCID: PMC6075859.

Downloaded from https://academic.oup.com/neuro-oncology/advance-article/doi/10.1093/neuonc/noag014/8455707 by guest on 04 April 2026

5. Louis DN, Perry A, Wesseling P, Brat DJ, Cree IA, Figarella-Branger D, Hawkins C, Ng HK, Pfister SM, Reifenberger G, Soffietti R, von Deimling A, Ellison DW. The 2021 WHO Classification of Tumors of the Central Nervous System: a summary. Neuro-Oncology. 2021;23(8):1231-51. doi: 10.1093/neuonc/noab106.

6. Perez A, Huse JT. The Evolving Classification of Diffuse Gliomas: World Health Organization Updates for 2021. Current Neurology and Neuroscience Reports. 2021;21(12):67. doi: 10.1007/s11910-021-01153-8.

7. Buczkowicz P, Hawkins C. Pathology, Molecular Genetics, and Epigenetics of Diffuse Intrinsic Pontine Glioma. Front Oncol. 2015;5:147. Epub 20150630. doi: 10.3389/fonc.2015.00147. PubMed PMID: 26175967; PMCID: PMC4485076.

8. Wu G, Diaz AK, Paugh BS, Rankin SL, Ju B, Li Y, Zhu X, Qu C, Chen X, Zhang J, Easton J, Edmonson M, Ma X, Lu C, Nagahawatte P, Hedlund E, Rusch M, Pounds S, Lin T, Onar-Thomas A, Huether R, Kriwacki R, Parker M, Gupta P, Becksfort J, Wei L, Mulder HL, Boggs K, Vadodaria B, Yergeau D, Russell JC, Ochoa K, Fulton RS, Fulton LL, Jones C, Boop FA, Broniscer A, Wetmore C, Gajjar A, Ding L, Mardis ER, Wilson RK, Taylor MR, Downing JR, Ellison DW, Zhang J, Baker SJ. The genomic landscape of diffuse intrinsic pontine glioma and pediatric non-brainstem high-grade glioma. Nat Genet. 2014;46(5):444-50. Epub 20140406. doi: 10.1038/ng.2938. PubMed PMID: 24705251; PMCID: PMC4056452.

9. Lee CH, Yu JR, Granat J, Saldaña-Meyer R, Andrade J, LeRoy G, Jin Y, Lund P, Stafford JM, Garcia BA, Ueberheide B, Reinberg D. Automethylation of PRC2 promotes H3K27 methylation and is impaired in H3K27M pediatric glioma. Genes Dev. 2019;33(19-20):1428-40. Epub 20190905. doi: 10.1101/gad.328773.119. PubMed PMID: 31488577; PMCID: PMC6771381.

10. Lewis PW, Müller MM, Koletsky MS, Cordero F, Lin S, Banaszynski LA, Garcia BA, Muir TW, Becher OJ, Allis CD. Inhibition of PRC2 activity by a gain-of-function H3 mutation found in

Downloaded from https://academic.oup.com/neuro-oncology/advance-article/doi/10.1093/neuonc/noag014/8455707 by guest on 04 April 2026

873 pediatric glioblastoma. Science. 2013;340(6134):857-61. Epub 20130328. doi:
874 10.1126/science.1232245. PubMed PMID: 23539183; PMCID: PMC3951439.
875 11. Silveira AB, Kasper LH, Fan Y, Jin H, Wu G, Shaw TI, Zhu X, Larson JD, Easton J, Shao
876 Y, Yergeau DA, Rosencrance C, Boggs K, Rusch MC, Ding L, Zhang J, Finkelstein D, Noyes
877 RM, Russell BL, Xu B, Broniscer A, Wetmore C, Pounds SB, Ellison DW, Zhang J, Baker SJ.
878 H3.3 K27M depletion increases differentiation and extends latency of diffuse intrinsic pontine
879 glioma growth in vivo. Acta Neuropathol. 2019;137(4):637-55. Epub 20190215. doi:
880 10.1007/s00401-019-01975-4. PubMed PMID: 30770999; PMCID: PMC6546611.
881 12. Chan KM, Fang D, Gan H, Hashizume R, Yu C, Schroeder M, Gupta N, Mueller S,
882 James CD, Jenkins R, Sarkaria J, Zhang Z. The histone H3.3K27M mutation in pediatric glioma
883 reprograms H3K27 methylation and gene expression. Genes Dev. 2013;27(9):985-90. Epub
884 20130419. doi: 10.1101/gad.217778.113. PubMed PMID: 23603901; PMCID: PMC3656328.
885 13. Krug B, De Jay N, Harutyunyan AS, Deshmukh S, Marchione DM, Guilhamon P,
886 Bertrand KC, Mikael LG, McConechy MK, Chen CCL, Khazaei S, Koncar RF, Agnihotri S, Faury
887 D, Ellezam B, Weil AG, Ursini-Siegel J, De Carvalho DD, Dirks PB, Lewis PW, Salomoni P,
888 Lupien M, Arrowsmith C, Lasko PF, Garcia BA, Kleinman CL, Jabado N, Mack SC. Pervasive
889 H3K27 Acetylation Leads to ERV Expression and a Therapeutic Vulnerability in H3K27M
890 Gliomas. Cancer Cell. 2019;35(5):782-97.e8. doi: 10.1016/j.ccell.2019.04.004. PubMed PMID:
891 31085178; PMCID: PMC6521975.
892 14. Stafford JM, Lee CH, Voigt P, Descostes N, Saldaña-Meyer R, Yu JR, Leroy G, Oksuz O,
893 Chapman JR, Suarez F, Modrek AS, Bayin NS, Placantonakis DG, Karajannis MA, Snuderl M,
894 Ueberheide B, Reinberg D. Multiple modes of PRC2 inhibition elicit global chromatin alterations
895 in H3K27M pediatric glioma. Sci Adv. 2018;4(10):eaau5935. Epub 20181031. doi:
896 10.1126/sciadv.aau5935. PubMed PMID: 30402543; PMCID: PMC6209383.
897 15. Harutyunyan AS, Chen H, Lu T, Horth C, Nikbakht H, Krug B, Russo C, Bareke E,
898 Marchione DM, Coradin M, Garcia BA, Jabado N, Majewski J. H3K27M in Gliomas Causes a

Downloaded from https://academic.oup.com/neuro-oncology/advance-article/doi/10.1093/neuonc/noag014/8455707 by guest on 04 April 2026

One-Step Decrease in H3K27 Methylation and Reduced Spreading within the Constraints of H3K36 Methylation. Cell Reports. 2020;33(7). doi: 10.1016/j.celrep.2020.108390.

16. Bender S, Tang Y, Lindroth Anders M, Hovestadt V, Jones David TW, Kool M, Zapatka M, Northcott Paul A, Sturm D, Wang W, Radlwimmer B, Højfeldt Jonas W, Truffaux N, Castel D, Schubert S, Ryzhova M, Şeker-Cin H, Gronych J, Johann Pascal D, Stark S, Meyer J, Milde T, Schuhmann M, Ebinger M, Monoranu C-M, Ponnuswami A, Chen S, Jones C, Witt O, Collins VP, von Deimling A, Jabado N, Puget S, Grill J, Helin K, Korshunov A, Lichter P, Monje M, Plass C, Cho Y-J, Pfister Stefan M. Reduced H3K27me3 and DNA Hypomethylation Are Major Drivers of Gene Expression in K27M Mutant Pediatric High-Grade Gliomas. Cancer Cell. 2013;24(5):660-72. doi: 10.1016/j.ccr.2013.10.006.

17. Xin DE, Liao Y, Rao R, Ogurek S, Sengupta S, Xin M, Bayat AE, Seibel WL, Graham RT, Koschmann C, Lu QR. Chaetocin-mediated SUV39H1 inhibition targets stemness and oncogenic networks of diffuse midline gliomas and synergizes with ONC201. Neuro-Oncology. 2023;26(4):735-48. doi: 10.1093/neuonc/noad222.

18. Larson JD, Kasper LH, Paugh BS, Jin H, Wu G, Kwon CH, Fan Y, Shaw TI, Silveira AB, Qu C, Xu R, Zhu X, Zhang J, Russell HR, Peters JL, Finkelstein D, Xu B, Lin T, Tinkle CL, Patay Z, Onar-Thomas A, Pounds SB, McKinnon PJ, Ellison DW, Zhang J, Baker SJ. Histone H3.3 K27M Accelerates Spontaneous Brainstem Glioma and Drives Restricted Changes in Bivalent Gene Expression. Cancer Cell. 2019;35(1):140-55.e7. Epub 20181227. doi: 10.1016/j.ccell.2018.11.015. PubMed PMID: 30595505; PMCID: PMC6570409.

19. Yu JR, LeRoy G, Bready D, Frenster JD, Saldaña-Meyer R, Jin Y, Descostes N, Stafford JM, Placantonakis DG, Reinberg D. The H3K36me2 writer-reader dependency in H3K27M-DIPG. Sci Adv. 2021;7(29). Epub 20210714. doi: 10.1126/sciadv.abg7444. PubMed PMID: 34261657; PMCID: PMC8279504.

20. Piunti A, Hashizume R, Morgan MA, Bartom ET, Horbinski CM, Marshall SA, Rendleman EJ, Ma Q, Takahashi YH, Woodfin AR, Misharin AV, Abshiru NA, Lulla RR, Saratsis AM, Kelleher

Downloaded from https://academic.oup.com/neuro-oncology/advance-article/doi/10.1093/neuonc/noag014/8455707 by guest on 04 April 2026

NL, James CD, Shilatifard A. Therapeutic targeting of polycomb and BET bromodomain proteins in diffuse intrinsic pontine gliomas. Nat Med. 2017;23(4):493-500. Epub 20170227. doi: 10.1038/nm.4296. PubMed PMID: 28263307; PMCID: PMC5667640.

21. Nikbakht H, Panditharatna E, Mikael LG, Li R, Gayden T, Osmond M, Ho C-Y, Kambhampati M, Hwang EI, Faury D, Siu A, Papillon-Cavanagh S, Bechet D, Ligon KL, Ellezam B, Ingram WJ, Stinson C, Moore AS, Warren KE, Karamchandani J, Packer RJ, Jabado N, Majewski J, Nazarian J. Spatial and temporal homogeneity of driver mutations in diffuse intrinsic pontine glioma. Nature Communications. 2016;7(1):11185. doi: 10.1038/ncomms11185.

22. Findlay IJ, De Iuliis GN, Duchatel RJ, Jackson ER, Vitanza NA, Cain JE, Waszak SM, Dun MD. Pharmaco-proteogenomic profiling of pediatric diffuse midline glioma to inform future treatment strategies. Oncogene. 2022;41(4):461-75. doi: 10.1038/s41388-021-02102-y.

23. Vinci M, Burford A, Molinari V, Kessler K, Popov S, Clarke M, Taylor KR, Pemberton HN, Lord CJ, Gutteridge A, Forshew T, Carvalho D, Marshall LV, Qin EY, Ingram WJ, Moore AS, Ng HK, Trabelsi S, H'Mida-Ben Brahim D, Entz-Werle N, Zacharoulis S, Vaidya S, Mandeville HC, Bridges LR, Martin AJ, Al-Sarraj S, Chandler C, Sunol M, Mora J, de Torres C, Cruz O, Carcaboso AM, Monje M, Mackay A, Jones C. Functional diversity and cooperativity between subclonal populations of pediatric glioblastoma and diffuse intrinsic pontine glioma cells. Nat Med. 2018;24(8):1204-15. Epub 20180702. doi: 10.1038/s41591-018-0086-7. PubMed PMID: 29967352; PMCID: PMC6086334.

24. Kluiver TA, Alieva M, van Vuurden DG, Wehrens EJ, Rios AC. Invaders Exposed: Understanding and Targeting Tumor Cell Invasion in Diffuse Intrinsic Pontine Glioma. Front Oncol. 2020;10:92. Epub 20200207. doi: 10.3389/fonc.2020.00092. PubMed PMID: 32117746; PMCID: PMC7020612.

25. Grasso CS, Tang Y, Truffaux N, Berlow NE, Liu L, Debily MA, Quist MJ, Davis LE, Huang EC, Woo PJ, Ponnuswami A, Chen S, Johung TB, Sun W, Kogiso M, Du Y, Qi L, Huang Y, Hütt-Cabezas M, Warren KE, Le Dret L, Meltzer PS, Mao H, Quezado M, van Vuurden DG, Abraham

Downloaded from https://academic.oup.com/neuro-oncology/advance-article/doi/10.1093/neuonc/noag014/8455707 by guest on 04 April 2026

J, Fouladi M, Svalina MN, Wang N, Hawkins C, Nazarian J, Alonso MM, Raabe EH, Hulleman E, Spellman PT, Li XN, Keller C, Pal R, Grill J, Monje M. Functionally defined therapeutic targets in diffuse intrinsic pontine glioma. Nat Med. 2015;21(6):555-9. Epub 20150504. doi: 10.1038/nm.3855. PubMed PMID: 25939062; PMCID: PMC4862411.

26. Lin GL, Wilson KM, Ceribelli M, Stanton BZ, Woo PJ, Kreimer S, Qin EY, Zhang X, Lennon J, Nagaraja S, Morris PJ, Quezada M, Gillespie SM, Duveau DY, Michalowski AM, Shinn P, Guha R, Ferrer M, Klumpp-Thomas C, Michael S, McKnight C, Minhas P, Itkin Z, Raabe EH, Chen L, Ghanem R, Geraghty AC, Ni L, Andreasson KI, Vitanza NA, Warren KE, Thomas CJ, Monje M. Therapeutic strategies for diffuse midline glioma from high-throughput combination drug screening. Sci Transl Med. 2019;11(519). doi: 10.1126/scitranslmed.aaw0064. PubMed PMID: 31748226; PMCID: PMC7132630.

