REVIEW ARTICLE

# A Tri-Phasic View of the Microenvironment in Breast Cancer Brain Metastasis

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## Abstract

**Breast cancer brain metastasis (BCBM) is driven by microenvironmental remodeling across three interconnected phases: the primary tumor microenvironment (TME) licenses dissemination, the pre-metastatic niche (PMN) primes the brain, and the established metastatic niche sustains outgrowth through central nervous system (CNS)-resident cell interactions. These phases are individually established; we integrate them through cross-phase signaling axes, exemplified by the proposed IL-6→extracellular vesicle (EV)→blood-brain barrier (BBB) disruption cascade, that link tumor-associated macrophage-driven epithelial-mesenchymal transition to PMN conditioning and niche maintenance. We integrate single-cell and spatial transcriptomic insights, discuss brain-specific mechanisms including astrocytic STAT3/connexin 43 signaling, neuronal contributions, and the neurovascular unit, and evaluate emerging therapies with emphasis on antibody-drug conjugates with intracranial activity. Subtype-specific differences between HER2-positive and triple-negative breast cancer brain metastases and key translational barriers are systematically addressed.**

**Keywords**: Breast cancer brain metastasis, Tumor microenvironment, Pre-metastatic niche, Metastatic niche, Blood-brain barrier.

## 1 Introduction

Among patients with breast cancer, distant metastasis remains the predominant factor leading to cancer-related death, with a 5-year relative survival rate of approximately 32% for metastatic disease [1]. Brain metastasis represents one of the most devastating complications: among patients with metastatic disease, the cumulative incidence of brain metastasis reaches approximately 31% in HER2-positive breast cancer (HER2+ BC) and 32% in triple-negative breast cancer (TNBC) [2]. Once

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**Table 1.** Key cross-phase mediators in BCBM.

| Mediator | Primary TME phase | PMN phase | Metastatic niche phase | Brain-specific evidence |
|---|---|---|---|---|
| IL-6 | TAM-driven EMT via JAK/STAT3 | Mobilizes myeloid cells; synergizes with EV signaling | Proposed to activate astrocytic STAT3; sustains immunosuppression | STAT3+ reactive astrocytes in BCBM [3] |
| miR-105 | Released by primary tumor EVs | Disrupts endothelial tight junctions (ZO-1); increases BBB permeability [4] | Facilitates sustained vascular leakiness | Demonstrated in brain endothelial cells [4] |
| miR-181c | Released by brain-tropic (already-seeded) tumor EVs[a] | Niche conditioning by already-seeded cells[a] | Disrupts BBB via PDPK1-mediated actin remodeling [5] | Derived from brain-metastatic cell lines [5] |
| LOX | HIF-1$\alpha$-induced in hypoxic primary TME | Crosslinks collagen IV; recruits CD11b+ BMDCs [6] | ECM stiffness sustains outgrowth | Lung PMN confirmed [6]; brain evidence emerging |
| CEMIP (exosomal) | Shed by brain-metastatic breast cancer cells[a] | Niche conditioning by already-seeded cells[a] | Promotes perivascular niche inflammation and vascular remodeling [7]; enhances colonization fitness | Brain-metastasis specific [7] |
| ANGPTL4 | TGF-$\beta$-induced in primary TME | Disrupts vascular integrity in distant organs [8] | Facilitates vascular co-option | Lung demonstrated [8]; brain under investigation |
| miR-122 | Packaged into tumor EVs | Reprograms glucose metabolism in PMN resident cells [9] | Increases nutrient availability | Demonstrated in brain and lung PMN [9] |

[a] miR-181c and CEMIP were identified in EVs from cells already selected for brain tropism or already seeded in the brain. They are therefore listed here for completeness of the cross-phase axis, but are best interpreted as niche-conditioning signals acting after arrival rather than as bona-fide pre-arrival priming factors (see Section 3.2).

brain metastasis occurs, median overall survival ranges from 6 to 12 months [10], reflecting limited therapeutic options and the protective barrier posed by the blood-brain barrier (BBB), which restricts the penetration of most systemic agents into the central nervous system (CNS) [11]. This review focuses on parenchymal brain metastases; leptomeningeal disease, a clinically and biologically distinct entity [10], is outside our scope.

The tumor microenvironment (TME) governs breast cancer brain metastasis (BCBM) across three temporally and spatially distinct yet mechanistically interconnected phases. Within the primary tumor, tumor-associated macrophages (TAMs) facilitate epithelial-mesenchymal transition (EMT) and tumor cell dissemination [12, 13], while secreted factors and extracellular vesicles (EVs) prime distal organs [14]. Prior to tumor cell arrival, primary tumor-derived signals establish a specialized pre-metastatic niche (PMN) in the brain through recruitment of bone marrow-derived cells (BMDCs), extracellular matrix (ECM) modification, and BBB disruption [15]. Finally, within established brain metastases, tumor cells engage in bidirectional crosstalk with CNS-resident populations, such as astrocytes, microglia, neurons, and cells of the neurovascular unit, creating an immunosuppressive ecosystem that supports outgrowth and confers therapeutic resistance [16].

