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REVIEW

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Immune niches in brain tumors: glial–immune cell interactions and spatial microdomains

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Abstract

The immune microenvironment of brain tumors is characterized by remarkable diversity, where immune cells are not randomly distributed but are systematically organized into specific spatial microdomains. This spatial order is primarily orchestrated by glial cells, including astrocytes, microglia, and oligodendrocyte-lineage cells, which actively shape the local immune landscape by determining the position and activation states of infiltrating immune populations. To reflect the latest conceptual advances, this review first details the complex interaction networks through which glial cells directly modulate T-cell function and shape myeloid cell properties via cytokine signaling and metabolic regulation. We then examine how these active cellular communications synergize with the temporal process of tumor immunoediting and physical microenvironmental stressors (such as hypoxia) to drive the formation and physical anchoring of these immune niches. Consequently, these structurally established domains mature into functionally distinct, heavily immunosuppressive hubs that foster local T-cell exhaustion and coordinated immune escape. Because these niches are dynamically evolving ecosystems rather than static entities, they present profound architectural barriers, directly contributing to the heterogeneous and often limited responses to current immunotherapies. By integrating these spatial and temporal dynamics into a comprehensive conceptual framework, this review highlights the urgent clinical need to shift toward multidimensional, niche-disrupting therapeutic strategies, ultimately aiming to improve immunotherapy efficacy and clinical outcomes for patients with brain tumors.

Keywords Glioblastoma, Tumor microenvironment, Immune niches, Glial–immune interactions, Spatial transcriptomics, Immunotherapy, Immunosuppression, Microenvironmental stressors, T cell exhaustion

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Introduction

Immune niches in brain tumors are spatially organized microenvironments in which interactions between glial cells and infiltrating immune cells shape local immune activity and influence disease progression. This spatial organization is particularly pronounced in glioblastoma (GBM) [1–3]. Numerous studies have identified myeloid-dominated immune niches within glial cell-rich regions, including macrophage-enriched and immunosuppressive microregions associated with tumor infiltration, immune escape, and therapy resistance [1, 4, 5]. Similar spatially organized immune-glia structures have also been described across different glioma subtypes and grades, and the localization patterns of immune cells relative to glial cell compartments correlate with functional immune status and clinical outcomes [2, 6]. These results indicate that the emergence of structured immune territories in brain tumors is a conserved process driven by coordinated glial–immune interactions. Although this review focuses primarily on gliomas, particularly GBM, which is the best-characterized model, the conceptual principles discussed here are likely applicable to other primary brain tumors.

Despite success in other malignancies, immune checkpoint blockade and other immunotherapies have largely failed in brain tumors [7]. This is primarily due to niche-level barriers, including severe immune exclusion, the dominance of suppressive immune niches, dense hypoxia, and abnormal vasculature [3, 8]. Furthermore, these tumor niches are not static; they continuously evolve during disease progression and under treatment pressure [9]. As the disease advances, chronic antigen exposure and metabolic stress continuously drive the microenvironment toward a progressively more immunosuppressive state [10]. Under the selective pressure of treatments, such as immune checkpoint blockade, these niches adaptively remodel their architecture by recruiting additional tolerogenic myeloid populations, upregulating alternative immune checkpoints, and reinforcing physical extracellular matrix (ECM) barriers [11, 12]. This directed spatial evolution shifts the niches into highly entrenched, therapy-resistant fortresses, which dynamically neutralizes immune attacks and directly underpins the acquired resistance and failure of current immunotherapies. Historically, traditional bulk tissue analyses have homogenized these complex tumors, completely obscuring the critical spatial relationships and localized cell states responsible for such therapy resistance. To accurately decipher these spatially restricted evasion mechanisms, it is essential to analyze the tumor microenvironment in its native architectural context. Fortunately, recent technological advances in spatial transcriptomics (ST) and single-cell RNA sequencing (scRNA-seq) have successfully bridged this gap. These innovative platforms have enabled high-resolution mapping of the cellular ecosystem across glioma tissue, revealing precisely how niche-specific regulatory programs and microenvironment dependencies dictate local immune responses [1, 4, 13, 14]. These approaches have resolved distinct cellular states in different anatomical regions, including oligodendrocyte progenitor–like cells within hypoxic tumor cores that confer specific metabolic niche vulnerabilities, and radial glia–like stem cells at invasive tumor fronts that contribute to distinct cellular heterogeneities [4]. Such findings demonstrate that complex cellular ecosystems are spatially organized into functionally specialized microregions [4]. These spatial programs (often reflecting broader transcriptional 'metaprograms') not only describe tissue topology, but also highlight specific microenvironmental states and immunological properties, including immunosuppressive signaling within perinecrotic zones and pro-inflammatory pathways in perivascular regions [15]. For instance, recent research by Greenwald et al. demonstrated that the organization of GBM cellular states is highly structured, strongly dichotomized into hypoxia-rich versus non-hypoxic regions [16].

Within these ecosystems, astrocytes, microglia and cells of the oligodendrocyte lineage play central regulatory roles [3, 6, 17]. Microglia and macrophages represent major immune-relevant cell populations in gliomas and are key regulators of tumor-associated inflammation and immunosuppression [5, 13, 18]. Reactive astrocytes further shape extracellular signaling networks and affect niche organization, while oligodendrocyte-like malignant cells drive specific metabolic adaptations and promote severe T-cell depletion within hypoxic tumor cores [19]. Spatial and single-cell studies also show that myeloid cells preferentially settle in glial cell-rich regions and develop niche-specific phenotypes associated with immunosuppression, tumor invasion and therapeutic resistance [1, 2, 4]. These findings underscore that the formation of immune niche in brain tumors is the result of coordinated glial-immune interactions that vary along spatial gradients.

However, a comprehensive conceptual framework linking glial cell-mediated immune organization, spatial microstructure, and functional immune states remains lacking. This review therefore aims to systematically synthesize current knowledge on the spatial organization of immune niches in brain tumors. In this review, we define immune niches as spatially restricted microenvironments characterized by specific cellular compositions and regulatory interactions. To provide a logical progression, we first outline the spatial composition of these niches, followed by a detailed examination of the underlying glial-immune cross-talk mechanisms. We then explore how these interaction networks synergize with

microenvironmental pressures to physically anchor and mature into immunosuppressive hubs. Finally, we discuss the profound clinical implications of this dynamic spatial architecture and highlight future directions for niche-disrupting immunotherapies. Understanding how these niches emerge, interact, and evolve is essential for interpreting immune heterogeneity and therapeutic responses in brain tumors.

Cellular composition and spatial organization of the brain tumor immune microenvironment

Immune cell populations and their spatial distribution patterns

Brain tumors have a unique immune microenvironment that is not haphazard, but rather well-organized and structured. In the case of gliomas, immune cells such as tumor-associated macrophages (TAMs), microglia residing in the brain, T lymphocytes, natural killer (NK) cells, and dendritic cells (DCs) play a dominant role. As illustrated in Fig. 1, these populations occupy specific spatial niches: the invasive margin is primarily surveyed by T cells, the perivascular niche serves as a diverse interaction hub recruiting macrophages, NK cells, and DCs, while the hypoxic and necrotic core is heavily dominated by immunosuppressive myeloid cells [2, 3]. These spatial patterns not only suggest a targeted recruitment of immune cells but also imply specialized functions within different compartments of the tumor.

Recent spatial transcriptomic and imaging studies have further refined this view by demonstrating that immune cells form distinct spatial neighborhoods rather than diffuse infiltrates. For example [1], identified spatially segregated immune microdomains in gliomas, characterized by region-specific transcriptional programs of myeloid cells and lymphocytes. Similarly [2], reported that immune cell states and functions vary systematically across anatomical regions, supporting the concept of organized immune territories within brain tumors.

