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Exploring the tumor microenvironment in diffuse intrinsic pontine glioma: immunological insights and therapeutic challenges

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ABSTRACT

Diffuse intrinsic pontine glioma (DIPG) is a rare and highly aggressive pediatric brain tumor with a median survival of less than 12 months. The tumor arises in the pons, making surgical resection unfeasible and limiting treatment options to palliative radiation, which offers minimal survival benefit. One of the major challenges in treating DIPG is the poorly understood tumor immune microenvironment, which has hindered the development of effective immunotherapies. DIPG tumors are considered to be immunologically cold with limited immune cell infiltration. Recent studies have begun to reveal the complex and heterogeneous immune landscape of DIPG, highlighting distinct immunological subgroups. This review aims to provide a comprehensive overview of the immune landscape of DIPG based on the latest insights, identify research gaps, and suggest potential areas for future investigation to improve treatment outcomes for patients with DIPG.

INTRODUCTION

Diffuse intrinsic pontine glioma (DIPG) is a rare but highly aggressive pediatric brain cancer that primarily affects children aged 5–7 years.[1] Despite its rarity, DIPG is the leading cause of cancer-related deaths in children, with a grim median survival of less than 12 months.[2] As a subset of diffuse midline gliomas (DMG), DIPG is located in the pons, a critical structure in the brainstem responsible for vital bodily functions. This location, combined with DIPG's diffuse and infiltrative growth pattern, makes surgical resection impossible. The current standard of care, fractionated radiotherapy, provides only temporary symptom relief, extending survival by approximately 3 months but offering no curative benefit.[3 4] While temozolomide is administered to around 44% of patients,[3] clinical studies have shown that adding temozolomide to radiotherapy does not improve overall survival or progression-free survival compared with radiotherapy alone.[5 6] Numerous other therapies have been tested, yet none have demonstrated a survival benefit. To date, over 250 clinical trials have failed to improve outcomes beyond those achieved with palliative radiation.[1]

Approximately 70–85% of DIPG cases are characterized by H3K27M alterations (lysine-to-methionine substitution at site 27 of histone 3), classifying them as "Diffuse Midline Glioma, H3 K27-altered" by the WHO in 2021.[7–9] These mutations, most commonly H3.3 (~60%) and less frequently H3.1 (~18%),[7] result in global loss of H3K27 trimethylation, disrupting normal gene regulation and impairing cell differentiation.[10 11] While the H3K27M mutation is considered a key tumor driver, it alone is insufficient for DIPG development. Common co-mutations include *TP53* mutations (75%),[12] *PDGFRA* amplifications (30%),[13] and *ACVR1* mutations (20–25%; which strongly co-segregate with H3.1 mutated tumors).[14 15]

Research indicates that DIPG arises from neural stem/progenitor cells in the developing pons, which express markers such as Sox2, nestin, and Olig2.[16] More specifically, single-cell RNA sequencing (RNA-seq) has identified oligodendroglial progenitor cells (OPCs) as the likely cell of origin for DIPG.[17–19] The presence of H3.3/H3.1K27M mutations in most DIPGs further supports this hypothesis, as these mutations are associated with global loss of H3K27 trimethylation and gain of H3K27 acetylation, which affects OPC differentiation.[20 21]

Despite significant advances in understanding DIPG genetics and biology, little is known about its tumor microenvironment (TME), a critical aspect for developing effective immunotherapy strategies. Access to tumor tissue remains limited due to DIPG's rarity, the lack of surgical resection as part

of standard treatment, and the historical reluctance to perform biopsies because of concerns about surgical risk. However, recent studies have demonstrated that stereotactic biopsies can be safely performed,[22] leading to their increasingly routine use. Despite these advances, tissue availability remains constrained in many settings. Traditional immune profiling using blood samples is inadequate for DIPG due to the presence of the blood-brain barrier, which hinders peripheral blood from accurately reflecting the immune profile within the tumor. In contrast, cerebrospinal fluid (CSF) biopsies, obtained through lumbar/ventricular puncture, offer a more direct reflection of the DIPG environment. CSF interacts with the brainstem and contains immune cells and immune-related biomarkers, such as cytokines and chemokines.[23 24] However, while CSF profiling shows promise for understanding the immune environment of DIPG,[25–27] it cannot capture immune cell infiltration within the tumor itself, and research in this area remains limited. As a result, the immune landscape of DIPG is still poorly understood.

