

Review Article

Breast cancer brain metastasis: mechanisms, emerging biomarkers, and therapeutic opportunities

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Abstract: Breast cancer brain metastasis (BCBM) is a clinically distinct form of metastatic breast cancer that causes substantial neurological morbidity and mortality. This review examines the biological bottlenecks that precede a detectable brain lesion: survival in the circulation, interaction with the cerebral endothelium, passage across the blood-brain barrier (BBB), persistence in neural tissue, and intracranial outgrowth. We emphasize how astrocytes, microglia, neurons, stromal cells, and the nutrient environment of the brain shape these events and influence treatment response. These biological features also shape disease assessment. Magnetic resonance imaging defines lesion burden but cannot reliably determine molecular status, whereas tissue sampling is invasive and plasma biomarkers may underrepresent disease confined to the central nervous system. We therefore examine the complementary roles and current limitations of tissue reassessment, blood- and cerebrospinal fluid-based liquid biopsy, molecular imaging, and artificial intelligence-assisted image analysis. Experimental evidence is interpreted according to the questions that individual models can answer. Spontaneous, intravascular, and intracranial models retain different stages of the metastatic cascade, while immunocompetent, patient-derived xenograft, and organoid systems differ in their ability to reproduce immune interactions and patient heterogeneity. Treatment is considered by subtype and clinical setting, including the need for immediate local control, intracranial disease burden, leptomeningeal involvement, previous therapy, and extracranial disease status. Clinical evidence is most developed for human epidermal growth factor receptor 2 (HER2)-positive BCBM, whereas treatment of hormone receptor-positive/HER2-negative disease and triple-negative breast cancer remains more dependent on local therapy and systemic indications derived from extracranial disease. Linking brain-specific mechanisms with biomarker selection, model interpretation, and subtype-guided treatment may improve the translation of experimental findings into clinically meaningful strategies.

Keywords: Breast cancer brain metastasis, blood-brain barrier, experimental models, subtype-guided treatment

Introduction

Breast cancer is the most commonly diagnosed malignancy among women worldwide [1]. It is generally classified into hormone receptor-positive (HR+)/human epidermal growth factor receptor 2 (HER2)-negative, HER2-positive, and triple-negative breast cancer (TNBC), among other molecular subtypes [2-4].

BCBM is among the most consequential forms of distant spread because it can impair

neurological function, quality of life, and survival [5, 6]. Its frequency and timing are subtype dependent: HER2-positive breast cancer and TNBC show the greatest brain-metastatic propensity, whereas HR-positive/HER2-negative tumors generally involve the brain less often and later in the disease course [5, 6]. Because asymptomatic patients are not routinely screened with brain imaging, the reported incidence probably underestimates the true burden [7].

Brain colonization is inefficient. Circulating tumor cells must lodge in cerebral microvessels, attach to brain endothelial cells, cross the BBB, and remain viable in the parenchyma [8-10]. Most cells are lost during or shortly after these steps. The minority that persists is selected by signals from endothelial cells, astrocytes, microglia, and neurons and by the immune and metabolic constraints of neural tissue [11]. BCBM therefore reflects continued selection within the brain, not simply the intracranial extension of systemic disease.

The same biology creates paired diagnostic and therapeutic problems. Magnetic resonance imaging (MRI) defines the location and burden of intracranial disease but does not directly reveal receptor or genomic status, and post-treatment change can resemble progression [7]. Intracranial biopsy provides tissue but is invasive; plasma assays may miss disease confined to the central nervous system, whereas cerebrospinal fluid (CSF) assays remain incompletely standardized [12, 13]. Drug access across the BBB and blood-tumor barrier, discordance between primary and metastatic tumors, and asynchronous intracranial and extracranial progression further complicate treatment selection [14-16].

Accordingly, this review connects the metastatic cascade with biomarker development, disease monitoring, and experimental-model selection. It then evaluates treatment by breast cancer subtype and intracranial setting, while distinguishing CNS-specific clinical evidence from conclusions derived mainly from extracranial disease or preclinical models.

The brain-specific metastatic cascade in BCBM

The formation of BCBM can be viewed as a sequence of bottlenecks. After leaving the primary tumor and surviving in blood, tumor cells arrest in cerebral capillaries, engage the endothelium, traverse the BBB, and enter neural tissue. Only a small fraction survives long enough to exploit brain-resident cells and local nutrients [17, 18]. The following sections consider these events as vascular adhesion and extravasation, early colonization, metastatic-niche remodeling, and metabolic adaptation.

Tumor dissemination, survival in the circulation, and BBB adhesion and extravasation

The steps preceding cerebral arrest are shared with metastasis to other organs. Breast cancer cells detach from the primary tumor, invade adjacent tissue, enter the vasculature, and tolerate loss of matrix attachment, hemodynamic shear, and immune attack [19, 20]. Epithelial-mesenchymal transition (EMT) can facilitate migration, invasion, and intravasation through loss of epithelial features such as E-cadherin and induction of regulators including ZEB1 and TWIST [21]. Because EMT is common to metastasis at many sites, it is best regarded as an upstream dissemination program rather than a brain-specific determinant [22]. Experimental studies also show that metastatic cells may reacquire epithelial and proliferative traits after extravasation, suggesting that reversible epithelial-mesenchymal plasticity is more relevant than a permanently mesenchymal state [23].

At the cerebral microvasculature, the BBB becomes the dominant physical constraint [24]. Brain microvascular endothelial cells are sealed by tight junctions and supported by pericytes, basement membrane, and astrocytic end-feet [25]. Claudins, occludin, and zonula occludens proteins limit paracellular movement and maintain CNS homeostasis [26]. Vascular arrest, stable endothelial adhesion, and transendothelial passage are therefore rate-limiting events in BCBM formation [26].

Successful entry into the brain requires molecular interactions with this barrier. ST6 N-acetylgalactosaminide alpha-2,6-sialyltransferase 5 (ST6GALNAC5) can increase the adhesion of brain-tropic breast cancer cells to brain endothelium [27], whereas L1 cell adhesion molecule (L1CAM) supports vascular attachment and perivascular positioning [28]. Cyclooxygenase-2 (COX-2) and heparin-binding EGF-like growth factor (HBEGF) can alter endothelial behavior and barrier permeability, enabling paracellular or transcellular passage [27, 29]. These mechanisms suggest that general invasive capacity alone is insufficient for BCBM. Tumor cells must also acquire molecular features that allow them to interact with and cross the cerebral vascular barrier. Most of the available evidence, however, comes from in vitro BBB systems and mouse models of brain metastasis. The relative contribution of these

