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Exploring glioblastoma microenvironment using organoids: opportunities, limitations, and emerging concepts

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ABSTRACT

Glioblastoma (GBM) remains the most lethal primary brain tumor in adults. These aggressive tumors evolve as dynamic, spatially organized ecosystems in which tumor cells continuously interact with the surrounding brain parenchyma and systemic environment. These reciprocal interactions actively drive invasion, therapeutic resistance, and ultimately, inevitable recurrence. Modelling this level of complexity has long required significant compromise. GBM organoids have emerged as a promising intermediate platform, bridging the gap between costly, low-throughput animal models and overly simplistic two-dimensional in vitro cultures. In this review, we summarize the diverse protocols currently used for GBM organoid derivation and long-term maintenance, focusing on the recapitulation of microenvironmental traits. We further discuss how these systems enable the investigation of tumor niche architecture and dynamic crosstalk with key components of the tumor microenvironment, including neural and immune elements, vascular-associated signals, and extracellular matrix cues. Although the inherent limitations of ex vivo systems must be carefully considered, increasingly advanced and well-designed protocols will enable robust interrogation of interactions within the GBM ecosystem and provide powerful platforms for therapeutic testing.

1. Introduction

Glioblastoma (GBM) is the most frequent and highest-grade malignant primary brain tumor in adults, characterized by diffuse infiltration, rapid proliferation, and near-universal recurrence regardless of aggressive therapy [1, 2]. Despite advances in surgery and supportive care, frontline treatment is still centered on maximal safe resection followed by radiotherapy with concomitant and adjuvant temozolomide (TMZ), resulting in a median overall survival of only 14–16 months, with a 5-year survival rate below 5% [3, 4]. The therapeutic failure in GBM stems from multimodal challenges, including intratumoral heterogeneity and cellular plasticity, conferring both intrinsic and adaptive resistance to treatment [5, 6]. In parallel, an immunosuppressive tumor microenvironment (TME) and the restrictive properties of the blood–brain barrier (BBB) further limit effective drug delivery and anti-tumor immunity, resulting in poor clinical outcomes [7, 8]. As a result, GBM continues to exhibit poor response to systemic therapies and immunotherapies, underscoring an urgent need for preclinical models that more accurately recapitulate its biological complexity.

A wide spectrum of preclinical models has been developed to investigate these mechanisms, yet no single system fully recapitulates the complexity of GBM biology [9]. In vitro approaches range from conventional 2D cell lines to 3D stem-like cultures that enable scalable perturbation, drug screening, and mechanistic discoveries [10, 11]. Importantly, these cultures lack key TME inputs and only partially reproduce native tissue architecture, diffusion-limited niches, and the multicompartment interactions that shape therapeutic response. In vivo models provide systemic context but introduce distinct trade-offs; immunocompetent syngeneic glioma models enable the study of anti-tumor immunity and therapy-induced immune dynamics [12, 13], whereas genetically engineered mouse models (GEMMs) offer controlled initiation, yet often under-represent patient-level diversity and evolutionary trajectories [14, 15]. Patient-derived orthotopic xenografts (PDOXs) retain key features of human GBM invasion and malignant cell-state programs [16, 17], but require immunodeficient hosts and progressively substitute human TME components with murine

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counterparts [18, 19]. Collectively, these limitations define a persistent "model gap" between experimental feasibility and clinical fidelity.

GBM organoid platforms have emerged as an intermediate solution that combines elements of in vivo-like tissue organization with the scalability and experimental control of ex vivo cultures, enabling preservation of patient-specific tumor features while allowing controlled perturbations [20]. In this review, we discuss how diverse GBM organoid platforms can be applied to model the GBM ecosystem, with particular emphasis on the reconstruction of key components of the TME for functional studies and assessment of TME-targeting therapies.

2. Glioblastoma, a heterogeneous and dynamic ecosystem

GBM is increasingly recognized as a dynamic, self-organizing ecosystem in which malignant cells co-opt the brain environment and attract additional immune components from the circulation to sustain growth, invasion, and therapeutic resistance (Fig. 1) [5, 7]. This ecological perspective emphasizes pronounced spatial and temporal heterogeneity, whereby distinct cellular states and microenvironmental niches coexist and continuously evolve under selective pressures imposed by therapy and local environmental cues.

2.1. Tumor cell heterogeneity and plasticity

GBM is characterized by marked molecular and functional heterogeneity and state plasticity, which together account for both the initial treatment resistance and recurrence observed clinically. Single-cell and spatial transcriptomic analyses have consolidated a view of GBM as a continuum of dynamic cell states rather than discrete subtypes. Within individual tumors, the classical, proneural, and mesenchymal expression programs coexist along a proneural–to–mesenchymal axis, where the overall equilibrium of states dictates GBM subtypes [21]. Malignant states, largely defined as neural progenitor-like (NPC-like), oligodendrocyte progenitor-like (OPC-like), astrocyte-like (AC-like), and mesenchymal-like (MES-like), create dynamic phenotypic equilibria shaped by genotype and environmental cues [22, 23]. Genetic drivers set the initial state composition, with EGFR amplifications enriching for AC-like programs, PDGFRA/CDK4 amplifications favoring OPC/NPC-like traits, and NF1 loss or inflammatory signaling promoting MES-like transitions [22]. Dynamic TME cues such as hypoxia, cytokines, and neuronal activity continuously reposition cells along this landscape, while developmental regulators (e.g., OLIG2, SOX2) and stress pathways (STAT3, FOSL1, NF-κB) reinforce lineage or resistance states [24]. Another functional gradient lies along the axis of glioma stem-like cells (GSCs) and more differentiated phenotypes, where GSCs reside in perivascular and hypoxic niches, and can be enriched in serum-free culture conditions [5, 25]. The general ability of GBM cells to regenerate a TME-specific equilibrium of states argues against a rigid, one-way hierarchy and supports a gradient-like, reversible organization of states [26]. Fidelity of molecular features and retention of such plastic potential in preclinical models is crucial to accurately investigate GBM biology, responses to treatment, and genetic perturbations. While conventional long-cultured GBM cell lines might evolve to less plastic phenotypes, with a higher dependence on the proliferation-sustaining growth pathways, advanced patient-derived models retain a higher degree of phenotypic plasticity, allowing modelling clinically relevant tumor biology and escape mechanisms better. The critical issue is therefore not merely whether a model preserves a given molecular

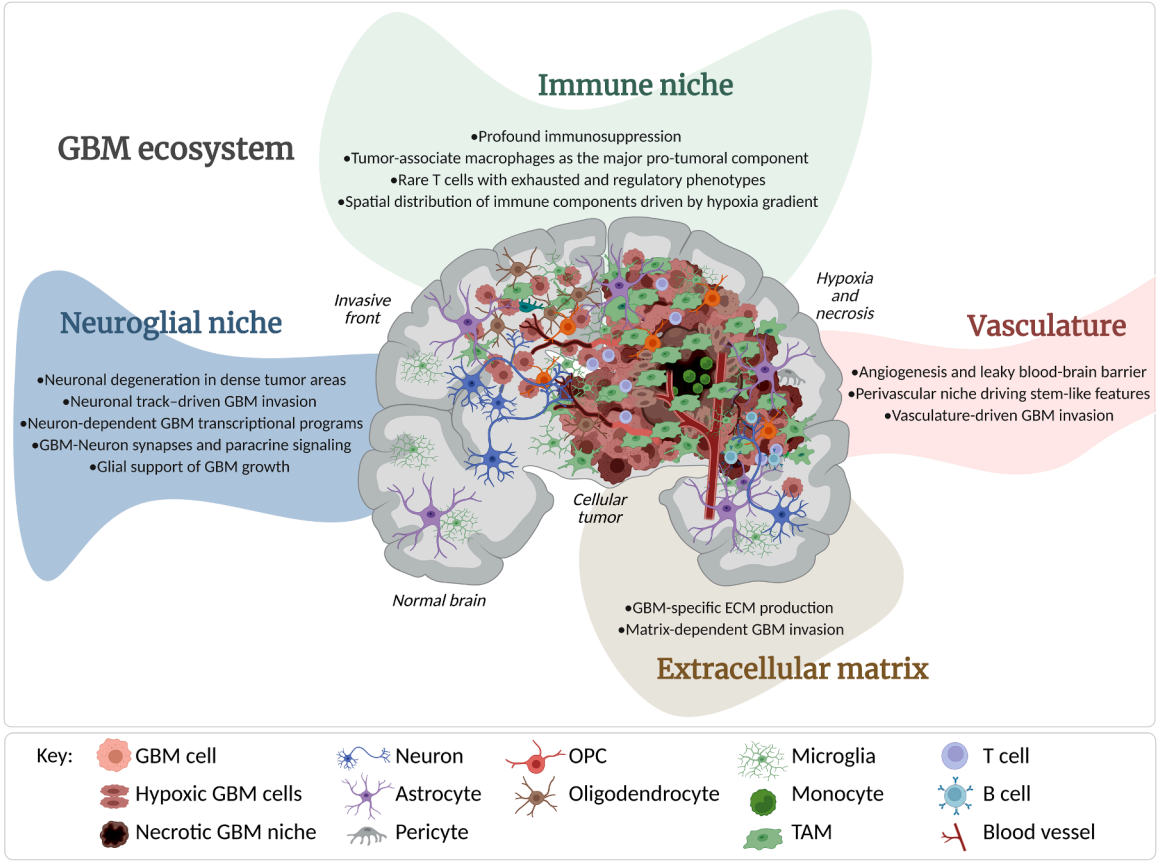

Fig. 1. The GBM ecosystem and its TME niches. Schematic representation of the GBM ecosystem illustrating spatial organization, creating a gradient of niches across hypoxia and necrosis, dense cellular tumor and invasive front, towards normal brain parenchyma. The diagram highlights key interconnected TME components that contribute to tumor progression, invasion, and adaptation through complex cellular and molecular crosstalk. Abbreviations: BBB: blood–brain barrier; ECM: extracellular matrix; GBM: glioblastoma; OPC: oligodendrocyte precursor cell; TAM: tumor-associated macrophage.

subtype, but whether it retains the capacity for reversible state transitions that drive adaptation under microenvironmental or therapeutic pressure.

