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Review

The Medulloblastoma Microenvironment: Developmental Niches, Cellular Crosstalk and Therapeutic Opportunities

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Simple Summary

Medulloblastoma is the most common malignant brain tumour of childhood and comprises several molecular subgroups with different biological and clinical behaviour. Tumour cells do not act in isolation but interact continuously with their surrounding microenvironment, including immune cells, astrocytes, blood vessels, the choroid plexus and cerebrospinal fluid. These interactions can influence tumour growth, spread through the cerebrospinal fluid, response to treatment and relapse. In this review, we summarise recent advances in understanding how these different microenvironmental components shape medulloblastoma biology and how their effects vary between tumour subgroups and anatomical niches. We also discuss emerging spatial and experimental models that allow these interactions to be studied more accurately. Finally, we consider how features of the tumour microenvironment may contribute to disease monitoring and prognosis, and how targeting immune, vascular, metabolic and astrocyte-related pathways could provide new therapeutic opportunities. A better understanding of these tumour–microenvironment interactions may help guide more precise and less toxic treatments for children with medulloblastoma.

Abstract

Medulloblastoma is increasingly best understood not simply as a cell-autonomous embryonal tumour, but as a family of molecularly and anatomically distinct diseases shaped by the developing cerebellum and posterior fossa. The four principal molecular subgroups—WNT, sonic hedgehog, Group 3 and Group 4—differ not only in genetics and outcome, but also in barrier permeability, immune composition, stromal crosstalk and routes of dissemination. Histopathological variation likewise reflects differences in tumour architecture, neuronal maturation and cytological aggressiveness that intersect with, but are not reducible to, molecular subgroup. This review takes a microenvironment-centred view of medulloblastoma biology. It asks how non-malignant cells, fluid compartments and physical interfaces—including immune cells, the choroid plexus and cerebrospinal fluid, the blood–brain and blood–tumour barriers, astrocytes and experimental model systems—modify tumour initiation, progression, therapy response and relapse. The central argument is that the medulloblastoma tumour microenvironment is not merely sparse or passive; instead, pathological, spatial and molecular analyses of human tumours, complemented by models that preserve the relevant niche, reveal a developmentally patterned, subgroup-specific and therapeutically tractable ecosystem.

Keywords: medulloblastoma; transcriptomics; tumour microenvironment; immune network; cerebrospinal fluid; astrocytes

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1. Introduction

Medulloblastoma (MB) is the most common malignant brain tumour of childhood and arises predominantly in the posterior fossa, with most cases presenting in young children. Contemporary diagnosis integrates molecular subgrouping—WNT-activated, sonic hedgehog (SHH)-activated, Group 3 and Group 4—with histopathological patterns comprising classic, desmoplastic/nodular and MB with extensive nodularity (MBEN), as well as large-cell/anaplastic morphology [1]. This classification has refined risk stratification: WNT tumours generally have excellent outcomes, whereas metastatic disease, MYC-amplified Group 3 tumours, SHH tumours with TP53 alteration and recurrent MB remain major therapeutic challenges. Standard treatment still relies on maximal safe resection followed, in children old enough to tolerate it, by craniospinal irradiation and multi-agent chemotherapy; infants and very young children are often treated with radiation-sparing approaches because of the vulnerability of the developing brain [2]. Although this multimodal strategy cures many patients, it does so at a substantial cost, including neurocognitive decline, endocrinopathy, hearing loss, impaired growth and other lifelong late effects. These limitations have driven efforts to move beyond tumour cell-intrinsic risk markers toward therapies that are more biologically selective and less damaging to children. In this context, recent insights into the MB tumour microenvironment (TME), including immune, astroglial, vascular, ventricular, meningeal, cerebrospinal fluid (CSF) and extracellular matrix niches, offer a framework for understanding progression and relapse, and for developing targeted interventions that may complement or de-intensify existing treatment. This review begins with the immune compartment, where subgroup- and niche-specific organisation has become most clearly defined.

2. The Immune Landscape of Medulloblastoma

MB, overall, has traditionally been regarded as an immunologically "cold" tumour, reflecting its relatively sparse lymphocyte infiltration and limited evidence of active anti-tumour immunity [3]. However, more recent subgroup-aware transcriptomic, pathological and single-cell studies have refined this view, revealing a sparse but heterogeneous immune landscape whose lineage composition and activation state vary with molecular subgroup and anatomical niche [4,5]. Although leukocytes remain relatively scarce compared with adult high-grade gliomas, transcriptomic "deconvolution," meaning extraction of cell-type signatures from bulk RNA using tools such as MCP-counter [6] and CIBERSORT [7], establishes a reproducible comparative pattern: SHH tumours are the most myeloid-rich, WNT tumours share part of that myeloid signal, Group 4 harbours the clearest cytotoxic-T-cell footprint, whereas Group 3 remains comparatively immune-poor (5). Numbers alone, however, do not reveal functional polarity, that is, whether a given infiltrate restrains or fuels tumour growth. A synthesis from the Wechsler–Reya laboratory identified six major barriers to effective anti-tumour immunity and, consequently, to successful immunotherapy in MB [8]: (i) an intact blood–brain barrier outside WNT tumours that limits lymphocyte trafficking, (ii) therapy-induced lymphopenia from craniospinal irradiation, alkylators and corticosteroids, (iii) intrinsically low tumour mutational burden that yields few neo-epitopes, (iv) frequent loss of human leucocyte antigen class 1 (HLA-I) and antigen-processing components, (v) secretion of immunosuppressive cytokines such as TGF-β, IL-10 and CCL2, and (vi) myeloid skewing toward PD-L1-expressing, tumour-supportive states observed in mouse models.

The vascular barrier is an important component of this immune-exclusion programme [8]. As discussed in detail below, WNT medulloblastomas remodel neighbouring endothelium into a plasmalemma vesicle-associated protein (PLVAP)-positive, fenestrated phenotype, whereas SHH, Group 3 and Group 4 tumours generally retain a more blood-

brain barrier (BBB)-like vascular state [9]. This genotype-dependent blood–tumour barrier is likely to influence immune-cell access as well as drug penetration [8,9]. However, vascular permeability alone is insufficient to generate a lymphocyte-rich microenvironment, since WNT tumours do not show the strongest cytotoxic T-cell signature [4,5]; chemokine signalling and antigen presentation therefore remain additional determinants of effective anti-tumour immunity [8].

Histopathological analysis of tumour tissue confirms the deconvolution findings. In more than fifty primary samples, SHH-MB tumours contain dense clusters of CD163 and CSF1R^{+} tumour-associated macrophages (TAMs), whereas the other subgroups are comparatively myeloid poor [10]. Bulk macrophage abundance, measured simply by counting CD163^{+} or CSF1R^{+} cells, does not correlate with progression-free or overall survival, even though SHH tumours contain far more of these cells than the other subgroups. This lack of prognostic power could be explained by the CD163 label masking a mixture of macrophage lineages and activation states: bone-marrow derived CCR2-positive cells that foster tumour growth [11], microglia-like or Lyve1^{HIGH} meningeal macrophages that restrain it [12], and transitional phenotypes that shift under therapy [11,13], so that the net numerical total is likely to average out opposing biological effects. Only when the populations are disentangled by ontogeny markers such as CCR2, by functional readouts such as TNF-α or arginase, or by spatial location within the posterior fossa, does macrophage biology reveal its influence on tumour behaviour (Figure 1A,B).