### 1.1 Conceptual Framework

The three phases described here, the primary TME, the PMN, and the established metastatic niche, are each well-established concepts in metastasis biology [15–17]. We do not claim novelty for the phases themselves; rather, our contribution is to (i) systematically integrate the signaling mediators that link these phases across time and space, and (ii) use these cross-phase axes to define concurrent, stage-specific therapeutic windows that can be targeted in parallel (Figure 1). On this basis we propose three organizing principles. First, each phase represents a mechanistically distinct but not mutually exclusive therapeutic window: preventing EMT-driven dissemination in the primary TME, intercepting EV-mediated conditioning in the PMN, and reprogramming the immunosuppressive CNS niche in established metastasis. Second, feed-forward signaling operates across phases: emerging evidence is consistent with a model in which TAM-derived IL-6 drives EMT while promoting the release of brain-tropic EVs (e.g., miR-105, miR-181c) that disrupt the BBB and condition the brain PMN, which in turn activates STAT3+ astrocytes that sustain the niche (Table 1). Third, this integrated view generates testable hypotheses: agents blocking cross-phase mediators (e.g., IL-6/JAK/STAT3 inhibitors) could concurrently suppress multiple stages of the cascade, and longitudinal liquid biopsy monitoring of EV cargo could identify patients transitioning from PMN

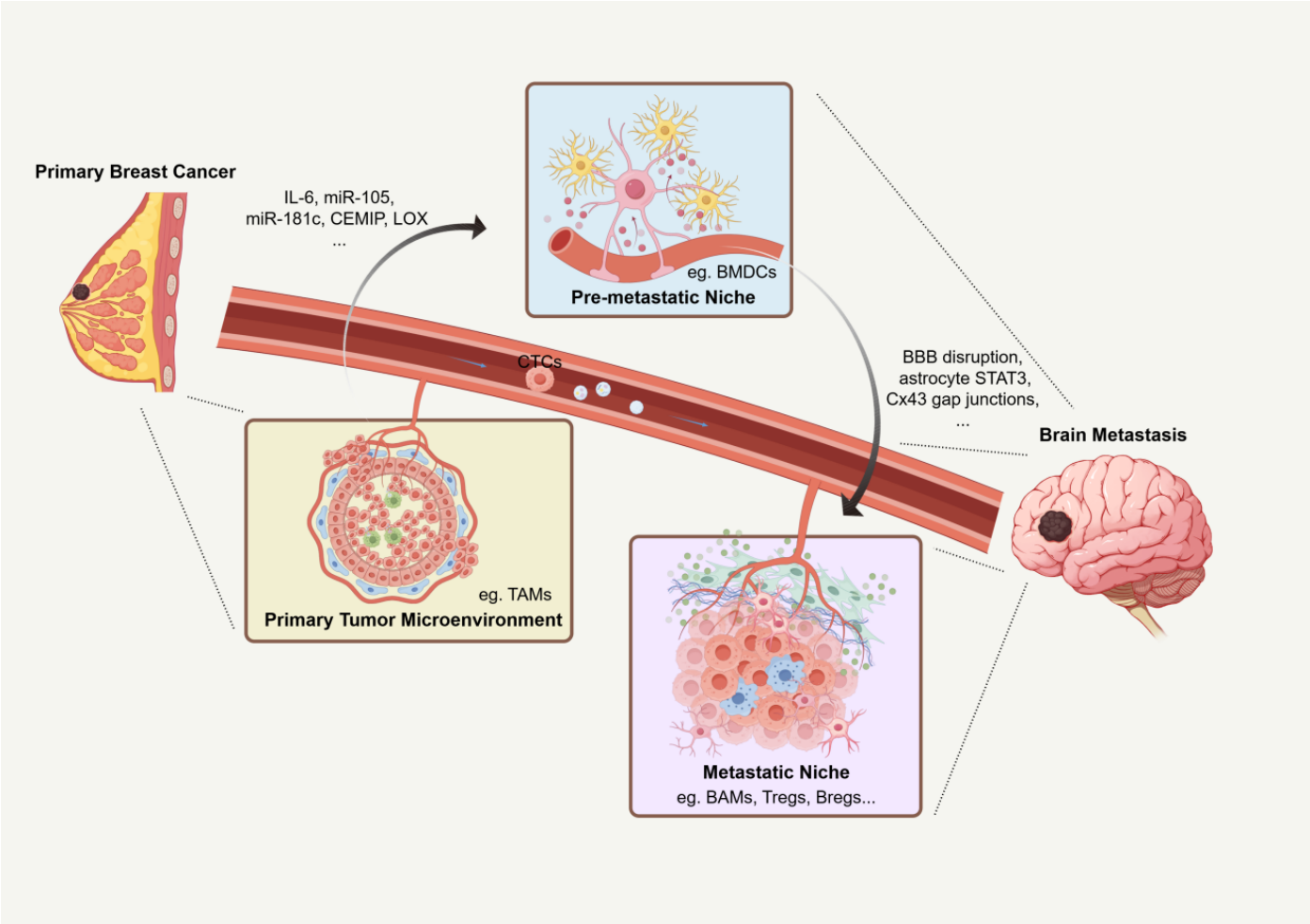


**Figure 1.** Tri-phasic microenvironmental remodeling in BCBM. Directional arrows indicate cross-phase signaling axes; representative molecular mediators are annotated at each transition. Representative cells are annotated at each phase. Created with Figdraw.

to established metastasis, enabling stage-specific intervention.

## 2 The Primary Tumor Microenvironment in Brain Metastasis

### 2.1 Tumor-Associated Macrophages in Promoting Metastasis via EMT

The TME is composed of tumor cells, stromal cells, immune cells, and non-cellular ECM components [17–19]. Among these, TAMs represent indispensable elements that actively promote metastatic progression. EMT, a dynamic process wherein cells lose epithelial polarity and intercellular adhesion while acquiring migratory and invasive properties, is a pivotal mechanism underlying metastatic capacity acquisition [12, 20, 21]. M2-polarized TAMs promote metastasis through multiple pro-tumorigenic paracrine programs, including EMT regulation via CXCL1/NF-$\kappa$B/SOX4 [21] and tumor angiogenesis via TAM-derived CCL18 secretion [22].