2.2. Microenvironmental Niches

The Ivy Glioblastoma Atlas Project provided a foundational molecular map of GBM, defining anatomy-linked compartments as leading edge, infiltrating tumor, cellular tumor, microvascular proliferation, and pseudopalisading cells around necrosis, whose cellular composition is strongly constrained by local perfusion, oxygenation, and tissue architecture [27]. Subsequent integrative spatial studies extended this framework by showing that GBM is organized by spatial segregation of lineage states and regionally adaptive programs, with hypoxic zones around necrosis displaying structured, multi-layered architectures whereas other tumor regions, including densely cellular but non-necrotic tumor and infiltrative edges, appear comparatively less structured [28, 29]. The perivascular niche represents a privileged signaling environment in which endothelial cells and associated mural elements reinforce self-renewal and treatment tolerance. Early work established that GSCs cluster around vessels and depend on endothelial-derived cues [30], including endothelial Notch ligands and perivascular nitric oxide [31, 32]. This crosstalk is bidirectional as GSCs may generate pericyte-like vascular support, strengthening vessel function and tumor growth [33]. In parallel, the hypoxic–necrotic niche imposes oxygen and nutrient gradients that drive stress programs, metabolic rewiring, and promotion of GSC and MES-like stress-associated states [28]. Conversely, the invasive niche is shaped by growth within brain parenchyma, where tumor cells adopt "brain-coupled" programs rather than angiogenic/hypoxic architectures [28]. Infiltrating GBM cells rely on less-cycling, developmentally biased OPC-/NPC-like programs while retaining plasticity toward stress-adapted MES-like states under inflammatory or therapeutic pressure [22, 34]. Beyond soluble cues, neuronal activity can directly support these edge ecosystems via activity–dependent neuron–glioma coupling, including glutamatergic neuron–glioma synapses, paracrine excitatory signaling, and tumor cell network formation through tumor microtubes [35]. Such a dynamic niche organization brings a particular challenge to preclinical modelling. While closest to the patient situation, intracranial PDOX models and GEMMs rarely recapitulate all TME hallmarks in one model, with visible inter-model diversity of angiogenesis and invasion potential. To what extent ex vivo and in vitro culture models can reconstitute such spatial diversity remain debatable. Consequently, model fidelity should be interpreted in a context-dependent manner, based on how effectively a given system recapitulates the niche-associated process under investigation, rather than on whether it reproduces the full spatial complexity of GBM.

2.3. GBM ecosystem at treatment and recurrence

Recurrence can be viewed as a reassembly of the GBM ecosystem under the combined selective pressure of extensive surgery, radiotherapy, and TMZ, combining clonal selection with state reprogramming. Longitudinal profiling of paired primary and recurrent tumors indicates that overall tissue architecture is largely preserved, and no single, common cellular or molecular trajectory has emerged across patients [36, 37]. Early driver alterations are largely retained, while subsequent evolution is dominated by patient-specific reshaping of subclonal representation and copy-number architecture, with therapy-induced mutational processes adding additional, partly stochastic variation [36, 37]. GBM intrinsic expression subtypes are conserved in the majority of cases at recurrence. When transitions occur, they are most often tracked toward mesenchymal/injury–inflammatory programs, with a smaller subset showing increased neuronal-like signatures [38]. Recurrent tumors that display enrichment of MES-like/injury-response programs are often linked to AP−1–associated stress circuitry and coordinated remodeling of inflammatory compartments, whereas neuronal-like signals at recurrence can reflect, at least in part, a compositional tilt toward a more glio-neural milieu alongside reduced malignant fraction [21, 23, 39, 40]. To assess treatment responses of tumor cells and associated TME components directly upon treatment, advanced preclinical models are essential. Comparative studies will be needed to reveal whether in vitro co-culture protocols can replace in vivo models to assess complex responses of the GBM ecosystem to treatment in time [41, 42]. Moreover, assessing treatments modulating TME components will require specific protocols, particularly for immunotherapies that involve the presence of both innate and adaptive immunity [7, 19, 43]. This underscores that treatment response should be considered at a dynamic ecosystem level, in which tumor-cell survival, state reprogramming, and TME remodeling may each contribute to recurrence and therapeutic failure.

3. GBM organoid platforms

The marked aggressiveness of GBM has catalyzed the development of diverse preclinical models using multiple protocols. In this context, GBM and other brain tumor organoid platforms have emerged as advanced systems that support the recapitulation of tumor heterogeneity, cellular plasticity, and TME niches [20]. Here, we review the major experimental routes used to generate GBM organoid models (Fig. 2**A**). In particular, we emphasize the strengths and limitations of diverse approaches in recapitulating TME cues (Fig. 2**B**), which we discuss in greater detail in the respective sections below.

3.1. Patient-derived GBM organoids

Patient-derived organoids (PDOs), also referred to as tumoroids or glioblastoma organoids (GBOs), aim to model GBM as small, self-organized heterogeneous tumor ecosystems rather than a uniform cell population, with the explicit goal of preserving spatial structure, cellular diversity, and diffusion-driven gradients. Building on the "minimal manipulation" principle, Jacob et al. introduced a rapid workflow in which fresh surgical specimens are processed rapidly to limit ischemia and stress by careful removal of necrotic/hemorrhagic regions, and mechanical fragmentation of viable regions into small pieces (sub-millimetre to a few millimetre) [44]. Such tissue fragments retain native cell organization and can be cultured in serum-free non-adherent conditions to limit differentiation cues and sustain heterogeneous populations [44, 45]. PDOs are maintained in low-adhesion conditions with gentle agitation to improve nutrient/oxygen exchange while still allowing physiologically relevant gradients (Fig. 2). More recent protocols report the use of additional supplements (bFGF, EGF, insulin) to boost indefinite growth of PDOs in vitro [46–51]. These protocols closely resemble the primary tissue fragment cultures in serum-containing conditions, historically defined as organotypic spheroids [43, 52, 53]. Both PDO pipelines provide a robust route for generating primary cultures and straightforward coupling to orthotopic xenografting. However, these approaches differ in their passaging principles. Serum-free protocols rely on in vitro "cut-and-grow" passaging through mechanical dissociation [47, 48, 50, 51]. Early-passage cryopreservation is routinely incorporated to limit clonal selection and culture drift. Serum-based protocols rely on serial transplantations in vivo to expand patient tumor material as PDOXs [16, 54]. These technical differences have a profound impact on the TME components: in vitro passaging inevitably leads to progressive loss of non-proliferative TME elements [55–58], whereas in vivo passages result in replacement of human TME components by murine counterparts [18, 59]. Early-passage PDOs can retain tumor-associated macrophages (TAMs), endothelial and pericyte cells from the remaining blood vessel structures, but these compartments are typically transient unless specifically supported.

PDOs can also be reconstituted from single cell suspensions after

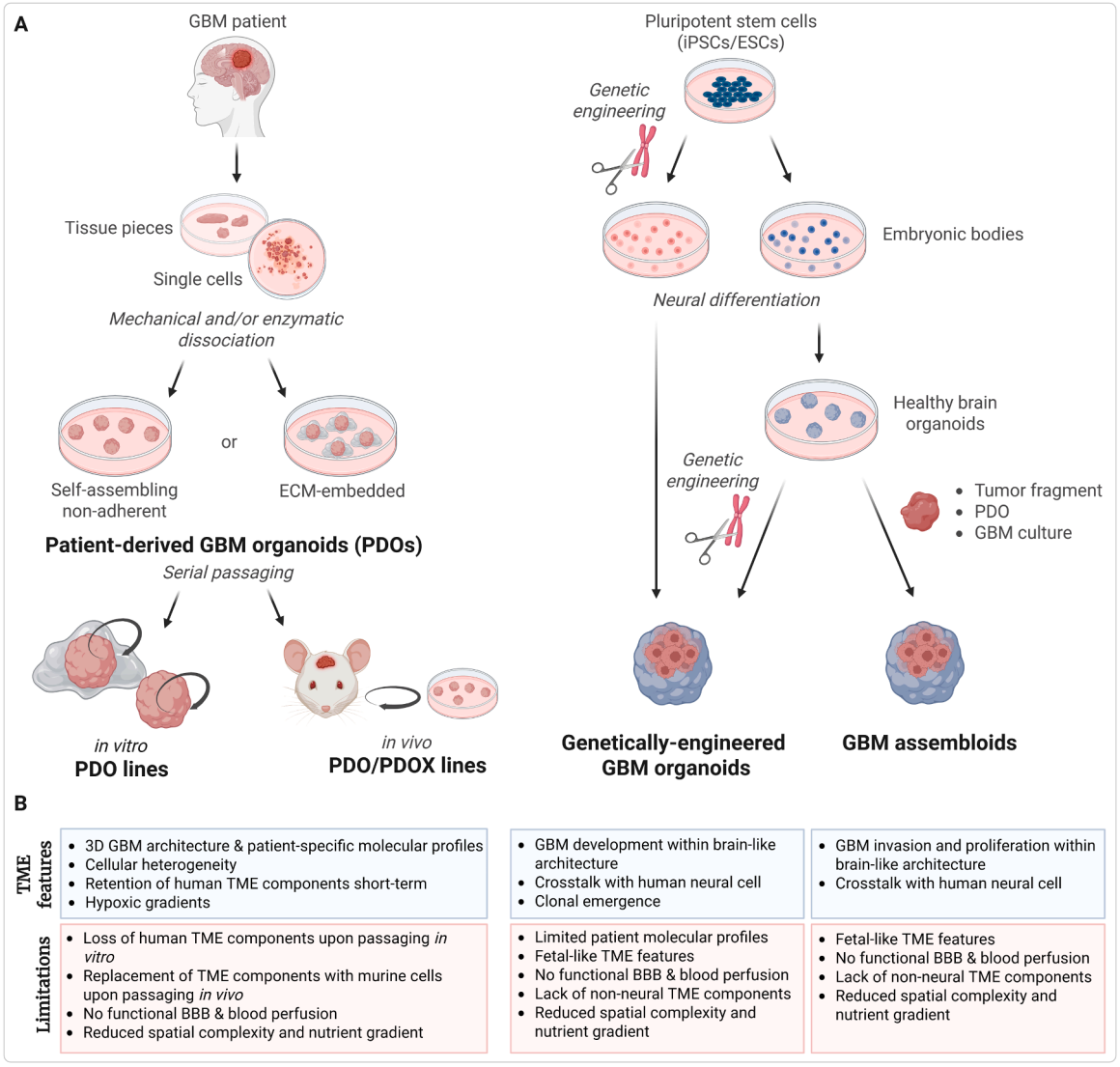

Fig. 2. GBM organoid platforms and their TME trade-offs. A. Schematic overview of major experimental protocols applied to generate GBM organoid models, including patient-derived GBM organoids, genetically engineered GBM organoids, and GBM assembloids. **B.** Comparative summary of key TME-related features and limitations of each platform, highlighting differences in patient specificity, tumor architecture, neural context, TME preservation, blood–brain barrier representation, perfusion, and spatial complexity. Abbreviations: ECM: extracellular matrix; GBM: glioblastoma; iPSCs/ESCs: induced pluripotent stem cells / embryonic stem cells; PDO: patient-derived organoid; PDOX: patient-derived orthotopic xenograft; TME: tumor microenvironment.