Complementary mouse genetic models uncover two macrophage lineages with opposite effects. In NeuroD2:SmoA1 tumours [14] resident microglia and a smaller bone marrow component release tumour-necrosing TNF-α and nitric oxide; depleting them with PLX3397, a brain-penetrant CSF1R inhibitor, accelerates growth [15]. In contrast, sporadic SmoM2 tumours recruit large numbers of CCR2-positive, CSF1R dependent macrophages together with Ly6G-positive neutrophils [11]. Eliminating these cells with PLX5622 removes neutrophil-attracting chemokines, doubles intratumoural CD8 T cells and shrinks or stabilises most enlarging lesions. Lyve1^{HIGH} meningeal macrophages illustrate the protective side of the balance. Endothelial Norrin signalling through Frizzled-4 maintains this subset; genetic loss of either component depletes Lyve1^{HIGH} cells, lowers their secretion of CXCL4, a chemokine that competes with the mitogen CXCL12 for CXCR4 on granule neuron progenitors, and doubles the incidence of preneoplastic nodules in *Ptch1* heterozygous and NeuroD2:SmoA1 mice (Figure 1B) [12]. These opposing outcomes show that microglia derived or Lyve1^{HIGH} meningeal macrophages can restrain tumour growth, whereas bone marrow macrophages and their neutrophil partners drive progression (Figure 1B). Children with germline *PTCH1*, *SUFU* or *TP53* mutations, which confer an inherited predisposition to SHH-MB, may therefore benefit from treatments that preserve meningeal-macrophage integrity rather than deplete the myeloid compartment indiscriminately.

Adaptive immunity is limited primarily by loss of antigen presentation. Two independent paediatric cohorts found virtually no PD-L1 protein on tumour cells or macrophages and little PD-1 on T cells [16,17]. Instead, tumours down-regulated MHC-I and the non-classical MHC molecule, CD1d, and up-regulated the granzyme inhibitors SERPINB1 and SERPINB4, molecular shields that bind and neutralise cytotoxic proteases [18]. In a predominantly adolescent series, more than half of the recurrent tumours expressed PD-L1, and those with high PD-L1 together with sparse CD8^{+} T-cell infiltration experienced the poorest survival, implying that checkpoint expression emerges during tumour evolution or in response to therapy [19]. Although generally T-cell poor, Group 3 tumours harbour a higher proportion of PD-1^{+} CD8^{+} T cells than SHH tumours in pre-clinical models, with PD-1 blockade, alone or combined with CTLA-4 inhibition, prolonging survival only in mice bearing Group 3 tumours [20]. The potential benefit of checkpoint blockade in MB

is therefore likely to depend on age, molecular subgroup and myeloid context, arguing against its uniform application.

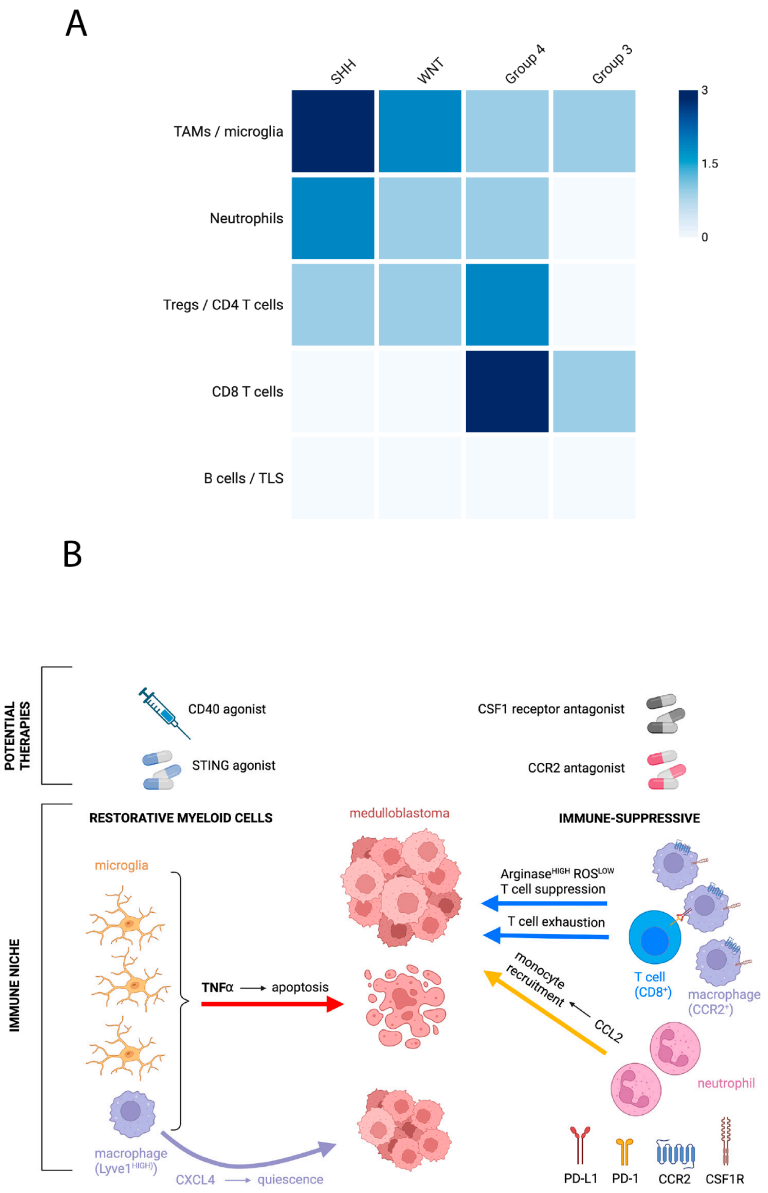

Figure 1. Immune composition and myeloid polarity in medulloblastoma (MB). (**A**) Subgroup-resolved immune landscape. Semi-quantitative heatmap (0 = absent, 3 = highest) summarising the relative abundance of TAMs/microglia, neutrophils, regulatory/helper T cells, cytotoxic CD8 T cells and B-cell/tertiary lymphoid structure (TLS) signatures across the four molecular subgroups. SHH tumours are myeloid-rich, WNT tumours retain a moderate myeloid signal, Group 4 shows the clearest CD8 imprint, and Group 3 remains largely "cold". (**B**) Dual myeloid niches and actionable targets. Left, 'restorative' compartment: resident microglia and Lyve1^{HIGH} meningeal macrophages release TNF-α (tumour apoptosis, red arrow) and CXCL4 (quiescence, purple arrow). These cells can be amplified with CD40 or STING agonists. Right, 'immune-suppressive' compartment: bone-marrow-derived CCR2^{+} macrophages secrete CCL2 (yellow arrow) which attracts additional monocytes and Ly6G^{+} neutrophils, up-regulate PD-L1 and maintain an arginase^{HIGH}/reactive oxygen species (ROS)^{LOW} state that suppresses and exhausts CD8 T cells (blue arrow). CSF1R or CCR2 antagonists may target this immunosuppressive myeloid compartment. Icons depict key ligands and receptors discussed in the text; pill or syringe symbols indicate drug classes in pre-clinical or early-phase clinical testing.

CSF1R inhibition revealed a latent CD8 response, providing a rationale for combination with additional immune stimulants. CD40 agonists activated antigen-presenting cells [21], STING ligands triggered type I interferon release [22], and hypofractionated radiation broadened the antigen repertoire [23], any of which could amplify the CD8 wave seen after treatment with the CSF1R inhibitor, PLX5622 [11]. Redox modulation offers an alternative approach; because reactive oxygen species can influence tumour-associated macrophage polarisation and function, pharmacological alteration of redox state may provide a means of reprogramming macrophages without depleting them [24,25].