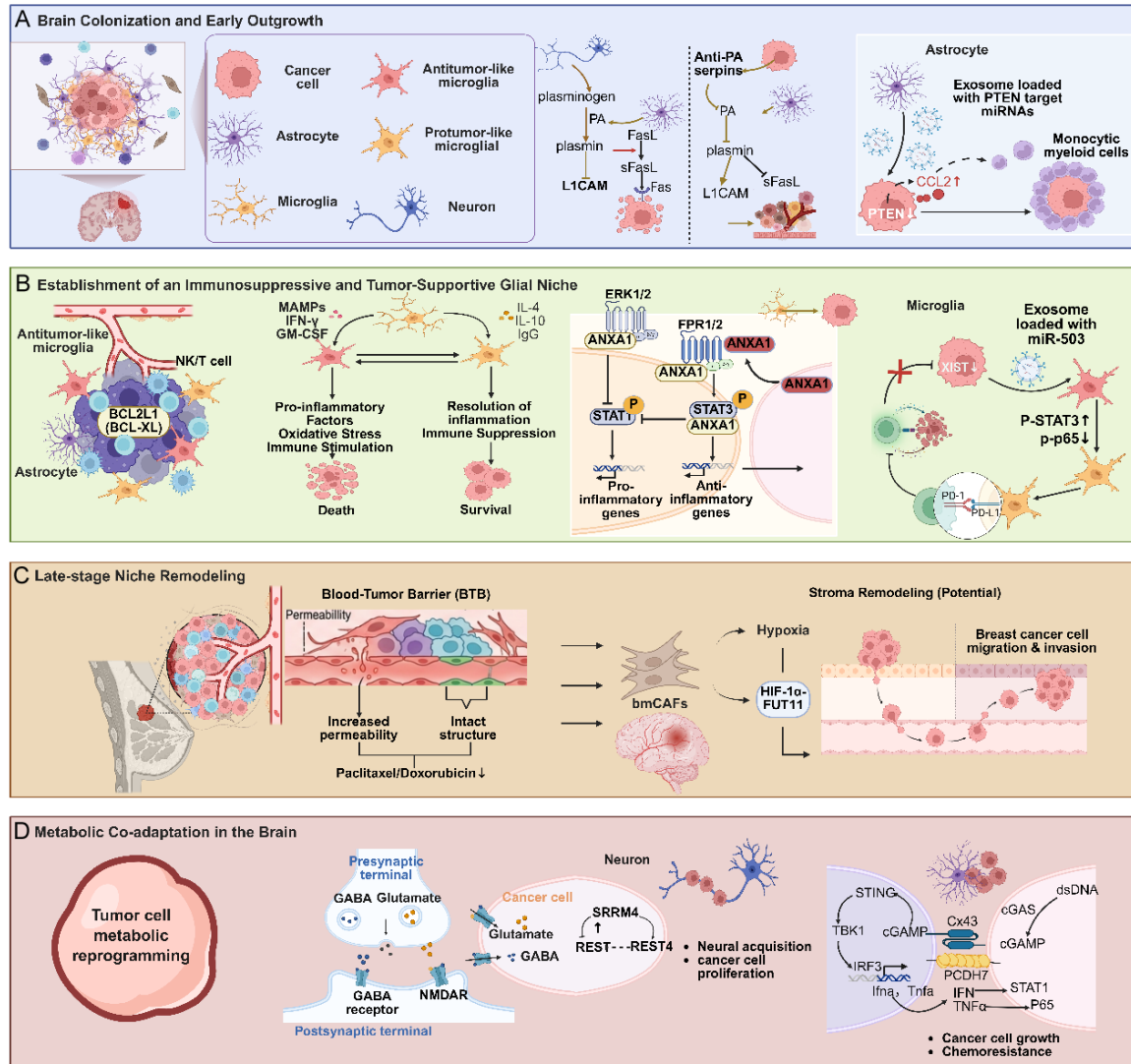


Figure 1. Cellular interactions in the BCBM microenvironment. A. Brain colonization and early outgrowth involving interactions among cancer cells, microglia, astrocytes, and neurons. B. Establishment of an immunosuppressive and tumor-supportive glial niche through interactions among tumor cells, glial cells, and immune cells. C. Late-stage niche remodeling involving changes in the blood-tumor barrier, hypoxia, and stromal remodeling. D. Metabolic and neuronal co-adaptation between breast cancer cells and the brain microenvironment. Abbreviations: ANXA1, annexin A1; ERK1/2, extracellular signal-regulated kinases 1 and 2; FPR1/2, formyl peptide receptors 1 and 2; GM-CSF, granulocyte-macrophage colony-stimulating factor; IRF3, interferon regulatory factor 3; NMDAR, N-methyl-D-aspartate receptor; PA, plasminogen activator; PCDH7, protocadherin 7; PTEN, phosphatase and tensin homolog; REST, RE1-silencing transcription factor; SRRM4, serine/arginine repetitive matrix 4; STING, stimulator of interferon genes; TBK1, TANK-binding kinase 1; XIST, X-inactive specific transcript.

molecules to different human BCBM subtypes remains to be established using patient specimens and spatial imaging approaches.

Brain colonization and early outgrowth

Once circulating breast cancer cells arrest within the cerebral microvasculature and cross the BBB, the process of brain colonization begins. Newly extravasated tumor cells encounter a restrictive perivascular microenviron-

ment composed of brain endothelial cells, pericytes, astrocytic endfeet, microglia, and other resident immune cells. Most disseminated tumor cells are eliminated at this stage, whereas a small subset manages to survive by resisting local defense mechanisms and taking advantage of inflammatory mediators, extracellular vesicles, and metabolic cues within the brain microenvironment [30]. The main mechanisms are summarized in **Figure 1**.

At this stage, successful brain colonization depends not simply on tumor cell survival, but on the gradual establishment of adaptive interactions between metastatic cells and brain-resident cells. Astrocytes can initially exert both antitumor and tumor-supportive effects. Reactive astrocytes can trigger tumor cell apoptosis through the plasmin-dependent release of soluble Fas ligand. Brain metastatic cells can counter this defense by expressing serine protease inhibitors, which suppress plasmin generation and thereby prevent Fas-mediated apoptosis while facilitating L1CAM-dependent vascular co-option [28]. At the same time, resident microglia may constrain early BCBM progression by promoting NK cell- and T cell-mediated antitumor responses [31]. Together, these interactions impose strong selective pressure during the early stages of brain colonization, favoring tumor cells that are better equipped to adapt to the brain microenvironment.

As tumor cells evade this initial clearance, their interactions with brain-resident cells gradually shift from a predominantly defensive response toward the establishment of a supportive niche. For instance, breast cancer-derived extracellular vesicles carrying miR-1290 can activate astrocytes through the FOXA2-ciliary neurotrophic factor (CNTF) pathway, leading to the production of astrocyte-derived signals that support the survival and proliferation of metastatic cells [32, 33]. In turn, astrocytes can provide additional trophic and metabolic support through the release of cytokines, exosomes, and unsaturated fatty acids [34]. Astrocyte-derived exosomal microRNAs can also induce a reversible loss of phosphatase and tensin homolog (PTEN) in metastatic cells, thereby further promoting their survival and early expansion within the brain [35].

Thus, early brain colonization is not simply a process of progressive tumor cell expansion, but rather a dynamic process of niche adaptation. Brain-resident cells initially form a defensive barrier that limits the survival of disseminated tumor cells. Those that escape clearance gradually exploit intercellular communication and locally available metabolic resources to gain a survival advantage, allowing them to persist in a dormant state or enter early micro-

metastatic growth. This adaptive process provides the basis for the subsequent reprogramming of brain-resident cells and the development of an immunosuppressive, tumor-supportive niche.