27. Ma X, Edmonson M, Yergeau D, Muzny DM, Hampton OA, Rusch M, Song G, Easton J, Harvey RC, Wheeler DA, Ma J, Doddapaneni H, Vadodaria B, Wu G, Nagahawatte P, Carroll WL, Chen IM, Gastier-Foster JM, Relling MV, Smith MA, Devidas M, Guidry Auvil JM, Downing JR, Loh ML, Willman CL, Gerhard DS, Mullighan CG, Hunger SP, Zhang J. Rise and fall of subclones from diagnosis to relapse in pediatric B-acute lymphoblastic leukaemia. Nat Commun. 2015;6:6604. Epub 20150319. doi: 10.1038/ncomms7604. PubMed PMID: 25790293; PMCID: PMC4377644.

28. Ransohoff RM, Engelhardt B. The anatomical and cellular basis of immune surveillance in the central nervous system. Nat Rev Immunol. 2012;12(9):623-35. Epub 20120820. doi: 10.1038/nri3265. PubMed PMID: 22903150.

29. Sandrone S, Moreno-Zambrano D, Kipnis J, van Gijn J. A (delayed) history of the brain lymphatic system. Nature Medicine. 2019;25(4):538-40. doi: 10.1038/s41591-019-0417-3.

30. Iliff JJ, Wang M, Liao Y, Plogg BA, Peng W, Gundersen GA, Benveniste H, Vates GE, Deane R, Goldman SA, Nagelhus EA, Nedergaard M. A Paravascular Pathway Facilitates CSF Flow Through the Brain Parenchyma and the Clearance of Interstitial Solutes, Including Amyloid

Downloaded from https://academic.oup.com/neuro-oncology/advance-article/doi/10.1093/neuonc/noag014/8455707 by guest on 04 April 2026

977 β. Science Translational Medicine. 2012;4(147):147ra11-ra11. doi:
978 doi:10.1126/scitranslmed.3003748.
979 31. Louveau A, Smirnov I, Keyes TJ, Eccles JD, Rouhani SJ, Peske JD, Derecki NC, Castle
980 D, Mandell JW, Lee KS, Harris TH, Kipnis J. Structural and functional features of central
981 nervous system lymphatic vessels. Nature. 2015;523(7560):337-41. doi: 10.1038/nature14432.
982 32. Aspelund A, Antila S, Proulx ST, Karlsen TV, Karaman S, Detmar M, Wiig H, Alitalo K. A
983 dural lymphatic vascular system that drains brain interstitial fluid and macromolecules. Journal
984 of Experimental Medicine. 2015;212(7):991-9. doi: 10.1084/jem.20142290.
985 33. Louveau A, Herz J, Alme MN, Salvador AF, Dong MQ, Viar KE, Herod SG, Knopp J,
986 Setliff JC, Lupi AL, Da Mesquita S, Frost EL, Gaultier A, Harris TH, Cao R, Hu S, Lukens JR,
987 Smirnov I, Overall CC, Oliver G, Kipnis J. CNS lymphatic drainage and neuroinflammation are
988 regulated by meningeal lymphatic vasculature. Nature Neuroscience. 2018;21(10):1380-91. doi:
989 10.1038/s41593-018-0227-9.
990 34. Hickey WF, Hsu BL, Kimura H. T-lymphocyte entry into the central nervous system.
991 Journal of Neuroscience Research. 1991;28(2):254-60. doi:
992 https://doi.org/10.1002/jnr.490280213.
993 35. Hickey WF, Vass K, Lassmann H. Bone Marrow-derived Elements in the Central
994 Nervous System: An Immunohistochemical and Ultrastructural Survey of Rat Chimeras. Journal
995 of Neuropathology & Experimental Neurology. 1992;51(3):246-56. doi: 10.1097/00005072-
996 199205000-00002.
997 36. Shechter R, Miller O, Yovel G, Rosenzweig N, London A, Ruckh J, Kim KW, Klein E,
998 Kalchenko V, Bendel P, Lira SA, Jung S, Schwartz M. Recruitment of beneficial M2
999 macrophages to injured spinal cord is orchestrated by remote brain choroid plexus. Immunity.
1000 2013;38(3):555-69. Epub 20130307. doi: 10.1016/j.immuni.2013.02.012. PubMed PMID:
1001 23477737; PMCID: PMC4115271.

Downloaded from https://academic.oup.com/neuro-oncology/advance-article/doi/10.1093/neuonc/noag014/8455707 by guest on 04 April 2026

1002 37. Poller WC, Downey J, Mooslechner AA, Khan N, Li L, Chan CT, McAlpine CS, Xu C,
1003 Kahles F, He S, Janssen H, Mindur JE, Singh S, Kiss MG, Alonso-Herranz L, Iwamoto Y, Kohler
1004 RH, Wong LP, Chetal K, Russo SJ, Sadreyev RI, Weissleder R, Nahrendorf M, Frenette PS,
1005 Divangahi M, Swirski FK. Brain motor and fear circuits regulate leukocytes during acute stress.
1006 Nature. 2022;607(7919):578-84. Epub 20220530. doi: 10.1038/s41586-022-04890-z. PubMed
1007 PMID: 35636458; PMCID: PMC9798885.
1008 38. Santos EN, Fields RD. Regulation of myelination by microglia. Science Advances.
1009 2021;7(50):eabk1131. doi: doi:10.1126/sciadv.abk1131.
1010 39. Stevens B, Allen NJ, Vazquez LE, Howell GR, Christopherson KS, Nouri N, Micheva KD,
1011 Mehalow AK, Huberman AD, Stafford B, Sher A, Litke Alan M, Lambris JD, Smith SJ, John
1012 SWM, Barres BA. The Classical Complement Cascade Mediates CNS Synapse Elimination.
1013 Cell. 2007;131(6):1164-78. doi: 10.1016/j.cell.2007.10.036.
1014 40. Schafer Dorothy P, Lehrman Emily K, Kautzman Amanda G, Koyama R, Mardinly
1015 Alan R, Yamasaki R, Ransohoff Richard M, Greenberg Michael E, Barres Ben A, Stevens B.
1016 Microglia Sculpt Postnatal Neural Circuits in an Activity and Complement-Dependent Manner.
1017 Neuron. 2012;74(4):691-705. doi: 10.1016/j.neuron.2012.03.026.
1018 41. Nimmerjahn A, Kirchhoff F, Helmchen F. Resting Microglial Cells Are Highly Dynamic
1019 Surveillants of Brain Parenchyma in Vivo. Science. 2005;308(5726):1314-8. doi:
1020 doi:10.1126/science.1110647.
1021 42. Persson ML, Douglas AM, Alvaro F, Faridi P, Larsen MR, Alonso MM, Vitanza NA, Dun
1022 MD. The intrinsic and microenvironmental features of diffuse midline glioma: Implications for the
1023 development of effective immunotherapeutic treatment strategies. Neuro Oncol.
1024 2022;24(9):1408-22. doi: 10.1093/neuonc/noac117. PubMed PMID: 35481923; PMCID:
1025 PMC9435509.
1026 43. Giles AJ, Hutchinson MND, Sonnemann HM, Jung J, Fecci PE, Ratnam NM, Zhang W,
1027 Song H, Bailey R, Davis D, Reid CM, Park DM, Gilbert MR. Dexamethasone-induced

Downloaded from https://academic.oup.com/neuro-oncology/advance-article/doi/10.1093/neuonc/noag014/8455707 by guest on 04 April 2026

immunosuppression: mechanisms and implications for immunotherapy. J Immunother Cancer. 2018;6(1):51. Epub 20180611. doi: 10.1186/s40425-018-0371-5. PubMed PMID: 29891009; PMCID: PMC5996496.

44. Lieberman NAP, DeGolier K, Kovar HM, Davis A, Hoglund V, Stevens J, Winter C, Deutsch G, Furlan SN, Vitanza NA, Leary SES, Crane CA. Characterization of the immune microenvironment of diffuse intrinsic pontine glioma: implications for development of immunotherapy. Neuro Oncol. 2019;21(1):83-94. Epub 2018/09/01. doi: 10.1093/neuonc/noy145. PubMed PMID: 30169876; PMCID: PMC6303470.

45. Lin GL, Nagaraja S, Filbin MG, Suvà ML, Vogel H, Monje M. Non-inflammatory tumor microenvironment of diffuse intrinsic pontine glioma. Acta Neuropathol Commun. 2018;6(1):51. Epub 20180628. doi: 10.1186/s40478-018-0553-x. PubMed PMID: 29954445; PMCID: PMC6022714.

46. Jha P, Manjunath N, Singh J, Mani K, Garg A, Kaur K, Sharma MC, Raheja A, Suri A, Sarkar C, Suri V. Analysis of PD-L1 expression and T cell infiltration in different molecular subgroups of diffuse midline gliomas. Neuropathology. 2019;39(6):413-24. Epub 20191017. doi: 10.1111/neup.12594. PubMed PMID: 31625205.

47. Haag D, Mack N, Benites Goncalves da Silva P, Statz B, Clark J, Tanabe K, Sharma T, Jäger N, Jones DTW, Kawauchi D, Wernig M, Pfister SM. H3.3-K27M drives neural stem cell-specific gliomagenesis in a human iPSC-derived model. Cancer Cell. 2021;39(3):407-22.e13. Epub 20210204. doi: 10.1016/j.ccell.2021.01.005. PubMed PMID: 33545065.

48. Monje M, Mitra SS, Freret ME, Raveh TB, Kim J, Masek M, Attema JL, Li G, Haddix T, Edwards MSB, Fisher PG, Weissman IL, Rowitch DH, Vogel H, Wong AJ, Beachy PA. Hedgehog-responsive candidate cell of origin for diffuse intrinsic pontine glioma. Proceedings of the National Academy of Sciences. 2011;108(11):4453-8. doi: doi:10.1073/pnas.1101657108.

49. Lindquist RA, Guinto CD, Rodas-Rodriguez JL, Fuentealba LC, Tate MC, Rowitch DH, Alvarez-Buylla A. Identification of proliferative progenitors associated with prominent postnatal

Downloaded from https://academic.oup.com/neuro-oncology/advance-article/doi/10.1093/neuonc/noag014/8455707 by guest on 04 April 2026

growth of the pons. Nat Commun. 2016;7:11628. Epub 20160518. doi: 10.1038/ncomms11628. PubMed PMID: 27188978; PMCID: PMC4873968.

50. Ballester LY, Wang Z, Shandilya S, Miettinen M, Burger PC, Eberhart CG, Rodriguez FJ, Raabe E, Nazarian J, Warren K, Quezado MM. Morphologic characteristics and immunohistochemical profile of diffuse intrinsic pontine gliomas. Am J Surg Pathol. 2013;37(9):1357-64. doi: 10.1097/PAS.0b013e318294e817. PubMed PMID: 24076776; PMCID: PMC3787318.

51. Tate MC, Lindquist RA, Nguyen T, Sanai N, Barkovich AJ, Huang EJ, Rowitch DH, Alvarez-Buylla A. Postnatal growth of the human pons: a morphometric and immunohistochemical analysis. J Comp Neurol. 2015;523(3):449-62. Epub 20141202. doi: 10.1002/cne.23690. PubMed PMID: 25307966; PMCID: PMC4270924.

52. Neftel C, Laffy J, Filbin MG, Hara T, Shore ME, Rahme GJ, Richman AR, Silverbush D, Shaw ML, Hebert CM, Dewitt J, Gritsch S, Perez EM, Gonzalez Castro LN, Lan X, Druck N, Rodman C, Dionne D, Kaplan A, Bertalan MS, Small J, Pelton K, Becker S, Bonal D, Nguyen QD, Servis RL, Fung JM, Mylvaganam R, Mayr L, Gojo J, Haberler C, Geyeregger R, Czech T, Slavc I, Nahed BV, Curry WT, Carter BS, Wakimoto H, Brastianos PK, Batchelor TT, Stemmer-Rachamimov A, Martinez-Lage M, Frosch MP, Stamenkovic I, Riggi N, Rheinbay E, Monje M, Rozenblatt-Rosen O, Cahill DP, Patel AP, Hunter T, Verma IM, Ligon KL, Louis DN, Regev A, Bernstein BE, Tirosh I, Suvà ML. An Integrative Model of Cellular States, Plasticity, and Genetics for Glioblastoma. Cell. 2019;178(4):835-49.e21. Epub 20190718. doi: 10.1016/j.cell.2019.06.024. PubMed PMID: 31327527; PMCID: PMC6703186.

53. Nagaraja S, Quezada MA, Gillespie SM, Arzt M, Lennon JJ, Woo PJ, Hovestadt V, Kambhampati M, Filbin MG, Suva ML, Nazarian J, Monje M. Histone Variant and Cell Context Determine H3K27M Reprogramming of the Enhancer Landscape and Oncogenic State. Mol Cell. 2019;76(6):965-80.e12. Epub 20191003. doi: 10.1016/j.molcel.2019.08.030. PubMed PMID: 31588023; PMCID: PMC7104854.

Downloaded from https://academic.oup.com/neuro-oncology/advance-article/doi/10.1093/neuonc/noag014/8455707 by guest on 04 April 2026

54. Jessa S, Mohammadnia A, Harutyunyan AS, Hulswit M, Varadharajan S, Lakkis H, Kabir N, Bashardanesh Z, Hébert S, Faury D, Vladoiu MC, Worme S, Coutelier M, Krug B, Faria Andrade A, Pathania M, Bajic A, Weil AG, Ellezam B, Atkinson J, Dudley RWR, Farmer JP, Perreault S, Garcia BA, Larouche V, Blanchette M, Garzia L, Bhaduri A, Ligon KL, Bandopadhayay P, Taylor MD, Mack SC, Jabado N, Kleinman CL. K27M in canonical and noncanonical H3 variants occurs in distinct oligodendroglial cell lineages in brain midline gliomas. Nat Genet. 2022;54(12):1865-80. Epub 20221205. doi: 10.1038/s41588-022-01205-w. PubMed PMID: 36471070; PMCID: PMC9742294.