Physical interventions that remodel the tumour niche further broaden the therapeutic repertoire. Proton FLASH irradiation delivered in microsecond pulses, which potentially spares cognitive function, reprogrammed macrophages by preventing oxidation of low-density lipoprotein, shutting down PPAR-γ signalling and converting CD206 and arginase-positive suppressors into CD86- and CD80-positive facilitators of T-cell entry. In a SHH-MB mouse model, this macrophage reprogramming sensitised tumours to intravenously administered GD2 CAR-T cell therapy [13]. Locoregional adoptive therapy provides another route to exploit an otherwise restricted T-cell pool. Donovan and colleagues [26] analysed more than one thousand MB and ependymoma transcriptomes and identified EPHA2, HER2 and IL13Rα2 as a trivalent antigen set absent from normal paediatric brain. A tricistronic CAR recognising all three targets eradicated both cerebellar masses and spinal leptomeningeal metastases when delivered into the ventricular system; mice re-challenged with tumour cells sixty days later remained tumour-free, indicating persistence of an anti-tumour response. For low EPHA2 expressors, pre-treatment with azacitidine, a DNA-methyltransferase inhibitor, demethylated the EPHA2 promoter, raised surface antigen and converted partial responses into cures, an approach termed epigenetic priming [27].

For clinical translation, the TaqMan Low-Density Array (TLDA) is a practical first-line assay. The microfluidic quantitative PCR (qPCR) card comes pre-loaded with dozens of probe-primer sets, profiles subgroup and immune-marker transcripts in a single run and delivers results in roughly two hours. The TLDA card flags SHH-MB tumours with heavy macrophage load and low antigen presentation, helping to select patients for macrophage-targeted or antigen-boosting strategies. CSF1R inhibitors warrant further study in progressive or relapsed SHH models where bone-marrow macrophages dominate; drug selectivity, brain penetration and continuous dosing must be optimised to prevent rapid microglial repopulation documented in adult mice [28]. Group 3 tumours with demonstrable PD-1$^+$ CD8 infiltrates provide a rationale for PD-1 blockade. FLASH irradiation combined with intrathecal multispecific CAR-T cells, possibly preceded by azacitidine priming, represents a plausible avenue for early-phase trials, with serum oxidised low-density lipoprotein (LDL) (and where feasible CSF oxidised LDL) or intratumoural PPAR-γ activity as exploratory pharmacodynamic readouts.

MB's immune microenvironment is therefore sparse but functionally diverse. Macrophage polarity, antigen-presentation loss and lipid driven metabolic rewiring are pivotal levers of immune escape, and their manipulation through selective depletion, metabolic tuning, checkpoint inhibition, ultrafast irradiation or locoregional CAR-T therapy offers potential routes toward improved outcomes across molecular subgroups. Given these barriers act in concert, durable benefit is more likely to come from rational combinations that address multiple immunosuppressive layers rather than from any single-agent approach.

3. Ventricular and Vascular Interfaces: How Choroid Plexus/CSF and Blood–Brain Barrier Niches Shape Medulloblastoma

The cerebrospinal fluid compartment has moved from the periphery of paediatric brain tumour research to centre stage because a series of recent investigations show that it

shapes MB biology at every step from tumour initiation to leptomeningeal spread. Collectively, these studies demonstrate that the ventricular system is a structured and reactive microenvironment whose composition is defined first by choroid plexus development and then remodelled by the tumour into a hypoxic, lipid-laden and immunosuppressed niche that supports stem-like persistence and therapeutic resistance.

A detailed review of choroid plexus ontogeny provides the developmental framework that underpins this concept [29]. The choroid plexus originates from roof plate neuroepithelium but rapidly diversifies into telencephalic, diencephalic and hindbrain domains, each controlled by distinct gradients of BMP and WNT morphogens and by lineage-specific transcription factors such as OTX2, LHX2, LMX1A and MAFB. Villous morphogenesis depends on reciprocal signalling between newly specified epithelial cells and fenestrated endothelial sprouts; epithelial WNT5A and SHH guide vascular invasion, while pericyte-derived cues consolidate tight junctions and blood–CSF barrier function. This developmentally regulated tissue secretes most of the CSF and distributes region-specific repertoires of proteins, metabolites and lipids into the ventricular system, establishing a baseline chemical landscape against which pathological changes can be measured.

The developmental biology of the choroid plexus is now complemented by evidence that this tissue can actively sustain tumour-initiating cells in Group-3 MB [30]. Using single-cell RNA-sequencing (RNA-seq) of human hindbrain, spatial in situ mapping and lineage-specific mouse models, Visvanathan and colleagues traced a population of Protogenin-positive ($PRTG^{+}$) MYC-high stem cells to the interface between the rhombic-lip ventricular zone and an 'interposed vascular plexus' that abuts the fourth-ventricle choroid plexus. In patient tumours, features of the same perivascular-choroid plexus niche architecture are re-capitulated, and paracrine signalling from choroid plexus endothelial cells drives cultured Group 3 cells toward a SOX2-high, tumour-propagating state; reciprocally, the tumour re-programmes adjacent endothelial cells into an immature, plexus-like phenotype. Genetic ablation or CAR-T targeting of the $PRTG^{high}$ compartment collapses both the aberrant vasculature and the stem-cell pool, demonstrating a functional dependency on this choroid plexus-adjacent niche. These findings position the choroid plexus not only as the developmental source of CSF but also as an active stromal partner that amplifies tumour-initiating capacity in aggressive Group 3 disease, reinforcing the concept that ventricular micro-anatomy and its specialised secretions are integral to MB biology.

One of the earliest functional demonstrations that CSF constituents control neural proliferation comes from work showing that embryonic CSF is rich in insulin-like growth factor-2 [31]. Radial-glial progenitors respond to IGF2 only when the IGF receptor is positioned at their apical surface, a process governed antagonistically by the polarity proteins PALS1 and PTEN. Removing IGF2 from the CSF or mis-localising its receptor curtails cortical expansion, whereas increased CSF-borne IGF2 has the opposite effect and promotes excessive proliferation. This study establishes the principle that ventricularly delivered growth factors act on brain cells in a spatially constrained and polarity-dependent manner.

A recent mechanistic study of high-risk, metastasis-prone MB extends these observations to the MB CSF niche. Svalina et al. [32] analysed lumbar CSF from children with metastatic Group 3 disease and detected markedly higher concentrations of IGF-1 together with its carrier IGFBP-3, IGFBP-3-cleaving proteases (MMP-9, tissue plasminogen activator) and their inhibitors TIMP-1 and PAI-1. Meningothelial cells, the first cellular layer encountered by tumour cells exiting the CSF, were shown to express the same proteases, providing a local source of IGF-1 release at the leptomeningeal surface. In MYC-amplified MB lines, liberated IGF-1 triggered robust IGF1R phosphorylation, enhanced migration in Boyden-chamber assays, and increased adhesion to fibronectin, laminin and cultured meningeal cells without requiring additional mitogens. Immunoblotting confirmed IGF1R

over-expression in all primary patient tumours surveyed, in matched spinal metastases, with expression also detectable in histologically uninvolved meninges, suggesting that both tumour and meningeal cells may respond to local IGF-1. A 60-compound viability screen identified three structurally distinct IGF1R inhibitors among the most potent single agents across four metastatic MB cultures, supporting IGF1R as a potential therapeutic target. Together, these data argue that the CSF/meningeal micro-niche not only supplies IGF-1 but also furnishes the proteolytic machinery needed to liberate the ligand, thereby creating a feed-forward axis in which IGF1R signalling drives migration, adhesion and outgrowth of metastatic MB cells (Figure 2A).

Three multi-omics analyses further extend the discussion from development to MB. By integrating CSF transcriptomes, metabolomes and lipidomes from forty patients, one study uncovered a hypoxia-like metabolic programme characterised by the accumulation of tricarboxylic-acid intermediates and triacylglycerols together with enrichment of TGF-β and NF-κB pathway transcripts [33]. Among the RNA species that drive this separation is circ_463 (also known as CDR1as/ciRS-7), the most abundant circular RNA (circRNA) detected in tumour CSF; its dozens of miR-7 binding sites and reported role in other cancers suggest it could modulate NF-κB or EGFR signalling, although no functional data were provided. These molecular alterations clearly distinguish tumour from control CSF but are not sufficient to resolve WNT, SHH, Group 3 or Group 4 subtypes, implying that hypoxic re-programming and circ_463 up-regulation are pan-MB features rather than subgroup-specific events. Importantly, the metabolite signature is dominated by energy-dense lipids, highlighting β-oxidation and lipid storage as potential therapeutic vulnerabilities.