This spatial order is particularly evident in the differential distribution of myeloid and lymphoid populations. Myeloid cells constitute the most abundant immune populations in gliomas, yet they exhibit distinct spatial preferences based on their origin and activation state. While homeostatic microglia tend to be broadly dispersed across the tumor tissue, disease-associated microglia (DAMs) and monocyte-derived TAMs preferentially accumulate within specific functional microdomains, such as perivascular niches, necrotic cores, and hypoxic regions [1, 2]. In contrast, T cells are relatively sparse and tend to localize at tumor margins and invasive fronts, where immune surveillance and tumor–immune interactions are more dynamic [10, 20]. Crucially, regulatory T cells (Tregs) are spatially enriched in the tumor core through CCL22-mediated recruitment by TAMs, where they are essential for coordinated immune escape [10, 13]. NK cells and DCs, although less abundant, have

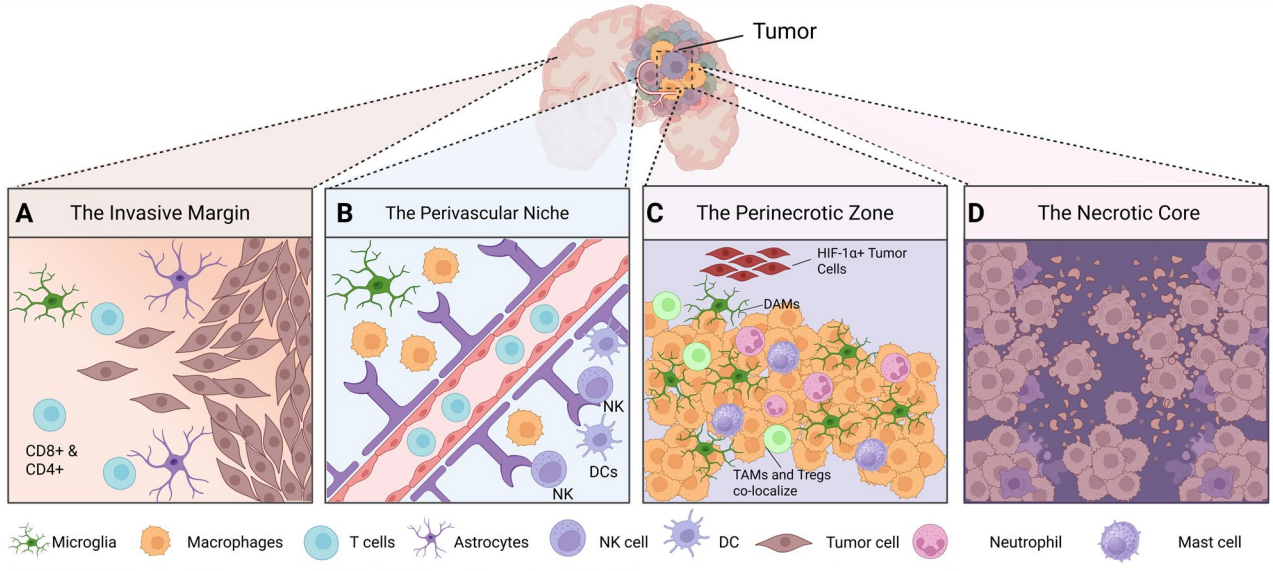

Fig. 1 Spatial Architecture of Immune Niches in Glioblastoma.The brain tumor immune microenvironment is highly compartmentalized. **A** The invasive margin acts as a dynamic interface for active immune surveillance by sparse CD8+ and CD4+T cells. **B** The perivascular niche serves as an immune-glial interaction hub where reactive astrocytes and endothelial structures recruit abundant macrophages, DCs, and NK cells. **C** The perinecrotic zone is a highly active immunosuppressive boundary characterized by HIF-1α + stressed tumor cells, disease-associated microglia (DAMs), and pronounced TAM-Treg co-localization. **D** The necrotic core represents a metabolically collapsed dead zone dominated by cellular debris and a severe exclusion of functional immune cells. Together, these gradients highlight that immune distribution is structurally organized by resident glial cells and microenvironmental stressors. Abbreviations: DAMs, disease-associated microglia; DCs, dendritic cells; HIF-1α, hypoxia-inducible factor 1-alpha; NK, natural killer; TAMs, tumor-associated macrophages; Tregs, regulatory T cells

also been detected in spatially restricted niches, particularly near vascular structures and immune cell aggregates [1, 2]. Additionally, tumor-infiltrating neutrophils and mast cells, both of which exert potent immunosuppressive functions, are prominently present in the perivascular and perinecrotic zones [3].

These findings indicate that immune cells in brain tumors exhibit highly structured spatial distributions, with distinct populations occupying specific tumor regions. This spatial organization forms a critical foundation for understanding how immune functions are locally regulated within the tumor microenvironment.

Glial cell subtypes and their spatial distribution

The spatial organization of immune cells in brain tumors is closely intertwined with the distribution and functional states of glial cells. Specifically, astrocytes, microglia, and oligodendrocyte-lineage cells not only constitute the structural framework of the central nervous system but also actively regulate immune responses within the tumor microenvironment [3, 17].

Astrocytes concentrate differentially and exhibit variable functional effects across specific anatomical zones. Spatial analyses demonstrate that they colocalize with immune-suppressive myeloid populations in regions such as the peritumoral border, the perivascular interface, and reactive gliosis zones surrounding the necrotic core [2, 21].

While often discussed interchangeably, microglia and monocyte-derived macrophages (MDMs) are distinct immune populations with different spatial distributions and transcriptional programs. As noted earlier, while homeostatic microglia broadly survey the tumor tissue, activation drives the accumulation of disease-associated microglia (DAMs) and MDMs near perivascular and perinecrotic zones [1, 2]. Notably, the distinct spatial patterning of these myeloid populations is strongly associated with differential immune profiles depending on their specific anatomical location.

Spatial transcriptomic studies have identified tumor cells with an oligodendrocyte precursor–like phenotype in hypoxic tumor cores and demonstrated their association with distinct immune landscapes [4, 6]. These findings confirm that oligodendrocyte-related cellular states are also spatially restricted and participate in shaping local environments.

Overall, the spatially patterned distributions of astrocytes, microglia, and oligodendrocyte-lineage cells form the cellular basis of immune organization. Their structural positioning creates a glial–immune framework. The specific molecular interaction networks that allow these cells to regulate immune recruitment and suppression will detail in the next section.

Spatial heterogeneity and structural foundations in brain tumors

The spatial organization described above is further shaped by the intrinsic structural heterogeneity of brain tumors. Gliomas exhibit distinct anatomical compartments, including perivascular regions, necrotic cores, hypoxic zones, and invasive margins, each characterized by unique cellular compositions, microenvironmental conditions, and distinct spatial gene signatures [4, 6]. These compartments serve as organizing scaffolds for both immune and glial cell localization.

Perivascular niches represent key hubs of immune–glial interaction. These regions are enriched with endothelial cells, astrocytic endfeet, and infiltrating immune cells, particularly macrophages and T cells, creating permissive environments for immune cell recruitment and signaling [3, 22]. In contrast, the perinecrotic zone (hypoxic border) and the necrotic core must be defined as separate compartments, as they are spatially and functionally distinct. The perinecrotic zone contains stressed tumor cells activating HIF-1α programs that interact with TAMs and microglia responding to hypoxia and tumor-derived cytokines. Meanwhile, the necrotic core is dominated by severe metabolic collapse and cellular death [2, 4].

The invasive tumor front constitutes another structurally distinct region, characterized by migrating tumor cells, reactive astrocytes, and spatially restricted immune surveillance. Specifically, active immune surveillance is spatially restricted to these outer margins, creating a stark difference in functional immune states between the highly suppressive tumor core and the somewhat more permissive invasive edge. Studies have shown that immune cell composition and activation states differ markedly between invasive edges and tumor cores, underscoring the spatial compartmentalization of immune responses [6, 10]. These compartment-specific immune–glial architectures collectively generate a heterogeneous landscape of immune microenvironments within a single tumor.

From a conceptual standpoint, these findings suggest that structural heterogeneity is not merely a passive consequence of tumor growth but actively shapes immune and glial cell positioning, thereby enabling the emergence of spatially distinct immune niches. Crucially, recent spatial transcriptomic research has shown that these tumor zones are not strictly discrete compartments [11, 15, 16]. Instead, immune cell composition, oxygen stress, glial activation states, and tumor cell transcriptional programs transition as a continuous spectrum across these spatial gradients. For instance, along the spatial trajectory from the invasive margin to the hypoxic core, malignant cells gradually shift from neural or oligodendrocyte progenitor-like states toward mesenchymal-like phenotypes

driven by increasing metabolic stress [6, 15]. Concurrently, the associated glial compartment smoothly transitions from border-associated reactive astrogliosis to a profoundly immunosuppressive, TAM-dominated network at the core [11, 16]. Exploring this gradient spatial architecture accurately captures the conceptual nuances of the tumor ecosystem and perfectly sets the stage for understanding the spatially graded functional immune states.