enzymatic tissue dissociation [60–63] with or without the support of the extracellular matrix (ECM) (Fig. 2). Foundational work by Hubert et al. established a matrigel-based 3D culture system that can reconstitute physiologically relevant proliferative–hypoxic compartmentalization and stem-like heterogeneity from GBM cell lines [64]. This protocol can also be applied to maintaining the growth of tissue fragments [46, 50]. Although ECM is not obligatory, as GBM fragments can self-organize, ECM embedding can be introduced deliberately to standardize morphology or support invasion-oriented readouts. How tissue dissociation and ECM influence the maintenance of the heterogeneous niches and TME components needs further investigation. In PDO systems, replicate-to-replicate variability may reflect the tumor region sampled, the initial proportion of malignant and non-malignant cells, organoid size, degree of dissociation, ECM exposure, culture duration, and passage number. Thus, variability between PDO replicates should be interpreted not only as technical noise, but also as a potential readout of regional heterogeneity and culture-driven selection.

Functionally, PDOs preserve intra-tumoral genetic and phenotypic heterogeneity arranged along hypoxia and nutrient gradients [45, 64]. Additional TME cues, such as hypoxia, can be externally imposed by incubating PDOs under controlled low-oxygen conditions (1–5% O_2) or chemical mimics (e.g., $CoCl_2$), driving mesenchymal transition and glycolysis and autophagy-associated survival mechanisms [55, 64–66]. In parallel, PDOs can also capture clinically relevant resistance phenotypes and standard-of-care responses (e.g., TMZ/radiotherapy) in a format that preserves inter-patient diversity and regional heterogeneity [16, 46, 55, 67] and reproduce therapy-induced DNA-damage signaling with limited impact on viability/invasion under TMZ and irradiation [48], thereby allowing mechanistic dissection of post-treatment persistence.

3.2. *Genetically-engineered GBM organoids*

Genetically engineered GBM organoids build GBM-like states de novo within human brain organoids derived from embryonic stem cells (ESCs) or induced pluripotent stem cells (iPSCs), shifting the modelling emphasis from faithful preservation of an individual patient tumor to genetic causality and isogenic control (Fig. 2). The core principle is to

trigger oncogenic transformation in an otherwise "normal" human neuroepithelial tissue context, typically by engineering mosaic driver activation and/or tumor-suppressor loss so that transformed tumor-like clones expand, invade, and remodel neighboring brain organoid tissue in a manner that more closely resembles clonal emergence than do uniformly transformed cultures [68, 69]. In practice, these models are based on cerebral or more committed dorsal forebrain and cortical organoids, generated from human ESCs or iPSCs, followed by targeted genetic perturbation using CRISPR/Cas9, transposon-based oncogene delivery, or combinatorial approaches coupled to fluorescent reporters for lineage tracing. A key design choice is when to introduce lesions: engineering at the pluripotent stage provides clean, clonally defined starting material for downstream organoid differentiation, enabling controlled comparisons across mutation sets [70], whereas editing within partially matured brain organoids better approximates tumor initiation within established brain tissue architecture and differentiation gradient [68]. Across engineered GBM organoid models, the normal compartment largely reflects the underlying cerebral organoid substrate, typically fetal-like cortical tissue containing neural stem/progenitor cells alongside immature neuronal and astrocytic lineages, whose proportions vary with organoid maturation and the duration of tumor outgrowth/colonization [68–70]. Accordingly, variability in genetically engineered GBM organoids can arise from the pluripotent stem cell line, timing and efficiency of genetic perturbation, degree of mosaicism, differentiation protocol, and maturation stage of the host neural tissue, all of which may influence tumor-like outgrowth and TME composition across organoid replicates.

Two foundational studies established the feasibility and logic of this platform. Ogawa et al. knocked an HRasˆG12V cassette into the TP53 locus to create mosaic transformed cells that invade within organoids and form tumors after xenotransplantation, with MES-like transcriptional features [68]. In parallel, Bian et al. developed "neoCORs" using Sleeping Beauty oncogene delivery with CRISPR tumor-suppressor perturbations, showing mutation-specific GBM-like neoplasms suitable for invasion assays and genotype-guided drug testing [69]. Subsequent work systematized this approach, for example an iPSC-clone-based "LEGO" approach defined tumor-suppressor combinations before differentiation to map mutation-dependent programs and drug-response states [70], while subtype-informed oncogenic programs profiled by single-cell/spatial transcriptomics recapitulated key GBM progression features after xenotransplantation [71]. Patient- or predisposition-informed iPSC models add additional context. For example, c-MET–mutant neuronal-like organoids show GBM-relevant signaling and altered TMZ sensitivity [72].

Notably, these genetically engineered GBM organoids inherently provide a human neural tissue context, but vascular and immune compartments are typically absent unless deliberately introduced, making them well-suited for dissecting tumor–brain interactions and genotype-driven trajectories while keeping TME complexity experimentally modular. Although further studies are required to characterize these models in greater depth, we anticipate that the composition of neural TME components depends on the maturation state of the original healthy brain organoids and the progression stage of tumor-like cells colonization.

3.3. GBM assembloids

In GBM assembloids, the organoid concept is applied to hybrid systems (Fig. 2) where patient-derived GBM material is combined with healthy ESC- or iPSC-derived brain organoids (cerebral or more committed cortical organoids) to model tumor–parenchyma interactions, particularly invasion and niche-driven phenotypic adaptation, in a human neural tissue context [73]. The guiding principle is modularity, where the brain organoid provides a reproducible, brain-like scaffold, while the malignant genotype and patient specificity are introduced through the GBM input, most commonly GSC cultures [74, 75], but increasingly also PDOs [73] or small tissue fragments [67]. A widely used implementation is the "GLICO" protocol, where GSC cultures home to and infiltrate cerebral organoids, forming invasive tumors that develop tumor microtube networks, enabling therapy testing in an organoid-resident setting [75–79].

Operationally, assembloids workflows typically converge on three main steps. First, brain organoids are created and developed until they exhibit stable neuronal and neuroepithelial compartments. Second, fluorescently traceable GBM cells are seeded onto brain organoids to create physical contact between the compartments. Integration is frequently visible within ~24–72 h, after which invasion and expansion proceed at rates reflecting patient-to-patient variations in proliferation and infiltrative capacity. Lastly, readouts are tailored for 3D architecture. Single-cell transcriptomics, tissue clearing, and high-resolution imaging are frequently used to measure invasion depth, tumor microtube formation, and tumor–host contacts [76]. In assembloids, additional variability arises from both compartments: the GBM input material and the host brain organoid. Differences in brain organoid maturity, size, cellular composition, tumor seeding position, and physical contact between the two components can affect integration efficiency, invasion depth, and tumor–host interaction readouts.

The platform has been formalized as an invasion and interaction assay rather than a purely descriptive co-culture thanks to several protocol-forward papers. Goranci-Buzhala et al. developed a set of standardized invasion assays that can support drug testing within the same experimental frame and differentiate invasive behaviors among GSC lines [80]. To maintain tumor cell fate heterogeneity while permitting invasion tracking, Azzarelli et al. developed a useful co-culture workflow that emphasizes repeatable assembloid production, tumor-cell labeling, and controlled co-incubation conditions [81]. More recently, more complex fusion-style co-culture formats incorporated GBM PDOs with cerebral organoids ("GCOA") to preserve intra-tumoral architecture while quantifying single-cell and collective invasion, intercellular tumor networks, and tumor–host interactions [73]. Further protocol refinements include "Flash Assembloids," where rapidly degradable oxidized alginate microgels are used to generate defined GBM–forebrain organoid interfaces for time-efficient modelling of early invasion, ECM remodelling, and tumor–host integration [82]. Similar assembloid principles have recently been extended to highly differentiated rat brain organoids as host tissue, enabling cross-species co--cultures with defined neuronal circuitry for functional readouts [83].

A recurring technical challenge is the accurate identification of GBM cells. Many studies label GBM cells with fluorescent or luminescent reporters (e.g., GFP, luciferase) before co-culture with brain organoids enabling live imaging and computational tracking of invasion dynamics, with recent FUCCI-based approaches further linking invasion trajectories to cell-cycle states [84], but this strategy is challenging when using intact primary tumor fragments. An elegant solution is the "IPTO" approach, in which tumor fragments are physically implanted into pre-labelled brain organoids, so that the host brain tissue, rather than the incoming tumor, is marked, enabling preservation of tumor structure and heterogeneity and patient-specific drug response prediction without genetic modification of the primary tissue [67]. Further on, this approach is highly adaptable and can include more specialized host environments and advanced functional measurements. For instance, a GBM–midbrain assembloid integrated with 3D electrode technology allowed for real-time electrophysiological monitoring during exposure to drugs [85]. Further advances now incorporate additional non-neuronal cell types, including microglia, immune cells, and vascular components, into brain organoids and PDO co-cultures to capture immune and stromal crosstalk, thereby expanding GBM assembloids toward more complete TME composition [86–88].