This TAM–EMT axis has direct relevance to brain tropism. TAM-derived IL-6 drives EMT [13], and we hypothesize that IL-6 may additionally promote the release of brain-tropic EV cargo (e.g., miR-105, miR-181c) that disrupts the BBB during the PMN phase. The individual links of this IL-6→EV→BBB axis are currently supported by discrete findings from separate experimental systems [5], and the keystone step — that TAM-derived IL-6 upregulates brain-tropic EV biogenesis — is cited to a review rather than to primary data [23]; integrated validation of the complete cascade in vivo therefore remains to be performed. We accordingly present the IL-6→EV→BBB cascade as a proposed, testable hypothesis rather than an established mechanism. Furthermore, in HER2+ BC, TAM infiltration correlates with trastuzumab resistance [24], and acquired trastuzumab resistance can drive EMT activation [25], a mechanism that may preferentially

predispose to CNS relapse given the brain's relative immune privilege.

### 2.2 Primary Tumor-Derived EVs in PMN Formation

As key mediators of intercellular crosstalk, EVs — including exosomes (30–150 nm), microvesicles, and apoptotic bodies — regulate remote microenvironments and drive organ-specific metastatic progression. Tumor-derived EVs are key drivers of PMN development, preparing sites at future metastatic locations before circulating tumor cell (CTC) arrival. Organotropism is governed by EV cargo, which reprograms recipient cells and constructs a favorable microenvironment that sustains colonization [23, 26, 27]. The specific EV cargoes that condition the brain PMN (CEMIP, annexin II, miR-122, miR-105, miR-181c) are summarized in Table 1 and their mechanisms are detailed in Section 3.2.

In the context of brain metastasis, several EV cargoes act on the brain vasculature and CNS-resident cells (Table 1). Exosomal integrins determine organotropism in a tissue-specific manner: integrin $\alpha6\beta4$ and $\alpha6\beta1$ (laminin-binding) were associated with lung-tropic metastasis, whereas integrin $\alpha v\beta5$ (fibronectin-binding) was associated with liver-tropic metastasis [27]. Although integrin profiling of circulating EVs holds promise for organ-specific liquid biopsy, the assignment of $\alpha v\beta5$ to brain tropism is not substantiated by that study and would require brain-specific validation. Of note, this integrin-organotropism study has recently received an Editorial Expression of Concern citing data integrity concerns [28], so these organ-specific associations should be interpreted with caution pending resolution. Collectively, these findings establish tumor-derived EVs as active agents of BBB disruption and brain PMN conditioning.

### 2.3 Angiogenesis and ECM Remodeling in Supporting Metastatic Events

Angiogenesis is critically involved in tumor metastasis, with the VEGF pathway serving as the central driver alongside contributions from FGF, PDGF, and TGF-$\beta$ [29, 30]. In the early stages, aberrant tumor-associated blood vessels serve as conduits for tumor cell escape. Upon arrival at distant organs, nascent tumor tissue stimulates angiogenesis to fuel overt metastatic growth. In the unique brain context, the neurovascular unit, comprising endothelial cells with tight junctions, pericytes, astrocytic end-feet, and a specialized basement membrane, imposes additional constraints on angiogenic signaling. Vascular co-option, by which tumor cells grow along pre-existing brain capillaries without inducing angiogenesis, partially explains the limited efficacy of anti-angiogenic agents in this setting [31].

ECM remodeling constitutes a major driving force for regional infiltration and intravascular spread. Pathways including TGF-$\beta$, Wnt/$\beta$-catenin, and Hippo control matrix metalloproteinases (MMPs), heparanases, and lysyl oxidase (LOX), in addition to regulating ECM protein composition [32, 33]. Brain-specific ECM components, such as hyaluronan, tenascin-C, and perlecan, interact uniquely with invading breast cancer cells and remain incompletely characterized in BCBM [33].

## 3 Mechanisms of PMN Formation in the Brain

Distant organs are not merely passive recipients of disseminated tumor cells (DTCs). Rather, active remodeling establishes a receptive microenvironment, namely the PMN, indispensable for metastatic colonization [15]. In this section, we focus on mechanisms specifically implicated in brain PMN formation, explicitly flagging extrapolations from other organ sites.

### 3.1 Tumor-Induced Conditioning of the Brain PMN

The establishment of a tumor-supportive PMN requires active crosstalk between cancer cells and distant organs, involving the systemic mobilization and accumulation of myeloid cells at future metastatic sites [15]. Hypoxia in the primary TME further promotes the conditioning of distant tissues via pro-tumorigenic factors. Hypoxically induced LOX accumulates in pre-metastatic lungs, mediates collagen crosslinking and ECM remodeling, and fosters CD11b+ myeloid cell mobilization that facilitates metastatic seeding [6]. LOX expression is tightly regulated by hypoxia-inducible factor 1$\alpha$ (HIF-1$\alpha$); HIF inhibition eliminated both PMN formation and subsequent metastatic colonization in breast cancer models [34]. It must be noted that the majority of LOX-mediated PMN studies have been conducted in lung metastasis models; direct evidence for LOX-dependent brain PMN formation remains incomplete.