Glial cell–immune cell interaction networks shaping immune niches

Interactions between glial cells and T cells

As depicted in the interaction networks of Fig. 2, glial cells constitute central regulators of T cell function within GBM immune niches, profoundly shaping T-cell fate by deploying immune checkpoint ligands for inhibitory signaling, altering local metabolic resources, and exhibiting defective or tolerogenic antigen presentation to actively drive T-cell exhaustion. Within this microenvironment, reactive astrocytes and microglia, and increasingly, oligodendrocyte-lineage cells, act as the primary cellular orchestrators of this local immune regulation [2, 10, 13, 23].

Specifically, reactive astrocytes significantly influence T-cell activation through regulation of immune checkpoint expression and cytokine production. In gliomas, reactive astrocytes upregulate PD-L1 (CD274), CD155 (TIGIT ligand), and galectin-9 in response to JAK/STAT pathway activation and interferon signaling. These changes affect the local immune environment and help form immunosuppressive regions in the tumor [24, 25]. Notably, astrocytic PD-L1 interacts with PD-1 expressed on microglia and T cells, attenuating inflammatory responses and promoting an immunosuppressive microenvironment [25]. Spatial analyses demonstrate that astrocyte-rich regions often co-localize with immunosuppressive myeloid populations, suggesting coordinated regulation of immune exclusion and tolerance [2].

Microglia, the resident immune cells of the brain, play a dual role as both innate immune effectors and modulators of T-cell responses within the tumor microenvironment. Single-cell studies have revealed that tumor-associated microglia exhibit transcriptional

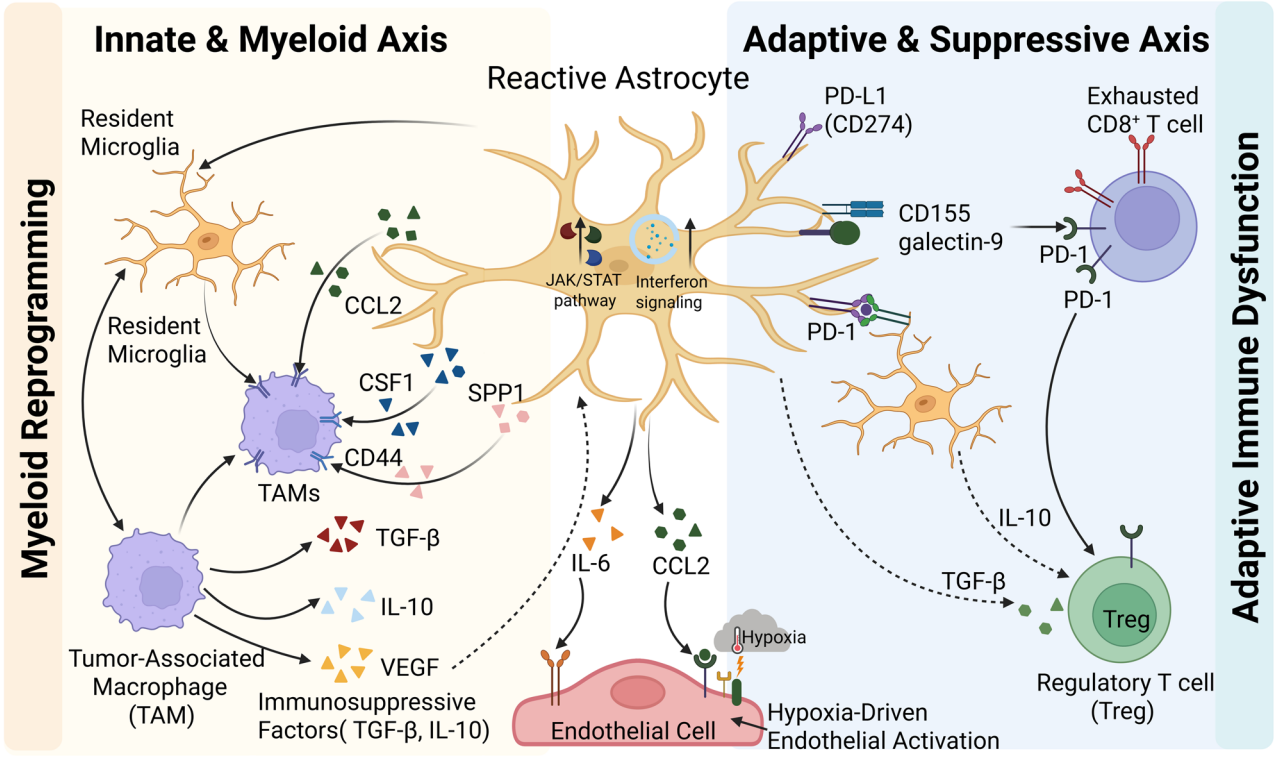

Fig. 2 Glial-Driven Molecular Networks Orchestrating Local Immunosuppression. Reactive astrocytes function as central organizers of the tumor microenvironment, engaging in bidirectional crosstalk with both innate and adaptive immune cells. (Left) Innate & Myeloid Axis: Astrocytes secrete CCL2, CSF1, and SPP1 to recruit and polarize TAMs. Notably, the SPP1-CD44 axis promotes TAM maintenance, leading to TGF-β, IL-10, and VEGF release. (Right) Adaptive & Suppressive Axis: Astrocytes deploy multiple checkpoint ligands (PD-L1, CD155, galectin-9) and cytokines to drive CD8+T cell exhaustion and promote Treg expansion, which is further reinforced by astrocytic PD-1 interactions with microglia. (Bottom) Additionally, astrocyte-derived IL-6 and hypoxia act on endothelial cells to regulate vascular activation, solidifying this suppressive niche. Abbreviations: CCL2, C-C motif chemokine ligand 2; CSF1, colony-stimulating factor 1; IL, interleukin; PD-1, programmed cell death protein 1; PD-L1, programmed death-ligand 1; SPP1, secreted phosphoprotein 1; TAMs, tumor-associated macrophages; TGF-β, transforming growth factor beta; Tregs, regulatory T cells; VEGF, vascular endothelial growth factor

programs distinct from peripheral macrophages and display region-specific phenotypes that directly dictate T-cell infiltration and exhaustion across tumor compartments [1, 2]. These spatially patterned microglial states are associated with defective antigen presentation capacity, actively failing to prime cytotoxic T cells effectively. Building on this defect, microglia-derived signals cooperate with astrocytes to establish a self-reinforcing immunosuppressive circuit that creates a hostile signaling environment, ultimately reducing T-cell activation and effector functions [24].

Oligodendrocyte-lineage cells, including both non-malignant progenitor cells and malignant tumor cells exhibiting Neftel OPC-like states, are increasingly recognized as contributors to T-cell modulation in gliomas. Spatial transcriptomic studies in hypoxic tumor cores and demonstrated their association with distinct immune landscapes, often characterized by severe T-cell depletion [4, 6]. These findings suggest that oligodendrocyte-related cellular states participate in shaping local immune environments, either directly or indirectly restricting T-cell access and survival through cross-talk with other immune and stromal cells. While their dominance relative to astrocytes is still being evaluated, their roles in T-cell modulation are primarily estimated through spatial transcriptomic co-localization analysis.

Through these mechanisms, reactive astrocytes, microglia, and oligodendrocyte-lineage cells actively regulate immune cell recruitment, activation, and suppression in brain tumors. Their spatially patterned distributions and functional states establish a glial–immune regulatory axis that underlies the organization of tumor immune microenvironments. Beyond their direct effects on T-cell activation, astrocyte-derived cytokines and chemokines also shape the recruitment and functional polarization of myeloid populations, thereby coordinating broader immunosuppressive networks within glioma niches.

Interactions between glial cells and myeloid cells

Although adaptive immune disorders manifest at the level of T cells, their persistence depends on coordinated reprogramming within myeloid populations orchestrated by the glial compartment. In GBM, microglia, which are the resident macrophages of the central nervous system, exhibits extensive transcriptional and functional plasticity that shapes the phenotypic spectrum of TAMs [1, 2, 13]. Within these defined microdomains, microglia and reactive astrocytes serve as central organizers that recruit, retain, and functionally polarize myeloid populations within specific spatial microdomains [24].