Collectively, these organoid platforms provide complementary strategies to model distinct aspects of GBM architecture and tumor–TME interactions. PDOs provide high patient-specific fidelity and can transiently retain endogenous TME features, whereas genetically-engineered

organoids and assembloids offer greater experimental control for modelling genotype-driven tumor development and tumor–brain interactions. Across these platforms, variability in TME recapitulation arises at several levels, including inter-patient and intra-tumoral heterogeneity, differences between independently generated organoid replicates, and protocol-dependent effects related to tissue dissociation, ECM embedding, media composition, passaging, organoid maturation, and co-culture design. Thus, platform selection should be guided by the biological or therapeutic mechanism under investigation, whether tumor-intrinsic drug sensitivity, invasion, immune engagement, or neural and vascular crosstalk. In this context, model fidelity should be balanced against reproducibility and scalability, therefore we advocate that the most appropriate organoid platform should be selected according to the biological question being addressed, rather than viewed as a universally superior model.

3.4. Advanced protocols and organ-on-chip technologies

Conventional GBM organoid platforms can reproduce several aspects of 3D tumor architecture, cellular heterogeneity, and diffusion-driven gradients; however, they remain limited in modelling dynamic tissue perfusion, vascular shear stress, and BBB-dependent transport [89]. Brain organoid-on-a-chip (BOoC) technologies address some of these limitations by combining brain organoids with microfluidic devices that provide controlled flow and brain-mimetic ECM cues, which can improve brain organoid survival, maturation, and reproducibility [90]. Neurovascular organoids have also been engineered using 3D-printed microfluidic chips, supporting the coupling of neural and vascular-like compartments [91]. Additional on-chip systems co-culture cerebral organoids with pre-formed vascular beds, enabling investigation of molecular cues that regulate organoid vascularization and neurovascular communication [92]. Modular BBB–brain chip platforms further provide a compartmentalized framework to model BBB and brain-tissue interactions and have been extended toward GBM-related drug-testing applications [93].

For GBM modelling, BOoC and related organ-on-chip systems are particularly relevant because they provide a route to integrate BBB-associated constraints with tumor–vascular and tumor–immune interactions. In a GBM BOoC workflow, PDOs, genetically engineered GBM organoids, or GBM assembloids could be positioned in a tissue chamber adjacent to perfused endothelial–pericyte–astrocyte channels, enabling analysis of drug penetration, endothelial activation, immune-cell trafficking, and tumor-cell response under dynamic exposure conditions. BBB-on-chip systems have already been used to model barrier dysfunction and immune-cell migration across endothelial–pericyte–astrocyte interfaces, supporting their potential integration into immune-competent GBM BOoC platforms [94]. Related GBM microfluidic platforms further illustrate how organ-on-chip strategies can reconstruct defined TME interfaces. For example, a multicompartment barrier-free device has been used to assess how ECM stiffening and temozolomide influence GBM invasiveness, immune-cell infiltration, and tumor–immune interactions under controlled microenvironmental conditions [95]. A microgravity-cultured GBM organoid platform integrated with a microfluidic chip further enabled dynamic evaluation of CAR-γδ T-cell cytotoxicity against PDOs [96]. Thus, BOoC approaches may bridge static organoid culture and in vivo models by combining human neural or tumor architecture with tunable vascular, BBB, ECM, and immune modules.

Nevertheless, BOoC models remain technically demanding and are not yet standardized for GBM. Device geometry, flow rate, ECM composition, vascular cell source, organoid maturation stage, and immune-cell compatibility can all influence experimental readouts, as microfluidic brain organoid studies show that flow conditions and brain-mimetic ECM can alter organoid survival, maturation, and reproducibility [90]. Vascularized BOoC platforms also remain constrained by the challenge of generating fully functional capillary networks in vitro, with on-chip cerebral organoid–vascular bed studies showing that neurovascular communication is complex and only partly recapitulated ex vivo [92]. Similarly, BBB-on-chip and modular BBB–brain platforms highlight the need to validate barrier integrity, compartmentalization, and transport function before these systems can be interpreted as physiological BBB models [93, 94]. Future GBM BOoC platforms should therefore prioritize transparent reporting of chip design, flow conditions, BBB validation, vascular-cell source, immune-cell source, and compatibility with longitudinal imaging, drug-delivery studies, and single-cell or spatial profiling.

4. Modeling TME interactions in GBM organoids

GBM organoid systems offer a versatile and human-relevant platform to reconstruct, in a modular manner, the diverse interactions between tumor cells and their surrounding TME. Rather than attempting to recapitulate the entire GBM ecosystem within a single model, current approaches selectively incorporate specific TME components, such as neuronal, glial, immune, and vascular elements, depending on the biological question (Fig. 3, Table 1). Here, we discuss how different platforms are leveraged to interrogate their functional impact on tumor progression and therapeutic response, and we outline key areas for further refinement that are driving the next generation of organoid development.

4.1. Neural crosstalk

Although progressing GBM tumor inevitably leads to the destruction of neuronal network, the axonal death signaling and remaining neuronal activity are non–cell autonomous regulators of GBM growth and invasion [35, 112]. In vivo studies established two key mechanisms to underpin this concept: activity-dependent secretion of neuroligin−3, which promotes glioma proliferation through oncogenic signaling activation, and direct formation of functional glutamatergic synapses between neurons and tumor cells, resulting in AMPA receptor–mediated depolarization and calcium influx that couples neuronal firing to tumor progression and invasion [35, 113, 114].

These neural interactions can be assessed functionally in GBM assembloids, since they provide a structured human neural context, allowing direct measurement of invasion, network architecture, and therapy response under synaptically active conditions. Comparisons of GSC cultures in vitro and within brain organoids demonstrated that the neural microenvironment enhances invasion-associated programs [75, 81]. Brain organoids also support tumor microtube network formation, linked by connexin 43 (Cx43)-positive gap junctions, which increases tolerance to irradiation and genotoxic stress [73, 75]. Assembloids preserve inter-patient diversity in GBM invasion, including single-cell and collective patterns [73, 83, 115]. Some differences between models also reflect the state of the brain organoid maturity rather than tumor-intrinsic behavior. Goranci-Buzhala et al. showed that GSC integration was slower in younger organoids and could be accelerated by exogenous neuroligin−3 [80]. This concept was extended further by Zhou et al., who applied highly differentiated rat brain organoids and found that organoid-derived extracellular vesicles promoted hypoxia/glycolysis-associated states, while AMPA receptor blockade with perampanel reduced single-cell invasion, supporting a role for mature neuron-associated signaling in GBM invasion dynamics [83]. Of note, invading GBM cells adopt distinct transcriptional programs in organoid-based neural environments [73], creating a higher diversity of GBM states [102], confirming a key role of brain milieu in maintaining transcriptomic gradients. Ge et al. further demonstrated that neural microenvironment could drive GBM state adaptation through receptor–ligand interactions, including neural TME-associated PTPRZ1 signaling [116]. Interestingly, the overall transcriptomic changes in the neurons appear less pronounced, although genes associated with glutamate receptor binding were downregulated [98]. In addition,

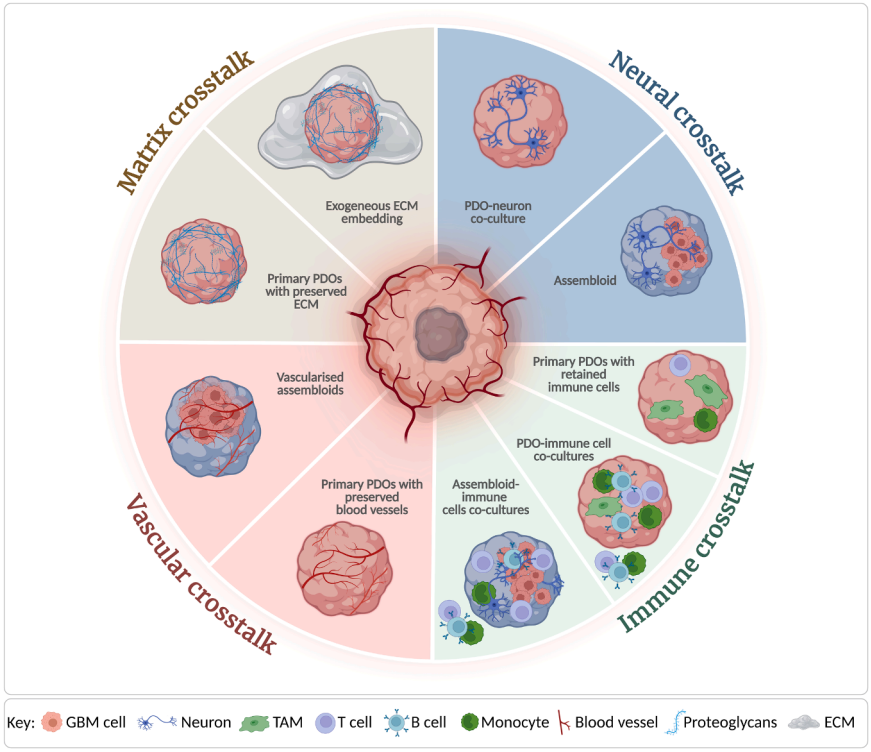

Fig. 3. Modular reconstruction of GBM TME crosstalk in organoid-based platforms. Schematic overview of how GBM organoids can be augmented by adding defined cell modules to study specific tumor–host interactions within the GBM tumor microenvironment. Abbreviations: ECM: extracellular matrix; PDOs: patient-derived organoids; TAM: tumor-associated macrophage.

assembloids also support an extracellular vesicle–mediated transfer of tumor-derived transcripts to neuroglial cells, indicating that neural crosstalk extends beyond classical synaptic wiring [102].