Early investigations into immunotherapeutic approaches for DIPG, mainly immune checkpoint inhibitors, have shown disappointing results.[12 28 29] Emerging strategies, such as cancer vaccines, adoptive T-cell therapy, chimeric antigen receptor T (CAR-T) cells, and autologous cell transfer therapy, hold promise but remain in early-stage trials.[27 30 31] However, no immunotherapy has yet advanced to a phase 3 clinical trial, partly due to the limited understanding of the tumor's immune landscape, which is one of the factors that complicates the development of effective therapeutic strategies.

This review will offer a comprehensive overview of the current understanding of the TME in DIPG. It will also highlight critical gaps in knowledge and pinpoint areas requiring further investigation, aiming to deepen insights into the complex DIPG TME and pave the way for more effective immunotherapy strategies.

Immune system in the brain

The brain's immune system differs significantly from other parts of the body due to its unique protective mechanisms. Historically considered an immune-privileged site, the brain is shielded by the blood-brain barrier, lacks conventional lymphatic drainage, and exhibits limited expression of major histocompatibility complex (MHC) molecules, all of which restrict immune cell entry and antigen presentation.[32 33] However, research has revealed that activated T cells can cross the intact blood-brain barrier.[34] Furthermore, the brain is now understood to have complex interactions with the immune system through specialized niches in the choroid plexus, meninges, and perivascular spaces, allowing for remote surveillance of the brain.[35] These areas, along with the newly discovered meningeal lymphatic system and skull microchannels, provide multiple routes for brain-immune communication.[35 36]

The brain's immune system involves complex interactions between resident and peripheral immune cells, playing crucial roles in development, homeostasis, and disease.[35] Research in healthy individuals has identified six main leukocyte types in the brain, including CD4 memory T cells, macrophages, and monocytes, with their proportions varying with age.[37] Microglia, the resident brain macrophages, originate from yolk sac progenitors that colonize the brain during early development and differentiate into adult cells in situ.[38] These highly dynamic cells continuously monitor their environment to maintain cellular, synaptic, and myelin homeostasis, yet paradoxically, can also contribute to certain pathologies.[39–41] Additionally, peripheral immune cells, including monocytes and lymphocytes, contribute to brain homeostasis and repair.[35]

The brain's immune system in children is even more complex and poorly understood, particularly during early development when many pediatric brain tumors arise.[42] The developing brain possesses unique immune characteristics influenced by ongoing processes like neurogenesis, synaptic pruning, and blood-brain barrier maturation. These developmental dynamics render immune activity in children distinct from adults, with children's immune systems characterized by reduced immune cell functionality, impaired inflammatory responses, and lower antibody activity.[43] Microglia are key in shaping neural circuitry and coordinating neurodevelopmental processes.[44 45] Furthermore, innate immune signaling pathways, such as those involving toll-like receptors (TLRs), cytokines, and inflammasomes, are critical for healthy brain development, and their dysfunction has been associated with neurodevelopmental disorders.[44]

Despite these insights, our understanding of the brain's immune system in healthy children remains limited, as much of neuro-immunological research has focused on adults. This knowledge gap is particularly problematic for children with DIPG, as the lack of foundational knowledge about the pediatric brain's immune landscape complicates efforts to understand tumor-immune interactions. Investigating the immune system in the developing brain, both in health and disease, could shed light on how immune activity changes during development and how these mechanisms might be harnessed to improve treatments for pediatric conditions like DIPG.

Immune system in DIPG

DIPG tumors are typically characterized by a non-inflammatory microenvironment and a low mutational burden, making them immunologically "cold" and challenging to target with immune-based therapies. However, recent findings reveal that DIPG is not a uniform disease but instead displays significant interpatient variability in its immune landscape.[12] Studies have identified distinct immunological subgroups within DIPG, providing valuable insights into potential therapeutic strategies. A comprehensive classification of immune profiles across various cancer types identified six immune subtypes:

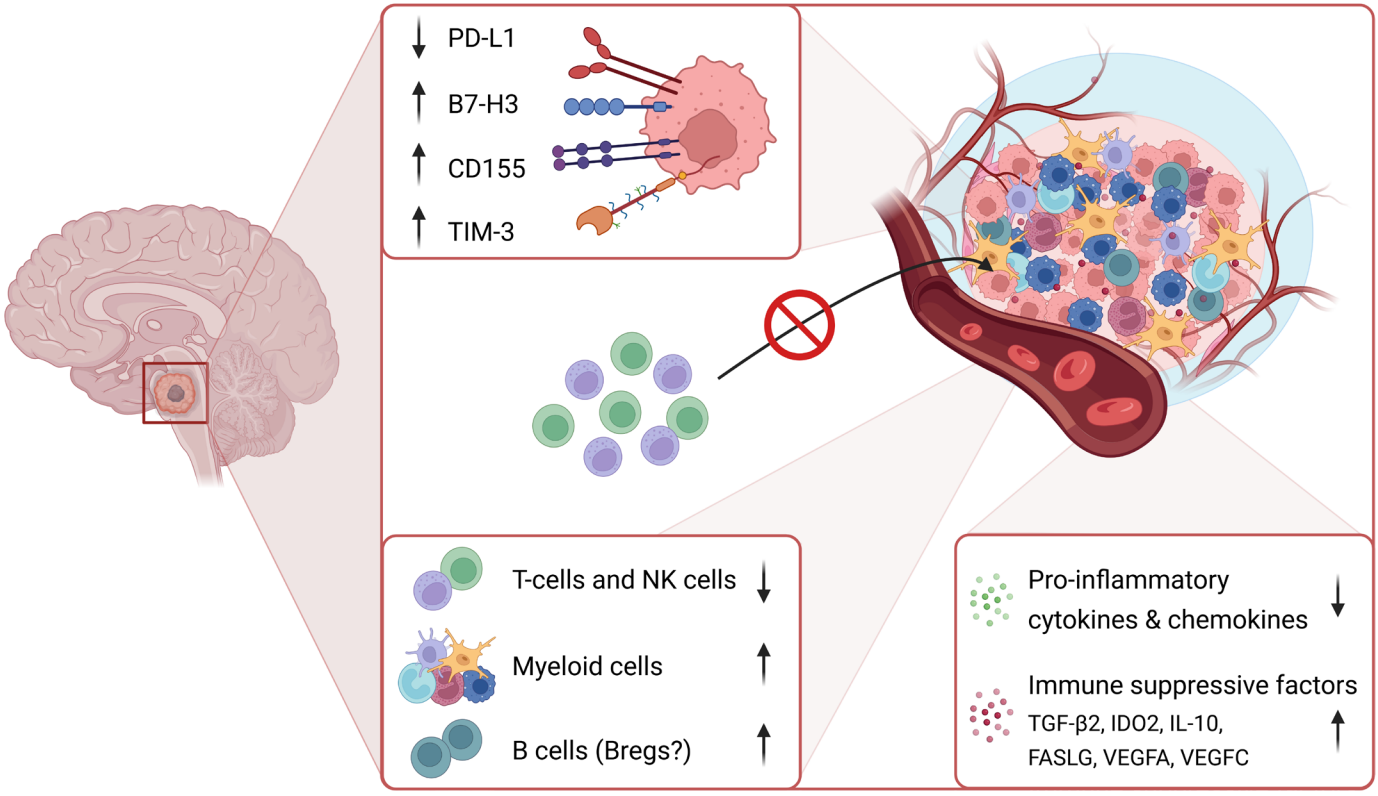

Figure 1 Immune profile of the DIPG tumor microenvironment. DIPG is classified as a "cold tumor", marked by minimal immune cell infiltration and low levels of inflammatory factors. The immune infiltrates are predominantly composed of myeloid cells, while T cells and natural killer cells are notably absent. DIPG tumors exhibit higher levels of immunosuppressive factors, including transforming growth factor beta 2 (TGF-β2), indoleamine 2,3-dioxygenase 2 (IDO2), interleukin-10 (IL-10), Fas ligand (FASLG), vascular endothelial growth factor A (VEGFA), and vascular endothelial growth factor C (VEGFC), alongside increased expression of immune checkpoint molecules B7-H3, CD155, and T-cell immunoglobulin and mucin domain-containing protein 3 (TIM-3). Bregs, regulatory B cells; DIPG, diffuse intrinsic pontine glioma; NK, natural killer; PD-L1, programmed death-ligand 1.

wound healing (C1), interferon (IFN)-gamma dominant (C2), inflammatory (C3), lymphocyte-depleted (C4), immunologically quiet (C5), and transforming growth factor-beta 2 (TGF-β) dominant (C6).[12 46] Building on this framework, Zhu *et al* categorized DIPG tumors into three immune subgroups: lymphocyte-depleted (50%), immunologically quiet (14%), and inflammatory (14%).[47] While most DIPG tumors belong to the "cold" categories—either lymphocyte-depleted or immunologically quiet—a small subset displays a more inflammatory profile with a higher mutational burden, potentially making them more susceptible to immunotherapy.[47]