Non-malignant cells within the primary TME also contribute to organ-specific metastasis priming. Beyond primary TME signals, disseminating tumor cells face distinct selective pressures at different metastatic sites; the molecular determinants that confer fitness specifically within the brain

microenvironment, as opposed to other organs, remain incompletely defined [35]. Gong et al. [8] demonstrated that astrocyte–tumor cell interactions in the brain promote breast cancer colonization through a TGF-$\beta$2/ANGPTL4 signaling axis, implicating ANGPTL4 as a mediator of the established brain metastatic niche. Whether ANGPTL4 additionally participates in pre-arrival brain PMN conditioning remains under investigation. The extent to which these CAF- and TGF-$\beta$-mediated priming mechanisms apply to brain metastasis therefore remains to be determined.

### 3.2 Exosome-Mediated Brain PMN Preparation

Tumor-derived exosomes (30–150 nm) contain DNA, RNA, proteins, and lipids that mediate intercellular communication between cancer cells and future metastatic sites, reshaping distant microenvironments. Exosomal integrin transfer contributes to organ-specific PMN constitution [27], while exosome internalization by macrophages can induce pro-inflammatory activation through Toll-like receptor 2-dependent NF-$\kappa$B signaling [36].

For brain metastasis, exosomal CEMIP promotes perivascular inflammation and vascular remodeling [7], and exosomal annexin II supports metastatic expansion via tissue plasminogen activator (tPA)-dependent angiogenesis [37]. Exosomal miR-122 reprograms glucose metabolism in the brain PMN [9], while miR-105 [4] and miR-181c [5] disrupt endothelial tight junctions and compromise vascular barrier integrity. miR-181c, identified in EVs from brain-metastatic breast cancer cell lines, disrupts the BBB through PDPK1 downregulation and actin cytoskeleton disorganization [5]. Because CEMIP and miR-181c derive from cells already selected for the brain, they are best interpreted as niche-conditioning signals acting after arrival, rather than bona-fide pre-arrival priming factors (Table 1). Whether HER2+ and TNBC primary tumors release EV cargoes that differentially condition the brain PMN — for example, subtype-specific miRNA or integrin repertoires — remains an open, testable question.

### 3.3 BBB Disruption During PMN Formation

A critical brain-specific aspect of PMN formation is BBB priming for subsequent tumor cell entry. The BBB, composed of specialized endothelial cells connected by tight junctions (claudins, occludin, ZO-1), pericytes, and astrocytic end-feet, represents both the principal barrier to brain metastasis and a key target of PMN conditioning signals [11]. Tumor-derived EVs actively disrupt these components: miR-105 downregulates ZO-1 expression [4]; miR-181c induces tight junction protein delocalization via PDPK1 [5]; and CEMIP promotes perivascular inflammation that increases endothelial permeability [7]. The primary tumor-derived cytokine milieu, particularly TNF-$\alpha$ and TGF-$\beta$, upregulates adhesion molecules (VCAM-1, ICAM-1) on brain endothelial cells, facilitating CTC arrest and extravasation [38, 39]. This BBB priming represents a brain-specific PMN mechanism not shared with other metastatic sites and marks the critical transition from the PMN to the established metastasis phase.

## 4 Cellular Components of the Established Brain Metastatic Niche

The brain metastatic microenvironment embodies far greater cellular and structural complexity than the primary TME. Only cancer cells capable of adapting to this unique ecological environment can establish intracranial metastases. The intrinsic cellular makeup of the brain, including neurons, astrocytes, microglia, oligodendrocytes, and the neurovascular unit, collectively shapes a distinct intracranial niche. The BBB, by restricting circulating immune cell entry into the CNS, also establishes the brain's distinctive immune microenvironment [40].

### 4.1 The Plastic Nature of Metastatic Cancer Cells

The cellular plasticity model—encompassing the reversible reprogramming of cell fate and identity in response to intrinsic and extrinsic signals, with epithelial–mesenchymal plasticity serving as a central driver of tumor malignancy [41]—has become a unifying framework for understanding phenotypic transitions in cancer progression. Throughout cancer progression, this plasticity equips tumor cells with the flexibility to dynamically transition between phenotypic states. The metastatic cascade is initiated by basement membrane breaching and intravasation [40]. Dissociated breast cancer cells evade environmental selection constraints and preserve metastatic potential through promotion of stemness, EMT induction, and metabolic reprogramming [42].

Molecular drivers of cellular plasticity differ between subtypes. In HER2+ BC, acquired trastuzumab resistance can trigger EMT toward a TNBC-like, invasive phenotype with mesenchymal characteristics [25]. More generally, cells transit

reversibly along a partial-to-full EMT continuum, with partial-EMT cells contributing to metastasis and chemoresistance and full-EMT cells enriched upon recurrence [20]. These differences partly explain the differential rates and latency of brain metastasis between subtypes [2]. Critically, tumor cell plasticity feeds directly into immunosuppressive niche establishment, as EMT transcription factors (SNAIL, ZEB1) upregulate immunosuppressive cytokines (TGF-$\beta$, IL-10) and immune checkpoint ligands (PD-L1), actively shaping the immune microenvironment that permits metastatic outgrowth [12].

### 4.2 The Immunosuppressive State of Adaptive Immune Cells

The marked immunosuppression typical of BCBM reflects an active state established via dynamic tumor cell–microenvironment interactions. Zou et al. [43] performed single-cell RNA sequencing (scRNA-seq) on 44,473 cells from liver and brain metastases of breast cancer, revealing extensive immunosuppressive cell population reprogramming. More recent comprehensive scRNA-seq and spatial transcriptomic analyses across multiple primary tumor types have demonstrated that the brain metastatic immune microenvironment is characterized by marked T cell exhaustion, expansion of immunosuppressive myeloid populations, and relative exclusion of effector lymphocytes compared to extracranial metastases [44].