Recent work has extended this framework beyond glutamatergic signaling to include cholinergic and other neuromodulatory circuits. Using an all-human co-culture model, Sun et al. showed that GBM PDOs receive cholinergic synaptic input from hiPSC-derived neurons, with CHRM3-mediated signaling functionally linking cholinergic activity to GBM phenotypes [99]. In parallel, the same group used assembloids formed by co-culturing GBM PDOs with sliced neocortical organoids and showed rapid tumor integration into human neocortical tissue together with increased migration in response to acetylcholine, supporting a role for cholinergic activity in shaping GBM invasion [100]. Horschitz et al. similarly demonstrated that a human cortical spheroid model can support neuron–glioma synaptic interactions, tumor microtube networks, and therapy-response studies [98]. Complementing these human organoid-based systems, co-cultures of GBM organoids with primary neonatal mouse cortical neurons have shown that therapy-induced stress can drive a feedback loop in which tumor-derived PGE2 induces neuronal senescence, and senescent neurons in turn promote GBM progression [101]. This suggests that treatment-damaged neural tissue can actively support tumor survival. 3D electrophysiological sensors integrated with GBM–midbrain assembloids further revealed that GBM cells increase electrophysiological activity, which can be attenuated by mTOR blocker everolimus, providing a dynamic functional readout beyond endpoint viability [85].

Despite these advances, iPSC/ESC-derived brain organoids remain developmentally immature relative to the adult cortex and typically lack long-range neuronal connectivity, which constrains mapping their activity patterns onto the adult human brain [75, 90, 102]. Most GBM assembloids also lack vascular and immune compartments, supporting isolated neural–GBM interactions without non-neural brain components [75, 87]. While genetic manipulation of the parental ESCs enables the investigation of altered GBM-brain crosstalk within human brain organoids [97], inducible and cell-type-specific systems will be required to selectively target defined TME cell types. Looking forward, the next step is to move beyond studying neuron–GBM coupling in isolation and to develop engineered niches that incorporate the currently missing compartments, along with functional readouts, as modular and controllable components. Evidence from brain organoid disease models indicates that incorporation of brain-resident immune cells reshapes inflammatory programs and influences synapse-related maturation [117, 118]. Translating these to GBM assembloids would enable direct interrogation of how GBM-mediated neuronal activity intersects with microglia or macrophage signaling to modulate invasion programs and treatment responses within a human neural context. In parallel, bioelectronic interfaces offer a route toward higher-resolution and longitudinal electrophysiological phenotyping [85, 119]. Finally, microfluidic neurovascular and BBB-on-chip platforms could provide a blueprint for introducing flow-controlled vascular modules to GBM assembloids, enabling simultaneous modelling of neural crosstalk and delivery constraints under defined exposure kinetics [87, 90].

4.2. *Astrocytic and oligodendroglial crosstalk*

Astrocytes and oligodendrocyte-lineage cells shape GBM progression through glial support programs that are mechanistically distinct from neuron-to-tumor synaptic coupling. Astrocytes promote tumor growth and invasion via inflammatory cytokine signaling (including IL−6–STAT3), metabolic coupling, and direct contact-dependent communication through Cx43-mediated gap junctions [120, 121]. In parallel, oligodendrocytes and white-matter environments provide structural and molecular guidance cues that bias tumor dissemination along fiber-like tracks and influence tumor differentiation trajectories at invasive borders [122, 123].

So far, the data assessing functional crosstalk between GBM cells and astrocytes have been largely obtained from in vivo tumors and classical co-culture protocols. For example, using GBM-astrocyte co-cultures and

Table 1
Selected GBM organoid protocols applied to investigate tumor-TME crosstalk axes.

Studied GBM-TME crosstalk	GBM source	Microenvironmental component	Protocol	Model type (name)	Main application or key readout	Reference
Neural	Patient tumor-derived primary cells	hESC-derived cortical organoids	Cortical organoids matured to ~W8–10 → GBM cells co-cultures (hanging-drop, 12 h) → analyzed over ~3–4 weeks	Assembloid ("iHOTT")	Identifies neural microenvironment-driven state adaptation and functional receptor–ligand interactions, including PTPRZ1 signaling	Ge et al. [97]
		hiPSC-derived cortical spheroids; enriched for neurons	Cortical spheroids matured to ~D40–50 → GBM cells co-cultures → invasion induced after EGF/FGF2 withdrawal → analyzed over ~4 weeks	Assembloid ("hGliCS")	Models tumor microtubes, neuron–glioma synapses, coordinated calcium signaling, and therapy response in a human neural niche	Horschitz et al. [98]
	PDOs	hiPSC-derived cerebral organoid	Cerebral organoids matured to 40 divisions → GBM PDO co-cultures, 72 h → analyzed over 14–30 days	Assembloid ("GCOA")	Models human brain-context invasion for tumor–parenchyma interactions and host-induced transcriptional changes	Kim et al. [73]
		hiPSC-derived cholinergic neurons	Neurons differentiated for ~W3 → PDOs dissociated into single cells and seeded onto neuron-containing coverslips → analyzed at D2 and D6	PDO co-culture	Interrogates cholinergic synapse formation and cholinergic signaling effects on tumor states, proliferation, and motility	Sun et al. [99]
		hiPSC-derived neocortical organoid slice cultures	Neocortical organoid matured to ≥D100 → sliced to 300 μm and maintained on air-liquid interface for 48 h → co-culture analyzed after ~3 days	Assembloid ("GBM–SNO")	Models human neuron–GBM circuit integration and neuronal activity-dependent GBM migration	Sun et al. [100]
		Primary neonatal mouse cerebral cortex-derived neurons	PDOs established → co-culture with primary neuron → exposed to therapy-conditioned perturbations	PDO co-culture	Dissects therapy-induced neuron–GBM reciprocity, including PGE2-linked neuronal senescence and tumor support	Zhao et al. [101]
	GSCs	hiPSC/hESC-derived cortical organoids	Cortical organoids matured to ~4–6 M → GSCs co-culture for 24–48 h → analyzed over 2 weeks	Assembloid ("GCO")	Recapitulates malignant cell-state heterogeneity and captures malignant-to-non-malignant intercellular transfer of RNA	Mangena et al. [102]
		hiPSC-derived cerebral organoids	Cerebral organoids matured to ~D42 → GSCs co-culture early during neural development → analyzed at D1 or D7	Assembloid	Models tumour complexity, heterogeneity, growth, and invasion in a developmental neural context	Azzarelli et al. [81]
		hiPSC-derived cerebral organoids	Brain organoids matured to ~D40–60 → GSC spheroid/single cell co-culture → downstream quantification over few days	Assembloid	Rapid quantification of GBM invasion and interaction with mature neurons in brain organoids	Goranci-Buzhala et al. [80]
		hESC-derived cerebral organoids	Cerebral organoids matured to ~D30–45 → GSCs co-culture → tumor growth and invasion monitored for up to D14	Assembloid ("GLICO")	Tests patient-specific invasion and proliferation in human brain-like tissue; tumor architecture and microtube network formation	Linkous et al. [75]
	PDOs/GSCs	Fetal rat brain-derived organoids ("rBO"); enriched for a mature neuronal and glial environment with microglia	Rat fetal brain cells aggregated into brain organoids and matured to D21 → PDOs or GSCs co-cultured with mature rBOs → invasion analyzed after 24–72 h	Assembloid	Quantifies GBM invasion, single-cell migration and tumor microtube formation within mature brain organoids and enables therapeutic response assessment	Zhou et al. [83]
	Human GBM line (U87 MG)	hiPSC-derived cortical organoids	Cortical organoids matured ≥D43 → high-density U87 cells encapsulated in 2% or 4% 5OA microgels → GBM/5OA microgels placed in direct contact with mature organoids → invasion monitored at 0–72 h	Assembloid ("Flash assembloid")	Rapid model of early GBM invasion at the tumor–brain organoid interface, enabling imaging-based quantification of cell dispersal	Liang et al. [82]
	Rat GBM lines (CNS1, 9 L)	Primary rat cortical microtissues containing neurons, astrocytes, and microglia	Primary rat cortical cells aggregated into agarose microwells to form 3D cortical microtissues (~24 h) → microtissues matured to D2–D14 → rat glioma cells introduced during formation or into pre-formed microtissues → GBM invasion analyzed after D1–7 of co-culture	Assembloid	Dissects GBM infiltration dynamics and tumor interactions with neurons, astrocytes, and microglia within multicellular brain microtissues	Calvao et al. [103]
Immune	Patient tumor-derived primary cells	hESC-derived cortical organoids + autologous PBMCs	Cortical organoids matured to ~W8–12 → GBM cells + matched PBMCs co-culture (hanging-drop, ~12–16 h) → suspension culture for 7 days	Assembloid ("iHOTT")	Models patient-specific tumor–immune interactions in human brain tissue and supports cytokine profiling, TCR analysis, and pembrolizumab response testing	Baisiwala et al. [88]

(*continued on next page*)

Table 1 (*continued*)