Reichl and colleagues [34] focused on relapsed disease and showed that CSF drawn from children with recurrent MB is depleted of canonical pro-inflammatory effectors such as S100A8/A9 and C-reactive protein but enriched for proteins, fibromodulin, ART3, GPNMB, which are typically produced by alternatively activated, or M2-polarised, macrophages (Figure 2A). Unlike classically activated M1 macrophages that generate cytotoxic nitric oxide and interleukin-1β, M2 macrophages secrete TGF-β, remodel extracellular matrix and suppress adaptive immunity, thereby creating a permissive setting for tumour survival. Metabolite profiling reinforces this picture; tryptophan and serine accumulate, consistent with an IDO (indoleamine 2,3-dioxygenase)/kynurenine axis that further blunts T-cell activity, while oxylipin analysis reveals a six-fold rise in 12,13-DiHOME, a soluble epoxide-hydrolase product of linoleic acid that stimulates fatty-acid uptake, boosts β-oxidation and induces mild mitochondrial uncoupling. Because 12,13-DiHOME is both a marker and driver of lipid-fuel metabolism, its elevation indicates that residual tumour cells and associated myeloid elements have shifted toward an oxidative, fatty acid dependent energy strategy. Taken together, the protein, amino acid and oxylipin data depict a relapse CSF niche that is immunologically tolerant, metabolically flexible and primed for efficient adenosine triphosphate (ATP) generation from lipids, conditions well suited for minimal residual MB to persist and evade standard therapies.

A mechanistic link between CSF chemistry and leptomeningeal spread was established in another study which isolated a MB subpopulation that strongly expresses γ-aminobutyric-acid transaminase (ABAT), the mitochondrial enzyme that initiates the gamma-amino butyric acid (GABA) shunt, a bypass pathway converting GABA to succinate and feeding it directly into the tricarboxylic acid cycle [35]. ABAT equips cells to generate ATP oxidatively under the nutrient-poor conditions that prevail in the ventricular and subarachnoid spaces. Loss-of-function clustered regularly interspaced short palindromic repeats (CRISPR) mediated knockdown of *Abat* left intracerebellar growth intact yet eliminated spinal leptomeningeal deposits, proving that GABA metabolism is dispensable in the primary site but critical in CSF. Conversely, gain-of-function retroviral overexpression

of ABAT in otherwise ABAT-low cells enabled long-term survival in artificial CSF, maintained tricarboxylic acid (TCA) cycle intermediates, and, in dual-luciferase competition assays, conferred a selective advantage after intracisternal injection, leading to robust spinal metastases. These data show that ABAT is both a metabolic switch and a dissemination driver; it allows MB cells to tap CSF GABA, while increased ABAT expression is associated with reduced proliferation and resistance to vincristine. Targeting *Abat* or depriving tumour cells of GABA therefore emerges as a niche-specific strategy to prevent or treat leptomeningeal MB.

While the ventricular CSF shapes MB from the luminal side, the parenchymal interface is influenced by BBB signalling, which, as Phoenix et al. demonstrated, is itself dictated by tumour genotype [9]. In genetically faithful mouse models and matched patient samples, they showed that WNT subgroup tumours dismantled the barrier, converting neighbouring endothelium into a PLVAP^{+}/SLC2A1^{-}, fenestrated state, whereas other MB subtypes preserved the opposite profile characteristic of an intact BBB. Tumour cell secretion of the WNT antagonists WIF1 and DKK1 silenced endothelial β-catenin/LEF1 signalling, reduced pericyte coverage, introduced nanoscale fenestrations in endothelial membranes and permitted entry of plasma proteins and large chemotherapeutic molecules such as vincristine into WNT tumours. Re-activating endothelial WNT signalling with WNT7A restored BBB characteristics and sharply lowered intra-tumoural vincristine exposure, whereas forced WIF1 plus DKK1 expression partially fenestrated SHH vasculature and increased drug penetration, underscoring endothelial WNT activity as the key regulator of vascular barrier permeability. Genotype-encoded vascular programming therefore joins choroid plexus secretion, CSF metabolic re-modelling and neurotransmitter scavenging as a fourth extrinsic axis that sculpts MB biology and treatment response.

Although these investigations targeted different niches, they converged on four common themes. First, the CSF is metabolically re-wired in MB, shifting toward hypoxia-associated intermediates, triacylglycerol accumulation and enhanced fatty-acid oxidation. Second, the fluid is immunologically re-programmed; pro-inflammatory signals decline while markers of M2-like macrophage activity rise, reducing immune surveillance. Third, niche-specific nutrients such as GABA can be exploited by minor tumour cell populations to seed and sustain leptomeningeal disease. Finally, genotype-dependent remodelling of the vascular barrier alters systemic drug exposure, as described above.

These findings potentially translate into several clinical opportunities. The developmental insight that IGF2 is a major CSF mitogen suggests that blocking IGF signalling, either by neutralising the ligand or interfering with apical receptor localisation, could limit tumour cell proliferation within the ventricular system. Metabolic interventions aimed at fatty acid oxidation or GABA catabolism offer niche-specific therapeutic leverage and might augment existing cytotoxic regimens. The BBB data add a vascular dimension; for non-WNT tumours which retain a tight SLC2A1-positive barrier, therapeutic penetration could be enhanced by transient barrier-opening approaches such as focused ultrasound with microbubbles, low-dose vascular-targeted irradiation, transporter mediated drug shuttles or pharmacological modulation of endothelial WIF1/DKK1–β-catenin signalling to induce temporary fenestration. Finally, the immunosuppressive shift observed in recurrent disease supports the exploration of macrophage re-polarisation strategies within the CSF.

These findings also define technical priorities. A lack of ventricle-specific *Cre* drivers still hampers precise genetic interrogation of choroid plexus regionalisation and, by extension, of ventricle-specific secretomes. Spatially resolved omics that correlate CSF composition with underlying choroid plexus transcriptomes and MB infiltrates are needed to map heterogeneity along the craniospinal axis. In vitro models that pair choroid-plexus organoids [36] with MB spheroids could dissect causal relationships and test candidate

therapeutics in a controllable setting. Equally, endothelial-selective tools that modulate β-catenin or WIF1/DKK1 in a temporally controlled manner are required to dissect genotype-dependent BBB permeability.