In that work, CD8+ T cells infiltrating brain metastases exhibited high expression of multiple immune checkpoints, such as PD-1, TIM-3, LAG-3, and TIGIT, with a transcriptional profile consistent with terminal exhaustion; the upregulation of alternative checkpoints, including LAG3–galectin-3 and TIM-3, represents a resistance mechanism to PD-1/PD-L1 monotherapy, and the degree of T cell exhaustion correlated with worse clinical outcomes [44]. This exhaustion program is enforced by chronic antigen stimulation in an immunosuppressive milieu rich in TGF-$\beta$ and IL-10.

A distinguishing feature of the brain metastatic niche is immune exclusion, whereby effector T cells are physically restricted to the perivascular space by dense reactive astrocytes forming a glial barrier and by chemokine dysregulation (e.g., downregulation of CXCL9/10/11 that recruit CXCR3+ effector T cells) [3]. The net effect is a profoundly immunosuppressive niche in which even tumor-antigen-specific T cells are functionally inert. Emerging evidence also implicates regulatory B cells (Bregs) and tertiary lymphoid structures (TLS) in brain metastases, although B cell-focused studies in BCBM specifically remain scarce [45].

### 4.3 The Multifaceted Roles of Innate Immune Cells

The brain microenvironment contains unique populations of resident innate immune cells. Brain macrophage populations comprise microglia (tissue-resident macrophages of CNS origin), border-associated macrophages (BAMs; meningeal, perivascular, and choroid plexus macrophages), and, in tumors, monocyte-derived macrophages recruited from the circulation [46].

These cells contribute to metastatic progression through proteolytic and pro-angiogenic programs; in particular, microglia- and macrophage-supplied cathepsin S cooperates with tumor-derived proteases to promote brain metastasis, and Tie2-expressing macrophages support tumor angiogenesis via VEGF [47, 48].

Recent spatial transcriptomic and imaging mass cytometry studies have revealed spatially organized zonation within brain metastases, with distinct transcriptional programs at the tumor periphery versus the tumor core [48]. Subtype-specific differences are notable: HER2+ BCBM tends toward pro-inflammatory, M1-like polarization potentially contributing to a T-cell-inflamed microenvironment, whereas TNBC brain metastases exhibit a predominantly immunosuppressive, M2-like myeloid infiltrate [44]; this dichotomy is based on limited subtype-stratified analyses and requires confirmation in larger cohorts. It should also be noted that the M1/M2 classification is a simplification: single-cell and spatial studies show that brain-metastasis-associated myeloid cells occupy a continuum of transcriptional states rather than two discrete polarization endpoints [48, 49].

Neutrophil extracellular traps (NETs) represent an emerging myeloid component of BCBM biology: in breast cancer, NETs have been implicated in pre-metastatic niche formation and the chemotactic attraction of circulating tumor cells, with NET-DNA acting via the CCDC25 receptor [50, 51], although the bulk of this evidence derives from liver- and lung-metastasis models and brain-BCBM-specific NET evidence is still emerging. Beyond T-cell checkpoints, myeloid and microglial checkpoints are increasingly relevant to brain tumors: the

CD47–SIRP$\alpha$ "don't-eat-me" axis represents an actionable phagocytosis checkpoint in breast cancer, with CD47-targeted therapeutics—including novel antibody–toxin conjugates—demonstrating antitumor activity [52], and TREM2 regulates myeloid function in CNS cancers [53]. The disease-specific remodeling of microglia and monocyte-derived macrophages in brain metastases [49] further supports the myeloid compartment as a therapeutic target.

### 4.4 Astrocyte-Tumor Interactions

Astrocytes, the most abundant glial cells in the CNS, play active roles in BCBM extending far beyond passive structural support. Priego et al. [3] demonstrated that a subpopulation of reactive astrocytes surrounding brain metastases exhibits STAT3 activation driven by tumor-secreted factors. These STAT3+ reactive astrocytes promote metastatic outgrowth through secretion of pro-survival factors (e.g., SerpinA3) that protect tumor cells from chemotherapy and through establishment of a physical barrier that excludes CD8+ T cells. Pharmacological STAT3 inhibition reduced brain metastatic burden preclinically. It remains to be determined whether the STAT3+ reactive-astrocyte barrier forms differentially across HER2+ versus TNBC brain metastases.

Chen et al. [54] identified a distinct mechanism involving connexin 43 (Cx43)-mediated gap junctions between brain-metastatic cancer cells and astrocytes. Brain-metastatic cancer cells transfer cGAMP through PCDH7/Cx43-containing gap junctions to adjacent astrocytes, where cGAMP activates STING signaling and induces the production of inflammatory cytokines, including IFN-$\alpha$ and TNF. These astrocyte-derived cytokines subsequently activate STAT1 and NF-$\kappa$B signaling in metastatic cancer cells, thereby promoting tumor growth and chemoresistance. Pharmacological disruption of gap-junction communication suppresses brain metastatic outgrowth in experimental models. Astrocyte metabolism is also remodeled in the niche: cGAMP transferred to astrocytes increases glutamate secretion while decreasing glutamine production [55]. Beyond astrocytic glutamine/glutamate metabolism, metabolic crosstalk in the brain niche extends to lactate-mediated immune evasion and glutamine oxidation, whereby metabolic diversity within brain-tropic cells determines metastatic fitness and residual disease [56], and to neuron–astrocyte glutamate/glutamine/lactate coupling, which breast cancer cells can hijack to promote colonization [57]. Lipid metabolism and ketone-body utilization represent additional axes that remain comparatively underexplored in BCBM.