55. Liu I, Jiang L, Samuelsson ER, Marco Salas S, Beck A, Hack OA, Jeong D, Shaw ML, Englinger B, LaBelle J, Mire HM, Madlener S, Mayr L, Quezada MA, Trissal M, Panditharatna E, Ernst KJ, Vogelzang J, Gatesman TA, Halbert ME, Palova H, Pokorna P, Sterba J, Slaby O, Geyeregger R, Diaz A, Findlay IJ, Dun MD, Resnick A, Suvà ML, Jones DTW, Agnihotri S, Svedlund J, Koschmann C, Haberler C, Czech T, Slavc I, Cotter JA, Ligon KL, Alexandrescu S, Yung WKA, Arrillaga-Romany I, Gojo J, Monje M, Nilsson M, Filbin MG. The landscape of tumor cell states and spatial organization in H3-K27M mutant diffuse midline glioma across age and location. Nature Genetics. 2022;54(12):1881-94. doi: 10.1038/s41588-022-01236-3.

56. Filbin MG, Tirosh I, Hovestadt V, Shaw ML, Escalante LE, Mathewson ND, Neftel C, Frank N, Pelton K, Hebert CM, Haberler C, Yizhak K, Gojo J, Egervari K, Mount C, van Galen P, Bonal DM, Nguyen Q-D, Beck A, Sinai C, Czech T, Dorfer C, Goumnerova L, Lavarino C, Carcaboso AM, Mora J, Mylvaganam R, Luo CC, Peyrl A, Popović M, Azizi A, Batchelor TT, Frosch MP, Martinez-Lage M, Kieran MW, Bandopadhayay P, Beroukhim R, Fritsch G, Getz G, Rozenblatt-Rosen O, Wucherpfennig KW, Louis DN, Monje M, Slavc I, Ligon KL, Golub TR, Regev A, Bernstein BE, Suvà ML. Developmental and oncogenic programs in H3K27M gliomas dissected by single-cell RNA-seq. Science. 2018;360(6386):331-5. doi: doi:10.1126/science.aao4750.

Downloaded from https://academic.oup.com/neuro-oncology/advance-article/doi/10.1093/neuonc/noag014/8455707 by guest on 04 April 2026

57. Jessa S, Blanchet-Cohen A, Krug B, Vladoiu M, Coutelier M, Faury D, Poreau B, De Jay N, Hébert S, Monlong J, Farmer WT, Donovan LK, Hu Y, McConechy MK, Cavalli FMG, Mikael LG, Ellezam B, Richer M, Allaire A, Weil AG, Atkinson J, Farmer J-P, Dudley RWR, Larouche V, Crevier L, Albrecht S, Filbin MG, Sartelet H, Lutz P-E, Nagy C, Turecki G, Costantino S, Dirks PB, Murai KK, Bourque G, Ragoussis J, Garzia L, Taylor MD, Jabado N, Kleinman CL. Stalled developmental programs at the root of pediatric brain tumors. Nature Genetics. 2019;51(12):1702-13. doi: 10.1038/s41588-019-0531-7.

58. Wei J, Barr J, Kong L-Y, Wang Y, Wu A, Sharma AK, Gumin J, Henry V, Colman H, Priebe W, Sawaya R, Lang FF, Heimberger AB. Glioblastoma Cancer-Initiating Cells Inhibit T-Cell Proliferation and Effector Responses by the Signal Transducers and Activators of Transcription 3 Pathway. Molecular Cancer Therapeutics. 2010;9(1):67-78. doi: 10.1158/1535-7163.Mct-09-0734.

59. Wu A, Wei J, Kong LY, Wang Y, Priebe W, Qiao W, Sawaya R, Heimberger AB. Glioma cancer stem cells induce immunosuppressive macrophages/microglia. Neuro Oncol. 2010;12(11):1113-25. Epub 20100728. doi: 10.1093/neuonc/noq082. PubMed PMID: 20667896; PMCID: PMC3098021.

60. Hara T, Chanoch-Myers R, Mathewson ND, Myskiw C, Atta L, Bussema L, Eichhorn SW, Greenwald AC, Kinker GS, Rodman C, Gonzalez Castro LN, Wakimoto H, Rozenblatt-Rosen O, Zhuang X, Fan J, Hunter T, Verma IM, Wucherpfennig KW, Regev A, Suvà ML, Tirosh I. Interactions between cancer cells and immune cells drive transitions to mesenchymal-like states in glioblastoma. Cancer Cell. 2021;39(6):779-92.e11. Epub 20210603. doi: 10.1016/j.ccell.2021.05.002. PubMed PMID: 34087162; PMCID: PMC8366750.

61. Schmitt MJ, Company C, Dramaretska Y, Barozzi I, Göhrig A, Kertalli S, Großmann M, Naumann H, Sanchez-Bailon MP, Hulsman D, Glass R, Squatrito M, Serresi M, Gargiulo G. Phenotypic Mapping of Pathologic Cross-Talk between Glioblastoma and Innate Immune Cells

Downloaded from https://academic.oup.com/neuro-oncology/advance-article/doi/10.1093/neuonc/noag014/8455707 by guest on 04 April 2026

by Synthetic Genetic Tracing. Cancer Discov. 2021;11(3):754-77. Epub 20201223. doi: 10.1158/2159-8290.Cd-20-0219. PubMed PMID: 33361384; PMCID: PMC7611210.

62. Sa JK, Chang N, Lee HW, Cho HJ, Ceccarelli M, Cerulo L, Yin J, Kim SS, Caruso FP, Lee M, Kim D, Oh YT, Lee Y, Her NG, Min B, Kim HJ, Jeong DE, Kim HM, Kim H, Chung S, Woo HG, Lee J, Kong DS, Seol HJ, Lee JI, Kim J, Park WY, Wang Q, Sulman EP, Heimberger AB, Lim M, Park JB, Iavarone A, Verhaak RGW, Nam DH. Transcriptional regulatory networks of tumor-associated macrophages that drive malignancy in mesenchymal glioblastoma. Genome Biol. 2020;21(1):216. Epub 20200826. doi: 10.1186/s13059-020-02140-x. PubMed PMID: 32847614; PMCID: PMC7448990.

63. Andrade AF, Annett A, Karimi E, Topouza DG, Rezanejad M, Liu Y, McNicholas M, Gonzalez Santiago EG, Llivichuzhca-Loja D, Gehlhaar A, Jessa S, De Cola A, Chandarana B, Russo C, Faury D, Danieau G, Puligandla E, Wei Y, Zeinieh M, Wu Q, Hebert S, Juretic N, Nakada EM, Krug B, Larouche V, Weil AG, Dudley RWR, Karamchandani J, Agnihotri S, Quail DF, Ellezam B, Konnikova L, Walsh LA, Pathania M, Kleinman CL, Jabado N. Immune landscape of oncohistone-mutant gliomas reveals diverse myeloid populations and tumor-promoting function. Nat Commun. 2024;15(1):7769. Epub 20240905. doi: 10.1038/s41467-024-52096-w. PubMed PMID: 39237515; PMCID: PMC11377583.

64. Ross JL, Puigdelloses-Vallcorba M, Piñero G, Soni N, Thomason W, DeSisto J, Angione A, Tsankova NM, Castro MG, Schniederjan M, Wadhwani NR, Raju GP, Morgenstern P, Becher OJ, Green AL, Tsankov AM, Hambardzumyan D. Microglia and monocyte-derived macrophages drive progression of pediatric high-grade gliomas and are transcriptionally shaped by histone mutations. Immunity. 2024. doi: 10.1016/j.immuni.2024.09.007.

65. Levine AB, Nobre L, Das A, Milos S, Bianchi V, Johnson M, Fernandez NR, Stengs L, Ryall S, Ku M, Rana M, Laxer B, Sheth J, Sbergio SG, Fedoráková I, Ramaswamy V, Bennett J, Siddaway R, Tabori U, Hawkins C. Immuno-oncologic profiling of pediatric brain tumors reveals major clinical significance of the tumor immune microenvironment. Nat Commun.

Downloaded from https://academic.oup.com/neuro-oncology/advance-article/doi/10.1093/neuonc/noag014/8455707 by guest on 04 April 2026

2024;15(1):5790. Epub 20240710. doi: 10.1038/s41467-024-49595-1. PubMed PMID: 38987542; PMCID: PMC11237052.

66. Larkin T, Das A, Bianchi V, Sudhaman S, Chung J, Alsafwani N, Negm L, Yachnis A, Blatt J, Hawkins C, Bouffet E, Tabori U, Gururangan S. Upfront Adjuvant Immunotherapy of Replication Repair-Deficient Pediatric Glioblastoma With Chemoradiation-Sparing Approach. JCO Precis Oncol. 2021;5:1426-31. doi: 10.1200/po.21.00153. PubMed PMID: 34994637.

67. Damodharan S, Shireman JM, Xie E, Distler E, Kendziorski C, Dey M. Transcriptomic and proteomic spatial profiling of pediatric and adult diffuse midline glioma H3 K27-Altered. Sci Rep. 2024;14(1):22668. Epub 20240930. doi: 10.1038/s41598-024-73199-w. PubMed PMID: 39349581; PMCID: PMC11443003.

68. Koussounadis A, Langdon SP, Um IH, Harrison DJ, Smith VA. Relationship between differentially expressed mRNA and mRNA-protein correlations in a xenograft model system. Sci Rep. 2015;5:10775. Epub 20150608. doi: 10.1038/srep10775. PubMed PMID: 26053859; PMCID: PMC4459080.

69. Mancusi R, Monje M. The neuroscience of cancer. Nature. 2023;618(7965):467-79. doi: 10.1038/s41586-023-05968-y.

70. Taylor KR, Barron T, Hui A, Spitzer A, Yalçin B, Ivec AE, Geraghty AC, Hartmann GG, Arzt M, Gillespie SM, Kim YS, Maleki Jahan S, Zhang H, Shamardani K, Su M, Ni L, Du PP, Woo PJ, Silva-Torres A, Venkatesh HS, Mancusi R, Ponnuswami A, Mulinyawe S, Keough MB, Chau I, Aziz-Bose R, Tirosh I, Suvà ML, Monje M. Glioma synapses recruit mechanisms of adaptive plasticity. Nature. 2023;623(7986):366-74. doi: 10.1038/s41586-023-06678-1.

71. Winkler F, Venkatesh HS, Amit M, Batchelor T, Demir IE, Deneen B, Gutmann DH, Hervey-Jumper S, Kuner T, Mabbott D, Platten M, Rolls A, Sloan EK, Wang TC, Wick W, Venkataramani V, Monje M. Cancer neuroscience: State of the field, emerging directions. Cell. 2023;186(8):1689-707. doi: 10.1016/j.cell.2023.02.002. PubMed PMID: 37059069; PMCID: PMC10107403.

Downloaded from https://academic.oup.com/neuro-oncology/advance-article/doi/10.1093/neuonc/noag014/8455707 by guest on 04 April 2026

72. Song K-W, Lim M, Monje M. Complex neural-immune interactions shape glioma immunotherapy. Immunity. 2025;58(5):1140-60. doi: 10.1016/j.immuni.2025.04.017.

73. Gibson EM, Purger D, Mount CW, Goldstein AK, Lin GL, Wood LS, Inema I, Miller SE, Bieri G, Zuchero JB, Barres BA, Woo PJ, Vogel H, Monje M. Neuronal Activity Promotes Oligodendrogenesis and Adaptive Myelination in the Mammalian Brain. Science. 2014;344(6183):1252304. doi: doi:10.1126/science.1252304.

74. Venkatesh HS, Johung TB, Caretti V, Noll A, Tang Y, Nagaraja S, Gibson EM, Mount CW, Polepalli J, Mitra SS, Woo PJ, Malenka RC, Vogel H, Bredel M, Mallick P, Monje M. Neuronal Activity Promotes Glioma Growth through Neuroligin-3 Secretion. Cell. 2015;161(4):803-16. Epub 20150423. doi: 10.1016/j.cell.2015.04.012. PubMed PMID: 25913192; PMCID: PMC4447122.

75. Venkatesh HS, Tam LT, Woo PJ, Lennon J, Nagaraja S, Gillespie SM, Ni J, Duveau DY, Morris PJ, Zhao JJ, Thomas CJ, Monje M. Targeting neuronal activity-regulated neuroligin-3 dependency in high-grade glioma. Nature. 2017;549(7673):533-7. Epub 20170920. doi: 10.1038/nature24014. PubMed PMID: 28959975; PMCID: PMC5891832.

76. Venkatesh HS, Morishita W, Geraghty AC, Silverbush D, Gillespie SM, Arzt M, Tam LT, Espenel C, Ponnuswami A, Ni L, Woo PJ, Taylor KR, Agarwal A, Regev A, Brang D, Vogel H, Hervey-Jumper S, Bergles DE, Suvà ML, Malenka RC, Monje M. Electrical and synaptic integration of glioma into neural circuits. Nature. 2019;573(7775):539-45. doi: 10.1038/s41586-019-1563-y.

77. Venkataramani V, Tanev DI, Strahle C, Studier-Fischer A, Fankhauser L, Kessler T, Körber C, Kardorff M, Ratliff M, Xie R, Horstmann H, Messer M, Paik SP, Knabbe J, Sahm F, Kurz FT, Acikgöz AA, Herrmannsdörfer F, Agarwal A, Bergles DE, Chalmers A, Miletic H, Turcan S, Mawrin C, Hänggi D, Liu H-K, Wick W, Winkler F, Kuner T. Glutamatergic synaptic input to glioma cells drives brain tumour progression. Nature. 2019;573(7775):532-8. doi: 10.1038/s41586-019-1564-x.