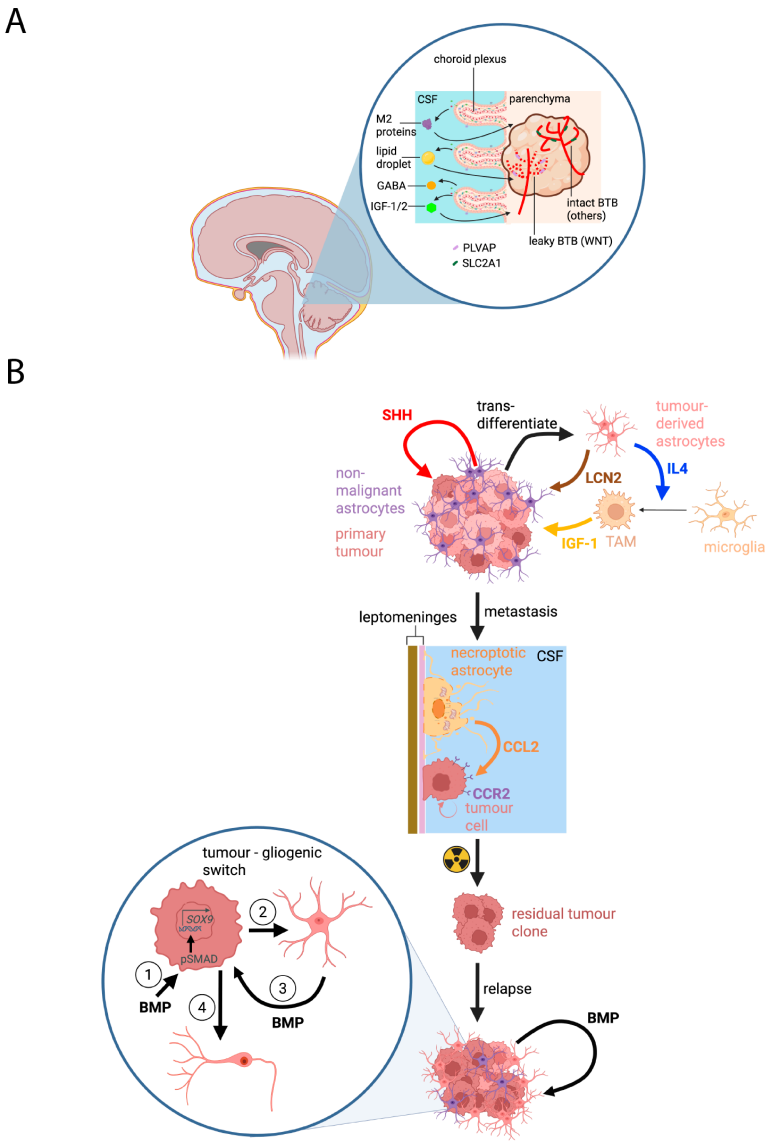

Figure 2. Anatomical niches and astrocyte-centred signalling loops that shape MB behaviour (**A**). Ventricular- and vascular-side cues. Magnified view of the fourth-ventricle wall shows choroid-plexus villi releasing CSF-borne cues—IGF-1/2, GABA, hypoxia-linked lipid droplets, and M2-skewed macrophage proteins—that diffuse across the ependyma into a periventricular tumour. On the parenchymal face, tumour vessels display either PLVAP^{+} fenestrated endothelium (leaky blood–tumour barrier [BTB], WNT subgroup) or SLC2A1^{+} tight endothelium (intact BTB, other subgroups). (**B**) Astrocyte programmes governing growth, dissemination and relapse. Within the primary tumour, host astrocytes sustain tumour expansion by providing SHH and LCN2. A small subset of tumour cells trans-differentiate into astrocytic derivatives that secrete interleukin-4 (IL-4); this cytokine in turn polarises nearby microglia/macrophages to release IGF-1, reinforcing proliferation. Tumour progression eventually results in metastasis, commonly involving the leptomeningeal compartment, where necroptotic astrocytes discharge CCL2, attracting CCR2^{+} tumour cells and supporting metastatic foci. Radiotherapy leaves behind a residual clone that can later seed relapse. Relapsed tumours contain predominantly tumour-derived astrocytes. Magnification inset illustrates the context-dependent effects of BMP signalling: BMP-SMAD-SOX9 (1) can drive tumour-cell trans-differentiation into relapse-associated astrocytes (2), whereas BMP-secreting astrocytic-like cells in MBEN (3) promote tumour cell differentiation and a more growth-restrained state (4).

In summary, the two fluid-based niches that bathe the tumour—CSF on the ventricular side and blood across the vascular border—are major extrinsic drivers of MB biology. Developmentally patterned choroid plexus secretions, most notably IGF-2, create a proliferative baseline; tumour-induced metabolic shifts and neurotransmitter utilisation generate a CSF environment that sustains dormancy, fuels dissemination and suppresses immunity. On the parenchymal side, tumour genotype-encoded signals determine whether the BBB remains intact or becomes fenestrated, thereby governing exposure to circulating metabolites and to systemically delivered drugs. Recognising and manipulating both the CSF and BBB landscapes offers complementary routes to improve outcomes across all molecular subgroups of MB.

4. Astrocyte–Medulloblastoma Crosstalk: Lineage Origins, Signalling Loops and Therapeutic Leverage

Astrocytes, once dismissed as passive scar tissue in paediatric brain tumours, have emerged as versatile contributors to MB progression. Multiple studies now show that MB-associated astrocytes can arise from pre-existing non-malignant astrocytes derived from the surrounding host cerebellar tissue [37], generated de novo through tumour cell transdifferentiation [38,39], or exist as malignant astrocytic-like populations [40], with these distinct origins associated with tumour-promoting or, in some settings, tumour-restraining functions. The first indication that astrocytes are not merely bystanders came from a *Math1-Cre/Ptch1*$^{flox/flox}$*/GFAP-TK* model in which systemic ganciclovir ablated GFAP^{+} astrocytes; their removal reduced extracellular Shh and Nestin expression, markedly decreased tumour cell proliferation and precipitated rapid tumour regression, even though those cells already lacked Ptch1 [37]. To explain this unexpected dependency, the authors proposed that alternative Shh receptors, Ptch2 or Boc, re-accumulated in the primary cilium and repressed Smo unless astrocytic Shh continuously bound and internalised these proteins, making astrocyte-derived ligand indispensable for sustaining oncogenic Hedgehog activity [37]. Further insight into the complex glia–tumour relationship came from subsequent lineage tracing by Yao et al. which revealed that ∼5% of SHH tumour cells transdifferentiated into bona fide astrocytes; these tumour-derived astrocytes secreted IL-4, polarised neighbouring microglia to an IGF-1-producing state, and thereby completed a self-sustaining IL-4 → IGF-1 feedback loop indispensable for proliferation [38]. A complementary irradiation-relapse model showed that while primary tumours are enveloped mainly by host astrocytes, the majority of astrocytes in recurrent lesions were derived from residual tumour clones that underwent a BMP-SOX9 transition; pharmacological BMPR1 blockade prevented this conversion and forestalled relapse without affecting the primary tumour mass [39]. Together, these lineage studies demonstrate that astrocytic populations may be either host- or tumour-derived and suggest that their origins may dictate therapeutic leverage points—SHH neutralisation in primary disease versus BMP inhibition after therapy (Figure 2B).

A series of murine SHH-MB studies now links astrocyte-derived signals to growth, invasion and relapse. Apart from astrocytic IL-4–IGF-1 and SHH signalling, context-specific mediators have been identified. In leptomeningeal deposits, astrocytes undergoing RIPK3–MLKL necroptosis released high levels of CCL2 that engaged CCR2 on stem-like tumour cells, to activate JAK2/STAT3 and Notch signalling, and maintained self-renewal; blocking necroptosis or CCR2 shrunk spinal burden and depleted the stem pool [41]. In cerebellar parenchyma, tumour-associated astrocytes accumulated at tumour margins and showed activation of JAK2/STAT3 signalling, which increased LCN2 secretion; LCN2 promoted MB cell proliferation and migration, whereas disrupting the STAT3-LCN2 axis in astrocytes reduced tumour volume and enhanced the anti-tumour activity of the Smo inhibitor, Sonidegib [42]. Therapy adds a layer of acute and chronic responses: soon after

irradiation or chemotherapy, tumour cells release IL-6 that draws astrocytes toward the lesion and may aid early immune clearance [43]; in other central nervous system (CNS) tumours, similar IL-6 surges are driven by alarmins such as HMGB1 and extracellular ATP released from dying cells [44,45], raising the possibility that the same damage-associated signals could operate here. Weeks later, a chronically stressed astrocyte subset re-engaged RIPK3-MLKL necroptosis, unleashing a second CCL2 wave that recruited $CCR2^{+}$ monocytes and polarised the niche toward immunosuppression and relapse, highlighting the need to blunt this secondary CCL2 spike while preserving the potentially beneficial early IL-6 response [43].