Lymphocytes

T cells

Recent studies on DIPG reveal a unique TME characterized by low lymphocyte infiltration[48–50] (figure 1), with no increase in $CD8^+$ T cells compared with healthy controls.[51] This observation holds true for both early post-mortem DIPG autopsy samples and pretreatment diagnostic biopsies, indicating that tumor-infiltrating lymphocytes are not a major factor in DIPG pathophysiology at any stage.[48]

Despite robust MHC class I expression, the presence of $CD8^+$ T-lymphocytes in DIPG is rare, especially when compared with adult glioblastomas (GBMs).[48] In fact, $CD3^+$ T lymphocytes represent only 1.72–2.65% of total $CD45^+$ leukocytes in DIPG, compared with 7.09–50.2% in GBM.[48]

Interestingly, analysis of raw RNA-seq data from patients using the CIBERSORT algorithm revealed that while $CD8^+$ T-cell infiltration is associated with improved survival outcomes in hemispheric pediatric high-grade gliomas (pHGGs), it correlates with significantly poorer survival in DIPG.[52] The mechanisms underlying this unexpected observation are yet to be determined. Furthermore, a study using CAR (chimeric antigen receptor) T cells demonstrated that immune synapse quality is impaired in DIPG. Specifically, calcium flux, a crucial signal for T-cell activation, was significantly reduced, and the recruitment of lysosomes to the immune synapse was diminished. This impairment led to reduced CAR-T cell activation and effector function, suggesting that DIPG tumors suppress immune responses at the immune synapse level.[53] While it remains unexplored whether similar dysfunction affects natural cytotoxic T cells, it is plausible that the limited lymphocytes present in DIPG are also unable to be activated due to this immune synapse dysfunction, an area that warrants further investigation.

Moreover, CIBERSORT analysis showed that DIPG exhibits higher levels of $CD4^+$ regulatory T cells compared with hemispheric HGGs.[52] While these cells are positively prognostic in hemispheric pHGGs, they do not provide a survival benefit in DIPG.[52]

Future research should focus on elucidating the mechanisms responsible for low lymphocyte infiltration and impaired T-cell activation in DIPG. The paradoxical correlation between increased $CD8^+$ T-cell presence and poor survival in patients with DIPG suggests a unique immune response that requires deeper investigation.

Natural killer cells

Recent studies have explored the role of natural killer (NK) cells in DIPG, revealing significant differences compared with other pediatric brain tumors. In the peripheral blood of patients with DIPG, NK cells are significantly reduced compared with healthy controls.[54] Furthermore, when compared with pediatric HGG, DIPG shows a lower infiltration of NK cells. CIBERSORT analysis on RNA-seq patient data revealed that NK cell infiltration is linked to better survival outcomes in pediatric hemispheric HGGs, but this correlation does not extend to patients with DIPG.[52]

Despite the overall scarcity of NK cell infiltration in DIPG, studies have demonstrated that NK cells can still be effective against DIPG cells in vitro. Lieberman *et al* found that NK cells outperformed T cells when directly in contact with DIPG cell cultures, likely due to the activation of NKG2D ligands, which trigger cytokine release and cytotoxic responses.[51] Furthermore, a recent study assessed the antitumor activity and safety of disialoganglioside (GD2)-CAR NK-92 cells in DIPG. In vitro, GD2-CAR NK-92 cells effectively killed high GD2-expressing DIPG cells, and in vivo, they inhibited tumor growth and prolonged survival in patient-derived xenograft mice. However, the study used severely immunodeficient NCG mice, which are far from an ideal model to study immune-based therapies.[55]

Traditionally, immunotherapy has focused primarily on activating T cells to target tumors. Recent results from phase 1 clinical trials of B7-H3 CAR T cells and GD2 CAR T-cell therapy have shown promising outcomes for patients with DIPG.[27 31] While these results are encouraging, research suggesting that NK cells are more effective than T cells in tumor killing,[51] combined with the success of CAR NK cell therapies in DIPG models, highlights the potential of NK cell-based therapy as an alternative or complementary approach. However, research on CAR NK cells is still in earlier stages compared with CAR T cells. Before NK cell-based therapies can be fully integrated into treatment strategies, further research is needed to understand the limitations of NK cell infiltration in DIPG and why increased infiltration does not correlate with improved survival. Understanding these mechanisms and identifying ways to overcome these barriers will be crucial for optimizing NK cell-based therapies for DIPG.