### 4.5 The Neurovascular Unit and Neuronal Contributions

The neurovascular unit, comprising brain endothelial cells, pericytes, astrocytic end-feet, vascular smooth muscle cells, and the basement membrane, represents the structural and functional interface between circulation and brain parenchyma [11]. In BCBM, the neurovascular unit actively participates in niche establishment, and the capacity of breast cancer cells to cross the BBB and colonize the brain is governed in part by intrinsic mediators such as ST6GALNAC5, COX2, and HBEGF [58]. Pericytes regulate BBB permeability during metastasis and influence tumor cell dormancy versus outgrowth decisions; pericyte coverage is reduced at extravasation sites, correlating with metastatic efficiency [11]. Astrocytic end-feet secrete Sonic hedgehog (Shh) and angiopoietin-1, which maintain BBB integrity under homeostatic conditions but are disrupted during PMN formation [11].

A notable recent finding is that neurons directly contribute to the metastatic niche. Zeng et al. [59] demonstrated that breast cancer cells metastasizing to the brain form pseudo-tripartite synapses with glutamatergic neurons, positioning themselves near synaptic clefts. Through N-methyl-D-aspartate receptor (NMDAR)-mediated signaling, tumor cells receive glutamatergic input that promotes proliferation and colonization. Complementing the pro-tumorigenic roles of neurons and astrocytes, brain-resident microglia exert tumor-suppressive functions in BCBM: using single-cell RNA sequencing and genetic mouse models, Evans et al. [60] demonstrated that microglia promote pro-inflammatory anti-tumor immunity, and that animals lacking microglia exhibit increased metastatic burden and impaired NK and T cell responses, identifying microglia as critical suppressors of BCBM outgrowth.

Tumor cell plasticity within brain metastatic niches is governed by niche-specific programs, including mechanosensitive YAP/MRTF signaling activated upon pericyte-like spreading [61]; whether inhibitory GABAergic signaling opposes these excitatory, tumor-promoting inputs remains to be determined. These findings open therapeutic avenues, including repurposing anti-epileptic drugs or NMDAR antagonists, but raise neurotoxicity concerns.

### 4.6 Organoid-Based Models and Spatial Transcriptomic Approaches

Recent technological advances have enabled unprecedented resolution in BCBM microenvironment characterization. Karimi et al. [48] used imaging mass cytometry to map the immune landscapes of primary and metastatic brain tumors, revealing spatially organized immune zonation with distinct cellular neighborhoods. Gonzalez et al. [44] profiled the cellular architecture of human brain metastases using scRNA-seq and spatial approaches, demonstrating conserved cellular neighborhoods across patients and tumor types. These studies have identified clinically relevant spatial biomarkers; more generally, immune checkpoint blockade induces prominent T cell infiltration into brain metastases and reduces a distinct CD206+ macrophage population in the perivascular space that may restrict T cell entry [62], providing a spatial mechanistic basis for the limited but real intracranial activity of ICIs.

Traditional in vivo models (intracardiac or intracarotid injection) and in vitro transwell BBB assays have limitations in recapitulating the full complexity of the human brain microenvironment. Patient-derived organoid co-culture systems, in which breast cancer cells or patient-derived organoids (PDOs) are co-cultured with cerebral organoids derived from human pluripotent stem cells, represent an emerging platform for studying BCBM [63]. These models recapitulate astrocyte reactivity, microglial recruitment, and BBB-like barrier formation, and have been used to screen for compounds with brain-penetrant activity. However, the lack of a functional immune system and vascular perfusion limits their utility for immunotherapy and pharmacokinetic studies, respectively.

### 4.7 Subtype-Specific Differences in the Brain Metastatic Niche

As noted throughout this review, HER2+ BC and TNBC exhibit markedly different behaviors in the brain metastatic context (Table 2).

## 5 Therapeutic Strategies by Targeting the Microenvironment

The tri-phasic framework highlights distinct therapeutic windows corresponding to each phase. However, translation of preclinical strategies to clinical benefit has been hampered by limited BBB penetration, the difficulty of targeting the PMN before it becomes clinically detectable, and profound immunosuppression of the established niche. Here we discuss both emerging opportunities and translational barriers, which are summarized in Table 3.

### 5.1 Targeting the Primary Microenvironment

#### *5.1.1 Inhibiting EMT*

EMT activation is promoted by different signaling pathways across breast cancer subtypes: TGF-$\beta$/SMAD–SNAIL in HER2+ BC, Wnt/$\beta$-catenin in TNBC [65]. Preclinically, TGF-$\beta$ inhibition sensitizes breast cancer cells to radiotherapy [66], and targeting LOX overcomes chemoresistance in TNBC [32]. As discussed in Section 4.1, acquired trastuzumab resistance can trigger EMT toward a TNBC-like phenotype, suggesting that EMT inhibition may re-establish anti-HER2 sensitivity [25]. Despite compelling preclinical evidence, no EMT-targeted agent has demonstrated clinical efficacy in BCBM, and this translational gap has a mechanistic basis. EMT is highly context-dependent and reversible, and colonization of distant organs is thought to require the reverse transition — mesenchymal-to-epithelial transition (MET) — to restore proliferative capacity [67]. Inhibiting EMT is therefore a double-edged sword: it may suppress dissemination and early invasion, yet it could also inadvertently impair, or paradoxically promote, the MET-dependent steps required for colonization. This therapeutic paradox, together with the lack of validated pharmacodynamic biomarkers that can resolve partial versus full EMT states [20], has hindered clinical development, and resolving the optimal timing of EMT inhibition relative to seeding versus colonization remains a central open question.