Astrocytes and microglia generate chemokine gradients that govern myeloid cell positioning and functional states. GBM-associated reactive astrocytes produce CCL2 and CSF1, which serve as precise navigational cues that recruit macrophages and induce their differentiation into tumor-promoting phenotypes [24]. In response, polarized macrophages secrete TGF-β, IL-10, and VEGF, supporting tumor growth and vascular remodeling. These cytokines actively help tumors survive by weakening the local immune defense, especially by inactivating cytotoxic T cells that would otherwise attack the tumor [14, 18]. Spatial mapping confirms that TAM-enriched domains closely correlate with reactive astrocyte signatures in hypoxic and perivascular regions [11, 24, 26], indicating bidirectional communication: glial-derived cues shape macrophage states, and macrophages reciprocally reinforce local immune suppression.

Astrocytes further integrate local stress signals to strengthen this suppressive myeloid circuitry. Reactive astrocytes release IL-6 and CCL2, molecules that not only recruit monocytes but also govern their initial entry and intra-tumoral positioning [3, 14]. Through these coordinated mechanisms, the glial compartment, mainly consisting of reactive astrocytes and microglia, modulates the intensity and spatial confinement of immune interactions.

Crucially, the biological impact of this glial–myeloid architecture extends beyond innate immunity to directly shape the local lymphoid landscape. Although regulatory T cells (Tregs) belong to the lymphoid lineage, their intra-tumoral accumulation and suppressive functions are profoundly dictated by the upstream glial–myeloid networks described above. Downstream of these interactions, Tregs constitute an additional layer of immunosuppressive regulation. High-dimensional analyses reveal significant expansion of $FOXP3^{+}$ Tregs within macrophage-rich regions, associated with elevated expression of CTLA-4, TIGIT, and ICOS [10, 13]. Astrocyte- and microglia-derived IL-10 and TGF-β promote Treg stability, while PD-L1 expression on glial cells further enhances their suppressive function [8, 24, 27]. Notably, single-cell TCR sequencing demonstrates clonal expansion of Tregs within tumors, indicating sustained, antigen-driven accumulation rather than transient infiltration [28, 29].

In turn, these accumulated Tregs are not merely passive recipients of these signals; they actively secrete high levels of their own TGF-β and IL-10 to lock local glial and myeloid cells into a continuously tolerogenic state [8, 27]. This reciprocal feedback establishes a self-amplifying loop, where glial cells recruit macrophages, macrophages stabilize Tregs, and Tregs reciprocally sustain glial-myeloid suppression, progressively elevating local immunosuppression and stabilizing regulatory dominance over effector immunity.

Other immune subpopulations within glial niches

While T cells and myeloid populations dominate the glial-regulated immune ecosystem, other immune subsets, including B cells, NK cells, and DCs, also populate the tumor microenvironment, though in smaller proportions and with less defined functional roles. Spatial transcriptomic analysis indicate that these populations are not diffusely scattered but are contextually localized. NK cells and DCs have been detected in spatially restricted niches, particularly near vascular structures and immune cell aggregates [2, 30]. Similarly, B cells occasionally form discrete clusters in perivascular regions, sometimes resembling tertiary lymphoid structures [1, 2]. These TLS-like aggregates are primarily found contextualized within less hypoxic, immune-permissive perivascular interfaces.

However, the precise functional role of these B-cell aggregates remains a subject of intense debate in current neuro-oncology. One perspective posits that these clusters represent early, functional tertiary lymphoid structures capable of locally orchestrating robust anti-tumor immunity. Conversely, an opposing view argues that within the hostile, glial-regulated GBM ecosystem, high concentrations of immunosuppressive cytokines such as TGF-β and IL-10, which are mainly derived from reactive astrocytes and microglia, can forcibly drive B cells to differentiate into immunosuppressive regulatory B cells, thus promoting immune escape [2, 13, 24].

Rather than acting as independent controllers of immune activity, these rarer immune subpopulations likely operate as secondary components within the broader glial-regulated ecosystem. Integrated spatial and functional analyses are urgently needed to explain whether these cells primarily enhance local immune activation or simply succumb to immunosuppression within these glial-defined niches.

Key signaling pathways and ligand–receptor axes

The cellular interactions described above converge on recurring ligand–receptor circuits through which glial cells coordinate immune regulation across spatial microdomains. The CSF1-CSF1R axis serves as the central engine for microglial and macrophage survival in GBM, with transcriptomic studies confirming heightened activity in myeloid-dominated microenvironments [1, 2, 13]. Critically, reactive astrocytes represent a major source of CSF1 in the glioma microenvironment, driving macrophage recruitment and pro-tumorigenic polarization [24].

Chemokine signaling pathways, particularly CXCL-CXCR and CCL2-CCR2, function as navigational control systems determining immune cell migration and positioning. These chemotactic gradients originate principally from reactive astrocytes and microglia, penetrating vascular structures and reinforcing spatial compartmentalization [1, 2, 13, 24]. Additionally, the SPP1-CD44/integrin axis is a major tumor-immune interaction pathway. It powerfully promotes the formation and maintenance of tumor-immune niches, particularly aiding TAM recruitment in hypoxic, perivascular, and invasive regions [11].

Immunomodulatory cytokines, notably TGF-β and IL-10, derive principally from reactive astrocytes and microglial populations in the GBM microenvironment [1, 2, 13, 24]. These molecules suppress T-cell cytotoxicity while promoting myeloid differentiation toward suppressor phenotypes. Immune checkpoint signaling, especially the PD-L1/PD-1 axis, also integrates glial and immune signals, with reactive astrocyte PD-L1 capable of modulating microglial activity [25]. The limited clinical success of checkpoint blockade in GBM likely reflects compensatory suppressive networks established by astrocytes and microglia [10, 23].

Rather than operating as independent modules, these signaling pathways form tightly interconnected networks. CSF1 signaling from reactive astrocytes can modulate PD-L1 expression in microglia, while TGF-β simultaneously inhibits T-cell activation and promotes macrophage reprogramming [24, 25]. This crosstalk stabilizes immunosuppression at the system level and positions reactive astrocytes and microglia as central architects of immune niche stability and plasticity in GBM.

Far from being static entities that emerge instantly, these interconnected signaling networks are progressively forged through continuous tumor-immune coevolution. To understand how these immunosuppressive hubs are physically anchored, we must shift our focus from this spatial blueprint to the temporal dynamics of their formation. The primary cellular sources, targets, and spatial contexts of these critical signaling axes are summarized in Table 1.

Formation of immune niches in brain tumors

Tumor initiation and the process of cancer immunoediting

Rather than emerging immediately, the establishment of these glial-immune interaction networks reflects the coevolution of tumor and immune cells during tumor initiation and progression. Increasing evidence suggests that this evolutionary process is not only temporal but also spatially organized, progressively shaping distinct immune microregions within the tumor microenvironment. As outlined in Fig. 3, this process unfolds through cancer immunoediting, classically defined by three phases: elimination, equilibrium, and escape [31]. Recent genomic and spatial studies have further refined this paradigm in brain tumors, revealing how each phase imprints distinct spatial organization upon the developing microenvironment.