B cells

Increased levels of B cells have been observed in DIPG samples, both at initial diagnosis and during disease progression.[54 56] Notably, B-cell levels in progressive DIPG are approximately three times higher compared with those at initial diagnosis.[56] The reason for this increase remains unclear, but it may be related to the expansion of regulatory B cells (Bregs), a subpopulation of B cells that play a suppressive role in the immune system by producing anti-inflammatory cytokines such as interleukin (IL)-10 and TGF-β.[57 58] These cytokines are also upregulated in the DIPG TME, making this a plausible explanation for the observed increase in B-cell levels.[52 59] However, identifying Bregs is challenging due to the lack of unique markers for this cell population.[57 60] Therefore, the role of B cells in DIPG warrants further investigation to better understand their contribution to tumor progression and immune evasion.

Myeloid cells

In DIPG tumors, the myeloid component is notably abundant (figure 1).[50 61] Flow cytometry studies by Lin *et al* revealed that the percentage of $CD11b^+$ myeloid cells is significantly higher in DIPG (94.8%) than in adult GBM (70.3%).[48] These myeloid cells are believed to play a crucial role in tumor growth, progression, and immunosuppression.[50 61 62] Specifically, macrophages and microglia have been identified as abundant populations within the DIPG TME,[2] both in post mortem and primary DIPG tumor tissue.[50 63]

DIPG tissue shows an increased presence of $CD45^+Iba1^+$ microglia, which exhibit an activated morphology with shorter processes and enlarged cell bodies compared with non-tumor control microglia.[48] Furthermore, these microglia show a significant downregulation of tumor necrosis factor (TNF) signaling, immune responses, and complement pathways, suggesting a shift toward a more immunosuppressive state.[50] Extending beyond DIPG, a recent study by Ross *et al* on DMG, including both pontine and thalamic tumors, identified four predominant microglial clusters: proliferating, homeostatic, IFN-responsive, and disease-associated microglia.[64] Notably, H3.3K27M-mutant DMGs had the highest proportion of disease-associated microglia (characterized by high expression of genes involved in glycolysis, oxidative phosphorylation, adipogenesis, and MTORC1 signaling) as well as proliferating microglia. These findings highlight the diversity of microglial populations in DMG, not limited to DIPG, and suggest that the H3.3K27M mutation may promote a more metabolically active and immunosuppressive microenvironment.[64]

Macrophages have traditionally been classified into M1 and M2 phenotypes, with M1 macrophages associated with antitumor immunity and M2 macrophages linked to protumorigenic functions. However, this binary classification fails to capture the full diversity of macrophage phenotypes, as studies show that tumor-associated macrophages,[65] including glioma-associated macrophages,

exhibit a spectrum of M1 and M2-like characteristics.[48 66] In line with this, analysis of DIPG-associated macrophages by Lin *et al* found no significant enrichment for either M1 or M2 gene signatures, indicating that these macrophages do not conform to the traditional M1/M2 classification.[48] Additionally, while CD163$^+$ macrophages, a marker for protumorigenic M2-like macrophages, are elevated in other pediatric gliomas—such as pediatric low-grade glioma (pLGG) and pHGG—DIPG samples do not show this increase. Lieberman *et al* observed a 10.4-fold and 5.9-fold increase in CD163$^+$ macrophages in pLGG and pHGG, respectively, compared with controls, whereas DIPG does not exhibit similar changes.[51] This suggests that DIPG does not effectively repolarize macrophages towards a more immunosuppressive phenotype.

Further refining this, Ross *et al* identified three predominant macrophage clusters in DMG (including both pontine and thalamic tumors), rather than the traditional M1/M2 polarization: pro-inflammatory, disease-associated, and proliferating macrophages.[64] Notably, H3.3K27M DMGs had the lowest proportion of pro-inflammatory macrophages (enriched for genes related to antigen presentation, IFN signaling, and TNF-α pathways) and the highest proportion of proliferating and disease-associated macrophages (showing reduced expression of MHC class II genes and enrichment for hypoxia-glycolysis and IL-2/Signal transducer and activator of transcription 5 (STAT5) signaling pathways). This reinforces the idea that the H3.3K27M mutation contributes to a more immunosuppressive environment in both microglia and macrophages in DMGs, including DIPG.[64] These findings highlight the unique macrophage landscape within DIPG, but further research is needed to characterize the full spectrum of macrophage activation states. Intermediate subtypes (M2a, M2b, M2c, M2d), each with distinct immunoregulatory properties, have been identified in other cancers, underscoring the need for deeper investigation into macrophage heterogeneity in DIPG.