Establishment of an immunosuppressive and tumor-supportive glial niche

As a small subset of tumor cells escapes early clearance and gives rise to micrometastatic lesions, the interactions between tumor cells and brain-resident glial cells gradually shift from passive adaptation toward active remodeling of the local microenvironment.

Reactive astrocytes can exert both tumor-supportive and immunomodulatory effects. When breast cancer cells are co-cultured with astrocytes, tumor cells upregulate survival-related genes, including BCL2-like 1 (BCL2L1), and become less sensitive to several chemotherapeutic agents [36]. In brain metastatic specimens from patients and in mouse models, a subset of reactive astrocytes also shows sustained activation of signal transducer and activator of transcription 3 (STAT3). These STAT3-positive astrocytes are associated with local immunosuppression and metastatic lesion growth [37].

Microglia, in addition to astrocytes, undergo substantial functional reprogramming during BCBM progression. Together with infiltrating monocyte-derived macrophages, microglia constitute the major myeloid populations within the evolving BCBM microenvironment, and their functions are shaped by disease stage, spatial localization, and tumor-derived signals [38]. Although microglia can restrict BCBM at early stages by supporting NK cell- and T cell-mediated antitumor responses [31], persistent signals from tumor cells can gradually drive specific microglial populations toward a tumor-supportive phenotype. In immunocompetent MMTV-Wnt1 and 4T1 models, as well as in cell-based experiments, tumor-derived annexin A1 stimulates microglial recruitment and STAT3 activation through formyl peptide receptors 1 and 2 (FPR1/2), thereby promoting tumor cell migration [39].

The coordinated reprogramming of astrocytes and microglia gradually transforms the brain metastatic niche from an initially restrictive

environment into one that supports tumor cell survival, migration, and immune evasion [40]. As metastatic lesions expand, this cellular remodeling progressively extends to stromal, vascular, and metabolic networks, facilitating the transition from micrometastatic lesions to more established and clinically apparent brain metastases.

Coordinated adaptation of stromal, vascular, and metabolic networks in advanced brain metastases

As micrometastatic lesions expand and develop into relatively mature metastatic lesions, regulation of the brain metastatic microenvironment gradually extends beyond local interactions between tumor cells and glial cells to the coordinated remodeling of stromal, vascular, and metabolic networks.

Stromal remodeling may contribute to the establishment of this more mature metastatic niche. Brain metastasis-associated cancer-associated fibroblasts (bmCAFs) may also participate in niche remodeling. Studies using patient-derived bmCAFs showed that hypoxia activates the hypoxia-inducible factor 1 α -fucosyltransferase 11 (HIF-1 α -FUT11) pathway, increasing the fucosylation and secretion of the poliovirus receptor (PVR/CD155) and subsequently enhancing breast cancer cell migration and invasion [41]. This mechanism is supported mainly by patient-derived cells and in vitro experiments. It has not yet been confirmed in immunocompetent mouse models or BCBM patient-derived xenograft (PDX) models. At present, bmCAFs should therefore be regarded as potential, rather than established, regulators of the BCBM niche.

As metastatic lesions expand, early vascular co-option becomes accompanied by remodeling of vascular structure and barrier function [28]. Brain metastatic lesions can develop a spatially heterogeneous blood-tumor barrier (BTB). Although some experimental BCBM lesions exhibit increased vascular permeability, drug delivery to most lesions remains insufficient, with only a small proportion reaching cytotoxic concentrations of paclitaxel or doxorubicin [42]. Thus, vascular remodeling not only influences the growth of established lesions but also determines the spatial heterogeneity

of drug exposure, which may further contribute to differences in therapeutic response.

In parallel with stromal and vascular changes, metastatic tumor cells undergo metabolic adaptation to the nutrient environment of the brain. After entering the brain parenchyma, breast cancer cells need to adjust neurotransmitter utilization, amino acid metabolism, and lipid synthesis to adapt to the unique metabolic conditions of brain tissue [43]. Some brain-metastatic breast cancer cells acquire a γ -aminobutyric acid (GABA)-utilizing phenotype and use GABA to support energy production and biosynthetic processes [44]. This phenotype has been observed in patient brain metastases and patient-derived cells, although whether it is broadly shared across breast cancer subtypes remains uncertain.

Brain-tropic TNBC cells can also increase the expression of 3-phosphoglycerate dehydrogenase (PHGDH), thereby enhancing de novo serine synthesis in the brain, where serine and glycine availability is limited. Pharmacological inhibition of PHGDH reduces brain metastatic burden in mouse models [45]. Brain metastatic cells may also increase the utilization of glutamine and branched-chain amino acids and activate gluconeogenic pathways to sustain nucleotide biosynthesis. Inhibition of fructose-1,6-bisphosphatase reduces the viability of brain metastatic cells [46]. These findings suggest that metabolic adaptation during brain metastasis involves coordinated reprogramming of carbon and amino acid metabolism rather than reliance on a single alternative nutrient source.

Lipid metabolism further illustrates the bidirectional metabolic adaptation between metastatic cells and the brain microenvironment. Astrocyte-derived polyunsaturated fatty acids can activate peroxisome proliferator-activated receptor γ (PPAR γ) signaling in brain metastatic cancer cells, thereby promoting metastatic growth [47]. At the same time, limited access to circulating lipids increases the dependence of metastatic cells on de novo fatty acid synthesis [48]. Analyses of patient brain metastases and xenograft studies have shown that inhibition of fatty acid synthase reduces intracranial tumor growth [43]. Increased expression of fatty acid-binding protein 7 (FABP7) in HER2-positive BCBM cells may further facilitate lipid

transport and lipid droplet storage, although its causal role is currently supported mainly by cell-based experiments and xenograft models [49].

Overall, the available evidence supports a model of metabolic adaptation driven by interactions with the microenvironment rather than by tumor cell-autonomous metabolic reprogramming alone. Stromal remodeling, vascular alterations, and metabolic adaptation may act together to sustain established BCBM and promote tumor proliferation, invasion, and therapeutic resistance. However, the temporal and spatial relationships among these mechanisms, as well as their causal interactions, remain to be determined in longitudinal models that preserve the immune, stromal, and vascular components of the metastatic microenvironment.

Biomarkers and diagnostic monitoring in BCBM

Accurate diagnosis of BCBM remains challenging and usually relies on a combination of radiological imaging, histopathological examination, molecular imaging, and cytological assessment [50]. No single method can fully determine the nature of an intracranial lesion, its molecular characteristics, and its response to treatment in every clinical setting [51]. Evaluation should therefore combine neuroimaging with selective tissue sampling and blood- or CSF-based liquid biopsy. The choice of method depends on the specific clinical question [52]. **Figure 2** summarizes the principal methods and the information each can provide.

Neuroimaging

Contrast-enhanced MRI is the preferred examination for suspected parenchymal BCBM [7]. Its soft-tissue contrast supports detection of small lesions and assessment of edema, hemorrhage, mass effect, and involvement of adjacent structures [53]. These features inform selection of surgery, stereotactic radiosurgery (SRS), whole-brain radiotherapy (WBRT), or CNS-active systemic treatment. Reported imaging differences among HER2-positive, TNBC, and other subtypes include enhancement, necrosis, edema, and lesion margins [54], but the distributions overlap and are influenced by lesion size, prior therapy, and acquisi-

tion parameters. Conventional MRI and radiomics cannot currently replace pathological or molecular testing for subtype assignment.