Table 1 Key glial-immune crosstalk pathways and ligand-receptor axes in GBM niches

Signaling Axis / Molecule	Interacting Cells (Source → Target)	Functional Consequence	Spatial Context & References
CSF1 / CSF1R	Reactive astrocytes → Microglia & Macrophages	Central engine for macrophage survival, recruitment, and pro-tumorigenic polarization	Heightened activity in hypoxic cores and myeloid-dominated niches [1, 13, 24]
CCL2 & CXCL	Astrocytes & Microglia → Monocytes & Macrophages	Act as precise navigational cues for immune cell migration and intra-tumoral positioning	Penetrate vascular structures, reinforcing spatial compartmentalization [1, 14]
TGF-β & IL-10	Astrocytes, Microglia & TAMs → T cells, Tregs, B cells	Suppress T-cell cytotoxicity; promote Treg/Breg stability and suppressive myeloid reprogramming	Highly enriched within established immunosuppressive hubs [8, 18]
PD-L1 / PD-1	Reactive astrocytes & Oligo-lineage cells → T cells, Microglia	Attenuate inflammatory responses, establishing a self-reinforcing suppressive circuit	Astrocyte-rich regions overlapping with suppressive myeloid populations [24, 25]
IL-6	Reactive astrocytes → Monocytes, Endothelial cells	Modulates blood–brain barrier permeability to govern immune cell entry	Perivascular niches and blood–brain barrier interfaces [3, 14]

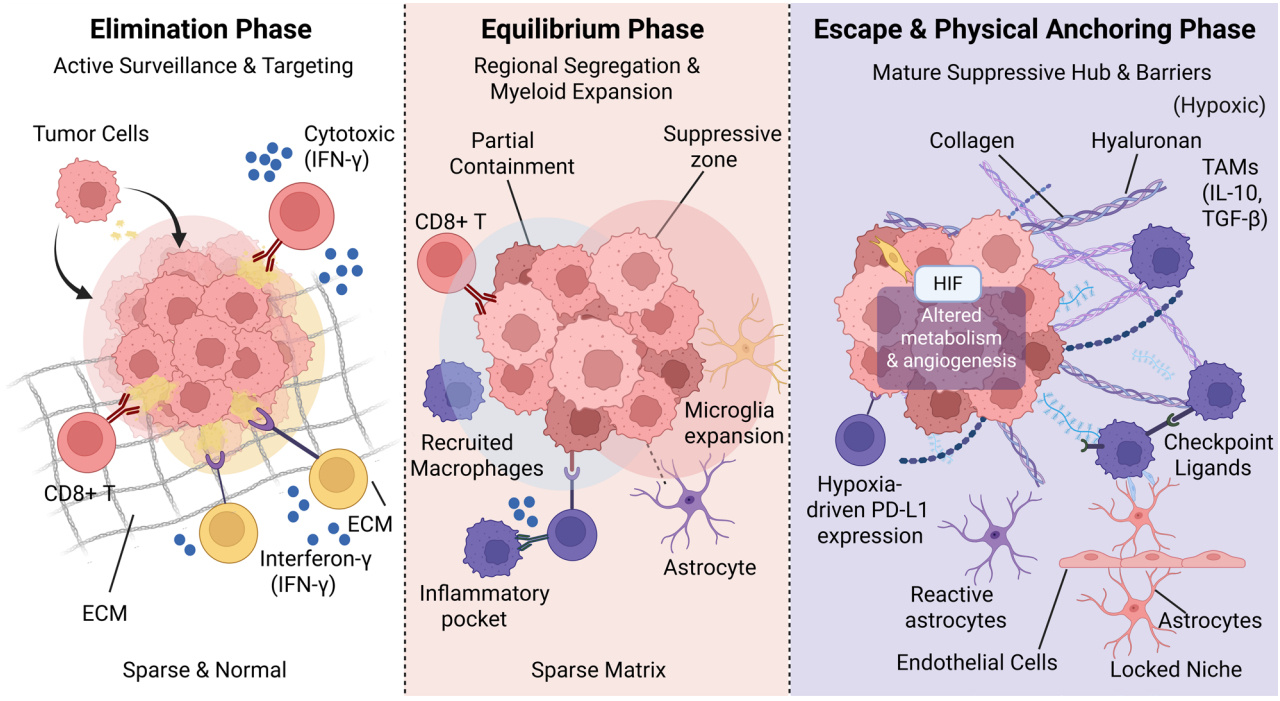

Fig. 3 Spatiotemporal Evolution and Physical Anchoring of Immunosuppressive Niches. The formation of immune niches follows the immunoediting trajectory—elimination, equilibrium, and escape—coupled with microenvironmental remodeling. (Left) In the elimination phase, cytotoxic CD8 + T cells destroy immunogenic tumor cells via IFN-γ signaling. (Center) The equilibrium phase is characterized by regional segregation: active immunity is restricted to inflammatory pockets, while microglia and macrophage expansion establishes early suppressive zones. (Right) During the escape phase, the niche matures into a firmly anchored, hypoxic immunosuppressive hub. Profound metabolic stress (HIF activation) and a dense ECM barrier (collagen and hyaluronan) synergistically restrict T-cell motility, promote suppressive TAM retention, and upregulate checkpoint ligands, locking the immunosuppressive architecture in place. Abbreviations: ECM, extracellular matrix; HIF, hypoxia-inducible factor; IFN-γ, interferon-gamma; IL-10, interleukin-10; PD-L1, programmed death-ligand 1; TAMs, tumor-associated macrophages; TGF-β, transforming growth factor beta

During the elimination phase, transformed cells carrying immunogenic neoantigens undergo immune-mediated negative selection. Pan-cancer analyses have shown that immune-dependent clonal reduction occurs in tumors with intact antigen presentation machinery [32, 33]. In gliomas specifically, transcriptomic analysis based on The Cancer Genome Atlas has identified subpopulations enriched with interferon-γ signaling and cytotoxic lymphocytes, suggesting heterogeneity in the immune response [34, 35]. Recent scRNA-seq further reveals that tumor cell states coexist with activated interferon response programs and infiltrating T cells, indicating that immune pressure acts at the level of cell states rather than uniformly across the tumor mass [6, 9].

However, in the central nervous system, complete elimination of tumor cells is rarely achieved. Instead, gliomas often enter an equilibrium phase characterized by partial immune containment. Deconvolution and single-cell studies consistently demonstrate an early expansion of macrophage and microglia populations that inversely

correlates with cytotoxic T-cell infiltration [1, 36]. This inverse correlation is likely influenced by additional factors beyond simple immune evasion, including physical anatomical barriers, reduced access via functional perivascular regions, and correspondingly higher hypoxia-rich zones that spatially exclude T cells. High-resolution ST further reveals that inflammatory and suppressive programs are regionally segregated rather than diffusely distributed [2, 11]. This spatial structuring of immune balance, where cytotoxic and regulatory signals occupy distinct microregions, marks the early formation of local suppressive niches.

Tumors progress to the escape phase, where TAMs predominate and exhibit transcriptional programs enriched in IL-10, TGF-β, and checkpoint ligands [2, 18]. Thus, immunoediting in gliomas is a temporally and spatially dynamic process: elimination phases shape clonal architecture, equilibrium phases enable regional expansion of regulatory myeloid programs, and escape phases consolidate suppressive regions into persistent niches. Consequently, as these immune-evasive tumor clones are selected and proliferate uncontrollably during the escape phase, they quickly exceed the local vascular supply, inevitably causing severe local hypoxia and metabolic stress.

Immune selection alone cannot fully explain the spatial stabilization of immune niches. Microenvironmental factors, which emerge directly from the rapid outgrowth of these tolerant clones, further reinforce these structures and shape immune cell behavior within specific tumor regions.

Regulation by metabolism, hypoxia, and the extracellular matrix

Driven by the uncontrolled proliferation discussed above, the physical stabilization of immune niches depends on metabolic competition, hypoxic stress, and extracellular matrix (ECM) remodeling. These factors collectively restrict immune cell infiltration and enhance local suppression, thereby consolidating the spatial patterns established during tumor-immune coevolution.

Metabolic diversity in gliomas is closely linked to hypoxic stress. Hypoxia stabilizes hypoxia-inducible factors, thus altering tumor metabolism, angiogenesis, and cytokine production. Single cell analyses show that hypoxia-associated tumor cell states are enriched in perinecrotic and perivascular regions, where they coexist with immunosuppressive myeloid cell populations [1, 6]. Functionally, hypoxia promotes PD-L1 expression on tumor cells and myeloid cells, impairs the function of cytotoxic T cells [10], and enhances the recruitment of macrophages via chemokines such as CCL2 and CSF1 [2, 14]. Spatial proteogenomics studies, such as the multiplexed imaging analyses by Coy et al. and Ravi et al., indicate that these macrophages directly co-localize with immunosuppressive T cells specifically within hypoxic and perivascular niches, where checkpoint ligands are highly enriched and functional T cells are severely reduced [11, 26]. Hypoxia thus acts as a spatial organizer that enhances the suppressive program initiated during immunoediting.