Furthermore, when compared with pediatric hemisphere HGG, DIPG shows higher proportions of eosinophils, activated dendritic cells (DCs), and neutrophils, suggesting a distinct myeloid composition in the DIPG TME.[52] While activated DCs and eosinophils are positively associated with prognosis in hemisphere HGG, they do not show a significant correlation with prognosis in DIPG. In contrast, neutrophils are negatively associated with prognosis in both hemisphere HGG and DIPG.[52]

Myeloid-derived suppressor cells (MDSCs)—(mainly) immature myeloid cells known to play a critical role in cancer progression by suppressing immune responses—warrant particular attention.[67 68] In various cancer types, MDSCs not only suppress immunity but also promote tumor angiogenesis, cancer stemness, and metastasis.[69] Studies have shown that GBM exposure can reprogram normal monocytes to acquire MDSC-like properties, such as elevated production of immunosuppressive cytokines and the ability to induce apoptosis in activated lymphocytes.[70] Furthermore, the density of MDSCs in gliomas correlates positively with tumor grade and negatively with patient survival.[71 72] Despite these insights from other gliomas, the role of MDSCs in DIPG remains largely unexplored. One study by Mueller *et al* demonstrated that lower circulating MDSC levels (CD33$^+$ CD11b$^+$ CD14$^+$ HLA-DR^{low}) in patients with DIPG are prognostic of better outcomes, including stronger CD8$^+$ T-cell responses and prolonged survival,[12 26] but their presence within the tumors was not examined. A recent study identified MDSCs in H3.3K27M DIPG mouse models, where they accounted for approximately 19% of CD45$^+$ cells,[50] highlighting their potential significance in the TME. Given their critical role in immune evasion and tumor progression in other cancers, further investigation into their function in DIPG could provide key insights into tumor immunity and uncover new therapeutic targets.

Given the diverse and immunosuppressive roles of myeloid cells in the DIPG TME, future research should prioritize understanding and strategically targeting these populations to improve therapeutic outcomes. Modulating myeloid cells—such as microglia, macrophages, and MDSCs—may help overcome immune suppression and enhance the effectiveness of current and emerging treatments. Ongoing research by Mishra *et al* is examining the specific phenotypes and functions of myeloid populations, evaluating changes in the proportions of myeloid cell subsets using flow cytometry (CD11b, Ly6c, Ly6G, MHC-II, F4/80, CD206, Arg1) in the bone marrow, peripheral blood, and TME throughout DIPG tumor progression. While results are still pending, the study is expected to yield important insights into the role of these cells in disease progression and offer guidance on optimal timing and approaches for therapeutic intervention.[61]

Immune checkpoint expression

Recent studies have provided new insights into immune checkpoint expression in DIPG. Unlike other tumor types, programmed death-ligand 1 and cytotoxic T-lymphocyte antigen 4 expression is not elevated in DIPG.[47 51 61] Instead, CD155 and B7-H3 (CD276) were identified as highly expressed checkpoint molecules on DIPG cells, with CD155 regulating both immune evasion and tumor growth.[73] Although the exact function of B7-H3 in DIPG remains unclear, it has been implicated in promoting tumor invasion and metastasis in several other cancers, including prostate, colon, pancreatic, renal, ovarian, and bladder cancers.[74] Moreover, Zhou *et al* demonstrated a correlation between B7-H3 expression and the malignancy grade of brainstem gliomas.[75] In patient-derived DIPG cultures, CAR T cells targeting B7-H3 produce IFN-γ, IL-2, and TNF-α, leading to tumor cell killing.[76] Although B7-H3 is expressed 2.4 times higher in DIPG compared with non-tumor tissue, its levels remain significantly lower than those observed in GBM cells.[49 51]

Additionally, T-cell immunoglobulin and mucin-domain containing-3 (TIM-3) is highly expressed on both DIPG tumor cells and within the TME, mainly on microglia/

macrophages.[2] TIM-3 acts as a negative regulator in monocytes and macrophages during innate immune responses, and its inhibition in microglia reduces the production of inflammatory factors.[77] In mouse models, TIM-3 inhibition has been shown to significantly extend survival. This effect was accompanied by an increase in both the number and the proliferative state of microglia, NK cells, DCs, and $CD8^+$ T cells, as well as elevated levels of IFN-γ, granzyme B, and TNF-α, indicative of activated NK and T-cell phenotypes.[2] This further emphasizes the crucial role of macrophages in DIPG.