Studied GBM-TME crosstalk	GBM source	Microenvironmental component	Protocol	Model type (name)	Main application or key readout	Reference
	PDOs	HBMECs + hPBMCs-derived $CD14^+$ monocytes + mouse BMECs	PDOs formed → TMZ treatment for 14 days → Transwell BBB co-culture with HBMECs in the insert + $CD14^+$ hPBMC-derived monocytes in the upper chamber (TMZ-treated PDOs in the lower compartment) for 24 h	PDO co-culture	Shows that TMZ-resistant GBM reprograms endothelial cells to facilitate monocyte-derived macrophage infiltration into GBM	Gao et al. [104]
		Endogenous TAMs	PDOs formed (D14–28, endogenous TAMs retained) → efferocytosis assessed ± TGM2 inhibitor	PDO	Models TAM-mediated efferocytosis and TGM2-dependent clearance of apoptotic cells within native GBM tissue architecture	Lui et al. [105]
		Endogenous T cells + TAMs	PDOs formed (D3–7, immune cells retained) → treated with IL−2 (4 h) or with anti-CSF1R inhibitor BLZ945 [4h–48 h]	PDO	Assesses short-term retention and drug responsiveness of endogenous intratumoral T cells and macrophages within GBM PDOs	Souberan et al. [49]
		Endogenous TAMs	PDOs formed (D14, TAMs retained) → treated with pexidartinib for 6 days	PDO ("GlioME")	Demonstrates depletion of endogenous TAMs in GBM organoids after CSF1R inhibition	Zheng et al. [50]
		Endogenous TAMs + exogenous CAR-γδ T cells	PDOs formed (D7–14, TAMs retained) → CAR-T co-culture for 48 h	PDO co-culture	Evaluates personalized CAR-γδ T-cell activity against heterogeneous GBM while retaining endogenous microenvironment features	Zhu et al. [106]
		Endogenous immune cells + exogenous CAR-γδ T cells	PDOs formed (D10–14) → transferred to microfluidic chip → CAR-γδ T-cells co-culture for 48 h	PDO co-culture	Dynamic immunotherapy testing in a perfused system designed to improve viability and immune-relevant readouts	Zhu et al. [106]
		Endogenous GAMs	PDOs formed (short culture, TAMs retained) → treated with CSF1R inhibitors (PLX3397, BLZ945, GW2580) for 48 h	PDO	Demonstrates that CSF1R-targeting can shift immunosuppressive TAMs toward a proinflammatory phenotype	Fermi et al. [107]
		Endogenous immune cells + EGFRvIII CAR-T cells	PDOs formed (~W2–4, immune cells retained) → EGFRvIII CAR-T cells co-culture for 5–7 days	PDO co-culture	Demonstrates the efficacy of EGFRvIII CAR-T cells against $EGFRvIII^+$ GBM cells	Jacob et al. [45]
		Autologous EGFR-IL13Rα2 CAR-T cells	PDOs formed (~W1–2)→ autologous CAR-T co-culture for 6 days	PDO co-culture	Demonstrates efficient GBM cell lysis in organoids exposed to dual EGFR–IL13Rα2 CAR-T cells that mirror patient CSF cytokine release and engraftment signals	Logun et al. [108]
		Allogeneic or partially HLA-matched immune cells (PBMCs and purified $CD3^+$/$CD4^+$/$CD8^+$ T-cells)	PDOs infected with adenovirus to induce CIITA expression → PBMCs or purified T-cells co-culture for 72 h	PDO co-culture	Tests whether induced CIITA and MHC-II expression sensitizes PDOs to T-cell-mediated killing	Salvato et al. [109]
Vascular	GSCs	hiPSC-derived cortico-endothelial assembloids, enriched for neural and vascular components	Assembloids matured to D60–70 → GSC co-culture for 3 weeks	Assembloid	Dissects GBM neuronal and vascular invasion routes and endothelial cell interaction	Bertacchi et al. [86]
	GSCs	hiPSC-derived cerebral organoids with hemato/endothelial cells creating vascular and glial niche	Assembloids matured to ~D40–100 → dissociated GSC co-culture for 14–45 days	Assembloid ("CCO")	Models vascular co-option, microglial reprogramming, and recurrence-associated behavior after radiotherapy in a vascularized brain organoid niche	Raguin et al. [110]
Matrix	GBM lines (JX6, U251)-derived organoids	Endogenous ECM (self-produced native tumor ECM)	JX6 GBM cells seeded → spheroid formation → transfer to bioreactor → PDO maturation (~D47–60) → ECM analyzed during growth (D7–21 / 1–2 mm stage)	PDO	Characterizes native ECM composition and matrix-rich GBM architecture relative to simpler spheroid systems	Avera et al. [111]

Abbreviations: 5OA: 5% oxidized alginate; BBB: blood-brain barrier; BLZ945: CSF1R inhibitor; BMECs: brain microvascular endothelial cells; CAR-γδ T cells: chimeric antigen receptor gamma-delta T cells; CCO: complex cerebral organoid; CIITA: Class II transactivator; CSF1R: colony stimulating factor−1 receptor; D: day; EGF: epidermal growth factor; EGFR: epidermal growth factor receptor; EGFRvIII: epidermal growth factor receptor variant III; FGF2: fibroblast growth factor 2; GBM: glioblastoma; GBM-SNO: Glioblastoma-sliced neurocortical organoid; GCO: glioma-cerebral organoid; GCOA: GBM tumor-cerebral organoid assembloid; GLICO: glioma-cerebral organoid model; GSCs: glioma stem-like cells; HBEMC: human brain microvascular endothelial cell; hESC: human embryonic stem cell; hGliCS: human Glioma-Cortical Spheroid; hiPSC: human induced pluripotent stem cell; HLA: human leukocyte antigen; hPBMC: human peripheral blood mononuclear cells; iHOTT: induced human organoid tumor–tissue assembloid; IL−2: interleukin−2; IL13Rα2: interleukin−13 receptor alpha−2; M: month; MHC-II: major histocompatibility complex class II; PBMC: peripheral blood mononuclear cell; PDO: patient-derived organoid; PGE2: prostaglandin E2; PTPRZ1: protein tyrosine phosphatase receptor type Z1; rBO: rat brain organoid; TAM: tumor-associated macrophage; TCR: T-cell receptor; TGM2: transglutaminase 2; TMZ: temozolomide; TME: tumor microenvironment; W: week.

brain slices, McCutcheon et al. showed that miRNA transfer and GBM invasion are substantially reduced in Cx43-deficient astrocyte environments, confirming that Cx43-mediated junctions between GBM cells and astrocytes at the tumor periphery can fuel tumor cell invasion [121]. Within in vivo mouse models and in vitro co-cultures, Watson et al. further reported mitochondria transfer from astrocytes to GBM cells mediated by a contact-dependent mechanism facilitated by $GAP43^+$ microtubes [124]. Astrocyte-derived mitochondria metabolically reprogram GBM cells towards increased proliferation. At the same time, in vivo xenografts revealed demyelination to be a specific event upon

GBM invasion to the white matter, associated with GBM state transition to a less proliferative, oligodendrocyte-like state [122].

To what extent crosstalk with mature glial cells can be assessed with GBM organoids is currently understudied. Although iPSC/ESC-derived brain organoids show spontaneous development of glial compartments, these often remain transcriptionally and functionally immature, with incomplete myelination and limited representation of reactive or disease-associated astrocyte states [125, 126]. This limitation reflects fundamental aspects of human neurodevelopment. Human astrocyte maturation occurs relatively late during cortical development and spans extended developmental windows that are difficult to access in primary human tissue. Studies using long-term cultures of human cortical organoids have shown that astrocyte differentiation typically begins only after ~80 days in culture and continues over many months before mature astrocyte states emerge [127]. Consequently, very long development timeframes [127] or specific protocols allowing for the advancement of iPSC-derived astrocytes from fetal to adult phenotypes [126] may be needed to investigate the functions recapitulative of the adult brain. Likewise, oligodendrocyte-lineage–inclusive systems such as "oligocortical spheroids" may be needed to generate not only OPCs but also myelinating oligodendrocytes in iPSC-derived contexts [125]. Optionally, more mature glial structures can be generated using rodent brain organoids. Initiation from fetal rodent brain tissue enables the formation of more adult-like architecture within shorter timeframes, owing to faster neurodevelopment in rodents compared to the human brain [83]. Using rat GBM assembloids, Calvao et al. showed that GBM cells attract host astrocytes and microglia, but not neurons to the 'microtumor' site, suggesting a functional crosstalk [103]. Incorporating glia-rich host tissues into GBM assembloids could help directly test how astrocytes and other glial cells influence tumor invasion and therapeutic responses.

4.3. Immune crosstalk

The recent interest in therapeutic modulation of tumor immunity has led to the development of a plethora of 3D co-culture systems, including immune-focused tumor organoid platforms [128, 129]. GBM is characterized by a profoundly immunosuppressive TME dominated by microglia and monocyte-derived TAMs, with limited and ineffective T cells [7, 43]. This immunosuppressive landscape is actively maintained through reciprocal molecular interactions between GBM cells and immune populations, including cytokine-mediated T-cell dysfunction and exhaustion, altered antigen-presentation programs, inflammatory mesenchymal-like transitions, and TAM-derived immunosuppressive signaling [7, 24, 130]. Mechanistically, these interactions involve both contact-dependent and soluble axes, including MHC-I–CD8 signaling, MIF–CD74 signaling, IL10-mediated T-cell dysfunction, AHR/CD39-dependent immunosuppression, and TAM-derived osteopontin/SPP1-associated T-cell exhaustion [7]. By adapting the immune components of GBM organoids, the current research aims to assess two complementary questions: how GBM instructs resident myeloid compartments, and under which conditions adaptive immune effectors can productively engage tumor cells in a patient-relevant context.

In practice, immune modelling in GBM organoid workflows generally follows two main strategies: (i) transient retention of autologous immune and TME elements from patient tissue, or (ii) controlled reconstitution of defined immune modules in co-culture systems. Minimal manipulation of primary GBM PDOs allows for transient retention of endogenous tumor-associated immune cells, enabling direct measurement of tumor–immune signaling and therapy-induced changes before immune compartments decay with time in culture [43, 45]. For instance, Souberan et al. reported retention of intratumoral T cells and TAMs within GBM PDOs, that remain responsive to IL2 and anti-CSF1R BLZ945 treatment, respectively [49]. Consistently, Fermi et al. observed TAMs responsive to CSF1R-targeting drugs, reporting their transition towards antitumour states within GBM PDOs [107], while Zheng et al. confirmed a reduction of endogenous TAMs in primary GBM PDOs upon treatment with CSF1R inhibitor pexidartinib [50]. Lui et al. used PDOs to show that endogenous TAMs mediate efferocytosis of apoptotic cells under physiologic oxygen conditions, and that this process is reduced by TGM2 inhibition, providing a mechanistic readout of how myeloid phagocytic programs operate in situ [105]. Complementary systems include GBM explant cultures, which are typically generated from larger tissue fragments than those used to establish primary GBM PDOs. Using tissue-based perfusion bioreactors, Shekarian et al. functionally assessed that dense tumor areas differ from peripheral tumor tissue in spatial immune composition and associated secretome, showing higher adaptive immune activity in the tumor center [131]. Among soluble proteins detected in the media, interferon-γ levels allowed distinguishing responder groups under immunotherapy exposure. Further data is needed to assess the functionality of tumor-associated immune cells in ex vivo cultures over time. Since such cultures were reported to exhibit higher hypoxia and cytokine-response signatures compared with matched patient tissues [132], potential changes in the functional crosstalk need to be taken into account.