Downloaded from https://academic.oup.com/neuro-oncology/advance-article/doi/10.1093/neuonc/noag014/8455707 by guest on 04 April 2026

78. Zhang X, Duan S, Apostolou PE, Wu X, Watanabe J, Gallitto M, Barron T, Taylor KR, Woo PJ, Hua X, Zhou H, Wei H-J, McQuillan N, Kang K-D, Friedman GK, Canoll PD, Chang K, Wu C-C, Hashizume R, Vakoc CR, Monje M, McKhann GM, II, Gogos JA, Zhang Z. CHD2 Regulates Neuron–Glioma Interactions in Pediatric Glioma. Cancer Discovery. 2024;14(9):1732-54. doi: 10.1158/2159-8290.Cd-23-0012.

79. Barron T, Yalçın B, Su M, Byun YG, Gavish A, Shamardani K, Xu H, Ni L, Soni N, Mehta V, Maleki Jahan S, Kim YS, Taylor KR, Keough MB, Quezada MA, Geraghty AC, Mancusi R, Vo LT, Castañeda EH, Woo PJ, Petritsch CK, Vogel H, Kaila K, Monje M. GABAergic neuron-to-glioma synapses in diffuse midline gliomas. Nature. 2025;639(8056):1060-8. doi: 10.1038/s41586-024-08579-3.

80. Labrakakis C, Patt S, Hartmann J, Kettenmann H. Functional GABA(A) receptors on human glioma cells. Eur J Neurosci. 1998;10(1):231-8. doi: 10.1046/j.1460-9568.1998.00036.x. PubMed PMID: 9753131.

81. Drexler R, Drinnenberg A, Gavish A, Yalçin B, Shamardani K, Rogers AE, Mancusi R, Trivedi V, Taylor KR, Kim YS, Woo PJ, Soni N, Su M, Ravel A, Tatlock E, Midler A, Wu SH, Ramakrishnan C, Chen R, Ayala-Sarmiento AE, Fernandez Pacheco DR, Siverts LA, Daigle TL, Tasic B, Zeng H, Breunig JJ, Deisseroth K, Monje M. Cholinergic neuronal activity promotes diffuse midline glioma growth through muscarinic signaling. Cell. 2025. doi: 10.1016/j.cell.2025.05.031.

82. Nejo T, Krishna S, Yamamichi A, Lakshmanachetty S, Jimenez C, Lee KY, Baker DL, Young JS, Chen T, Phyu SSS, Phung L, Gallus M, Maldonado GC, Okada K, Ogino H, Watchmaker PB, Diebold D, Choudhury A, Daniel AGS, Cadwell CR, Raleigh DR, Hervey-Jumper SL, Okada H. Glioma-neuronal circuit remodeling induces regional immunosuppression. Nature Communications. 2025;16(1):4770. doi: 10.1038/s41467-025-60074-z.

83. Bernstock JD, Hoffman SE, Kappel AD, Valdes PA, Essayed W, Klinger NV, Kang KD, Totsch SK, Olsen HE, Schlappi CW, Filipski K, Gessler FA, Baird L, Filbin MG, Hashizume R,

Downloaded from https://academic.oup.com/neuro-oncology/advance-article/doi/10.1093/neuonc/noag014/8455707 by guest on 04 April 2026

1234 Becher OJ, Friedman GK. Immunotherapy approaches for the treatment of diffuse midline
1235 gliomas. Oncoimmunology. 2022;11(1):2124058. Epub 20220926. doi:
1236 10.1080/2162402x.2022.2124058. PubMed PMID: 36185807; PMCID: PMC9519005.
1237 84. Kline C, Liu SJ, Duriseti S, Banerjee A, Nicolaides T, Raber S, Gupta N, Haas-Kogan D,
1238 Braunstein S, Mueller S. Reirradiation and PD-1 inhibition with nivolumab for the treatment of
1239 recurrent diffuse intrinsic pontine glioma: a single-institution experience. J Neurooncol.
1240 2018;140(3):629-38. Epub 20180911. doi: 10.1007/s11060-018-2991-5. PubMed PMID:
1241 30206764.
1242 85. Dunkel IJ, Doz F, Foreman NK, Hargrave D, Lassaletta A, André N, Hansford JR,
1243 Hassall T, Eyrich M, Gururangan S, Bartels U, Gajjar A, Howell L, Warad D, Pacius M, Tam R,
1244 Wang Y, Zhu L, Cohen K. Nivolumab with or without ipilimumab in pediatric patients with high-
1245 grade CNS malignancies: Safety, efficacy, biomarker, and pharmacokinetics-CheckMate 908.
1246 Neuro Oncol. 2023;25(8):1530-45. doi: 10.1093/neuonc/noad031. PubMed PMID: 36808285;
1247 PMCID: PMC10398811.
1248 86. Walia P, D'Arcy C, Hawkins C, Warren KE. IMMU-25. PROGRAMMED CELL DEATH-
1249 LIGAND 1 (PD-L1) IS NOT EXPRESSED IN DIFFUSE INTRINSIC PONTINE GLIOMA (DIPG)
1250 TUMOR CELLS. Neuro Oncol. 2018;20(Suppl 6):vi126. Epub 20181105. doi:
1251 10.1093/neuonc/noy148.528; PMCID: PMC6216217.
1252 87. Ausejo-Mauleon I, Labiano S, de la Nava D, Laspidea V, Zalacain M, Marrodán L,
1253 García-Moure M, González-Huarriz M, Hervás-Corpión I, Dhandapani L, Vicent S, Collantes M,
1254 Peñuelas I, Becher OJ, Filbin MG, Jiang L, Labelle J, de Biagi-Junior CAO, Nazarian J,
1255 Laternser S, Phoenix TN, van der Lugt J, Kranendonk M, Hoogendijk R, Mueller S, De Andrea
1256 C, Anderson AC, Guruceaga E, Koschmann C, Yadav VN, Gállego Pérez-Larraya J, Patiño-
1257 García A, Pastor F, Alonso MM. TIM-3 blockade in diffuse intrinsic pontine glioma models
1258 promotes tumor regression and antitumor immune memory. Cancer Cell. 2023;41(11):1911-

Downloaded from https://academic.oup.com/neuro-oncology/advance-article/doi/10.1093/neuonc/noag014/8455707 by guest on 04 April 2026

26.e8. Epub 20231005. doi: 10.1016/j.ccell.2023.09.001. PubMed PMID: 37802053; PMCID: PMC10644900.

88. Jiang Z, Sun H, Yu J, Tian W, Song Y. Targeting CD47 for cancer immunotherapy. J Hematol Oncol. 2021;14(1):180. Epub 20211030. doi: 10.1186/s13045-021-01197-w. PubMed PMID: 34717705; PMCID: PMC8557524.

89. Gholamin S, Mitra SS, Feroze AH, Liu J, Kahn SA, Zhang M, Esparza R, Richard C, Ramaswamy V, Remke M, Volkmer AK, Willingham S, Ponnuswami A, McCarty A, Lovelace P, Storm TA, Schubert S, Hutter G, Narayanan C, Chu P, Raabe EH, Harsh Gt, Taylor MD, Monje M, Cho YJ, Majeti R, Volkmer JP, Fisher PG, Grant G, Steinberg GK, Vogel H, Edwards M, Weissman IL, Cheshier SH. Disrupting the CD47-SIRPα anti-phagocytic axis by a humanized anti-CD47 antibody is an efficacious treatment for malignant pediatric brain tumors. Sci Transl Med. 2017;9(381). doi: 10.1126/scitranslmed.aaf2968. PubMed PMID: 28298418.

90. Prasad V. Immunotherapy: Tisagenlecleucel - the first approved CAR-T-cell therapy: implications for payers and policy makers. Nat Rev Clin Oncol. 2018;15(1):11-2. Epub 20171004. doi: 10.1038/nrclinonc.2017.156. PubMed PMID: 28975930.

91. Cappell KM, Kochenderfer JN. Long-term outcomes following CAR T cell therapy: what we know so far. Nat Rev Clin Oncol. 2023;20(6):359-71. Epub 20230413. doi: 10.1038/s41571-023-00754-1. PubMed PMID: 37055515; PMCID: PMC10100620.

92. Rafiq S, Hackett CS, Brentjens RJ. Engineering strategies to overcome the current roadblocks in CAR T cell therapy. Nat Rev Clin Oncol. 2020;17(3):147-67. Epub 20191217. doi: 10.1038/s41571-019-0297-y. PubMed PMID: 31848460; PMCID: PMC7223338.

93. Thomas BC, Staudt DE, Douglas AM, Monje M, Vitanza NA, Dun MD. CAR T cell therapies for diffuse midline glioma. Trends Cancer. 2023;9(10):791-804. Epub 20230803. doi: 10.1016/j.trecan.2023.07.007. PubMed PMID: 37541803.

Downloaded from https://academic.oup.com/neuro-oncology/advance-article/doi/10.1093/neuonc/noag014/8455707 by guest on 04 April 2026

94. Uribe ML, Marrocco I, Yarden Y. EGFR in Cancer: Signaling Mechanisms, Drugs, and Acquired Resistance. Cancers. 2021;13(11). Epub 20210601. doi: 10.3390/cancers13112748. PubMed PMID: 34206026; PMCID: PMC8197917.

95. Li G, Mitra SS, Monje M, Henrich KN, Bangs CD, Nitta RT, Wong AJ. Expression of epidermal growth factor variant III (EGFRvIII) in pediatric diffuse intrinsic pontine gliomas. J Neurooncol. 2012;108(3):395-402. Epub 20120302. doi: 10.1007/s11060-012-0842-3. PubMed PMID: 22382786; PMCID: PMC3368992.

96. O'Rourke DM, Nasrallah MP, Desai A, Melenhorst JJ, Mansfield K, Morrissette JJD, Martinez-Lage M, Brem S, Maloney E, Shen A, Isaacs R, Mohan S, Plesa G, Lacey SF, Navenot JM, Zheng Z, Levine BL, Okada H, June CH, Brogdon JL, Maus MV. A single dose of peripherally infused EGFRvIII-directed CAR T cells mediates antigen loss and induces adaptive resistance in patients with recurrent glioblastoma. Sci Transl Med. 2017;9(399). doi: 10.1126/scitranslmed.aaa0984. PubMed PMID: 28724573; PMCID: PMC5762203.

97. Goff SL, Morgan RA, Yang JC, Sherry RM, Robbins PF, Restifo NP, Feldman SA, Lu YC, Lu L, Zheng Z, Xi L, Epstein M, McIntyre LS, Malekzadeh P, Raffeld M, Fine HA, Rosenberg SA. Pilot Trial of Adoptive Transfer of Chimeric Antigen Receptor-transduced T Cells Targeting EGFRvIII in Patients With Glioblastoma. J Immunother. 2019;42(4):126-35. doi: 10.1097/cji.0000000000000260. PubMed PMID: 30882547; PMCID: PMC6691897.

98. Vitanza NA, Johnson AJ, Wilson AL, Brown C, Yokoyama JK, Künkele A, Chang CA, Rawlings-Rhea S, Huang W, Seidel K, Albert CM, Pinto N, Gust J, Finn LS, Ojemann JG, Wright J, Orentas RJ, Baldwin M, Gardner RA, Jensen MC, Park JR. Locoregional infusion of HER2-specific CAR T cells in children and young adults with recurrent or refractory CNS tumors: an interim analysis. Nat Med. 2021;27(9):1544-52. Epub 20210712. doi: 10.1038/s41591-021-01404-8. PubMed PMID: 34253928.

99. Ahmed N, Brawley V, Hegde M, Bielamowicz K, Kalra M, Landi D, Robertson C, Gray TL, Diouf O, Wakefield A, Ghazi A, Gerken C, Yi Z, Ashoori A, Wu MF, Liu H, Rooney C, Dotti G,

Downloaded from https://academic.oup.com/neuro-oncology/advance-article/doi/10.1093/neuonc/noag014/8455707 by guest on 04 April 2026

Gee A, Su J, Kew Y, Baskin D, Zhang YJ, New P, Grilley B, Stojakovic M, Hicks J, Powell SZ, Brenner MK, Heslop HE, Grossman R, Wels WS, Gottschalk S. HER2-Specific Chimeric Antigen Receptor-Modified Virus-Specific T Cells for Progressive Glioblastoma: A Phase 1 Dose-Escalation Trial. JAMA Oncol. 2017;3(8):1094-101. doi: 10.1001/jamaoncol.2017.0184. PubMed PMID: 28426845; PMCID: PMC5747970.

100. Brown CE, Aguilar B, Starr R, Yang X, Chang WC, Weng L, Chang B, Sarkissian A, Brito A, Sanchez JF, Ostberg JR, D'Apuzzo M, Badie B, Barish ME, Forman SJ. Optimization of IL13Rα2-Targeted Chimeric Antigen Receptor T Cells for Improved Anti-tumor Efficacy against Glioblastoma. Mol Ther. 2018;26(1):31-44. Epub 20171005. doi: 10.1016/j.ymthe.2017.10.002. PubMed PMID: 29103912; PMCID: PMC5763077.

101. Brown CE, Alizadeh D, Starr R, Weng L, Wagner JR, Naranjo A, Ostberg JR, Blanchard MS, Kilpatrick J, Simpson J, Kurien A, Priceman SJ, Wang X, Harshbarger TL, D'Apuzzo M, Ressler JA, Jensen MC, Barish ME, Chen M, Portnow J, Forman SJ, Badie B. Regression of Glioblastoma after Chimeric Antigen Receptor T-Cell Therapy. N Engl J Med. 2016;375(26):2561-9. doi: 10.1056/NEJMoa1610497. PubMed PMID: 28029927; PMCID: PMC5390684.