The immune checkpoint profile in DIPG differs significantly from other cancers, necessitating a tailored approach to immunotherapy for this tumor type. Previous immune checkpoint inhibitor strategies were based on GBM rationales and have failed to improve survival in DIPG.[12 28 29] Instead, immune checkpoints such as CD155, B7-H3, TIM-3 and CD47 appear to play a more critical role in DIPG, suggesting that future immunotherapy should focus on these targets. However, further research into these immune checkpoint inhibitors is essential to understand their roles and mechanisms in DIPG progression before advancing to clinical trials.

Cytokine and chemokine profile

The TME of DIPG is characterized by low expression of inflammatory cytokines and chemokines by tumor cells, resulting in a low inflammatory environment.[48 61] At the transcriptional level, patient-derived DIPG cell cultures show little to no expression of cytokine genes, expressing only a limited array of chemokines and growth factors that may play a role in immune cell infiltration within the tumor.[48 78] One example is IL-2, a key cytokine whose reduced expression in DIPG may contribute to the lack of T-lymphocyte infiltration seen in DIPG.[48 78] Additionally, immune cells in the DIPG microenvironment, such as macrophages, secrete lower levels of cytokines and chemokines—including IL-6, IL-1A, IL-1B, CCL3, and CCL4—compared with macrophages in adult HGG, further contributing to the low inflammatory nature of the tumor.[48] However, recent clinical data suggest that locoregional immunotherapy can partially overcome this immunosuppressive milieu. In the phase 1 clinical trial of B7-H3-directed CAR T cells delivered intraventricularly in patients with DIPG (NCT04185038), serial CSF samples revealed post-infusion increases in several key cytokines, including IFN-γ, granulocyte-macrophage colony-stimulating factor, and CXC motif chemokine ligand 10.[27] These elevations indicate active CAR T-cell engagement and cytotoxicity within the CNS compartment, despite DIPG's baseline low-inflammatory state. These findings demonstrate that while DIPG typically lacks a proinflammatory environment, therapeutic interventions such as CAR T-cell therapy can transiently induce inflammatory signaling within the TME, potentially enhancing antitumor immune responses.

The expression of TGF-β2, an immune suppressive cytokine, is elevated in DIPG compared with normal tissue and low-grade gliomas, reaching levels similar to those seen in pediatric GBM.[59] This is consistent with the fact that TGF-β2 is frequently expressed by tumor-associated macrophages, which are abundant in DIPG tumors.[79] Since members of the TGF-β family are known to suppress NK cell function, these elevated TGF-β2 levels may contribute to immune evasion in DIPG.[80] Supporting this, Uckun *et al* found higher levels of TGF-β2 in newly diagnosed DIPG tumor samples compared with control pons samples.[81] TGF-β2 was identified as a poor prognostic indicator for overall survival, with high levels correlating with significantly shorter survival rates.[81 82] Conversely, TGF-β2 messenger RNA levels did not serve as a poor prognostic marker for patients with pediatric GBM or for patients with pediatric DMG with tumors outside the pons/brainstem. Additionally, Quasi *et al* discovered that patients with high TGF-β2 expression combined with low interferon gamma receptor 2 (IFNGR2) expression had the worst survival outcomes. These findings suggest that targeting TGF-β2, particularly in tumors with low IFNGR2 expression, could offer a promising therapeutic approach.[82] Notably, recent findings from the phase 1 clinical trial (NCT04196413) involving GD2-CAR T cells in patients with DIPG revealed that elevated TGF-β1 levels in CSF early after infusion were associated with disease progression, suggesting that TGF-β signaling may limit the efficacy of the immunotherapy.[31]

Furthermore, IL-8 expression is significantly elevated in DIPG compared with non-tumor tissue.[48 51] IL-8, also known as neutrophil chemotactic factor, is associated with promoting cell proliferation, invasion, and vascular mimicry—a process in which tumor cells drive the formation of new blood vessels—in GBM.[83 84] Further research is required to elucidate the specific role of IL-8 in DIPG and evaluate its potential as a therapeutic target.

RNA-seq data analysis of immunosuppressive gene expression and cytokine secretion in DIPG revealed higher levels of indoleamine 2,3-dioxygenase 2 (IDO2), IL-10, Fas ligand, IL-6, vascular endothelial growth factor A, and vascular endothelial growth factor C in K27M patient with DIPG samples compared with wildtype hemispheric HGG samples. Interestingly, in DIPG, IL-10 and IDO2 were significantly correlated with improved survival.[52]

The absence of inflammatory mediators in DIPG poses a challenge for the development of immunotherapies, as the tumor lacks the chemokines and inflammatory cytokines required to recruit immune cells to the TME. Nonetheless, recent CAR T-cell studies have shown that it is possible to induce localized immune activity within the DIPG TME, offering hope for future strategies aimed at reshaping the immune landscape to enhance therapeutic efficacy. However, further research into the TME—particularly the role of immunosuppressive factors—is essential and may uncover new opportunities for innovative treatment approaches.