Since endogenous immune compartments are often short-lived in conditions sustaining GBM growth and are highly variable across primary GBM organoids [44, 60, 132], many systems instead introduce defined immune populations to generate controlled and interpretable immune activation and cytotoxicity readouts. We have set up a co-culture protocol allowing for interrogation of the crosstalk between GBM PDOs and PBMC-derived donors or autologous T cells [109]. We showed that immunosuppression of T cells mediated directly via GBM cells can be reverted via adenovirus gene therapy, leading to CIITA expression in GBM cells and a consequent T cell-specific killing [43, 109]. Comparable autologous immune–tumor systems have also been developed. Stavrakaki et al. established an ex vivo co-culture model combining GSC cultures with autologous PBMCs maintained through cytokine supplementation with IL−2, IL−15 and M-CSF, allowing assessment of GBM-mediated immunosuppression in various immune populations, including T cells, NK cells and macrophages. In this system, they showed that oncolytic virus infection mediates immune cells activation and increase release of pro-inflammatory cytokines and chemokines [133], a functional behavior recently reported in patient tumors [134]. Related co-culture approaches have also been used to dissect how non-immune stromal elements regulate immune entry into GBM. Gao et al. showed that endothelial cells exposed to signals from TMZ-resistant GBM became functionally altered and promoted infiltration of monocyte-derived macrophages into vascularized GBM organoids, highlighting an active role for the endothelial compartment in shaping myeloid recruitment rather than serving only as a passive barrier [104]. By co-culturing exogenous T cells with GBM tissue slices, Ravi et al. revealed CD8 + T cells exhaustion under chronic IL−10 exposure derived from TAMs and mesenchymal-like GBM cells [130].

More complex organoid immune ecosystems have also been developed to capture interactions between tumor, neural and immune compartments. Baisiwala et al. implemented an organoid co-culture system, integrating patient-derived GBM cells and autologous PBMCs within human cortical organoids [88]. Assessment of cell states and cytokine release confirmed recapitulation of immunosuppressive cues, while treatment with pembrolizumab (anti-PD1) revealed increased immune engagement and lymphocyte activation, in particular expansion of stem-like CD4 T cell clones with patient-specific repertoires. Interestingly, the fetal origin of rat brain organoids allows for the derivation of intra-organoid microglia [83], which was shown to respond to rat GBM cells by increased proliferation [103].

Co-culture systems can also be directly used for testing the efficacy of engineered T cells. E.g. Jacob et al., showed the efficacy of EGFRvIII CAR-T cells against EGFRvIII^{+} GBM cells within primary PDOs, depending on their genetic background [45]. Efficient GBM cell lysis was also observed in PDOs upon exposure to dual EGFR–IL13Rα2 CAR-T cells [108]. More recently, Zhu et al. developed GBM PDO co-culture

systems with CAR-γδ T cells, including both a TAM-retaining organoid platform [106] and a microfluidic perfusion format, enabling functional assessment of immune-cell infiltration, cytotoxicity and short-term viability

Importantly, immune organoid platforms are shaped by two recurrent constraints. Immune persistence depends strongly on media and stimulation conditions, creating trade-offs between functional longevity and physiological activation states. As a result, many readouts are confined to short experimental windows and are highly protocol-dependent [128, 129]. In addition, HLA compatibility and donor choice may critically influence adaptive immune readouts, as alloreactivity can obscure tumor-specific responses when immune cells are not autologous or HLA-matched [129]. While autologous systems are preferable, access to patient blood is limited; therefore, robust and sustainable protocols based on donor immune cells will be required. The future advancements in co-culture protocols will need to incorporate both innate and adaptive immune cells, allowing for efficient antigen presentation ex vivo for efficient T cell priming. These protocols will need to deliver reproducible outcomes, as current complex co-cultures often exhibit substantial inter-organoid variability in immune composition and in the extent of tumor-immune crosstalk. On the technical site, across predominantly non-brain tumor types, the air–liquid interface organoid format has demonstrated that culture geometry and media can sustain infiltrating immune populations, including preservation of tumor-associated TCR repertoires [135]. Although not yet applied for GBM, this concept underscores that immune retention is not "all-or-nothing" and depends strongly on platform design rather than simple component addition [135]. These constraints do not diminish the value of immune organoid platforms; rather, they define the parameters that must be engineered and reported to ensure that immune phenotypes are reproducible and comparable across studies. Additional comparative studies are needed to assess whether transient immune retention from patient tissue or controlled immune add-back.

Together, these GBM organoid-based studies demonstrate that patient-derived 3D platforms can be used to test immune engagement, cytokine signaling, immune-cell infiltration, and cytotoxic function. A remaining challenge is to connect these functional readouts more systematically with immune cell identity, spatial organization, and clinically meaningful outcomes. Although biologically distinct from GBM, breast cancer brain metastasis models illustrate such an integrated strategy: Jassowicz et al. recently combined spatial tissue assessment with functional validation in PDOs to link CD8 tissue-resident memory-like T-cell states and tertiary lymphoid structures with ex vivo tumor-cell killing and patient prognosis [136]. For GBM, such multimodal framework could inspire immune-organoid studies that move beyond measuring immune-cell presence alone and instead link immune composition, spatial organization, functional anti-tumor activity, and clinical relevance. Future immune-organoid studies may also benefit from complementary technologies such as MicroFAST, a lightning optofluidic platform that isolates antigen-specific CD8 T cells based on function. Although not yet adapted to organoid-based tumor models, this approach could help distinguish truly tumor-reactive T cells from bystander immune cells in complex co-cultures [137].

4.4. Vascular and endothelial crosstalk

Vascular interactions impose fundamental constraints in GBM, shaping oxygen and nutrient delivery, hypoxia-driven metabolic stress, invasion routes, immune accessibility, and drug exposure. Endothelial cells also provide paracrine and metabolic support and contribute to perivascular niches that sustain stem-like states and resistance phenotypes [30]. Such vascular support relies on endothelial–tumor crosstalk mechanisms, including NOTCH activation, nitric oxide signaling, vascular co-option, and pericyte-like transitions, which collectively regulate GBM invasion, stemness, and therapy resistance [8, 31–33]. In parallel, the functional BBB further constrains immune-cell trafficking and mediates drug delivery and efflux in GBM [138].

In organoid systems, similarly to immune cells, the vascular components may arise from the initial vasculature present in patient tumor tissue or can be reincorporated either by inducing endothelial differentiation within the organoid or by adding defined vascular modules [86, 139]. In general, primary PDOs retain the original vascular structures of the GBM tumor tissue [16, 45, 140], though the functionality ex vivo is compromised long-term and the vasculature is replaced by the rodent components upon xenografting [59]. Endothelial cells can also be further incorporated into PDOs in co-cultures. E.g., we have assessed the impact of the TME components isolated from tumors with different vascular architectures on the growth of GBM PDOs, demonstrating that a GBM-primed angiogenic TME can stimulate the growth of PDOs that do not typically recruit highly aberrant vasculature [59]. Recently, Bertacchi et al., proposed cortico-endothelial assembloids in which matched iPSC-derived cortical and endothelial organoids form BBB-like vascular networks, allowing discrimination between neural and vascular routes of GBM invasion mediated by differential CELF2 expression and state transitions to neural and mesenchymal phenotypes, respectively [86]. Similarly, Raguin et al. further showed that vascularized complex cerebral organoids when co-cultured with GSCs reproduced vascular co-option, microglial reprogramming toward TAM-like states, and recurrence after radiotherapy [110]. Together, these studies suggest that vascularized organoids can model not only endothelial structure but also functionally relevant neurovascular routes of tumor progression. A complementary strategy is endogenous vascularization of the host organoid itself. In the ETV2 hybrid system, a subset of hESCs is engineered to express the endothelial regulator ETV2 during early stages of differentiation, promoting vascular-like structure formation and BBB-like maturation within cortical organoids [87]. Such assembloids appear more complex and physiologically relevant than classical BBB-like co-culture systems based on cell lines [33] or vasculature based on non-brain HUVEC cells [139].

However, endothelial networks in vascularized organoids often remain partially immature and lack functional flow. As a result, vessels are not stably perfused and do not experience physiological hemodynamic forces, which limits the modeling of vascular crosstalk as well as transport-dependent processes such as drug delivery, vascular normalization, and long-timescale vascular remodeling compared with in vivo models. More advanced implementations will likely require integrating perfusion through microfluidic platforms, enabling controlled delivery of oxygen, nutrients, and therapeutics under exposure regimes that cannot be reproduced in static cultures, moving beyond from simple vascular presence toward neurovascular unit–like function [87, 90]. Integration of vascular and immune modules within the same platform will be further essential to directly test how the BBB barrier influences immune access and immunotherapy response under human-relevant delivery conditions [141].

4.5. Other TME niche cues and extracellular matrix crosstalk

ECM composition and mechanics critically influence GBM invasion and therapy tolerance by regulating adhesion, force sensing, and migration behavior in the brain-like microenvironment [142]. In parallel, diffusion-driven oxygen and nutrient limitations create metabolic constraints that promote stress programs and adaptive states in 3D tumor tissues [67]. Together, ECM and diffusion-driven niches form an integrated physical-metabolic layer of crosstalk that can impact how GBM cells respond to therapy.

In organoid-based modelling, the ECM axis is most commonly addressed through matrix-embedded workflows, although it is currently unclear whether embedding PDOs in ECM influences particular GBM traits compared to PDOs derived as free-floating 3D structures embedded in a 'natural' ECM derived from tumor cells and TME components. As shown by Avera et al., GBM cells can progressively elaborate their own native ECM without exogenous matrix encapsulation, shifting

from relatively uniform spheroids toward more tissue-like, matrix-rich structures as they grow [111]. While external ECM can stabilize PDO growth and facilitate matrix-resisted invasion readouts, it introduces a non-brain-like non-human ECM rich in basement-membrane proteins that may bias adhesion signaling and state composition relative to the native hyaluronan-rich brain milieu. More data are needed to understand the role of matrix composition and its stiffness on the functional properties of GBM cells. Mechanistically, hyaluronic acid–rich matrices have been linked to CD44-dependent mechanosensing programs that drive invasive motility, establishing a direct ECM-to-behavior pathway relevant to brain tissue [142]. Moreover, ECM stiffness has been shown to regulate GBM metabolism and functional properties: embedding GSC cultures with soft hydrogels favored aerobic glycolysis and invasion, whereas stiff matrix promoted oxidative phosphorylation and proliferation [143]. Furthermore, it remains unclear to what extent the ECM properties of the healthy brain organoids at different stages of differentiation may influence properties of GBM cells beyond the neural crosstalk. For example, while rat brain organoids were reported to show similar stiffness to the mature brain [103], reports differ regarding the influence of the organoid maturity status on GBM cell invasion [83, 103]. Moreover, oxygen and nutrient gradients depend not only on matrix properties but also on culture geometry and exposure conditions, rendering metabolic niche readouts inherently platform-dependent unless carefully benchmarked. Future models will need to combine invasion systems that allow control of ECM properties with vascular or immune modules to better capture the TME complexity [141]. At the same time, these platforms should remain compatible with analyses of tumor cell states, allowing treatment-induced tumor dynamics to be monitored over time [67]. Transitioning toward synthetic or hybrid hydrogels with precisely defined mechanical and biochemical properties will further enhance reproducibility and enable causal testing of mechanics-dependent GBM cell invasion and adaptation.