Astrocytes can also facilitate invasion. Conditioned medium from primary neonatal rat astrocytes re-programmed SHH MB lines to upregulate the pentaspan protein CD133, boosting migration, adhesion and neurosphere formation; CD133 knock-down reduced these phenotypes, supporting a functional role for CD133 in astrocyte-induced plasticity [46]. Parallel work showed that astrocyte-derived extracellular matrix rich in fibronectin and collagen supported MB cell proliferation in patient-derived "tumoroids," while blockade of β1 and other integrins significantly reduced this ECM-dependent growth [47]. These findings extend the influence of astrocytes beyond soluble ligands to extracellular matrix support of tumour growth.

Not every astrocytic programme promotes malignancy. In MB with extensive nodularity (MBEN), single-nucleus and spatial transcriptomics uncovered an astrocytic-like malignant population at the nodule–internodule interface that carries the same SHH pathway mutation as the bulk tumour cells and showed a strong BMP-related transcriptional signature, potentially driving differentiation of proliferative cells into benign nodular compartments (Figure 2B) [40]. This raises the possibility that astroglial differentiation may contribute to the relatively benign phenotype of MBEN and therapeutically promoting similar differentiation programmes could merit further investigation.

These mechanistic insights have already begun to shape translational thinking. Astrocyte-tumour signalling pathways discussed above including SHH, CCL2/CCR2 and STAT3/LCN2, represent potential therapeutic targets [37,41,42,48]. Their convergence with microglia-derived IGF-1 and other TME signals suggests that targeting a single node may be insufficient for durable control. Effective treatment is therefore expected to require coordinated interventions that address both tumour-intrinsic drivers and astrocyte-orchestrated extrinsic support.

In sum, astrocytes integrate developmental, injury and therapy-related signals to sculpt MB behaviour. They supply growth ligands (SHH), survival cytokines (IL-4, CCL2), metabolic mediators, such as the iron-binding protein LCN2 and biomechanical scaffolds such as fibronectin-collagen ECM, while also participating in paracrine circuits that induce microglial IGF-1 production. Moreover, they possess the capacity to adopt potentially tumour-restraining, differentiation-promoting BMP-rich astroglial states. Therapies that remove deleterious astrocytic signalling, by blocking SHH, IL-4, CCL2 or STAT3, while preserving or inducing differentiation-promoting BMP outputs, combined with modalities that enhance immune penetration, emerge as a rational path toward durable control. Future pre-clinical models must therefore retain native astrocyte populations or faithfully recreate them, ensuring that candidate drugs are tested against the full complexity of the astrocyte–tumour alliance before reaching the clinic.

5. Spatially Resolved Organisation of the Medulloblastoma Microenvironment

Spatial cellular and molecular mapping is now beginning to convert the cellular inventories described above into anatomical maps. Early single-cell and spatial transcrip-

tomic analysis of the postnatal cerebellum and *Ptch1*+/− MB showed that transformed granule-lineage cells resembled developing granule neuron states but displayed a reduced tendency to differentiate, establishing a developmental template for interpreting later tumour datasets [49]. In MBEN, multimodal single-cell and spatial transcriptomics resolved the characteristic biphasic histology into proliferative internodular cerebellar granule neuron progenitor (CGNP)-like malignant cells admixed with stromal, vascular and immune elements, nodular postmitotic differentiated tumour cells, and an astrocytic-like malignant population positioned near the transition zone between these compartments [40]. Spatial mapping in SHH patient-derived orthotopic xenografts extended this principle to therapy response. Palbociclib reduced proliferation and promoted neuronal differentiation across much of the tumour, but a tumour–brain interface enriched for astrocytes and microglia retained proliferative activity, identifying the boundary niche as a potential reservoir of treatment persistence [50].

Subgroup-spanning human multi-omics has now shown that progenitor-like, cycling and differentiated malignant programmes are geographically organised into stem-like and mature regions, with spatial trajectories accompanied by changing microenvironmental components that may maintain stemness or promote differentiation [51]. A directly TME-focused preprint using Visium spatial transcriptomics across paediatric MBs further identified tumour-associated astrocyte, macrophage, stromal and vascular endothelial regions, dense non-cycling progenitor-like tumour-cell neighbourhoods enriched in high-risk disease, and expansion of astrocytic, macrophage and dysregulated vascular components at relapse [52]. In chromothriptic SHH-MB, spatial and temporal transcriptomics also linked regional clone architecture to differentiation, proliferation and immune infiltration, with multiple clones contributing to treatment resistance and relapse [53]. At the protein level, multiplex immunofluorescence in a large paediatric cohort confirmed subgroup-associated immune architecture, including differences in immune-cell densities, and B-cell infiltration, and identified early tertiary lymphoid structures [54], while membrane-focused spatial proteomics identified CD155 and Endophilin-A1 as mediators of MB growth and invasion [55]. Together, these studies argue that the MB microenvironment is not only subgroup-specific but spatially partitioned: vascular, immune, stromal and astroglial programmes cluster around defined malignant-cell states and anatomical interfaces, where they may modulate differentiation, invasion, drug response and relapse.

6. Potential Diagnostic, Prognostic and Disease-Monitoring Value of Tumour Microenvironment Characteristics

Although MB diagnosis and risk stratification remain principally defined by histopathological, molecular and clinical features, emerging TME characteristics may provide complementary information on disease state and outcome. Importantly, simple quantification of immune-cell abundance is unlikely to be sufficient; bulk $CD163^+$ or $CSF1R^+$ macrophage density does not correlate with progression-free or overall survival [10], consistent with the coexistence of tumour-promoting and tumour-restraining myeloid populations. More informative assessments may incorporate lineage, activation state and spatial context. In recurrent medulloblastoma, high PD-L1 expression accompanied by sparse $CD8^+$ T-cell infiltration has been associated with poorer survival [19]. In the large paediatric multiplex-immunofluorescence cohort discussed above, several immune-cell populations were also associated with outcome, with B-cell infiltration emerging as an independent adverse prognostic factor [54]. These observations suggest that the composition and spatial organisation of the immune microenvironment may ultimately prove more informative than individual cell counts alone.

CSF profiling offers a complementary, minimally invasive approach to disease monitoring. A multiomic study identified tumour-associated RNA, metabolite and lipid signatures that distinguished CSF in MB from controls, although these profiles do not yet reliably discriminate WNT, SHH, Group 3 and Group 4 disease [33]. Analysis of recurrent-disease CSF additionally identified an immunosuppressive and metabolically altered milieu characterised by M2-associated proteins, amino acid changes and altered oxylipins [34]. Longitudinal combinations of such markers could potentially complement imaging for detection of recurrence or leptomeningeal dissemination. At present, however, these TME-derived signatures remain investigational and have not been validated as independent diagnostic, prognostic or staging biomarkers in prospective clinical cohorts.

7. Clinical and Therapeutic Translation of the Medulloblastoma Microenvironment

The advances discussed above identify several routes by which TME biology could be translated into therapeutic strategies. Importantly, these approaches are likely to require selection according to molecular subgroup, anatomical compartment and microenvironmental state rather than uniform application across medulloblastoma. In SHH tumours, the abundance and functional polarity of myeloid populations provide a rationale for selectively targeting tumour-supportive CSF1R- or CCR2-dependent macrophage programmes while avoiding indiscriminate depletion of tumour-restraining microglial and meningeal macrophage populations [11,12,15]. In Group 3 disease, the presence of PD-1-positive CD8 T cells in pre-clinical models provides a rationale for checkpoint inhibition; PD-1 blockade, alone or combined with CTLA-4 blockade, prolonged survival in Group 3 tumour-bearing mice but not SHH tumour-bearing mice [20]. Other approaches seek to remodel immune access more directly; proton FLASH irradiation reprogrammed tumour macrophages towards pro-inflammatory states and, in a SHH-MB model, increased the tumour infiltration and therapeutic efficacy of intravenously administered GD2 CAR-T cells [13], while locoregional delivery of EPHA2/HER2/IL13Rα2-directed CAR-T cells offers a complementary strategy for targeting both intracranial and leptomeningeal disease [26].