#### *5.1.2 Targeting Extracellular Matrix Remodeling*

ECM remodeling contributes to metastatic progression through MMPs and LOX family members. The clinical failure of first-generation broad-spectrum MMP inhibitors (marimastat, batimastat, prinomastat) provides a cautionary lesson [74, 75]: these agents failed due to insufficient efficacy and dose-limiting musculoskeletal toxicities arising from excessive inhibition of physiological MMP activity. Future ECM-targeted therapies must therefore shift toward precise target selection (e.g., selective inhibition of MMP-2/MMP-9, or targeting LOX/LOXL2) and biomarker-guided patient stratification. LOX-mediated collagen crosslinking drives fibrosis-enhanced metastasis [68]; however, clinical translation of this target has proven difficult, as exemplified by the lack of efficacy of anti-LOXL2

**Table 2.** Subtype-specific features of the BCBM microenvironment.

| Feature | HER2+ BCBM | TNBC BCBM | References |
|---|---|---|---|
| CNS tropism | High (~31%) | High (~32%) | [2] |
| Latency to brain metastasis | Longer (often late recurrence) | Shorter (often early recurrence) | [2, 64] |
| BBB penetration | BBB partially disrupted at macrometastases | Similar, but more vascular co-option | [11, 31] |
| Immune microenvironment | More T-cell-inflamed; tumour-infiltrating lymphocytes (TILs) present (tentative) | Immunosuppressive; M2-dominant myeloid infiltrate (tentative) | [44] |
| Key signaling pathways | HER2/EGFR–PI3K–mTOR; TGF-$\beta$/SMAD–SNAIL (EMT) | Wnt/$\beta$-catenin; HIF-1$\alpha$ (hypoxia-driven); Notch | [65] |
| Therapeutic vulnerabilities | Anti-HER2 ADCs (T-DXd); PI3K/mTOR inhibitors | PARP inhibitors (germline *BRCA*-mutated); immune checkpoint blockade | Section 5 |
| Astrocyte interactions | STAT3+ astrocyte barrier prominent | Cx43 gap junctions–cGAMP/STING-mediated chemoresistance | [3, 54, 55] |
| EV cargo | HER2-containing EVs; miR-105 | miR-122 (metabolic reprogramming); miR-181c (BBB disruption) | [4, 5, 9] |

**Table 3.** Clinical versus preclinical evidence for selected microenvironment-targeted strategies in BCBM.

| Strategy | Preclinical evidence | Clinical evidence | Key gap |
|---|---|---|---|
| EMT inhibition | Strong; multiple pathways (TGF-$\beta$ [66], Wnt [65]) | None; no BCBM-specific trials | EMT–MET reversibility paradox; risk of impairing or promoting colonization; no clinical-grade inhibitor [67] |
| LOX/LOXL2 inhibition | Prevents lung PMN [6]; limited brain data | Negative (simtuzumab in fibrosis) [68] | Brain-specific PMN evidence needed |
| Anti-VEGF (bevacizumab) | Reduced angiogenesis; tumor vessel normalization | Phase I signal [69], but no further progression | Vascular co-option bypass [31]; pseudoprogression |
| T-DXd (HER2 ADC) | Intracranial activity in xenografts | Positive: DESTINY-Breast03 [70], TUXEDO-1 [71] | Durability; sequencing with SRS |
| Immune checkpoint blockade | Modest as monotherapy; synergy with SRS in models [72] | Modest in BCBM; better in melanoma brain metastases | T cell exclusion; low TMB [44] |
| CSF-1R inhibition | Reprograms macrophages in glioma models [73]; BCBM-specific preclinical data lacking | Limited; no BCBM-specific data | CNS penetration; compensatory mechanisms |

antibodies (simtuzumab) in fibrotic indications, underscoring the broader challenge of translating ECM-targeted approaches to the clinic.

#### *5.1.3 Targeting Angiogenesis*

Bevacizumab, a monoclonal antibody against VEGF, received FDA accelerated approval in 2008

for metastatic HER2-negative breast cancer; this indication was withdrawn in November 2011 after the confirmatory trials AVADO [76] and RIBBON-1 [77] failed to reproduce the progression-free-survival benefit observed in E2100 [78], and bevacizumab use in breast cancer is therefore off-label in the United States. In the BCBM setting, bevacizumab combined with trastuzumab and lapatinib achieved disease stabilization for at least six months in heavily pretreated HER2+ BCBM patients [69]. Despite this early signal, anti-angiogenic strategies have not progressed further for several reasons: vascular co-option represents a VEGF-independent mode of brain metastasis growth [31]; anti-angiogenic therapy can paradoxically increase tumor hypoxia and promote a more invasive phenotype [79]; and clinical management is complicated by pseudoprogression on magnetic resonance imaging (MRI) and rebound edema upon treatment discontinuation.

### 5.2 Targeting PMN Formation

#### *5.2.1 Early Detection Using Exosomal Biomarkers*

EVs are ideally suited for liquid biopsy applications due to their availability, abundance, and stability in the circulatory system [80]. Beyond the well-characterized miR-105 [4], miR-181c [5], and miR-122 [9], recent work has identified novel EV cargoes with brain-metastasis specificity. Curtaz et al. [81] identified hsa-miR-576-3p (upregulated) and hsa-miR-130a-3p (downregulated) as candidate prognostic markers in serum exosomes of BCBM patients, with potential utility for early risk stratification. The exosomal integrin repertoire reflects organotropism, with distinct integrin subsets associated with lung versus liver tropism [27]; whether a brain-specific integrin signature exists remains to be established. Beyond circulating EVs, proteomic profiling of the experimental brain-metastatic microenvironment has nominated additional candidate proteins, including tenascin-C [82]. Emerging lipid signatures (phosphatidylserine externalization, specific ceramide species) and engineered small EVs as BBB-penetrant drug-delivery vehicles represent future directions [83].