Together, these TME-focused organoid platforms demonstrate that GBM models are no longer limited to reproducing tumor growth in 3D. They can now be tailored to test how specific microenvironmental components shape invasion, immune engagement, drug exposure, and therapy resistance. This functional dimension is central for translation, because clinical failure in GBM rarely depends on tumor-cell intrinsic drug sensitivity alone, but also on the neural, immune, vascular, and physical niches in which residual tumor cells persist.

5. Translational and clinical applications

GBM organoids are increasingly positioned as translational platforms for precision neuro-oncology because they preserve patient-specific tumor features while remaining experimentally accessible for therapeutic testing [20]. However, their clinical relevance depends on selecting the appropriate model complexity for the therapeutic question being asked. Tumor-only organoids may be informative for direct tumor-cell vulnerabilities, whereas models incorporating immune, vascular, neural, or ECM components become necessary when these compartments are expected to modify therapeutic response.

5.1. Tumor-intrinsic therapeutic testing and functional precision oncology

A major application of GBM organoids is the functional testing of therapies that primarily target tumor-intrinsic vulnerabilities. Tumor-only PDOs are informative when the aim is to assess direct tumor-cell sensitivity, adaptation, or regrowth after treatment, including responses to DNA-damaging agents, targeted drugs, repurposed compounds, and drug combinations [48]. They are particularly useful for scalable pharmacological testing and for readouts such as cytotoxicity, DNA-damage signaling, growth arrest, cell-state shifts, and residual or persistent tumor-cell populations after therapy [60].

GBM PDOs can also complement genomic profiling by providing functional evidence of drug sensitivity or resistance, particularly when actionable mutations alone are insufficient to predict therapeutic response. Bioinformatic drug prediction, pharmacogenomic analysis, and multi-omics-guided target nomination can identify candidate vulnerabilities, but functional perturbation assays are needed to test whether these predicted dependencies translate into measurable treatment response [67, 70]. This concept is supported by recent single-cell-resolved ex vivo screening in patient GBM 2D tumor cell cultures, which integrated pharmacology, molecular profiling, and computational drug prediction to identify repurposable neuroactive drugs with anti-GBM activity [144].

At the same time, functional screening in 3D requires careful assay design and interpretation. PDOs remain highly useful for scalable tumor-intrinsic drug testing, but organoid size, growth kinetics, baseline viability, drug penetration, and image-based quantification need to be normalised to generate interpretable results [145]. These technical constraints do not reduce the value of PDOs for drug testing, but highlight the need for robust, standardized and sensitive 3D readouts. These would be of particular importance for discriminating responses in tumor and TME cells in the 3D context in co-clinical functional precision oncology studies. So far, such clinical efforts are based on short-term 2D tumor cultures, allowing for image-based discrimination between tumor and TME cells, or 3D GSC cultures depleted from TME components [144, 146].

Experience from other cancer organoid fields illustrates both the promise and the limitations of functional drug testing. In colorectal cancer, organoid biobanks have established a framework for personalized therapy design [147]. In metastatic gastroesophageal cancers, PDO pharmacotyping can correlate with selected clinical responses [148]. Similar translational frameworks have been developed in pancreatic and rectal cancer organoids, where pharmacotyping and chemoradiation-response assays have been linked to patient treatment outcomes [149, 150]. For GBM, prospective validation should account not only for drug-induced cytotoxicity, but also for diffuse invasion, recurrence, treatment-induced persistence, and the clinical feasibility of generating interpretable functional data within actionable timeframes.

5.2. Advanced organoid models for TME-dependent therapeutic testing

The need for TME-containing models becomes critical when non-malignant compartments are expected to modify therapeutic outcome or are directly a subject of the treatment. Co-culture or advanced organoid systems are required to determine whether therapeutic response is maintained in the presence of immune cells, vascular barriers, neural or glial support, ECM mechanics, or brain-resident myeloid populations. Immune co-cultures are most relevant for therapies involving T-cell engagement, macrophage reprogramming, CAR-T activity, oncolytic virus responses, or checkpoint blockade [109]. Vascularized organoids and BBB-on-chip systems are better suited to assess drug penetration, endothelial activation, immune-cell trafficking, or vascular co-option [110], while neural, glial, and ECM-defined models become relevant when invasion, niche-dependent survival, or mechanics-driven adaptation are central to the therapeutic question [73, 143].

These added components also create readout-specific challenges. Bulk readouts may reflect combined effects on malignant cells, stromal or immune compartments, and changes in their relative proportions. Therefore, therapeutic testing in these systems should incorporate compartment-resolved readouts, such as tumor-cell-specific viability, spatial imaging, flow cytometry, or single-cell profiling, to distinguish direct tumor-cell sensitivity from TME-dependent modulation.

This principle is especially important for immunotherapies and cellular therapies. GBM organoid co-cultures have already been used to test T-cell-mediated killing, CIITA-driven immune activation, CAR-T responses, CAR-γδ T-cell activity, and oncolytic virus-induced immune activation [106, 109]. However, clinically meaningful interpretation requires readouts that distinguish immune-cell infiltration from stable

tumor engagement, acute cytotoxicity from durable tumor control, and tumor-cell death from broader TME remodeling. Air–liquid interface organoids from other tumor types provide a useful technical precedent by preserving endogenous stromal and immune compartments, including tumor-infiltrating lymphocytes and tumor-specific TCR repertoires [135], but their direct implementation remains challenging because immune cells are often short-lived in tumor-supportive culture conditions, autologous immune material is limited, HLA mismatch can confound readouts, and brain-resident myeloid populations are difficult to preserve or reconstruct.

Overall, GBM organoids should be viewed as functional avatars that complement histopathology, molecular diagnostics, imaging, and computational prediction. Their strongest near-term translational value may lie in modular therapeutic testing, drug repurposing, pharmacogenomic studies, and preclinical validation of advanced therapies, particularly in recurrent disease. Prospective co-clinical studies will be required to determine whether organoid-derived responses correlate with treatment exposure, radiological response, progression-free survival, and recurrence patterns. Such validation will be essential before GBM organoids can be incorporated into routine clinical decision-making, but their capacity to link patient-specific tumor biology with functional treatment response already makes them a promising platform for precision neuro-oncology.

6. Challenges and perspectives

Viewing GBM through an ecosystem lens makes it clear why progress has been slow when models capture either tumor-intrinsic programs or single TME inputs in isolation. Organoid-based platforms have already become a practical way to keep patient-relevant organization and heterogeneity while allowing controlled perturbation, and their real value lies in how flexibly they can be tailored to the specific crosstalk being tested. At the same time, these systems still retain inherent limitations: neural and vascular compartments are often developmentally immature, immune components are typically short-lived or artificially engineered, and ECM and barrier properties only partly recapitulate those of the adult brain. These limitations may be addressed by using more mature brain organoid substrates, defined immune or vascular add-back strategies, brain-mimetic ECMs, and perfusable organ-on-chip systems when these components are essential to the biological question being tested. Consequently, no single model fully captures the complexity of the GBM ecosystem.

Looking ahead, we envisage that the most impactful advances will prioritize better experimental control over added complexity. Multi-compartment integration will be most informative when it is functionally justified by the biological question, for example, when modelling immune-cell trafficking, BBB-dependent drug delivery, vascular co-option, or neuron–glioma communication. A key challenge will be to determine whether adding new compartments improves biological relevance or instead introduces additional variability. This can be addressed through systematic benchmarking against patient tissue, orthotopic models, and clinical datasets, as well as by using high-resolution readouts capable of tracking cell-type composition, spatial organization, and state transitions over time. It will be essential to determine whether added complexity improves biological or translational relevance. Across all GBM organoid platforms, improved reporting of replicate number, organoid size, passage, culture duration, maturation stage, ECM and media conditions, and the distinction between biological and technical replicates will be essential for improving reproducibility and cross-study comparison.

Progress in other cancer organoid fields, particularly epithelial tumor organoids used for pharmacotyping and treatment-response prediction, illustrates the translational potential of patient-derived models. However, GBM organoids face additional barriers, including diffuse invasion, limited tissue sampling, BBB-restricted drug exposure, and strong dependence on neural, immune, and vascular niches. Overcoming these barriers will require GBM-specific validation strategies, including co-clinical studies that integrate organoid drug testing with molecular profiling, imaging features, treatment exposure, and patient outcome data. As these refinements mature, GBM organoids should increasingly support more rigorous testing of therapies targeting not only tumor-intrinsic vulnerabilities but also TME-driven circuits that sustain resistance and recurrence. This progress will further consolidate their role in translational research and, where feasible, in the development of patient-matched therapeutic strategies.

7. Conclusions

GBM organoid models range from 3D tumor cultures to modular platforms, allowing for dissecting how the TME shapes invasion, plasticity, immune escape, and therapy resistance. Their greatest strength lies not in reproducing the entire GBM ecosystem, but in enabling controlled reconstruction of defined tumor–TME interactions in a human context. Future progress will depend on matching each platform to the biological or translational question, while improving reproducibility, functional readouts, and clinical benchmarking. In this way, GBM organoids can advance both mechanistic understanding and precision-oriented therapeutic testing.

Author contribution

All authors have collected the literature, reviewed manuscript versions and approved the final version.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Data availability

No data was used for the research described in the article.

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