Therapeutic opportunities also extend beyond the immune compartment. The CSF and meningeal niches expose potentially actionable metabolic and growth-factor dependencies; IGF1R signalling promoted migration and adhesion of high-risk metastatic MB cells [32], whereas ABAT-dependent utilisation of CSF GABA provides a selective metabolic advantage during leptomeningeal dissemination [35]. Subgroup-specific vascular biology provides a further translational opportunity because WNT tumours possess a relatively permeable, PLVAP-positive blood–tumour barrier whereas other molecular subgroups retain a more restrictive BBB-like vasculature, with corresponding consequences for systemic drug delivery [9]. Astrocyte–tumour signalling similarly identified context-dependent therapeutic targets, including CCL2–CCR2 signalling in disseminated disease [41] and LCN2-mediated tumour support [42]. BMP signalling may also require context- specific manipulation: BMPR1 receptor blockade suppressed relapse-associated astrocytic transdifferentiation in an irradiated SHH model [39], whereas BMP activity in MBEN was associated with tumour differentiation [40]. Most of these TME-directed strategies remain pre-clinical in medulloblastoma (summarised in Table 1); their translational value therefore lies not simply in identifying additional drug targets, but in defining which microenvironmental dependency is present in a particular subgroup, anatomical niche and disease stage so that rational combinations can be prioritised for clinical evaluation.

Table 1. Potential tumour-microenvironment-targeted therapeutic strategies in medulloblastoma.

TME Target/Pathway	Potential Therapeutic Strategy	Relevant Subgroup/Niche	Pre-Clinical Rationale/Effect
CSF1R-dependent tumour-associated macrophages	CSF1R inhibition (e.g., PLX5622)	SHH medulloblastoma; myeloid-rich tumours	In the SmoM2 model, CSF1R inhibition depleted tumour-associated macrophages, reduced neutrophil-attracting chemokines, increased intratumoural CD8+ T cells and shrank or stabilised enlarging lesions [11].
PD-1/CTLA-4 immune checkpoints	PD-1 blockade alone or combined with CTLA-4 blockade	Group 3 medulloblastoma	Group 3 tumours showed a higher proportion of PD-1+ CD8+ T cells than SHH tumours in pre-clinical models; PD-1 blockade, alone or with CTLA-4 inhibition, prolonged survival in mice bearing Group 3 but not SHH tumours [20].
Suppressive macrophage state and immune exclusion	Proton FLASH irradiation combined with intravenous GD2 CAR-T cells	SHH medulloblastoma	FLASH reprogrammed tumour macrophages towards a more pro-inflammatory state and increased tumour infiltration and therapeutic efficacy of intravenously administered GD2 CAR-T cells [13].
EPHA2/HER2/IL13Rα2 tumour antigens and CSF access	Locoregional intraventricular trivalent CAR-T-cell therapy	Group 3 medulloblastoma; primary and leptomeningeal metastatic disease	Intraventricular delivery eradicated cerebellar masses and spinal leptomeningeal metastases in pre-clinical models; mice re-challenged with tumour cells later remained tumour-free, indicating persistence of an anti-tumour response [26].
IGF-1/IGF1R signalling	IGF1R inhibition	High-risk metastatic medulloblastoma; CSF/meningeal niche	CSF- and meningeal-derived IGF-1 activated IGF1R and enhanced tumour-cell migration and adhesion; multiple IGF1R inhibitors were among the most active compounds in a viability screen of metastatic medulloblastoma cultures [32].
ABAT-dependent GABA metabolism	ABAT inhibition or disruption of GABA utilisation	Leptomeningeal dissemination/CSF niche	ABAT enabled tumour cells to exploit CSF GABA as a metabolic substrate. Abat loss left intracerebellar growth largely intact but eliminated spinal leptomeningeal deposits in experimental models [35].
Astrocyte CCL2–CCR2 signalling	CCR2 blockade or inhibition of astrocyte necroptosis	Disseminated/leptomeningeal medulloblastoma	Necroptotic astrocytes released CCL2 to sustain stemness via CCR2/JAK2-STAT3/Notch signalling; blocking CCR2 or necroptosis reduced stemness and disseminated tumour growth [41].

Table 1. *Cont.*

TME Target/Pathway	Potential Therapeutic Strategy	Relevant Subgroup/Niche	Pre-Clinical Rationale/Effect
Genotype-dependent blood–tumour barrier	Barrier-aware drug delivery; experimental transient modulation of endothelial WNT signalling	Non-WNT tumours with relatively intact BBB-like vasculature	WNT tumours possess a fenestrated, drug-permissive vasculature, whereas other subgroups retain a tighter barrier. Experimental WIF1/DKK1 expression partially fenestrated SHH vasculature and increased vincristine penetration, providing proof of principle for barrier modulation [9].
Astrocyte-derived LCN2/JAK2-STAT3 signalling	Disruption of LCN2/downstream signalling	SHH medulloblastoma; invasive tumour–brain interface	Tumour-associated astrocyte-derived LCN2 promoted MB cell proliferation and migration; disruption of this axis reduced tumour volume and increased sensitivity to Smoothened inhibition in a pre-clinical model [42].
BMP-dependent astroglial plasticity	Context-dependent BMP pathway modulation, including BMPR1 blockade after irradiation	Irradiated SHH medulloblastoma/MBEN	BMP signalling promoted relapse-associated astrocytic differentiation, which was suppressed by BMPR1 blockade, whereas in MBEN it was associated with tumour differentiation [39,40].

Note: *The specific TME-directed applications summarised here are predominantly pre-clinical in medulloblastoma. Where a proposed strategy is based mainly on mechanistic proof-of-principle rather than direct therapeutic efficacy testing in medulloblastoma, this is indicated in the rationale column.*

8. Advanced Modelling of Medulloblastoma and Brain-Tumour Niches

Experimental models that reproduce the physical and cellular complexity of brain-tumour niches have advanced rapidly and now span organoid, micro-engineered and humanised-mouse formats. Human induced pluripotent stem cell (iPSC) derived organoids represent a major advance. Van Essen et al. fused two long-established MB (MB) cell lines (ONS-76 and DAOY) with 35-day cerebellar organoids composed of all major cerebellar cell types including granule lineage neurons, glia and roof-plate epithelium to simulate the non-malignant microenvironment; single-cell RNA-seq of several thousand cells showed that the non-malignant tissue environment alone was sufficient to re-programme tumour phenotypes along subtype-specific trajectories [56]. ONS-76 cells up-regulated *NEUROD1* and other granule neuron differentiation genes, whereas DAOY cells gained a stem-like signature marked by *SOX2* and other stemness genes, state changes not observed in monoculture. Building on this work, van Essen et al. and Ballabio et al. separately used human cerebellar organoids to model MB within simulated cerebellar tissue contexts in vitro. van Essen et al. introduced a *PTCH1* loss-of-function mutation into iPSC-derived cerebellar organoids, which recapitulated in vivo developmental cues and revealed early MB tumorigenesis emerging in a cerebellum-like microenvironment [57]. Similarly, Ballabio et al. drove Group 3 MB formation by overexpressing OTX2 and c-MYC in organoids, yielding MB-like tumours that even exhibited DNA methylation profiles clustering with those of patient Group 3 tumours [58]. Both studies advanced MB modelling by embedding tumour or pre-tumour states within three-dimensional (3D) cerebellar microenvironments that closely reflected the in vivo composition and organisation of the cerebellum, thus

providing more physiologically relevant in vitro models of MB. The findings validate cerebellar organoids as a tractable, human-specific substrate for probing stromal control of MB lineage plasticity.