102. Mount CW, Majzner RG, Sundaresh S, Arnold EP, Kadapakkam M, Haile S, Labanieh L, Hulleman E, Woo PJ, Rietberg SP, Vogel H, Monje M, Mackall CL. Potent antitumor efficacy of anti-GD2 CAR T cells in H3-K27M(+) diffuse midline gliomas. Nat Med. 2018;24(5):572-9. Epub 20180416. doi: 10.1038/s41591-018-0006-x. PubMed PMID: 29662203; PMCID: PMC6214371.

103. Nazha B, Inal C, Owonikoko TK. Disialoganglioside GD2 Expression in Solid Tumors and Role as a Target for Cancer Therapy. Front Oncol. 2020;10:1000. Epub 20200707. doi: 10.3389/fonc.2020.01000. PubMed PMID: 32733795; PMCID: PMC7358363.

104. Monje M, Mahdi J, Majzner R, Yeom KW, Schultz LM, Richards RM, Barsan V, Song K-W, Kamens J, Baggott C, Kunicki M, Rietberg SP, Lim AS, Reschke A, Mavroukakis S, Egeler E, Moon J, Patel S, Chinnasamy H, Erickson C, Jacobs A, Duh AK, Tunuguntla R, Klysz DD,

Downloaded from https://academic.oup.com/neuro-oncology/advance-article/doi/10.1093/neuonc/noag014/8455707 by guest on 04 April 2026

1335 Fowler C, Green S, Beebe B, Carr C, Fujimoto M, Brown AK, Petersen A-LG, McIntyre C,
1336 Siddiqui A, Lepori-Bui N, Villar K, Pham K, Bove R, Musa E, Reynolds WD, Kuo A, Prabhu S,
1337 Rasmussen L, Cornell TT, Partap S, Fisher PG, Campen CJ, Grant G, Prolo L, Ye X, Sahaf B,
1338 Davis KL, Feldman SA, Ramakrishna S, Mackall C. Intravenous and intracranial GD2-CAR T
1339 cells for H3K27M+ diffuse midline gliomas. Nature. 2024. doi: 10.1038/s41586-024-08171-9.
1340 105. Lin FY, Stuckert A, Tat C, White M, Ruggieri L, Zhang H, Mehta B, Lapteva N, Mei Z,
1341 Major A, Thakkar S, Shum T, Parikh K, Wu MF, Lindsay HB, Scherer L, Shekar M, Baxter P,
1342 Wang T, Grilley B, Moeller K, Hicks J, Roy A, Anastas J, Malbari F, Aldave G, Chintagumpala M,
1343 Blaney S, Parsons DW, Brenner MK, Heslop HE, Rooney CM, Omer B. Phase I Trial of
1344 GD2.CART Cells Augmented With Constitutive Interleukin-7 Receptor for Treatment of High-
1345 Grade Pediatric CNS Tumors. J Clin Oncol. 2024;42(23):2769-79. Epub 20240521. doi:
1346 10.1200/jco.23.02019. PubMed PMID: 38771986; PMCID: PMC11305939.
1347 106. Shum T, Omer B, Tashiro H, Kruse RL, Wagner DL, Parikh K, Yi Z, Sauer T, Liu D,
1348 Parihar R, Castillo P, Liu H, Brenner MK, Metelitsa LS, Gottschalk S, Rooney CM. Constitutive
1349 Signaling from an Engineered IL7 Receptor Promotes Durable Tumor Elimination by Tumor-
1350 Redirected T Cells. Cancer Discov. 2017;7(11):1238-47. Epub 20170822. doi: 10.1158/2159-
1351 8290.Cd-17-0538. PubMed PMID: 28830878; PMCID: PMC5669830.
1352 107. Maachani UB, Tosi U, Pisapia DJ, Mukherjee S, Marnell CS, Voronina J, Martinez D,
1353 Santi M, Dahmane N, Zhou Z, Hawkins C, Souweidane MM. B7-H3 as a Prognostic Biomarker
1354 and Therapeutic Target in Pediatric central nervous system Tumors. Transl Oncol.
1355 2020;13(2):365-71. Epub 20191227. doi: 10.1016/j.tranon.2019.11.006. PubMed PMID:
1356 31887631; PMCID: PMC6938869.
1357 108. Vitanza NA, Wilson AL, Huang W, Seidel K, Brown C, Gustafson JA, Yokoyama JK,
1358 Johnson AJ, Baxter BA, Koning RW, Reid AN, Meechan M, Biery MC, Myers C, Rawlings-Rhea
1359 SD, Albert CM, Browd SR, Hauptman JS, Lee A, Ojemann JG, Berens ME, Dun MD, Foster JB,
1360 Crotty EE, Leary SES, Cole BL, Perez FA, Wright JN, Orentas RJ, Chour T, Newell EW,

Downloaded from https://academic.oup.com/neuro-oncology/advance-article/doi/10.1093/neuonc/noag014/8455707 by guest on 04 April 2026

1361 Whiteaker JR, Zhao L, Paulovich AG, Pinto N, Gust J, Gardner RA, Jensen MC, Park JR.
1362 Intraventricular B7-H3 CAR T Cells for Diffuse Intrinsic Pontine Glioma: Preliminary First-in-
1363 Human Bioactivity and Safety. Cancer Discov. 2023;13(1):114-31. doi: 10.1158/2159-8290.Cd-
1364 22-0750. PubMed PMID: 36259971; PMCID: PMC9827115.
1365 109. Vitanza NA, Ronsley R, Choe M, Seidel K, Huang W, Rawlings-Rhea SD, Beam M,
1366 Steinmetzer L, Wilson AL, Brown C, Beebe A, Lindgren C, Gustafson JA, Wein A, Holtzclaw S,
1367 Hoeppner C, Goldstein HE, Browd SR, Hauptman JS, Lee A, Ojemann JG, Crotty EE, Leary
1368 SES, Perez FA, Wright JN, Alonso MM, Dun MD, Foster JB, Hurst D, Kong A, Thomsen A,
1369 Orentas RJ, Albert CM, Pinto N, Annesley C, Gardner RA, Ho O, Pattabhi S, Gust J, Wendler
1370 JP, Park JR, Jensen MC. Intracerebroventricular B7-H3-targeting CAR T cells for diffuse intrinsic
1371 pontine glioma: a phase 1 trial. Nature Medicine. 2025;31(3):861-8. doi: 10.1038/s41591-024-
1372 03451-3.
1373 110. Ochs K, Ott M, Bunse T, Sahm F, Bunse L, Deumelandt K, Sonner JK, Keil M, von
1374 Deimling A, Wick W, Platten M. K27M-mutant histone-3 as a novel target for glioma
1375 immunotherapy. Oncoimmunology. 2017;6(7):e1328340. Epub 20170512. doi:
1376 10.1080/2162402x.2017.1328340. PubMed PMID: 28811969; PMCID: PMC5543817.
1377 111. Mueller S, Taitt JM, Villanueva-Meyer JE, Bonner ER, Nejo T, Lulla RR, Goldman S,
1378 Banerjee A, Chi SN, Whipple NS, Crawford JR, Gauvain K, Nazemi KJ, Watchmaker PB,
1379 Almeida ND, Okada K, Salazar AM, Gilbert RD, Nazarian J, Molinaro AM, Butterfield LH, Prados
1380 MD, Okada H. Mass cytometry detects H3.3K27M-specific vaccine responses in diffuse midline
1381 glioma. J Clin Invest. 2020;130(12):6325-37. doi: 10.1172/jci140378. PubMed PMID: 32817593;
1382 PMCID: PMC7685729.
1383 112. Khuong-Quang DA, Buczkowicz P, Rakopoulos P, Liu XY, Fontebasso AM, Bouffet E,
1384 Bartels U, Albrecht S, Schwartzentruber J, Letourneau L, Bourgey M, Bourque G, Montpetit A,
1385 Bourret G, Lepage P, Fleming A, Lichter P, Kool M, von Deimling A, Sturm D, Korshunov A,
1386 Faury D, Jones DT, Majewski J, Pfister SM, Jabado N, Hawkins C. K27M mutation in histone

Downloaded from https://academic.oup.com/neuro-oncology/advance-article/doi/10.1093/neuonc/noag014/8455707 by guest on 04 April 2026

H3.3 defines clinically and biologically distinct subgroups of pediatric diffuse intrinsic pontine gliomas. Acta Neuropathol. 2012;124(3):439-47. Epub 20120603. doi: 10.1007/s00401-012-0998-0. PubMed PMID: 22661320; PMCID: PMC3422615.

113. Wu G, Broniscer A, McEachron TA, Lu C, Paugh BS, Becksfort J, Qu C, Ding L, Huether R, Parker M, Zhang J, Gajjar A, Dyer MA, Mullighan CG, Gilbertson RJ, Mardis ER, Wilson RK, Downing JR, Ellison DW, Zhang J, Baker SJ. Somatic histone H3 alterations in pediatric diffuse intrinsic pontine gliomas and non-brainstem glioblastomas. Nat Genet. 2012;44(3):251-3. Epub 20120129. doi: 10.1038/ng.1102. PubMed PMID: 22286216; PMCID: PMC3288377.

114. Schwartzentruber J, Korshunov A, Liu XY, Jones DT, Pfaff E, Jacob K, Sturm D, Fontebasso AM, Quang DA, Tönjes M, Hovestadt V, Albrecht S, Kool M, Nantel A, Konermann C, Lindroth A, Jäger N, Rausch T, Ryzhova M, Korbel JO, Hielscher T, Hauser P, Garami M, Klekner A, Bognar L, Ebinger M, Schuhmann MU, Scheurlen W, Pekrun A, Frühwald MC, Roggendorf W, Kramm C, Dürken M, Atkinson J, Lepage P, Montpetit A, Zakrzewska M, Zakrzewski K, Liberski PP, Dong Z, Siegel P, Kulozik AE, Zapatka M, Guha A, Malkin D, Felsberg J, Reifenberger G, von Deimling A, Ichimura K, Collins VP, Witt H, Milde T, Witt O, Zhang C, Castelo-Branco P, Lichter P, Faury D, Tabori U, Plass C, Majewski J, Pfister SM, Jabado N. Driver mutations in histone H3.3 and chromatin remodelling genes in paediatric glioblastoma. Nature. 2012;482(7384):226-31. Epub 20120129. doi: 10.1038/nature10833. PubMed PMID: 22286061.

115. Mendez-Gomez HR, DeVries A, Castillo P, von Roemeling C, Qdaisat S, Stover BD, Xie C, Weidert F, Zhao C, Moor R, Liu R, Soni D, Ogando-Rivas E, Chardon-Robles J, McGuiness J, Zhang D, Chung MC, Marconi C, Michel S, Barpujari A, Jobin GW, Thomas N, Ma X, Campaneria Y, Grippin A, Karachi A, Li D, Sahay B, Elliott L, Foster TP, Coleman KE, Milner RJ, Sawyer WG, Ligon JA, Simon E, Cleaver B, Wynne K, Hodik M, Molinaro AM, Guan J, Kellish P, Doty A, Lee J-H, Massini T, Kresak JL, Huang J, Hwang EI, Kline C, Carrera-Justiz S, Rahman M, Gatica S, Mueller S, Prados M, Ghiaseddin AP, Silver NL, Mitchell DA, Sayour EJ. RNA

Downloaded from https://academic.oup.com/neuro-oncology/advance-article/doi/10.1093/neuonc/noag014/8455707 by guest on 04 April 2026

aggregates harness the danger response for potent cancer immunotherapy. Cell. 2024;187(10):2521-35.e21. doi: https://doi.org/10.1016/j.cell.2024.04.003.

116. Qdaisat S, Wummer B, Stover BD, Zhang D, McGuiness J, Weidert F, Chardon-Robles J, Grippin A, DeVries A, Zhao C, Marconi C, Karachi A, Xie C, Jobin G, Liu R, Michel S, Ma X, Moor RSF, von Roemeling C, Nguyen DT, Elliott L, Thomas N, Barpujari A, Geffrard H, Campaneria Y, Ogando-Rivas E, Rabideau C, Soni D, Huang J, Carrera-Justiz S, Fredenburg K, Silver NL, Sawyer WG, Rahman M, Ligon JA, Flores CT, Lee J-H, Mitchell DA, Castillo P, Mendez-Gomez HR, Sayour EJ. Sensitization of tumours to immunotherapy by boosting early type-I interferon responses enables epitope spreading. Nature Biomedical Engineering. 2025;9(9):1437-52. doi: 10.1038/s41551-025-01380-1.

117. Kaufman HL, Kohlhapp FJ, Zloza A. Oncolytic viruses: a new class of immunotherapy drugs. Nature reviews Drug discovery. 2015;14(9):642-62. doi: 10.1038/nrd4663. PubMed PMID: 26323545; PMCID: PMC7097180.

118. Totsch SK, Schlappi C, Kang KD, Ishizuka AS, Lynn GM, Fox B, Beierle EA, Whitley RJ, Markert JM, Gillespie GY, Bernstock JD, Friedman GK. Oncolytic herpes simplex virus immunotherapy for brain tumors: current pitfalls and emerging strategies to overcome therapeutic resistance. Oncogene. 2019;38(34):6159-71. Epub 20190709. doi: 10.1038/s41388-019-0870-y. PubMed PMID: 31289361; PMCID: PMC6771414.

119. Seymour LW, Fisher KD. Oncolytic viruses: finally delivering. Br J Cancer. 2016;114(4):357-61. Epub 20160114. doi: 10.1038/bjc.2015.481. PubMed PMID: 26766734; PMCID: PMC4815777.

120. Ghajar-Rahimi G, Kang KD, Totsch SK, Gary S, Rocco A, Blitz S, Kachurak K, Chambers MR, Li R, Beierle EA, Bag A, Johnston JM, Markert JM, Bernstock JD, Friedman GK. Clinical advances in oncolytic virotherapy for pediatric brain tumors. Pharmacol Ther. 2022;239:108193. Epub 20220426. doi: 10.1016/j.pharmthera.2022.108193. PubMed PMID: 35487285; PMCID: PMC9709696.