Computed tomography (CT) is most useful when MRI is contraindicated or unavailable, particularly for rapid evaluation of hemorrhage, marked edema, hydrocephalus, or mass effect [55, 56]. It is less sensitive than contrast-enhanced MRI for small lesions, posterior fossa disease, and leptomeningeal involvement. When MRI cannot distinguish recurrent tumor from treatment-related change such as radiation necrosis, radiolabeled amino-acid positron emission tomography (PET) can provide complementary metabolic information [57]. By comparison, ^{18}F -fluorodeoxyglucose (^{18}F -FDG) PET is limited by high physiological glucose uptake in normal brain [58]. PET is therefore better suited to selected diagnostically difficult cases than to routine initial detection of BCBM.

Tissue biopsy

Tissue biopsy can confirm whether an intracranial lesion represents metastatic breast cancer and can provide information about histological type, hormone receptor status, HER2 status, and other molecular alterations [59]. It is particularly important when imaging findings are inconclusive or when the pathological result is likely to change treatment. The method used to obtain tissue should be selected according to lesion location, size, associated symptoms, resectability, and the purpose of sampling. For deep lesions, lesions close to functionally important brain regions, or lesions that cannot be safely resected but require pathological confirmation, neuronavigation-guided stereotactic biopsy can provide tissue with less surgical disruption than open resection [60]. Biopsy nevertheless remains invasive and may cause hemorrhage, infection, neurological injury, or complications related to anesthesia [61]. A sample from one region may also fail to capture spatial heterogeneity within the same lesion or among different metastatic lesions. In addition, the risks and practical limitations of repeated intracranial biopsy make it unsuitable for continuous monitoring of treatment response [62]. Tissue sampling should therefore be reserved for questions that are likely to alter diagnosis or management, and the find-

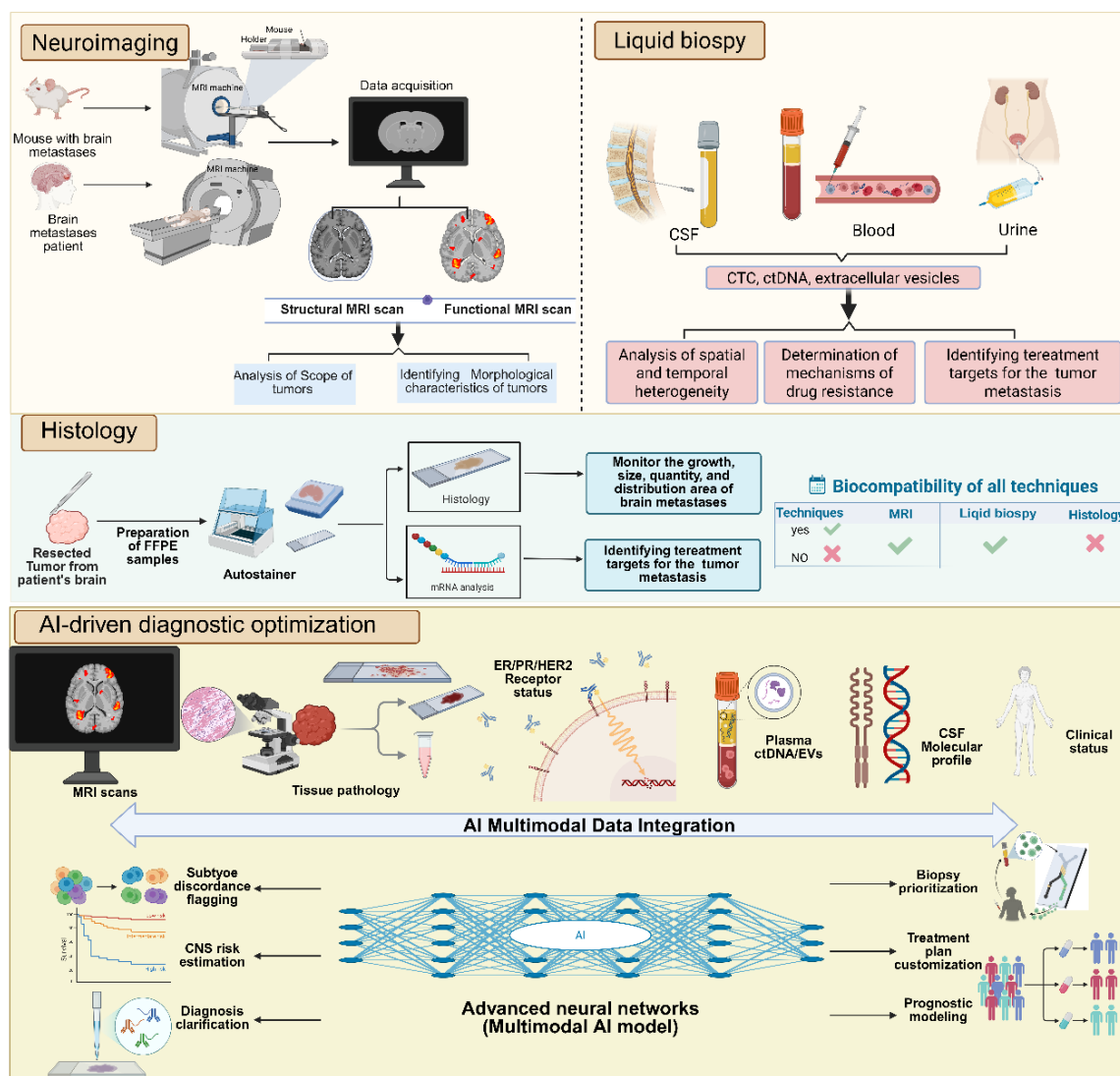


Figure 2. Multimodal approaches for BCBM assessment and management. Neuroimaging, liquid biopsy, histology, and artificial intelligence (AI) can be integrated to characterize brain metastases, assess tumor heterogeneity and molecular features, identify therapeutic targets, and support risk stratification, treatment planning, and prognostic modeling. Abbreviations: AI, artificial intelligence; CSF, cerebrospinal fluid; FFPE, formalin-fixed paraffin-embedded; MRI, magnetic resonance imaging.

ings should be interpreted together with neuroimaging and liquid biopsy results [61, 62].

Blood-based liquid biopsy

Blood-based liquid biopsy can detect circulating tumor cells (CTCs), circulating tumor DNA (ctDNA), circulating RNA, and extracellular vesicles (EVs). These tumor-derived components provide a relatively minimally invasive means of studying molecular characteristics and monitoring changes during treatment [63, 64].