Significantly, this hypoxia-driven metabolic shift and the resulting vascular abnormalities directly exacerbate ECM remodeling, smoothly combining biochemical stress with physical barriers. Abnormal deposition of collagen, tenascin C and hyaluronan is observed in GBM, which alters tissue stiffness and interstitial pressure [3, 37]. Spatial analyses show that ECM-rich regions tend to exclude cytotoxic lymphocytes and promote the proliferation of macrophages with a tissue-remodeling phenotype [11, 13]. Mechanistically, increased stiffness activates integrin signaling, promotes the production of immunosuppressive cytokines and restricts T-cell motility [38]. Additionally, hyaluronan-rich perivascular niche additionally hinders lymphocyte infiltration while simultaneously supporting macrophage retention [2]. The metabolic gradients establish biochemical barriers, hypoxia enhances checkpoint-mediated suppression and myeloid recruitment, and ECM architecture restricts immune cell access. The synergy of these interactions stabilizes immune niches within specific anatomical regions.

Glial-stromal networks anchor immune populations within spatial niches

While environmental gradients such as hypoxia and ECM architecture constrain immune cell infiltration, the precise spatial organization of immune niches ultimately depends on active cellular networks discussed in Sect. 3. Within these permissive environments, TAMs, reactive astrocytes, and endothelial cells function as central structural anchors.

Rather than randomly diffusing throughout the tumor, myeloid populations and Tregs are precisely positioned into hypoxic and perivascular domains through the glial-derived chemokine gradients and ligand-receptor axes (e.g., CSF1 and CCL2) detailed previously [1, 2, 14]. Importantly, endothelial cells and reactive astrocytes integrate these immune signals with local stromal stress. By responding to hypoxia and altering blood–brain barrier permeability, these stromal elements physically restrict immune cell entry and promote the retention of suppressive phenotypes [3, 10, 26].

Consequently, the biochemical barriers of metabolism, the physical constraints of the ECM, and the active spatial tethering by glial-immune cross-talk synergize. These reciprocal interactions create a self-amplifying loop that locks regulatory cells into place, effectively stabilizing the immunosuppressive niches and embedding therapeutic resistance within the tumor microenvironment [24, 27].

Spatial functional immune states in brain tumors

Immune activation within the tumor microenvironment

Through the spatial organization and maturation processes described above, the embedding of suppressive circuits gives rise to region-specific functional immune states. Distinct combinations of immune cell populations, metabolic conditions, and stromal signals within these niches collectively determine whether local immune responses become activated, suppressed, or excluded. The glioma immune microenvironment is not uniformly suppressive but encompasses functionally distinct territories ranging from inflammatory to immunosuppressive states. Spatial transcriptomic and imaging studies have identified inflammatory microdomains characterized by activated interferon signaling, antigen presentation machinery, and cytotoxic lymphocyte infiltration [1, 2, 13]. These regions typically localize near perivascular interfaces, where endothelial activation and chemokine expression facilitate immune cell migration [2, 23].

Myeloid cell populations within these inflammatory areas do not remain in static pro- or anti-inflammatory states but exhibit context-dependent plasticity. Under specific environmental influences, these cells retain the capacity to support antigen presentation and T-cell activation [1, 13]. This suggests that inflammatory microdomains represent poised immune environments with latent antitumor capacity, rather than merely transient or ineffective immune responses.

Consistent with this plastic immune landscape, single-cell transcriptomic and epigenetic analyses reveal that a subset of $CD8^+$ T cells within gliomas retain activation markers and cytotoxic molecules, indicating preserved effector functions despite partial exhaustion signatures [6, 10]. Moreover, neoadjuvant immune checkpoint blockade in both preclinical models and human patients can restore T-cell function, particularly when combined with strategies that enhance antigen presentation or reduce myeloid suppression [23, 39]. These findings collectively demonstrate that inflammatory niches in gliomas are reversibly suppressed rather than irreversibly dysfunctional, providing a spatial substrate for therapeutic reactivation.

Despite this residual immune activity, inflammatory microdomains are inherently unstable and progressively transition toward immunosuppressive states through continuous antigen exposure and metabolic stress. Single-cell trajectory analyses reveal that chronic stimulation drives T cells along a differentiation path toward terminal exhaustion, with gene signatures of dysfunction increasing over time and spatially overlapping with macrophage-dense regions [2, 10, 26, 40]. This spatial gradient of exhaustion, extending from inflammatory peripheries to suppressive cores, suggests that T-cell dysfunction propagates locally through cytokine diffusion and metabolic constraints rather than occurring uniformly across the tumor.

These observations indicate that inflammatory niches represent dynamic immune territories that can either sustain antitumor activity or gradually evolve into suppressive microenvironments depending on local regulatory signals.

Immunosuppressive microenvironments and coordinated immune escape

As inflammatory niches progressively transition toward suppressive states, T-cell exhaustion emerges as a central axis of adaptive immune failure. Recent scRNA-seq and multiplex imaging consistently demonstrate that T cells infiltrating GBM persistently express inhibitory receptors including PD-1, TIM-3, and LAG-3, while simultaneously exhibiting reduced effector cytokine production and proliferative capacity [10, 13, 41]. Importantly, these exhausted states are spatially enriched in regions characterized by high antigen burden and suppressive cytokine signaling, indicating that exhaustion is locally induced rather than systemic [2].

Parallel to adaptive immune dysfunction, innate immune reprogramming occurs results in a continuous spectrum of macrophages and microglia activation states [1, 2, 6]. Hypoxic cores and necrotic regions preferentially harbor myeloid populations expressing robust immunoregulatory and tissue-remodeling programs, whereas perivascular areas may retain partially inflammatory subpopulations [2, 13]. This spatial stratification demonstrates that myeloid phenotypes are dynamically shaped into highly specific local configurations. Such macrophage-enriched suppressive niches frequently overlap with mesenchymal-like tumor regions [1, 6].

These resulting adaptive and innate immune dysfunctions are not independent processes but form integrated networks that consolidate immune escape. Driven by the complex glial-immune cross-talk established earlier, exhausted T cells amplify suppressive myeloid phenotypes, while tolerogenic macrophages reciprocally reinforce T-cell dysfunction. These interactions generate spatially confined immunosuppressive hubs and create a self-sustaining feedback loop resistant to spontaneous immune reactivation [1, 2]. The establishment of such compartmentalized immune dysfunction dictates that systemic immune activation may fail to penetrate suppressive hubs, necessitating strategies that simultaneously disrupt these localized cellular networks and metabolic barriers. To provide a holistic conceptual framework, the entire orchestration of clonal evolution, localized fate transitions, and the multi-barrier lockdown that stabilizes these immunosuppressive architectures is comprehensively integrated and summarized in Fig. 4.