Downloaded from https://academic.oup.com/neuro-oncology/advance-article/doi/10.1093/neuonc/noag014/8455707 by guest on 04 April 2026

121. Friedman GK, Bernstock JD, Chen D, Nan L, Moore BP, Kelly VM, Youngblood SL, Langford CP, Han X, Ring EK, Beierle EA, Gillespie GY, Markert JM. Enhanced Sensitivity of Patient-Derived Pediatric High-Grade Brain Tumor Xenografts to Oncolytic HSV-1 Virotherapy Correlates with Nectin-1 Expression. Sci Rep. 2018;8(1):13930. Epub 20180917. doi: 10.1038/s41598-018-32353-x. PubMed PMID: 30224769; PMCID: PMC6141470.

122. Friedman GK, Langford CP, Coleman JM, Cassady KA, Parker JN, Markert JM, Yancey Gillespie G. Engineered herpes simplex viruses efficiently infect and kill CD133+ human glioma xenograft cells that express CD111. J Neurooncol. 2009;95(2):199-209. Epub 20090612. doi: 10.1007/s11060-009-9926-0. PubMed PMID: 19521665; PMCID: PMC4574288.

123. Coukos G, Makrigiannakis A, Montas S, Kaiser LR, Toyozumi T, Benjamin I, Albelda SM, Rubin SC, Molnar-Kimber KL. Multi-attenuated herpes simplex virus-1 mutant G207 exerts cytotoxicity against epithelial ovarian cancer but not normal mesothelium and is suitable for intraperitoneal oncolytic therapy. Cancer Gene Therapy. 2000;7(2):275-83. doi: 10.1038/sj.cgt.7700130.

124. Friedman GK, Johnston JM, Bag AK, Bernstock JD, Li R, Aban I, Kachurak K, Nan L, Kang KD, Totsch S, Schlappi C, Martin AM, Pastakia D, McNall-Knapp R, Farouk Sait S, Khakoo Y, Karajannis MA, Woodling K, Palmer JD, Osorio DS, Leonard J, Abdelbaki MS, Madan-Swain A, Atkinson TP, Whitley RJ, Fiveash JB, Markert JM, Gillespie GY. Oncolytic HSV-1 G207 Immunovirotherapy for Pediatric High-Grade Gliomas. N Engl J Med. 2021;384(17):1613-22. Epub 20210410. doi: 10.1056/NEJMoa2024947. PubMed PMID: 33838625; PMCID: PMC8284840.

125. Bernstock JD, Bag AK, Fiveash J, Kachurak K, Elsayed G, Chagoya G, Gessler F, Valdes PA, Madan-Swain A, Whitley R, Markert JM, Gillespie GY, Johnston JM, Friedman GK. Design and Rationale for First-in-Human Phase 1 Immunovirotherapy Clinical Trial of Oncolytic HSV G207 to Treat Malignant Pediatric Cerebellar Brain Tumors. Hum Gene Ther. 2020;31(19-

Downloaded from https://academic.oup.com/neuro-oncology/advance-article/doi/10.1093/neuonc/noag014/8455707 by guest on 04 April 2026

20):1132-9. Epub 20200817. doi: 10.1089/hum.2020.101. PubMed PMID: 32657154; PMCID: PMC7585605.

126. Jiang H, Gomez-Manzano C, Aoki H, Alonso MM, Kondo S, McCormick F, Xu J, Kondo Y, Bekele BN, Colman H, Lang FF, Fueyo J. Examination of the therapeutic potential of Delta-24-RGD in brain tumor stem cells: role of autophagic cell death. J Natl Cancer Inst. 2007;99(18):1410-4. Epub 20070911. doi: 10.1093/jnci/djm102. PubMed PMID: 17848677.

127. Fueyo J, Gomez-Manzano C, Alemany R, Lee PS, McDonnell TJ, Mitlianga P, Shi YX, Levin VA, Yung WK, Kyritsis AP. A mutant oncolytic adenovirus targeting the Rb pathway produces anti-glioma effect in vivo. Oncogene. 2000;19(1):2-12. doi: 10.1038/sj.onc.1203251. PubMed PMID: 10644974.

128. Lang FF, Conrad C, Gomez-Manzano C, Yung WKA, Sawaya R, Weinberg JS, Prabhu SS, Rao G, Fuller GN, Aldape KD, Gumin J, Vence LM, Wistuba I, Rodriguez-Canales J, Villalobos PA, Dirven CMF, Tejada S, Valle RD, Alonso MM, Ewald B, Peterkin JJ, Tufaro F, Fueyo J. Phase I Study of DNX-2401 (Delta-24-RGD) Oncolytic Adenovirus: Replication and Immunotherapeutic Effects in Recurrent Malignant Glioma. J Clin Oncol. 2018;36(14):1419-27. Epub 20180212. doi: 10.1200/jco.2017.75.8219. PubMed PMID: 29432077; PMCID: PMC6075856.

129. Martínez-Vélez N, Garcia-Moure M, Marigil M, González-Huarriz M, Puigdelloses M, Gallego Pérez-Larraya J, Zalacaín M, Marrodán L, Varela-Guruceaga M, Laspidea V, Aristu JJ, Ramos LI, Tejada-Solís S, Díez-Valle R, Jones C, Mackay A, Martínez-Climent JA, García-Barchino MJ, Raabe E, Monje M, Becher OJ, Junier MP, El-Habr EA, Chneiweiss H, Aldave G, Jiang H, Fueyo J, Patiño-García A, Gomez-Manzano C, Alonso MM. The oncolytic virus Delta-24-RGD elicits an antitumor effect in pediatric glioma and DIPG mouse models. Nat Commun. 2019;10(1):2235. Epub 20190528. doi: 10.1038/s41467-019-10043-0. PubMed PMID: 31138805; PMCID: PMC6538754.

Downloaded from https://academic.oup.com/neuro-oncology/advance-article/doi/10.1093/neuonc/noag014/8455707 by guest on 04 April 2026

130. Gállego Pérez-Larraya J, Garcia-Moure M, Labiano S, Patiño-García A, Dobbs J, Gonzalez-Huarriz M, Zalacain M, Marrodan L, Martinez-Velez N, Puigdelloses M, Laspidea V, Astigarraga I, Lopez-Ibor B, Cruz O, Oscoz Lizarbe M, Hervas-Stubbs S, Alkorta-Aranburu G, Tamayo I, Tavira B, Hernandez-Alcoceba R, Jones C, Dharmadhikari G, Ruiz-Moreno C, Stunnenberg H, Hulleman E, van der Lugt J, Idoate M, Diez-Valle R, Esparragosa Vázquez I, Villalba M, de Andrea C, Núñez-Córdoba JM, Ewald B, Robbins J, Fueyo J, Gomez-Manzano C, Lang FF, Tejada S, Alonso MM. Oncolytic DNX-2401 Virus for Pediatric Diffuse Intrinsic Pontine Glioma. N Engl J Med. 2022;386(26):2471-81. doi: 10.1056/NEJMoa2202028. PubMed PMID: 35767439.

131. Mackay A, Burford A, Carvalho D, Izquierdo E, Fazal-Salom J, Taylor KR, Bjerke L, Clarke M, Vinci M, Nandhabalan M, Temelso S, Popov S, Molinari V, Raman P, Waanders AJ, Han HJ, Gupta S, Marshall L, Zacharoulis S, Vaidya S, Mandeville HC, Bridges LR, Martin AJ, Al-Sarraj S, Chandler C, Ng HK, Li X, Mu K, Trabelsi S, Brahim DH, Kisljakov AN, Konovalov DM, Moore AS, Carcaboso AM, Sunol M, de Torres C, Cruz O, Mora J, Shats LI, Stavale JN, Bidinotto LT, Reis RM, Entz-Werle N, Farrell M, Cryan J, Crimmins D, Caird J, Pears J, Monje M, Debily MA, Castel D, Grill J, Hawkins C, Nikbakht H, Jabado N, Baker SJ, Pfister SM, Jones DTW, Fouladi M, von Bueren AO, Baudis M, Resnick A, Jones C. Integrated Molecular Meta-Analysis of 1,000 Pediatric High-Grade and Diffuse Intrinsic Pontine Glioma. Cancer Cell. 2017;32(4):520-37.e5. Epub 20170928. doi: 10.1016/j.ccell.2017.08.017. PubMed PMID: 28966033; PMCID: PMC5637314.

132. Hersh DS, Wadajkar AS, Roberts N, Perez JG, Connolly NP, Frenkel V, Winkles JA, Woodworth GF, Kim AJ. Evolving Drug Delivery Strategies to Overcome the Blood Brain Barrier. Curr Pharm Des. 2016;22(9):1177-93. doi: 10.2174/1381612822666151221150733. PubMed PMID: 26685681; PMCID: PMC4900538.

133. Chen SR, Chen MM, Ene C, Lang FF, Kan P. Perfusion-guided endovascular super-selective intra-arterial infusion for treatment of malignant brain tumors. J Neurointerv Surg.

Downloaded from https://academic.oup.com/neuro-oncology/advance-article/doi/10.1093/neuonc/noag014/8455707 by guest on 04 April 2026

2022;14(6):533-8. Epub 20211125. doi: 10.1136/neurintsurg-2021-018190. PubMed PMID: 34824133.

134. Chastkofsky MI, Pituch KC, Katagi H, Zannikou M, Ilut L, Xiao T, Han Y, Sonabend AM, Curiel DT, Bonner ER, Nazarian J, Horbinski CM, James CD, Saratsis AM, Hashizume R, Lesniak MS, Balyasnikova IV. Mesenchymal Stem Cells Successfully Deliver Oncolytic Virotherapy to Diffuse Intrinsic Pontine Glioma. Clin Cancer Res. 2021;27(6):1766-77. Epub 20201203. doi: 10.1158/1078-0432.Ccr-20-1499. PubMed PMID: 33272983; PMCID: PMC7956061.

135. Carceller F, Aleu A, Casasco A, Guimaraens L, López-Pino MA, Madero L, Ramírez M. Superselective intracerebral catheterization for administration of oncolytic virotherapy in a case of diffuse intrinsic pontine glioma. J Pediatr Hematol Oncol. 2014;36(7):e430-2. doi: 10.1097/mph.0000000000000084. PubMed PMID: 24327128.

136. Chohan KL, Siegler EL, Kenderian SS. CAR-T Cell Therapy: the Efficacy and Toxicity Balance. Curr Hematol Malig Rep. 2023;18(2):9-18. Epub 20230210. doi: 10.1007/s11899-023-00687-7. PubMed PMID: 36763238; PMCID: PMC10505056.

137. Morris EC, Neelapu SS, Giavridis T, Sadelain M. Cytokine release syndrome and associated neurotoxicity in cancer immunotherapy. Nat Rev Immunol. 2022;22(2):85-96. Epub 20210517. doi: 10.1038/s41577-021-00547-6. PubMed PMID: 34002066; PMCID: PMC8127450.

138. Siegler EL, Kenderian SS. Neurotoxicity and Cytokine Release Syndrome After Chimeric Antigen Receptor T Cell Therapy: Insights Into Mechanisms and Novel Therapies. Front Immunol. 2020;11:1973. Epub 20200828. doi: 10.3389/fimmu.2020.01973. PubMed PMID: 32983132; PMCID: PMC7485001.

139. Mahdi J, Dietrich J, Straathof K, Roddie C, Scott BJ, Davidson TB, Prolo LM, Batchelor TT, Campen CJ, Davis KL, Gust J, Lim M, Majzner RG, Park JR, Partap S, Ramakrishna S, Richards R, Schultz L, Vitanza NA, Wang LD, Mackall CL, Monje M. Tumor inflammation-

Downloaded from https://academic.oup.com/neuro-oncology/advance-article/doi/10.1093/neuonc/noag014/8455707 by guest on 04 April 2026

associated neurotoxicity. Nat Med. 2023;29(4):803-10. Epub 20230406. doi: 10.1038/s41591-023-02276-w. PubMed PMID: 37024595; PMCID: PMC10166099.

140. Warren KE. Beyond the Blood:Brain Barrier: The Importance of Central Nervous System (CNS) Pharmacokinetics for the Treatment of CNS Tumors, Including Diffuse Intrinsic Pontine Glioma. Front Oncol. 2018;8:239. Epub 20180703. doi: 10.3389/fonc.2018.00239. PubMed PMID: 30018882; PMCID: PMC6037693.

141. Warren KE. Diffuse intrinsic pontine glioma: poised for progress. Front Oncol. 2012;2:205. Epub 20121228. doi: 10.3389/fonc.2012.00205. PubMed PMID: 23293772; PMCID: PMC3531714.

142. Wei X, Meel MH, Breur M, Bugiani M, Hulleman E, Phoenix TN. Defining tumor-associated vascular heterogeneity in pediatric high-grade and diffuse midline gliomas. Acta Neuropathol Commun. 2021;9(1):142. Epub 20210823. doi: 10.1186/s40478-021-01243-1. PubMed PMID: 34425907; PMCID: PMC8381557.

143. Arms LM, Duchatel RJ, Jackson ER, Sobrinho PG, Dun MD, Hua S. Current status and advances to improving drug delivery in diffuse intrinsic pontine glioma. J Control Release. 2024;370:835-65. Epub 20240520. doi: 10.1016/j.jconrel.2024.05.018. PubMed PMID: 38744345.

144. Rechberger JS, Power BT, Power EA, Nesvick CL, Daniels DJ. H3K27-altered diffuse midline glioma: a paradigm shifting opportunity in direct delivery of targeted therapeutics. Expert Opin Ther Targets. 2023;27(1):9-17. Epub 20230212. doi: 10.1080/14728222.2023.2177531. PubMed PMID: 36744399; PMCID: PMC10165636.