CTCs: CTCs are intact tumor cells that have entered the peripheral blood from a primary or metastatic lesion. Their number, phenotype, and genomic features may reflect the capacity of the tumor to disseminate as well as its clonal heterogeneity. CTCs have therefore been investigated as markers of BCBM risk and treatment response [65]. Zhang et al. isolated a population of CTCs from the peripheral blood of patients with breast cancer that lacked epithelial cell adhesion molecule (EpCAM) but expressed HER2, epidermal growth factor

receptor (EGFR), heparanase (HPSE), and NOTCH1. These cells showed a strong capacity to form brain metastases in xenograft models, suggesting that this phenotype may enrich for brain-tropic metastasis-initiating cells [66]. The reported detection rate of CTCs depends heavily on the enrichment method. In one BCBM study using an EpCAM-independent approach, CTCs were detected in 32.6% of the entire cohort and in 70.0% of the TNBC subgroup [67]. EpCAM-independent enrichment may therefore be more effective for capturing CTCs with mesenchymal features. However, the 70.0% value was obtained from a specific, small cohort and should not be interpreted as an established diagnostic sensitivity. Genomic analysis of CTCs may also reveal copy-number alterations and patterns of clonal evolution associated with brain metastasis [68]. Whether these measurements can consistently predict brain metastatic risk or guide treatment remains to be tested prospectively.

ctDNA: ctDNA consists of tumor-derived DNA fragments within the broader pool of cell-free DNA (cfDNA) [69]. It can contain somatic mutations, copy-number alterations, insertions and deletions, gene rearrangements, and abnormal methylation patterns [69]. These features make ctDNA a potential source of molecular information when tumor tissue is unavailable.

Plasma ctDNA may nevertheless underestimate intracranial tumor burden, particularly when there is no active extracranial disease. Plasma ctDNA was analyzed in a single-arm phase II study of palbociclib combined with trastuzumab in patients with HER2-positive BCBM. The findings did not establish that plasma ctDNA could reliably monitor intracranial disease [70]. Plasma ctDNA may therefore be more informative about changes in systemic disease and cannot replace intracranial imaging or CSF analysis.

Extracellular vesicles: EVs are membrane-bound particles released by cells. They carry DNA, RNA, proteins, lipids, and metabolites and contribute to communication between tumor cells and their surrounding environment [71]. The lipid membrane protects their molecular contents from degradation, which may make EV-associated molecules more stable than unprotected circulating nucleic acids. EVs

are therefore being investigated as biomarkers for BCBM detection and risk assessment.

EVs released by brain-tropic and non-brain-tropic breast cancer cells may differ in their microRNA (miRNA) and protein profiles [72]. In patient serum, increased EV-associated hsa-miR-576-3p and decreased hsa-miR-130a-3p have also been associated with breast cancer brain metastasis [73]. These findings, however, come mainly from small patient cohorts or pre-clinical studies. The tissue of origin of circulating EVs is often uncertain, and the reproducibility of current assays has not been established. It is also unclear whether EV-based measurements provide additional clinical information beyond existing tests. EVs and their miRNA cargo should therefore be regarded as candidate biomarkers rather than established diagnostic tools for BCBM.

CSF-based liquid biopsy

CSF is in closer anatomical contact with intracranial lesions than peripheral blood and generally contains less background cfDNA. It may therefore provide a more direct representation of the molecular features of CNS tumors [74]. This advantage may be particularly relevant in patients with leptomeningeal metastasis, predominantly intracranial progression with stable extracranial disease, or low levels of plasma ctDNA [75].

The modified Fast Aneuploidy Screening Test-Sequencing System (mFAST-SeqS) detects genome-wide aneuploidy and can be used to estimate the presence of tumor-derived DNA. In a study of 121 patients with suspected leptomeningeal metastasis from breast cancer, mFAST-SeqS produced analyzable results for 112 samples [76]. Aneuploidy was detected in some patients with negative CSF cytology who were subsequently confirmed to have CNS progression on the basis of imaging or clinical findings [77].

Ultra-low-pass whole-genome sequencing (ULP-WGS) can estimate the fraction of ctDNA in CSF and thereby provide a quantitative measure of disease burden. In patients with leptomeningeal metastasis from breast cancer, suppression of the CSF ctDNA fraction during treatment was associated with better treatment response and survival, whereas an in-

crease in ctDNA sometimes preceded clinically evident progression [78]. Beyond measuring tumor burden, CSF ctDNA sequencing can identify tumor clones and potentially targetable alterations that are absent from the primary tumor or extracranial metastases. It may therefore help characterize the molecular evolution of BCBM [79]. Siravegna et al., for example, used droplet digital polymerase chain reaction (ddPCR) and whole-exome sequencing to analyze CSF from a patient with HER2-positive BCBM. They identified TP53 and PIK3CA mutations together with ERBB2 and MYC amplification. Changes in CSF ctDNA reflected the intracranial disease course, whereas changes in plasma ctDNA were more closely related to extracranial disease [80].

CSF-based liquid biopsy also has important limitations. Lumbar puncture is invasive, the amount of available material is often small, and tumor DNA release may vary among patients and over time. Preanalytical procedures and analytical methods have not yet been standardized. CSF ctDNA should therefore be used as a complement to MRI and CSF cytology rather than as a replacement. It may be most useful when leptomeningeal metastasis is suspected, intracranial and extracranial disease show discordant changes, plasma ctDNA is negative despite intracranial progression, or brain tissue cannot be obtained safely and information about intracranial molecular alterations is needed.

AI-assisted multimodal assessment and clinical decision support in BCBM

Current applications of AI in BCBM assessment include lesion detection and segmentation, differential diagnosis, inference of molecular characteristics, prognostic stratification, and evaluation of treatment response. Many published studies have enrolled patients with brain metastases from several primary cancers. Their results therefore require separate validation before they can be applied specifically to BCBM.

For lesion detection and segmentation, contrast-enhanced MRI serves as the model input, and the output consists of automatically identified and delineated metastatic lesions. Luo et al. developed a model using 10,338 brain metastases from 488 patients and evaluated it in a prospective multireader study

conducted at five centers [81]. Assistance increased the median Dice coefficient from 0.87 to 0.92 and reduced contouring time by about 42%, indicating gains in both agreement and efficiency. The AURORA study subsequently evaluated the cross-center performance of the model in 348 patients from six institutions [82]. Neither study, however, reported performance separately for the BCBM subgroup. The clinical benefit for patients with breast cancer therefore remains uncertain.

Models developed for differential diagnosis generally use radiomic features extracted from MRI as input and produce classifications such as brain metastasis versus glioblastoma or predictions of the primary origin of a metastatic lesion [83]. Available studies suggest that AI can assist in distinguishing brain metastases from glioblastoma, but its ability to determine the primary origin of a brain metastasis remains limited. Training and validation cohorts frequently include metastases from several primary tumor types, and independent external validation in BCBM cohorts is uncommon. The additional clinical value of these models for patients with an established diagnosis of breast cancer is therefore limited at present.