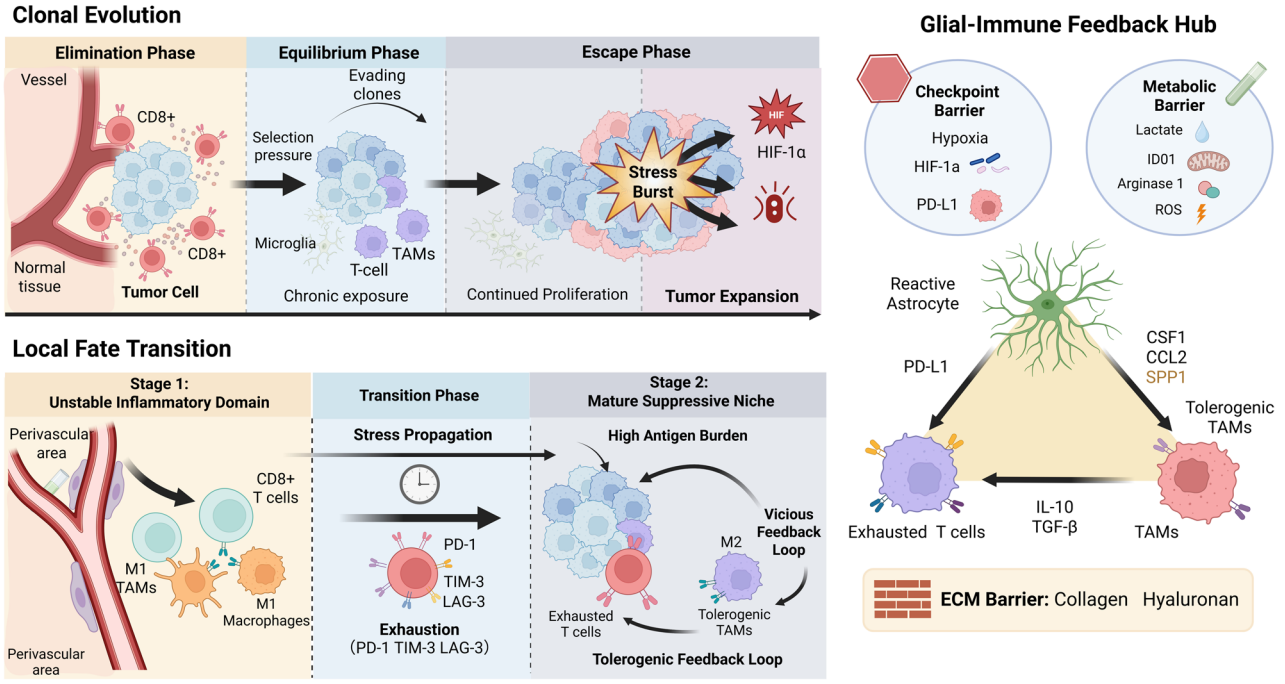

Fig. 4 Spatiotemporal Evolution and the Glial-Immune Feedback Hub in Glioblastoma. **A** Clonal Evolution: Immunoediting drives the progression from active immune surveillance to the selection of evading clones, culminating in tumor expansion and metabolic stress pathway activation (e.g., HIF-1α) during the escape phase. **B** Local Fate Transition: Unstable inflammatory microdomains progressively transition into mature suppressive states on a temporal scale. Chronic stress drives severe T-cell exhaustion (PD-1, TIM-3, LAG-3) and establishes a tolerogenic feedback loop dominated by TAMs. **C** Glial-Immune Feedback Hub: The immunosuppressive phenotype is stabilized by the synergy between glial-immune crosstalk (via SPP1, CSF1, IL-10, TGF-β) and a multi-barrier system. This includes an ECM barrier restricting immune access, a metabolic barrier, and hypoxia-induced checkpoint suppression (PD-L1), collectively locking the niche into an immune-evasive state

Spatial architecture as a determinant of immunotherapy responsiveness

Traditional interpretations of immunotherapy resistance in GBM have emphasized systemic immunodeficiency; however, growing recognition of intra-tumoral immune heterogeneity necessitates a spatially informed perspective [13, 23]. Specifically, perivascular regions with functional vasculature permit immune cell access and may respond to checkpoint inhibition, whereas hypoxic, necrotic, or metabolically restricted areas remain dominated by suppressive myeloid cells and lymphocyte depletion [1, 2].

Spatial transcriptomic analyses further reveal that expression of immune checkpoint ligands, cytokine gradients, and metabolic stress signatures vary widely across tumor regions, creating local treatment barriers even within single lesions [1, 2]. Consequently, systemic immune activation does not guarantee spatially uniform or effective intratumoral responses. This spatial heterogeneity explains the inconsistent and locally limited radiographic and molecular responses to immune checkpoint blockade observed in clinical trials.

These observations indicate that therapeutic responsiveness is influenced not only by the overall immune composition of the tumor but also by the spatial organization of immune niches within the tumor microenvironment. Longitudinal spatial profiling reveals that immune niches dynamically evolve under treatment pressure, leading to shifting regional immune states over time [15, 42, 43]. Understanding the cellular interaction networks that establish and maintain these spatial immune architectures is therefore essential for interpreting treatment resistance and guiding the development of more effective immunomodulatory strategies.

Clinical implications of immune ecological niches and therapeutic targeting strategies

Immune niches as biomarkers: prognostic and therapeutic implications

The spatial organization of immune niches in GBM reflects the cumulative effects of the cellular interactions and immunoregulatory mechanisms described above, and therefore constitutes a biologically meaningful unit with significant clinical relevance. Traditional immune typing methods based on bulk transcriptomics or single-marker staining have given unreliable results for predicting prognosis. This is mainly because they do not consider spatial heterogeneity [1, 34]. In contrast, spatial analyses using proteomic profiling (such as multiplexed IHC) and spatial transcriptomics datasets (like Visium)

show that the immune system has a clear compartmentalized structure. Usually, the core area is dominated by macrophages, while the outer area is more permissive to immune cells [2, 11].

Spatial transcriptomic analyses provide strong biological evidence for this compartmentalization. They show that mesenchymal regions rich in macrophages, which represent the suppressive myeloid patterns mentioned earlier, are linked to worse outcomes than neural progenitor–like regions, establishing spatial organization as an independent prognostic factor [2, 6, 44]. Critically, hypoxia- and TGF-β–associated programs do not work alone as molecular markers. Instead, they work together in specific spatial areas that are related to poor prognosis [11, 42]. Thus, the architecture of immune niches itself provides prognostic information beyond individual molecular signatures. Furthermore, single-cell and spatial profiling have identified specific biomarkers, such as S100A4, as a spatially defined immunosuppressive TAM marker in human GBM, presenting a promising targeted immunotherapy candidate [5].

Beyond myeloid populations, this spatial arrangement directly affects how T cells work, consistent with the spatial immune states described in Sect. 5. T cells that only exist around blood vessels have weak cytotoxic ability and are related to worse survival [10, 23]. However, when organized immune aggregates appear, even rarely, they are associated with stronger immune activation and improve outcomes [1].

These biological observations demonstrate that patient prognosis depends less on absolute immune cell numbers and more on the structural organization of immune niches. This indicates that spatial context, rather than mere immune cell presence, must guide therapeutic strategies.

Based on this spatial biological findings, clinical evaluations further confirm the important role of niche architecture. While, immune checkpoint blockade has not worked very well in most GBM patients [7], neoadjuvant PD-1 inhibition can successfully change the local immune remodeling in a specific subset of responders [45]. Spatial profiling shows that patients who respond well usually harbor pre-existing immune-permissive microdomains, typically located near functional perivascular interfaces. Rather than reversing the profound immunosuppression within hypoxic tumor cores, checkpoint blockade effectively capitalizes on the baseline inflammatory signatures and active antigen presentation already present within these specific permissive niches to drive localized T-cell clonal expansion [11, 23, 45]. Consequently, the initial structure of the immune niche may predict the response of immunotherapy better than just PD-L1 expression alone. Emerging computational methods further translate spatial architecture into quantitative biomarkers: spatial entropy metrics and immune exclusion indices are being evaluated for treatment response prediction [46, 47]. Although these approaches require prospective validation, they represent a conceptual shift from descriptive immune typing to spatially informed predictive stratification.

Therapeutic targeting of immune niches

The robust immunosuppressive infrastructure within GBM tumors necessitates therapeutic approaches that remodel tumor architecture rather than merely inhibiting individual signaling pathways. Spatial multi-omics analyses often find that tumor cores are rich in macrophages, with high CSF1R activity, hypoxia-related gene expression, and lipid metabolism features [2, 11, 13]. These compartments act as independent metabolic and gene-expression hubs. They limit the function of T cells by taking away nutrients and causing oxidative stress. Thus, immune resistance in GBM comes from these spatially organized, self-sustaining suppressive regions.

Early macrophage-targeting approaches illustrate the limitations of neglecting spatial structure. Although CSF1R inhibition initially changed TAM reprogramming and slowed tumor growth in preclinical models [48], resistance quickly developed through alternative survival signaling and macrophage flexibility [12]. Spatial profiling reveals that residual macrophages reaccumulate in tumor cores, maintaining local immunosuppression despite reduced cell numbers [42]. These results demonstrate that reducing macrophage counts without altering tumor architecture permits suppressive niche reconstitution.

Checkpoint blockade provides a parallel illustration. The phase III CheckMate-143 trial showed that nivolumab improved survival only slightly in patients with recurrent GBM [7], even though studies found that neoadjuvant PD-1 inhibition can activate local interferon signaling and help T cells expansion [23, 45].Spatial analyses indicate that such immune activation remains confined to pre-existing immune-permissive regions, while macrophage-rich cores retain resistance [11]. Therefore, activating the immune system throughout the body is not enough to break through the strong suppressive hubs.

These findings collectively support a strategic shift toward therapies that target and dismantle immune niche architecture. Vascular normalization through VEGF signaling can temporarily improve T-cell infiltration [49]. Spatial studies further show that genes related to angiogenesis and myeloid pathways are often enriched together in the same regions, suggesting that dual targeting may be needed to reduce niche stability [42]. Similarly, modulation of hypoxia and lipid metabolism pathways has partially restored T-cell cytotoxicity in macrophage-dense regions in experimental models, highlighting the therapeutic potential of metabolic niche remodeling.