Cancer-associated fibroblasts

Cancer-associated fibroblasts (CAFs) play a pivotal role in shaping the immune landscape within the TME. As key regulators of cancer progression, CAFs are among the most abundant cellular components in the TME across various cancer types.[85] They contribute to immune suppression through various mechanisms, including the secretion of cytokines, chemokines, and metabolites.[86 87] CAFs facilitate T-cell exclusion and exhaustion, induce protumoral phenotypic shifts in macrophages and neutrophils, and support tumor progression through multiple pathways.[88–90] Additionally, CAFs physically impede immune cell infiltration through extracellular matrix production.[85 87]

Recent research has identified CAFs in GBM, challenging previous assumptions about their absence in brain tumors. Single-cell RNA-seq and spatial transcriptomics confirmed the presence of CAFs in patient GBMs, particularly in the subventricular zone.[90] CAFs were found to promote tumor growth, induce immunosuppression, and contribute to therapeutic resistance.[90–92]

Despite their well-established role in other cancers, CAFs remain unexplored in the context of DIPG. Given the increasing interest in targeting the extracellular matrix as a therapeutic strategy, investigating the role of CAFs in DIPG could provide valuable insights into the tumor's immune dynamics and uncover potential therapeutic targets.

Tertiary lymphoid structures

Recent research has highlighted the importance of tertiary lymphoid structures (TLS) in cancer. TLS are ectopic lymphoid aggregates that form at sites of chronic inflammation, including tumors.[93] They play a crucial role in orchestrating local and systemic antitumor immune responses, with high TLS densities correlating with improved patient survival in various cancer types.[93 94]

TLS are particularly intriguing in the context of DIPG, where tumor-infiltrating lymphocytes are inefficient, and the blood-brain barrier further limits immune cell access. In adult-type diffuse gliomas, Cakmak *et al* identified TLS in 15% of tumors using a multimodal approach that combined RNA-seq with spatial transcriptome and proteome profiling. The presence of TLS correlated with improved overall survival and was associated with a remodeled perivascular space characterized by transcriptional upregulation and spatial redistribution of collagens linked to barrier functions. TLS maturation into sites of dynamic adaptive immune responses, including clonal T and B-cell expansion and plasma-cell formation, was driven by effective early T-cell recruitment to the perivascular space.[95] Further research categorized gliomas into three subtypes based on TLS gene expression profiles, revealing notable prognostic differences and underscoring the heterogeneity of TLS in gliomas.[96]

For pediatric DIPG, TLS remain unexplored but represent a promising therapeutic target. Strategies to promote TLS formation, such as chemokines, cytokines, antibodies, and cancer vaccines, have shown potential in enhancing antitumor immunity.[97 98] In glioma-bearing mice, TLS formation was successfully induced by intracranial administration of TLR agonists (OK-432, TLR2/4/9 agonist) and glioma antigens (ie, αTLR-mix).[99] This approach increased lymphocyte infiltration within the glioma microenvironment and improved prognosis. Notably, post-TLS formation, $CD4^+$, and $CD8^+$ T cells—but not $CD19^+$ B cells—were identified as key contributors to anti-glioma immunity.[99] These findings suggest that inducing TLS in the brain is feasible and warrants further investigation in DIPG, where TLS could unlock novel therapeutic opportunities.

CONCLUSION

DIPG remains a highly aggressive pediatric brain tumor with a dismal prognosis and limited treatment options. Despite advances in understanding the genetic and molecular landscape of DIPG, the TME remains poorly understood. DIPG's non-inflammatory TME presents significant challenges for immunotherapy, with low lymphocyte infiltration and a high myeloid cell presence contributing to its immunologically "cold" profile. To address this, future research should prioritize comprehensive profiling of the pediatric immune system in both health and disease to better understand the immune interactions specific to DIPG. Additionally, exploring the mechanisms that regulate myeloid cell function, immune checkpoints, and cytokine signaling in DIPG will be crucial. Furthermore, newer concepts in cancer biology, such as the role of CAFs and TLS, should be investigated in the context of DIPG, as they have been shown to be pivotal in other cancers. Advancing these areas of research will provide the foundation for innovative immunotherapies that could significantly improve outcomes for patients with DIPG.

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