For BCBM, inference of the molecular characteristics of the intracranial lesion may be more clinically relevant than prediction of the primary tumor site. Cho et al. used preoperative MRI radiomic features and the subtype of the primary breast tumor as inputs to classify brain metastases into four molecular subtypes [84]. The model was developed in 102 patients with BCBM and achieved an accuracy of 0.902 in the external validation set. This was higher than the accuracy of 0.765 obtained by simply assuming that the molecular subtype of the brain metastasis was identical to that of the primary tumor. The model may therefore help identify subtype discordance between primary and intracranial disease. Other studies have used MRI radiomic features to predict estrogen receptor (ER), progesterone receptor (PR), and HER2 status in brain metastases [85]. Most of these studies are retrospective and involve small cohorts, with limited independent external validation. They cannot yet replace intracranial tissue biopsy or reassessment of receptor status. More recently, Sun et al. developed a

multimodal deep learning framework integrating three-dimensional MRI features, radiomics, and lesion location to predict ER, PR, and HER2 status in BCBM, with AUCs of 0.862, 0.899, and 0.876, respectively [86]. Nevertheless, these models remain exploratory and cannot yet replace intracranial tissue assessment when pathological confirmation is clinically indicated.

Prognostic models usually take clinicopathological variables - including age, performance status, molecular subtype, and intracranial and extracranial disease burden - as inputs and return survival probabilities at predefined time points. Li et al. trained an extreme gradient boosting (XGBoost) model using data recorded between 2010 and 2019 in the Surveillance, Epidemiology, and End Results (SEER) database. The model predicted survival at 6 months and at 1, 2, and 3 years in patients with BCBM and was externally evaluated in an independent hospital cohort [87]. It may assist with individualized risk stratification and identified lung metastasis as an adverse prognostic factor. However, the model did not include MRI features and was not adequately compared with established clinical prognostic scores. It should therefore be regarded as a clinical risk model rather than an imaging-clinical multimodal decision tool.

Studies of treatment response have focused mainly on local outcomes after SRS. Deep learning models use serial MRI examinations as input and generate predictions of lesion response [88]. Radiomics models instead use post-treatment MRI features to distinguish tumor progression from radiation necrosis [89, 90]. Most of these studies included patients with brain metastases from different primary tumors and have not undergone adequate independent validation in BCBM cohorts.

One proof-of-concept study enrolled 39 patients with BCBM and used pretreatment multiparametric MRI together with radiation dose maps as model inputs. The predicted output was the pattern of MRI change after SRS [91]. Although the study showed that early prediction of local response may be feasible, it had a small sample size and did not include external validation. Its findings are therefore insufficient to support routine clinical use.

Beyond single-task image analysis, the most clinically consequential role of AI in BCBM may be multimodal and longitudinal decision support [92]. A future system could integrate serial multiparametric MRI features with digital pathology, ER/PR/HER2 status, genomic and transcriptomic alterations, plasma- and CSF-derived circulating tumor DNA, circulating tumor cells and extracellular vesicles, as well as neurological symptoms, performance status, previous treatment, and extracranial disease control [92, 93]. Rather than returning only a segmentation mask, molecular classification, or survival score, such a system could estimate the individual risk of CNS involvement, identify suspected discordance between the primary tumor and intracranial lesion [84, 86], prioritize cases in which tissue or CSF confirmation is likely to alter management [93], and distinguish patterns more consistent with treatment response, radiation necrosis, or tumor progression [88, 90].

At the treatment stage, multimodal AI could support - not autonomously determine - the comparison of clinically relevant options, including surgery, SRS, WBRT, CNS-active systemic therapy, and clinical-trial enrollment [92]. Longitudinal integration of MRI, liquid-biopsy biomarkers, neurological status, and extracranial disease could continuously update these estimates and alert the multidisciplinary team when intracranial and extracranial disease follow divergent courses [88, 93, 94]. Such a framework would connect detection, molecular characterization, treatment selection, and response monitoring within a single clinical pathway rather than treating them as separate analytical tasks [92].

Overall, automated segmentation is the most mature AI application in brain-metastasis imaging. Subtype inference, prognosis, and post-SRS response prediction remain exploratory in BCBM. The evidence base is limited by retrospective designs, small and heterogeneous cohorts, variable MRI protocols, and sparse external validation; many datasets also combine metastases from different primary tumors. This multimodal framework remains aspirational because current BCBM models are predominantly retrospective, task specific, and trained on relatively small or heterogeneous datasets. Before AI output can influence clinical

cal management, models will require harmonized imaging and molecular data, multicenter external and prospective validation, assessment of calibration and clinical net benefit, transparent reporting of uncertainty, evaluation across demographic and molecular subgroups, and integration into multidisciplinary workflows. Ultimately, AI should function as an auditable decision-support layer that prioritizes and contextualizes clinical options rather than replacing pathological confirmation or specialist judgment.

Experimental models for studying BCBM

Preclinical models preserve different portions of the metastatic cascade and should be selected accordingly. Spontaneous models retain primary-tumor dissemination, whereas carotid, left ventricular, and intracranial injection successively bypass earlier steps. Immune-competent systems, patient-derived xenografts (PDXs), and organoid platforms add distinct information about host immunity or human-tumor heterogeneity. **Table 1** compares the biological stages retained by each model, their immune context, and the main limitations affecting interpretation [47, 49, 95-109]. **Table 2** lists representative BCBM studies and specifies the conclusions that can and cannot be supported by the models used [28, 35, 39, 47, 49, 97-99, 102, 110-120].

Spontaneous brain metastasis models

Spontaneous brain metastasis models are usually established by implanting breast cancer cells or PDX tissue orthotopically into the mammary fat pad. Tumor cells must then undergo local invasion, intravasation, survival in the circulation, extravasation from the cerebral vasculature, and colonization of the brain. These models are therefore useful for studying the acquisition of brain tropism, formation of the premetastatic niche, and clonal selection during dissemination.

Serial in vivo selection can be used to generate cell sublines with a greater propensity to metastasize to the brain. Lorusso et al. derived the spontaneously brain-metastatic 4T1-BM2 subline from 4T1 cells and used it to identify a role for connexin-focal adhesion kinase (FAK) signaling in brain colonization [95]. Echeverria et al. combined TNBC PDX models with molecu-

lar barcoding and found evidence of clonal selection between primary and metastatic tumors. The same dominant clones could contribute to metastases in the lung, liver, and brain [96]. These models also have practical limitations. Brain metastases occur at a relatively low frequency, latency is often long, and the number and timing of lesions vary among animals. Some mice reach experimental endpoints because of extracranial disease before detectable brain metastases develop. In addition, PDX models generally require immunodeficient hosts and are therefore unsuitable for assessing a complete adaptive immune response [97]. Spontaneous models are valuable for studying the initiation and evolution of brain metastasis but are less suitable for pharmacological experiments that require intracranial treatment responses to be measured within a fixed period.

Intravascular injection models

Internal carotid artery and left ventricular injection are both experimental metastasis methods used in preclinical mouse studies. They bypass primary tumor formation, local invasion, and natural intravasation. The main difference between them is the extent to which injected tumor cells are directed toward the cerebral circulation rather than distributed throughout the body.

Internal carotid artery injection: Internal carotid artery (ICA) injection delivers breast cancer cells directly into the cerebral circulation and reduces their initial entrapment in peripheral organs. The model retains vascular arrest, adhesion to brain endothelial cells, BBB extravasation, and subsequent colonization of the brain [35, 99]. It is therefore commonly used to investigate how breast cancer cells cross the BBB and adapt to the brain microenvironment.