Spatial evolution of immune niches: the driver of therapeutic resistance

The targeted therapeutic strategies discussed previously often face eventual resistance because they treat the tumor microenvironment as a static entity. In reality, immune niches in GBM are dynamically changing ecological units that develop through tumor plasticity, undergo adaptive changes in immune function, and are gradually reshaped under therapeutic selection. Single-cell lineage tracing demonstrates malignant cells dynamically switch between proneural, classical, and mesenchymal programs in response to environmental stress [6]. Spatial transcriptomic analyses further indicate that mesenchymal-like tumor regions are more likely to be enriched with macrophages and express higher levels of immunomodulatory cytokines [6, 11]. These observations support that tumor state transitions and macrophage recruitment develop together, forming structurally stable immunosuppressive regions.

Within these established niches, continuous antigen exposure drives progressive gradual T-cell dysfunction. Exhaustion-associated gene signatures increase over time and spatially overlap with macrophage-dense regions [2, 10]. Trajectory modeling suggests that chronic stimulation drives T cells toward terminal dysfunction along a clear differentiation path [40, 50]. Spatial analyses reveal exhaustion gradients extending from immunosuppressive cores, supporting the idea that T-cell dysfunction spreads locally through cytokine diffusion and metabolic restrictions, rather than appearing uniformly across the tumor.

Under therapeutic and temporal pressure, these niches become more stable through evolutionary selection. Comparative studies of primary and recurrent GBM show that recurrent tumors have a higher proportion of myeloid cells and lower cytotoxic T-cell signatures [11, 51]. Recent integrated datasets suggest that recurrence-associated tumor clones strengthen suppressive cytokine networks, leading to a more stable tolerogenic structure [11]. Similarly, while neoadjuvant PD-1 blockade causes temporary interferon signaling and T-cell clonal expansion [23, 45], compensatory myeloid expansion and alternative checkpoint upregulation frequently restore the immunosuppressive balance [2, 42].

This inherent capability of immune niches to dynamically evolve explains why purely static, single-timepoint therapeutic targeting frequently fails. Viewing these niches as adaptive ecosystems dictates that single-snapshot evaluations are fundamentally insufficient. This dynamic evolution underscores an urgent clinical need to shift toward longitudinal and multidimensional monitoring, paving the way for the future methodological breakthroughs discussed in Sect. 7.

Current limitations and future breakthroughs in immune niche research

Major limitations of current research

Although spatial multi-omics technologies have significantly improved our understanding of the immune system in GBM, significant methodological and conceptual challenges remain. Early ST platforms only provided transcription data at the multicellular level, which limited accurate cell type identification and interpretation of cell-cell interactions [52, 53]. While, computational deconvolution methods that integrating scRNA-seq partially mitigate this [54, 55], accuracy highly depends on reference data and is compromised by the remarkable transcriptional plasticity of TAMs and malignant cells [2, 6]. High-resolution platforms such as Slide-Seqv2 and Merfish improve spatial accuracy, but often sacrifice transcriptome coverage and scalability [56, 57]. Consequently, current ST techniques effectively capture structural patterns but may insufficiently resolve transitional cell states crucial for immune niche dynamics. To address these limitations, advanced spatial transcriptomic platforms like Xenium, utilizing prime 5 K and custom add-on panels, have recently emerged to offer subcellular localization at a near-whole transcriptome resolution. This provides unprecedented transcriptome coverage to flawlessly resolve complex cell states within these niches [58].

Beyond resolution, fundamental limitations in temporal inference and causal interpretation remain. Most spatial analyzes rely on single-timepoint surgical specimens, providing static snapshots of dynamic ecosystems [1, 11]. Although trajectory modeling of scRNA-seq data estimates pseudo timelines for T cell exhaustion and macrophage reprogramming [2, 10], it cannot measure actual temporal changes.

A more fundamental challenge is that spatial colocalization does not prove causality, leading to a profound "chicken-and-egg" dilemma regarding niche formation. For instance, mesenchymal tumor regions are rich in macrophages and associated with poor prognosis [6, 44], yet whether macrophage accumulation drives tumor state transitions or represents a secondary response remains unresolved. One perspective posits that tumor-intrinsic genetic or epigenetic shifts initiate the mesenchymal transition, which subsequently recruits macrophages to establish a suppressive niche.

An emerging opposing view suggests that macrophage-driven inflammatory stress actively forces adjacent tumor cells into a mesenchymal state. Preclinical perturbation experiments, including CSF1R inhibition [12, 48], provide partial mechanistic insights but cannot fully resolve this casual ambiguity human tumors.

These interpretation challenges are further complicated by discrepancies between animal models and the human

tumor microenvironment. Genetically engineered mouse models and human GBM diverge significantly in immune ontogeny, antigenicity, and myeloid cell composition [18, 59]. Comparative single-cell analyses reveal distinct macrophage transcriptional programs across species, and preclinical immunotherapy responses often fail to translate clinically [2, 7]. Progress from descriptive mapping to mechanistic understanding requires overcoming these interrelated limitations.

Key breakthrough directions for future research

Overcoming these limitations requires methodological integration and conceptual expansion. Multi-layer spatial profiling is an essential starting point. Because protein abundance and metabolic states often diverge from RNA levels due to post-transcriptional regulation and stress response [60], integrating high-dimensional spatial proteomics [61, 62] and emerging spatial metabolomics [63] can reveal how metabolic stress, cytokine signaling, and cellular topology interact to stabilize inhibitory niches in GBM.

To resolve the temporal and causal controversies discussed above, longitudinal studies represent a critical breakthrough. Paired pre-and post-treatment sampling has revealed therapy-induced immune remodeling [23, 45]. Combining scRNA-seq with ST over time enables direct tracking of immune state transitions, which improves causal inferences by linking structural changes to treatment outcomes.

Given the complexity of spatial and longitudinal datasets, analytical innovation becomes essential. Artificial intelligence and systems biology approaches increasingly model cellular interaction networks and quantify spatial entropy [62, 64, 65]. Machine learning models that integrate spatial imaging and transcriptome data show promising results in predicting response to immunotherapies in solid tumors [66], paving the way for predictive ecosystem modeling in GBM.

Ultimately, clinical translation relies on precision strategies targeting immune microenvironment remodeling. Combination therapies that simultaneously block myeloid signaling, normalize vascular structure, and enhance T-cell activation show promising results in preclinical research systems [67]. Spatially informed biomarkers could soon guide patient selection, as initial evidence confirms immune niche topology influences treatment efficacy and durability [11, 68]. Integrating these spatial profiles into adaptive trial designs will propel research from structural characterization to rational, niche-disrupting clinical application.

Conclusion

This review highlights that antitumor immunity in gliomas is not diffusely distributed but is spatially organized into structured immune microregions. These niches arise through dynamic, reciprocal interactions between glial cells and infiltrating immune populations. Astrocytes, microglia, and other glial cells actively dictate the localization and functional status of immune cells, synergizing with metabolic stressors to stabilize formidable immunosuppressive hubs within the tumor microenvironment. These findings collectively support a critical paradigm shift: from linear, pathway-centered models to a holistic ecosystem approach that integrates spatial topology, cellular cross-talk, and metabolic constraints. The true potential of immunotherapy in GBM will not be realized by merely amplifying systemic immune responses, but by strategically dismantling the spatial and metabolic fortresses erected by these glial-immune interactions. While advances in ST and single-cell profiling have illuminated the architecture of these ecosystems, moving forward requires transitioning from static descriptive mapping to dynamic mechanistic interventions. By combining longitudinal multi-omics, spatially informed biomarkers, and adaptive clinical trials, future research can translate our understanding of immune niche topology into precision, patient-specific immunotherapeutic strategies, ultimately offering renewed hope for patients with refractory brain tumors.

Abbreviations

DCs	Dendritic cells
ECM	Extracellular matrix
GBM	Glioblastoma
NK	Natural killer (cells)
scRNA-seq	Single-cell RNA sequencing
ST	Spatial transcriptomics
TAMs	Tumor-associated macrophages
Tregs	Regulatory T cells

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