#### *5.2.2 Early Intervention by Disrupting PMN Formation*

Disrupting PMN formation holds potential to intercept metastasis at its earliest stages. ATP release activates P2Y purinoceptor 2 (P2RY2), triggering the HIF-1$\alpha$–LOX axis; pharmacological P2RY2 inhibition prevents PMN establishment preclinically [84]. A fundamental challenge is that the PMN phase is subclinical, that is, patients are identified only after brain metastases become radiographically detectable. This necessitates either preventive strategies in high-risk populations (requiring large, long-duration trials) or ultra-early liquid biopsy detection, which remains under development.

### 5.3 Targeting the Established Metastatic Niche

The highly orchestrated immunosuppressive network in BCBM can be approached at three levels: reversing immunosuppression, reprogramming the microenvironment, and establishing effective combination therapies.

#### *5.3.1 Immune Checkpoint Blockade*

Alternative immune checkpoints, such as LAG3–galectin-3 and TIM-3, are markedly activated in BCBM, positioning them as complements to PD-1/PD-L1 blockade [43, 85]. However, clinical efficacy of immune checkpoint inhibitors (ICIs) has been modest, reflecting profound T cell exclusion, low tumor mutational burden (TMB), and BBB restriction of therapeutic antibody access. Stereotactic radiosurgery (SRS) may synergize with ICIs, though the efficacy/toxicity balance remains under investigation in BCBM [72].

#### *5.3.2 Antibody-Drug Conjugates with Intracranial Activity*

A critical advance in HER2+ BCBM is trastuzumab deruxtecan (T-DXd), which achieved clinically meaningful intracranial responses in an exploratory analysis of patients with baseline brain metastases in the DESTINY-Breast03 trial [70] and, prospectively, in the TUXEDO-1 phase 2 trial conducted specifically in patients with active brain metastases [71]. This intracranial activity reflects properties that earlier systemic agents lacked: the DXd payload is membrane-permeable, enabling diffusion to neighboring antigen-negative tumor cells (bystander killing), and the high drug-to-antibody ratio delivers a potent cytotoxic load even at the limited drug concentrations achieved across the BBB. This bystander effect is especially relevant to the intratumoral HER2 heterogeneity observed in brain metastases. Emerging resistance mechanisms include HER2-low/heterogeneous antigen expression that reduces antibody-drug conjugate (ADC) binding, drug efflux at the BBB (e.g., P-glycoprotein/breast cancer resistance protein, BCRP) that limits parenchymal payload delivery, and acquired payload resistance [86]. Other ADCs, including sacituzumab

govitecan (anti-Trop-2) for TNBC and datopotamab deruxtecan, are under active investigation [87]; collectively, ADCs with membrane-permeable, bystander-active payloads represent a notable advance as the first systemic therapy class with reliable intracranial activity.

#### 5.3.3 *Targeting Immunosuppressive Populations*

Depletion of intratumoral regulatory T cells (Tregs) through anti-CCR4 antibodies [88] or low-dose cyclophosphamide [89] may undermine tumor immune evasion. Reprogramming microglia and BAMs from an immunosuppressive toward a pro-inflammatory phenotype using CSF-1R inhibitors (pexidartinib) has shown preclinical activity in glioma models [73]—a brain-resident myeloid reprogramming principle that is now being explored in the context of brain metastasis. Combination therapies hold the greatest promise, as the cooperativity of immunosuppressive networks (Treg–Breg axis, astrocytic barrier with T cell exclusion) means single-target interventions are prone to adaptive resistance [40].

### 5.4 Translational Challenges

#### 5.4.1 *BBB Drug Delivery*

Despite partial BBB disruption at macrometastatic sites, the BBB remains a significant barrier at the micrometastatic and invasive front [11]. Emerging strategies include focused ultrasound with microbubbles [90], receptor-mediated transcytosis (transferrin receptor-targeted nanoparticles [91]), and membrane-permeable payloads as exemplified by T-DXd [71].

#### 5.4.2 *Animal Model Limitations*

Most preclinical BCBM studies rely on intracardiac or intracarotid injection in immunodeficient mice, bypassing early metastatic cascade steps and lacking functional immune systems. Syngeneic and humanized mouse models are needed to enable meaningful preclinical testing of immunotherapies [92].

## 6 Conclusion

BCBM development depends on three interconnected microenvironments — the primary TME, the PMN, and the established niche — each representing a distinct therapeutic window linked by proposed feed-forward signaling axes such as IL-6→EV→BBB disruption. Important gaps remain: much PMN literature derives from extracranial models, studies rarely stratify by breast cancer subtype, and immunodeficient xenograft models dominate. Priority questions include identifying truly brain-specific PMN signals, developing liquid biopsy-based pre-radiographic detection, understanding neuronal modulation of metastatic outgrowth, and determining the optimal sequencing of ADCs with radiotherapy and immunotherapy. Addressing these gaps through multi-modal single-cell and spatial omics, organoid co-cultures, and longitudinal liquid biopsy studies will be essential to translating mechanistic insight into stage- and subtype-specific therapies for BCBM.

## Data Availability Statement

Not applicable.

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## Conflicts of Interest

The authors declare no conflicts of interest.

## AI Use Statement

The authors declare that DeepSeek-V4-Pro was used for language editing and grammar refinement of the manuscript. The authors have carefully reviewed, revised, and verified the AI-assisted output and take full responsibility for the content of the manuscript.

## Ethical Approval and Consent to Participate

Not applicable.

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