Studies using ICA injection have shown that astrocyte-derived exosomal miRNAs can induce reversible PTEN loss in brain-metastatic cells [35]. Another preclinical study found that phosphorylated enhancer of zeste homolog 2 (EZH2) promoted the recruitment of immunosuppressive neutrophils and supported the growth of brain metastases [113]. The efficiency and distribution of tumor formation in ICA models, however, depend on the injection site and cerebral blood flow [98]. Mechanisms iden-

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Table 1. Research question-model matching for preclinical BCBM studies

Research question	Recommended model	Retained stages	Bypassed stages	Immune context	Interpretive limitation	References
Complete metastatic cascade, acquisition of brain tropism, clonal selection, and premetastatic niche formation	Spontaneous brain metastasis model following orthotopic implantation into the mammary fat pad	Primary tumor growth, local invasion, intravasation, survival in the circulation, cerebral vascular extravasation, brain colonization, and metastatic outgrowth	Cell-line implantation does not reproduce spontaneous tumor initiation; otherwise, most stages of the metastatic cascade are retained	Syngeneic models retain an intact immune system; human cell line- or PDX-based models usually require immunodeficient mice	Brain metastasis incidence is low, latency is long, and inter-animal variability is substantial. Extracranial metastases may reach experimental endpoints before detectable brain metastases develop. This model alone cannot determine the specific stage at which a tested molecule acts.	[95-97]
Cerebral vascular arrest, endothelial adhesion, BBB extravasation, and early brain colonization	Internal carotid artery injection model	Cerebral arterial circulation, arrest and adhesion within the brain microvasculature, BBB extravasation, and early colonization	Primary tumor formation, local invasion, spontaneous intravasation, and most peripheral-organ selection	Depends on the cell-host combination. Syngeneic models can retain immune competence, whereas human xenografts usually require immunodeficient hosts	The procedure is technically demanding, and injection site or altered cerebral blood flow may affect model establishment. It cannot be used to study dissemination from the primary tumor. Treatment initiated immediately before or after tumor-cell injection mainly evaluates metastasis prevention rather than therapy of established lesions.	[98-100]
Organ tropism, multi-organ distribution, and post-intravasation brain metastasis	Left ventricular injection model	Systemic distribution, arrest within the microvasculature of different organs, BBB extravasation, brain colonization, and metastatic outgrowth	Primary tumor formation, local invasion, and spontaneous intravasation	Depends on the cell-host combination	Extracranial metastases and inaccurate injection can increase experimental variability. A reduction in brain metastatic burden may result from effects on systemic distribution, extravasation, or colonization and therefore cannot be interpreted directly as inhibition of BBB crossing.	[47, 101, 102]
Intracranial growth, local niche interactions, and treatment response of established lesions	Stereotactic intracranial injection model	Growth within the brain parenchyma, local tumor-brain cell interactions, and drug response of established lesions	Primary tumor dissemination, survival in the circulation, cerebral vascular adhesion, and BBB extravasation	Syngeneic or xenograft models may be used; the immune context depends on the host	Injection-related injury may cause local inflammation and BBB disruption. The model usually produces a single localized lesion and cannot demonstrate natural brain metastasis, BBB crossing, or drug penetration across an intact BBB.	[49, 103]
Patient tumor heterogeneity, molecular subtype differences, and treatment sensitivity	BCBM PDX models established through carotid artery, left ventricular, or intracranial inoculation, depending on the research question	Retains part of the histological and molecular heterogeneity of the patient tumor; the metastatic stages retained depend on the inoculation route	Depends on the inoculation route	Usually requires immunodeficient mice	These models cannot establish complete T-cell-mediated mechanisms. Engraftment and serial passaging may cause clonal selection, and drug activity in a PDX model cannot be equated directly with clinical benefit in patients.	[104-107]
Tumor-brain tissue interactions and in vitro drug screening	PDX-derived organoids or breast cancer cell-brain organoid coculture platforms	Local invasion, growth, inter-cellular interactions, and in vitro drug responses	Dissemination from the primary tumor, circulation, a perfused BBB, and systemic pharmacokinetics	Usually lacks an intact immune system	These platforms cannot assess BBB penetration after systemic administration, pharmacokinetics, or the complete brain metastatic cascade. Screening results require validation in in vivo models.	[108, 109]

Abbreviations: BBB, blood-brain barrier; BCBM, breast cancer brain metastasis; PDX, patient-derived xenograft.

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Table 2. Representative BCBM studies and model-supported conclusions

Biological question	Model and inoculation route	Subtype/host	Conclusion supported	Conclusion not supported	References
Does α B-crystallin promote spontaneous brain metastasis?	MDA-MB-231 cells; orthotopic implantation into the mammary fat pad	TNBC; human xenograft	The overall effect of α B-crystallin on brain metastasis formation across the metastatic cascade, including dissemination from the primary tumor	The model cannot identify whether α B-crystallin acts mainly during intravasation, survival in the circulation, BBB extravasation, or brain colonization. It also cannot assess complete adaptive immune mechanisms.	[97]
Does SRC contribute to cerebral vascular extravasation and early colonization?	BT474M1 and MDA-MB-231Br cells; right carotid artery injection	HER2-positive and TNBC; human xenografts	The effects of SRC on cerebral microvascular arrest, BBB extravasation, and early brain colonization	The model cannot demonstrate a role for SRC in primary tumor invasion or spontaneous intravasation and cannot directly evaluate treatment responses in established lesions.	[112]
Does FABP7 promote intracranial adaptation and growth of BCBM cells?	BT474-Br and HCC1954 cells; intracardiac and intracranial injection	HER2-positive; human xenografts	The two inoculation routes allow assessment of FABP7 in post-intravasation brain colonization and the growth of established intracranial lesions	The models do not reproduce spontaneous dissemination from the primary tumor and cannot establish immune-dependent mechanisms.	[49]
Does HDAC activity contribute to spontaneous BCBM in an immunocompetent setting?	4T1Br4 cells; orthotopic implantation into the mammary fat pad	TNBC; syngeneic immunocompetent BALB/c model	The overall effect of HDAC activity on brain metastasis formation across the complete metastatic cascade in an intact immune environment	Brain metastatic burden alone cannot determine the specific stage of the metastatic cascade affected by HDAC activity.	[98]
Does YTHDF3 promote brain metastasis after tumor cells enter the circulation?	4T1-Br and MDA-MB-231Br cells; intracardiac injection	TNBC; syngeneic and human xenograft models	The effects of YTHDF3 on systemic distribution, cerebral vascular extravasation, and brain colonization after intravasation	The model cannot demonstrate a role in local invasion or spontaneous intravasation from the primary tumor. A single inoculation route cannot clearly distinguish effects on extravasation from those on intracranial growth.	[99]
Does the brain microenvironment induce reversible PTEN downregulation in metastatic cells?	MDA-MB-231, HCC1954, BT474, MDA-MB-435, and 4T1 cells; carotid artery injection	TNBC and HER2-positive; xenograft and syngeneic models	The relationship between PTEN downregulation, BBB extravasation, early brain colonization, and exposure to the brain microenvironment	The models cannot show that PTEN downregulation drives dissemination from the primary tumor or establish its prevalence among patients with different BCBM subtypes.	[35]
Does pY696-EZH2 regulate different stages of brain metastasis and the immune niche?	4T1 and MDA-MB-231 cells; right common carotid artery, left ventricular, and intracranial injection	TNBC; syngeneic and human xenograft models	Comparison across inoculation routes can assess its effects on brain extravasation, colonization, and established intracranial growth. The 4T1 model can support immune-related conclusions.	The models do not reproduce spontaneous dissemination from the primary tumor. Human xenografts cannot establish adaptive immune mechanisms.	[113]
Does EGFR/HER2 signaling promote brain metastasis after intravasation?	MDA-MB-231Br cells; left ventricular injection	TNBC background; human xenograft	The overall effect of EGFR/HER2 signaling on cerebral extravasation and brain colonization after entry into the systemic circulation	The model cannot demonstrate involvement in spontaneous intravasation or determine whether the observed effect occurs during extravasation or subsequent intracranial growth.	[114]
Does L1CAM contribute to cerebral vascular adhesion and brain colonization?	MDA-MB-231 BrM2 cells; left ventricular injection	TNBC; human xenograft	The effects of L1CAM on cerebral vascular arrest, perivascular growth, and brain colonization after intravasation	The model cannot demonstrate a role in primary tumor invasion or spontaneous intravasation and cannot evaluate its function in an intact immune environment.	[28]
Does Cx43/PCDH7-mediated tumor-astrocyte communication promote brain colonization and treatment resistance?	MDA-MB-231 BrM2 cells; left ventricular injection	TNBC; human xenograft	The effects of Cx43/PCDH7 on brain colonization, tumor-brain cell communication, and treatment protection after tumor cells enter the circulation	The model cannot establish involvement in dissemination from the primary tumor. An immunodeficient host cannot support conclusions about complete adaptive immune responses.	[115]