145. Tosi U, Souweidane M. Convection Enhanced Delivery for Diffuse Intrinsic Pontine Glioma: Review of a Single Institution Experience. Pharmaceutics. 2020;12(7). Epub 20200714. doi: 10.3390/pharmaceutics12070660. PubMed PMID: 32674336; PMCID: PMC7407112.

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146. Kuzan-Fischer CM, Souweidane MM. The intersect of neurosurgery with diffuse intrinsic pontine glioma: JNSPG 75th Anniversary Invited Review Article. Journal of Neurosurgery: Pediatrics PED. 2019;24(6):611-21. doi: https://doi.org/10.3171/2019.5.PEDS18376.

147. Heiss JD, Jamshidi A, Shah S, Martin S, Wolters PL, Argersinger DP, Warren KE, Lonser RR. Phase I trial of convection-enhanced delivery of IL13-Pseudomonas toxin in children with diffuse intrinsic pontine glioma. J Neurosurg Pediatr. 2019;23(3):333-42. Epub 20181207. doi: 10.3171/2018.9.Peds17225. PubMed PMID: 30544335; PMCID: PMC7266009.

148. Kobus T, Zervantonakis IK, Zhang Y, McDannold NJ. Growth inhibition in a brain metastasis model by antibody delivery using focused ultrasound-mediated blood-brain barrier disruption. J Control Release. 2016;238:281-8. Epub 20160803. doi: 10.1016/j.jconrel.2016.08.001. PubMed PMID: 27496633; PMCID: PMC5014601.

149. Kinoshita M, McDannold N, Jolesz FA, Hynynen K. Noninvasive localized delivery of Herceptin to the mouse brain by MRI-guided focused ultrasound-induced blood-brain barrier disruption. Proc Natl Acad Sci U S A. 2006;103(31):11719-23. Epub 20060725. doi: 10.1073/pnas.0604318103. PubMed PMID: 16868082; PMCID: PMC1544236.

150. Timbie KF, Mead BP, Price RJ. Drug and gene delivery across the blood-brain barrier with focused ultrasound. J Control Release. 2015;219:61-75. Epub 20150908. doi: 10.1016/j.jconrel.2015.08.059. PubMed PMID: 26362698; PMCID: PMC4656107.

151. Bunevicius A, McDannold NJ, Golby AJ. Focused Ultrasound Strategies for Brain Tumor Therapy. Oper Neurosurg (Hagerstown). 2020;19(1):9-18. doi: 10.1093/ons/opz374. PubMed PMID: 31853548; PMCID: PMC7293897.

152. Butterfield LH, Najjar YG. Immunotherapy combination approaches: mechanisms, biomarkers and clinical observations. Nature Reviews Immunology. 2024;24(6):399-416. doi: 10.1038/s41577-023-00973-8.

153. Koschmann C, Al-Holou WN, Alonso MM, Anastas J, Bandopadhayay P, Barron T, Becher O, Cartaxo R, Castro MG, Chung C, Clausen M, Dang D, Doherty R, Duchatel R, Dun

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M, Filbin M, Franson A, Galban S, Garcia Moure M, Garton H, Gowda P, Marques JG, Hawkins C, Heath A, Hulleman E, Ji S, Jones C, Kilburn L, Kline C, Koldobskiy MA, Lim D, Lowenstein PR, Lu QR, Lum J, Mack S, Magge S, Marini B, Martin D, Marupudi N, Messinger D, Mody R, Morgan M, Mota M, Muraszko K, Mueller S, Natarajan SK, Nazarian J, Niculcea M, Nuechterlein N, Okada H, Opipari V, Pai MP, Pal S, Peterson E, Phoenix T, Prensner JR, Pun M, Raju GP, Reitman ZJ, Resnick A, Rogawski D, Saratsis A, Sbergio SG, Souweidane M, Stafford JM, Tzaridis T, Venkataraman S, Vittorio O, Wadden J, Wahl D, Wechsler-Reya RJ, Yadav VN, Zhang X, Zhang Q, Venneti S. A road map for the treatment of pediatric diffuse midline glioma. Cancer Cell. 2024;42(1):1-5. doi: https://doi.org/10.1016/j.ccell.2023.11.002.

154. Castel D, Philippe C, Calmon R, Le Dret L, Truffaux N, Boddaert N, Pagès M, Taylor KR, Saulnier P, Lacroix L, Mackay A, Jones C, Sainte-Rose C, Blauwblomme T, Andreiuolo F, Puget S, Grill J, Varlet P, Debily MA. Histone H3F3A and HIST1H3B K27M mutations define two subgroups of diffuse intrinsic pontine gliomas with different prognosis and phenotypes. Acta Neuropathol. 2015;130(6):815-27. Epub 20150923. doi: 10.1007/s00401-015-1478-0. PubMed PMID: 26399631; PMCID: PMC4654747.

155. Hoeman CM, Cordero FJ, Hu G, Misuraca K, Romero MM, Cardona HJ, Nazarian J, Hashizume R, McLendon R, Yu P, Procissi D, Gadd S, Becher OJ. ACVR1 R206H cooperates with H3.1K27M in promoting diffuse intrinsic pontine glioma pathogenesis. Nat Commun. 2019;10(1):1023. Epub 20190304. doi: 10.1038/s41467-019-08823-9. PubMed PMID: 30833574; PMCID: PMC6399349.

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Figures Legends:

Figure 1, Anatomical and Molecular Characteristics of DMG

A) DMG predominantly arises in the midline structures of the brain and brainstem.

B) The K27M substitution in the tail of H3 leads to a global epigenetic dysregulation.

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Figure 2, Evolution of the WHO Classification of DMG.

In 2007, DIPG/DMG was not recognized as a distinct entity. These tumors were histologically classified as glioblastomas (GBM, grade IV), anaplastic astrocytomas (AA, grade III), low-grade gliomas (primarily diffuse astrocytomas, LGG-DA, or grade II). By 2016, most cases were classified as DMG, H3K27M-mutant tumors. However, H3K27 WT DMGs remained unclassified. The 2021 WHO CNS Classification integrated all pediatric DMG cases with H3K27 alterations as including the loss of H3 K27 trimethylation.

GBM, glioblastoma; AA, anaplastic astrocytoma; LGG, low-grade glioma; DA; diffuse astrocytoma; IDHwt, IDH wild-type; NOS, not otherwise specified; DMG, diffuse midline glioma; DIPG, diffuse intrinsic pontine glioma.

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Figure 3, Somatic mutation profiling of H3.1K27M versus H3.327M DMGs.

Venn diagram summarizing common somatic mutations between DMGs driven by either H3.1K27M (blue) and H3.3K27M (orange) or both (red). Text boxes highlight effects specific to the respective driver mutation.

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Figure 4, The Hallmarks of the DMG TME.

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Cartoon schematic showcases the three major characteristics of the tumor microenvironment (TME) of diffuse midline glioma (DMG): 1) Scarce lymphocytic infiltration, 2) increased myeloid cell populations, 3) an intact blood-brain barrier (BBB), and 4) neuron–glioma interactions. Created in BioRender. Shah, A. (2025) https://BioRender.com/fc7bo2f

Figure 5, Overcoming the DMG TME.

A summary of current approaches to remodel the tumor microenvironment and improve immune response in DMG, including immunogenic priming, multi-antigen and combination immunotherapies, and techniques to bypass the blood–brain barrier for targeted delivery. Created in BioRender. Shah, A. (2025) https://BioRender.com/awne5ez

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Table Legends (for Online Supplement):

Table 1. Immune landscape by DMG molecular subtype.

Known immunological characteristics of DMG molecular subtypes.

Table 2. Recent Immune Checkpoint Blockade Trials in DIPG/DMG

* denotes a combinatorial regimen.

Table 3. Recent Adoptive Cell Transfer Trials in DIPG/DMG

* denotes a combinatorial regimen.

Table 4. Recent Vaccine Trials in DIPG/DMG

* denotes a combinatorial regimen.

Table 5. Recent Oncolytic Virus Trials in DIPG/DMG

* denotes a combinatorial regimen.

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Figure 1

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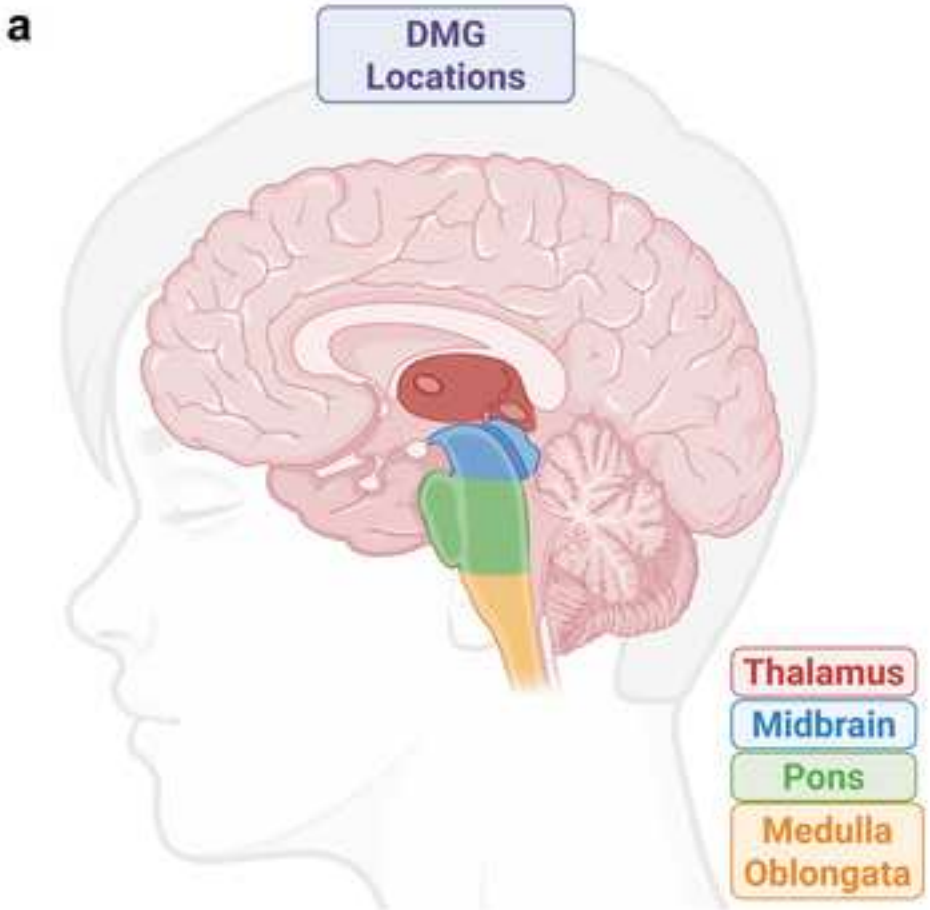