Although the focus here remains on MB, glioblastoma provides a useful methodological comparator because organoid, micro-engineered and humanised-mouse approaches have often matured first in that field. Patient-derived glioblastoma organoids (GBOs) conserve intratumour heterogeneity and respond to treatment in ways that predict clinical outcome; in one study, GBOs treated with the same autologous CAR-T products given to patients mirrored clinical responses, including antigen loss and cytolysis, while cytokine release kinetics paralleled those measured in cerebrospinal fluid, functioning as real-time therapy avatars [59]. Yet, organoids, cerebellar or cerebral, share key deficiencies; they are avascular spheroids, their neuronal elements resemble those of the mid-gestation brain and there is no cerebrospinal fluid flow, meningeal barrier or shear stress. These limitations hamper studies of blood–tumour barrier permeability, immune trafficking and leptomeningeal dissemination. Encouragingly, some of these gaps are beginning to close; immune elements are being incorporated as well. Furthermore, iPSC-derived microglia seeded into cerebral organoids survive for weeks and adopt a more homeostatic transcriptional profile, opening avenues to study tumour–microglia crosstalk under human genetic control [60].

Bioengineering is also beginning to address some of the other limitations of organoid tissues. Vascularised human cortical organoids created by ectopic expression of ETV2 in mesodermal progenitors develop lumenised microvessels that acquired tight junctions, nutrient transporters and trans-endothelial electrical resistance characteristic of the embryonic BBB [61]. Such vasculature enhances neuronal maturation and offers a platform for studying genotype-driven barrier modulation, e.g., WIF1/DKK1-mediated fenestration in WNT-MB vessels. As a proof of principle for brain-tumour barrier modelling, microfluidic glioblastoma multiforme (GBM)-on-a-chip devices go further by embedding spheroids next to self-assembled endothelial-astrocyte-pericyte networks; nanoparticle trafficking and drug efficacy in the chip tracked closely with matched in vivo data, underscoring its predictive power for barrier-limited therapies [62]. Similar platforms, adapted with cerebellar microvessels, could interrogate Group 3 MB adhesion to meningeal surfaces or test transporter-based drug shuttles for SHH tumours that have an intact blood-tumour barrier [9].

At the organismal scale, human-immune xenograft platforms evolved in stepwise fashion from early athymic "nude" hosts through severe combined immunodeficient (SCID) and finally NSG strains, each successive line deleting additional lymphoid and cytokine pathways to improve human-tumour take-rates [63]. Engrafting peripheral blood mononuclear cells (PBMCs) into these mice yields rapid T-cell reconstitution but fatal graft-versus-host disease (GVHD) within weeks, whereas $CD34^+$ haematopoietic-stem-cell (HSC) engraftment generates multi-lineage human immunity but usually requires sublethal irradiation. More complete immune reconstitution may require additional human cytokine support, often provided by cytokine-transgenic or knock-in strains, which adds cost and complexity. The practical outcome is that most patient-derived xenografts (PDXs) still grow in profoundly immunodeficient animals and thus cannot be used to evaluate immunotherapies. Humanised DRAG mice are based on NOD.RagKO.IL2RγcKO (NRG) hosts that express human HLA-DR4. After engraftment with HLA-$DR4^+$ $CD34^+$ HSCs these mice developed mutilineage human immunity, including T, B, NK myeloid and dendritic cells [64]. In a GBM proof-of-principle study, patient-derived neurospheres implanted intracranially reproduced hallmark tumour histology and, crucially, developed a functional human immune microenvironment; infiltrating $CD4^+$/$CD8^+$ T cells, macrophages and regulatory T cells resembled the immune composition of human GBM [65]. Within the

same study, anti-PD-1 treatment remodelled these infiltrates, reducing the proportion of immunosuppressive cells in the TME. Comparable humanised platforms for paediatric MB remain an unmet need but would be invaluable for evaluating CSF1R inhibitors or multi-antigen CAR-T cells under paediatric immune conditions.

To move beyond the limits of monolayer culture and free-floating organoids, ex vivo "hybrid" platforms that combine intact brain tissue with micro-engineered systems now deliver the spatial fidelity and real-time resolution needed to watch tumour-niche interactions unfold. Organotypic mouse cerebellar slices, maintained either on an air-liquid interface [66] or, in microfluidic formats, under continuous perfusion with oxygenated medium [67], permitted direct imaging of MB invasion in an architecturally intact milieu. In this setting, exogenous EGF accelerated the invasiveness of SHH-subtype cells, whereas Group 3 cells remained intrinsically aggressive, illustrating how slice models reveal subgroup-specific micro-environmental cues [66]. Although developed largely in glioma systems, three-component hydrogel co-cultures illustrate principles directly relevant to MB TME modelling: defined cellular ratios, controllable matrix composition and interstitial fluid flow with associated shear forces [68]. A complementary hydrogel model that pairs glioma stem cells with M2-polarised macrophages identified macrophage-secreted TGFBI as a driver of GBM stem cell self-renewal, mesenchymal transition and invasion [69,70]; neutralising TGFBI markedly suppressed invasion, highlighting actionable TME signals revealed only in multicellular settings [70].

Each platform therefore captures a different dimension of the brain-tumour niche. Organoids are invaluable platforms to study lineage and paracrine dynamics; microfluidic chips recreate barrier and flow; organotypic slices preserve native cytoarchitecture; humanised mice add systemic immunity. Integrating these models in an iterative pipeline of organoid discovery, chip-based validation under flow and in vivo confirmation offers an attractive experimental pathway for defining how MB exploits its microenvironment, while adapting transferable advances from the more mature glioblastoma modelling field where appropriate.

9. Conclusions

Collectively, the studies reviewed here reposition the MB tumour microenvironment as a set of developmentally patterned, anatomically discrete and therapeutically malleable niches rather than as a uniformly sparse bystander compartment. Human tumour pathology, transcriptomic deconvolution, single-cell approaches, CSF profiling and mechanistic models converge on the same principle: subgroup identity shapes not only malignant cell programmes, but also the composition and function of immune, ventricular, vascular, meningeal and astrocytic interfaces. This complexity has important therapeutic implications. Future strategies should not aim simply to activate immunity or disrupt stromal support indiscriminately; instead, they will need to preserve tumour-restraining components, such as protective myeloid or differentiation-promoting astroglial states, while selectively disabling tumour-supportive macrophage, neutrophil, astrocyte, extracellular-matrix and barrier programmes that promote immune exclusion, invasion, leptomeningeal dissemination and relapse. Further progress will depend on pairing longitudinal human sampling of tumour, CSF and recurrent disease with spatially resolved molecular pathology and niche-preserving models that incorporate cerebellar development, barrier function, CSF exposure, mechanical forces and paediatric immunity. Such systems should allow candidate therapies—including myeloid reprogramming, CSF1R/CCR2 inhibition, checkpoint combinations, barrier-aware drug delivery, ultra-high-dose-rate FLASH radiotherapy to enhance immune access, epigenetically primed or multi-antigen CAR-T approaches and astrocyte/ECM modulation—to be tested as rational combinations matched to subgroup,

anatomical compartment and disease stage. In this framework, the MB microenvironment becomes not an obstacle to be removed, but a set of context-dependent circuits that can be measured, modelled and therapeutically rewired to improve tumour control while reducing the toxicity that currently limits cure in children.

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