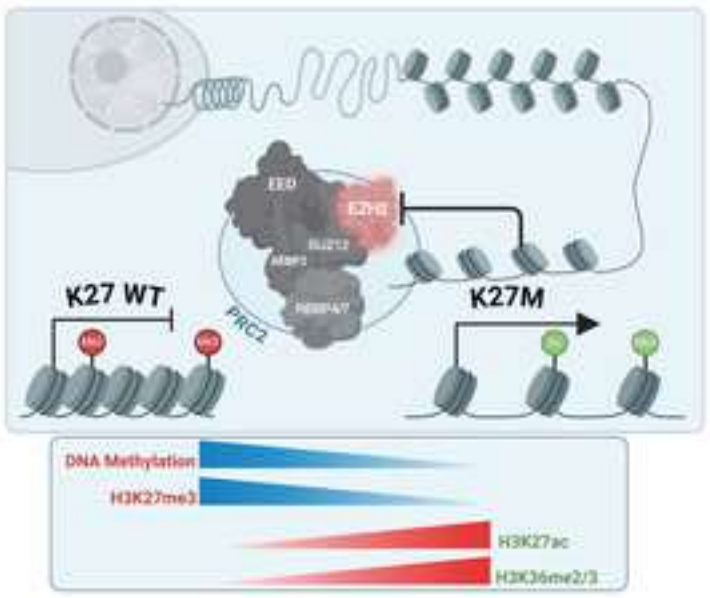

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Figure 2

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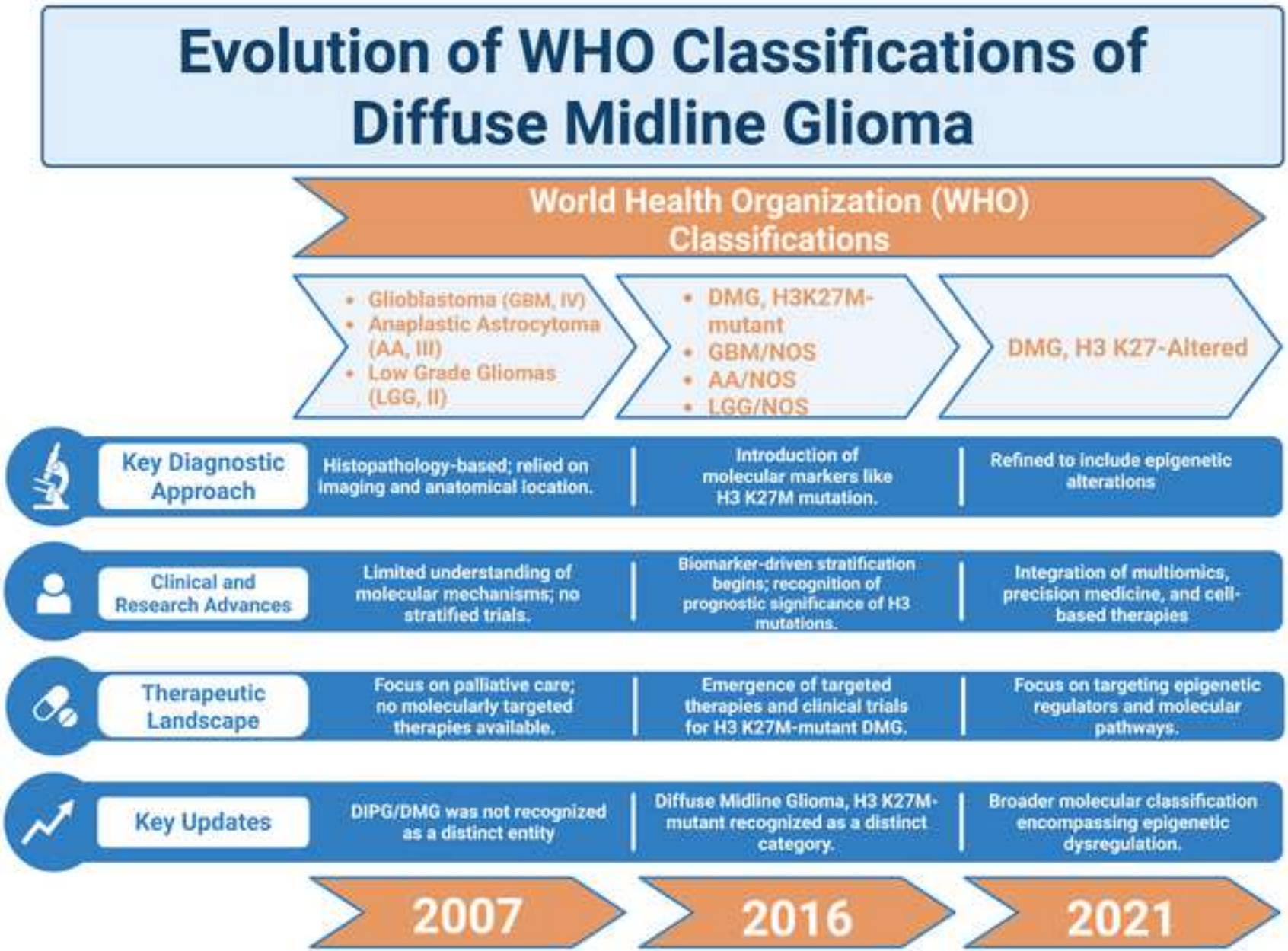

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Figure 3

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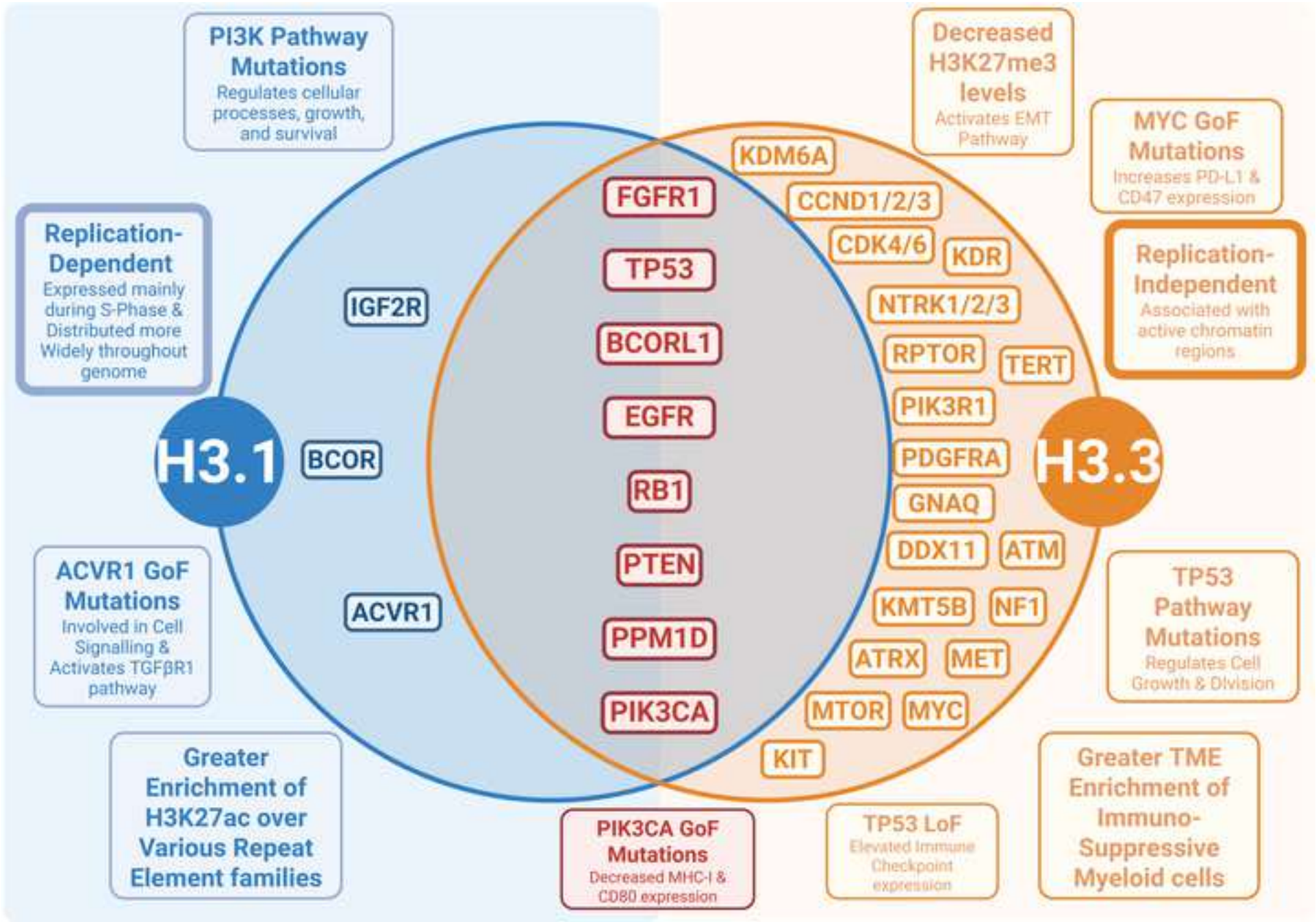

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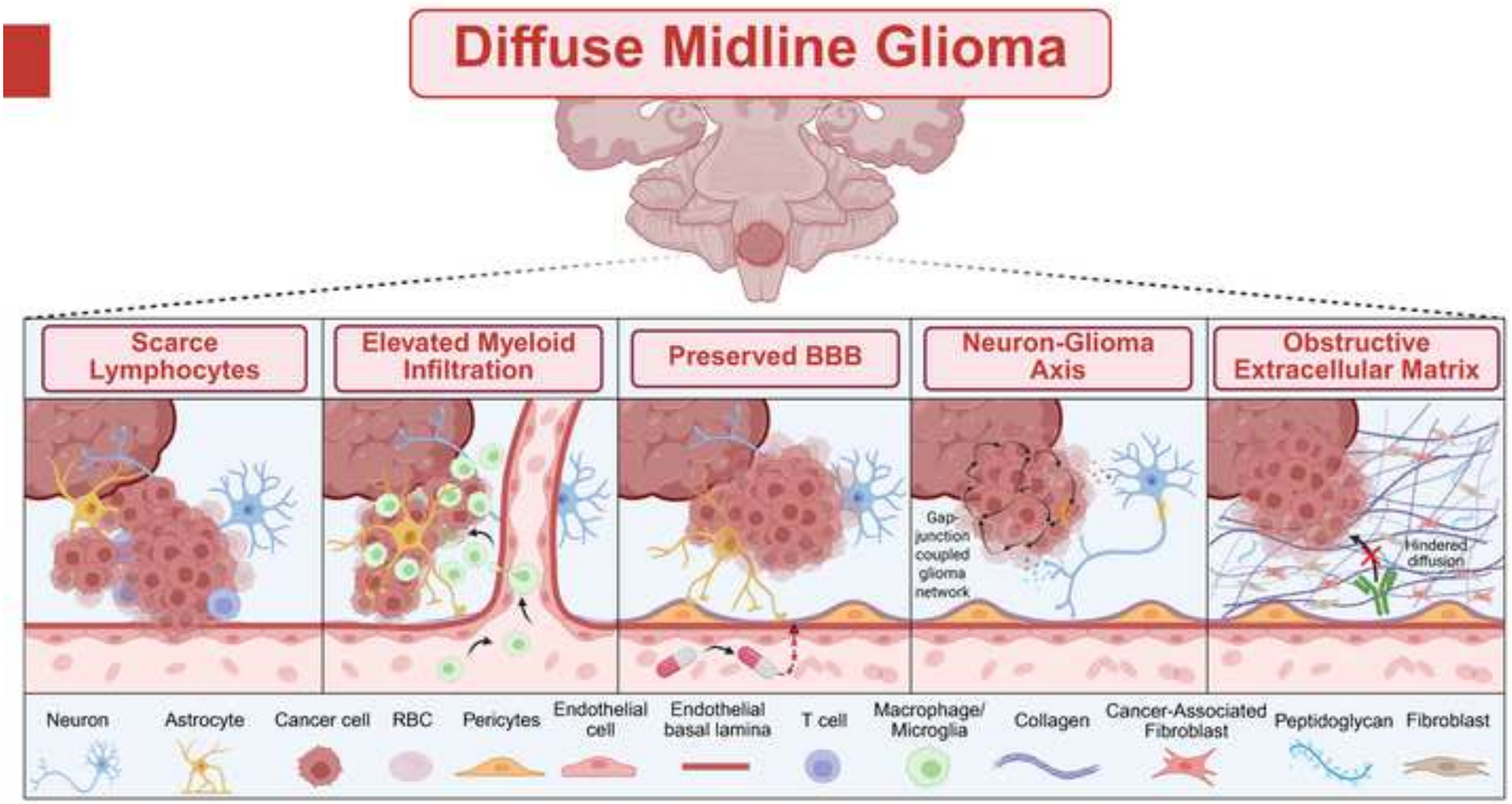

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Figure 5

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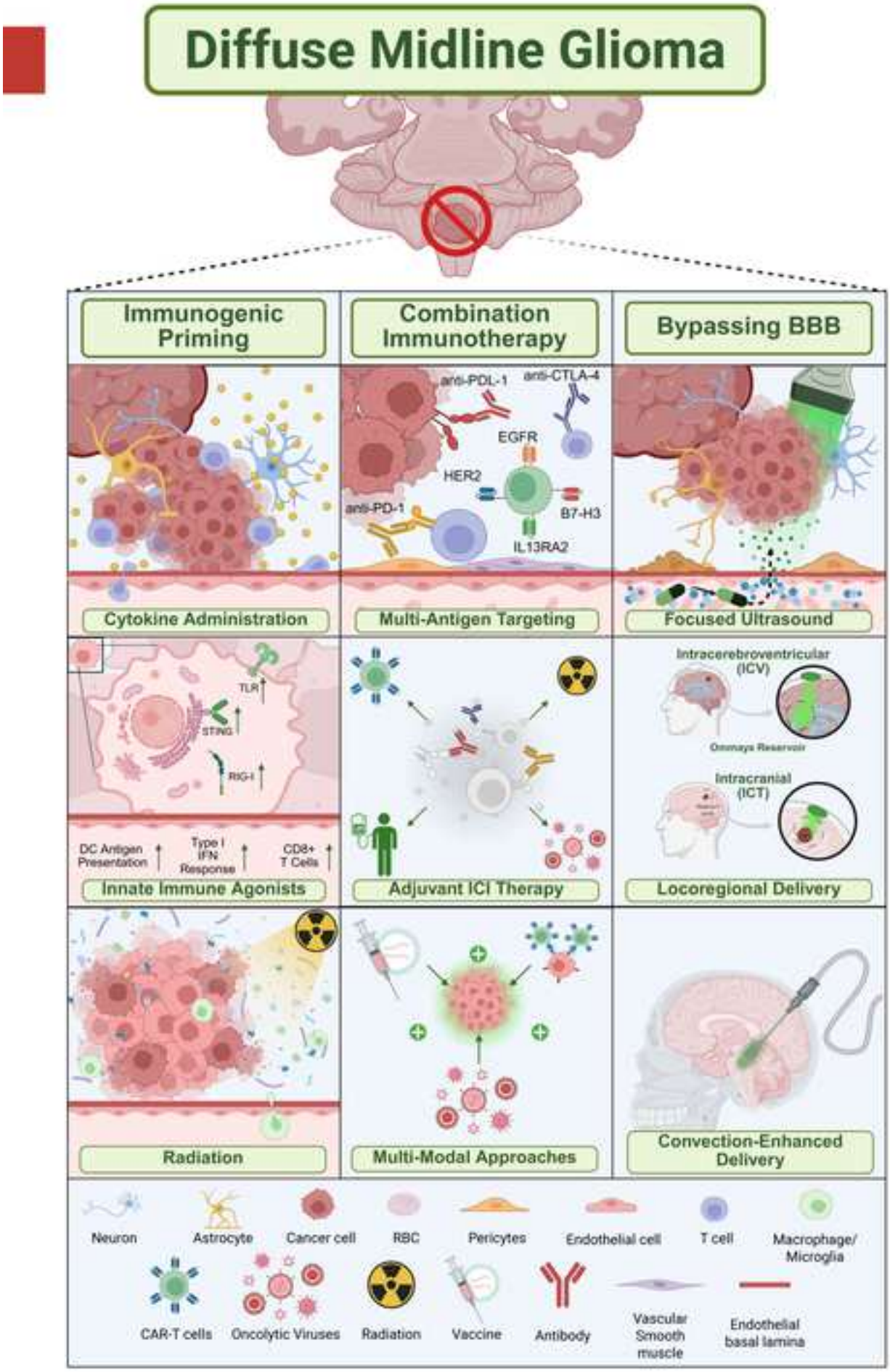

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