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Does PPAR γ contribute to metabolic adaptation and brain colonization?	MDA-MB-231 BrM cells; left ventricular injection	TNBC; human xenograft	The overall effects of PPAR γ on post-intravasation brain colonization and adaptation to the brain microenvironment	The model cannot demonstrate a role in primary tumor invasion or spontaneous intravasation and cannot independently distinguish effects on extravasation from those on intracranial growth.	[47]
Does CCR2 regulate brain metastasis formation in an immunocompetent setting?	E0771 BrM cells; left ventricular injection	TNBC-like murine breast cancer; syngeneic immunocompetent model	The effects of CCR2 on post-intravasation brain metastasis formation and immune-cell regulation	The model cannot assess dissemination from the primary tumor. Without additional experiments, it cannot determine whether CCR2 mainly affects cerebral extravasation or the growth of established lesions.	[102]
Does annexin A1 promote early brain metastasis through microglia?	4T1 cells; carotid artery injection	TNBC; syngeneic immunocompetent BALB/c model	The effects of annexin A1 on microglial responses, cerebral extravasation, and early colonization after tumor cells enter the cerebral circulation	The model cannot demonstrate involvement in spontaneous intravasation from the primary tumor or directly evaluate therapeutic effects on established brain metastases.	[39]
Does CSF1R-related myeloid signaling promote brain metastatic colonization?	MDA-BrM cells; intracardiac injection	TNBC; human xenograft	The effects of CSF1R-related myeloid components on brain metastasis formation after intravasation	The model cannot demonstrate involvement in primary tumor dissemination. The immunodeficient host cannot support conclusions about T-cell-dependent mechanisms or clinical responses to immunotherapy.	[117]
Does NMDAR signaling enable BCBM cells to use neural signals within the brain?	MDA-MB-231 BrM cells; left ventricular injection	TNBC; human xenograft	The effects of NMDAR on post-intravasation brain colonization, intracranial growth, and tumor-neuron interactions	The model cannot demonstrate involvement in primary tumor invasion or spontaneous intravasation and cannot establish its role in an intact immune environment.	[118]
Does SRRM4 promote brain tropism and colonization across breast cancer subtypes?	SKBR3, BT474, and MDA-MB-231 cells; intracardiac injection	HER2-positive and TNBC; human xenografts	The effects of SRRM4 on brain metastasis formation after entry into the circulation and its role across different breast cancer subtypes	The model cannot demonstrate involvement in spontaneous dissemination from the primary tumor and cannot determine whether SRRM4 acts mainly during BBB extravasation or intracranial growth.	[119]
Is IL13R α 2 a potential target for post-intravasation BCBM formation and treatment?	MDA-MB-231 Br, JIMT-1Br-3, and BT474M1 Br cells; left ventricular injection	HER2-positive and TNBC; human xenografts	The effects of IL13R α 2 on cerebral extravasation, brain colonization, and responses to targeted treatment in preclinical models	The model cannot demonstrate involvement in primary tumor dissemination. Drug activity in these models does not establish clinical efficacy in patients or therapeutic effects in an intact immune environment.	[120]

Abbreviations: CCR2, C-C motif chemokine receptor 2; CSF1R, colony-stimulating factor 1 receptor; Cx43, connexin 43; EGFR, epidermal growth factor receptor; EZH2, enhancer of zeste homolog 2; FABP7, fatty acid-binding protein 7; FAK, focal adhesion kinase; HDAC, histone deacetylase; HER2, human epidermal growth factor receptor 2; IL13R α 2, interleukin-13 receptor subunit alpha 2; NMDAR, N-methyl-D-aspartate receptor; PCDH7, protocadherin 7; PPAR γ , peroxisome proliferator-activated receptor gamma; PTEN, phosphatase and tensin homolog; SRC, SRC proto-oncogene non-receptor tyrosine kinase; SRRM4, serine/arginine repetitive matrix 4; TNBC, triple-negative breast cancer; YTHDF3, YTH domain family member 3.

tified using ICA injection should be confirmed in spontaneous metastasis models and patient BCBM samples. The timing of treatment also affects how the results should be interpreted. When an intervention is administered immediately before or after tumor cell injection, the experiment mainly evaluates the prevention of vascular arrest, extravasation, or early colonization. It does not demonstrate efficacy against an established brain metastasis. Using this model, our group has evaluated several candidate agents for the prevention or treatment of brain metastases, including BCBM [121-125]. These studies provide additional preclinical evidence for potential therapeutic approaches but do not by themselves establish clinical efficacy.

Left ventricular injection: Left ventricular injection introduces breast cancer cells into the systemic arterial circulation, allowing them to reach the brain, bone, and other organs. The method is useful for studying organ tropism, selection of brain-metastatic populations, and the distribution of tumor cells across multiple organs during the later stages of metastasis [28, 113, 114].

Because the model includes systemic circulation, organ-specific arrest, BBB extravasation, and brain colonization, a reduction in intracranial tumor burden may arise from an effect on any of these stages. It should not automatically be interpreted as evidence that BBB crossing has been inhibited. Experimental outcomes may also be affected by extracranial metastases that reach endpoint earlier than brain lesions, inaccurate placement of the injection, and selection of particular tumor subpopulations during serial in vivo passage [114]. Although repeated in vivo selection increases the efficiency of brain metastasis formation, it may produce a cell population that differs substantially from the original patient tumor [47]. Variation in whether cells are accurately delivered into the left ventricle is another important source of experimental variability [115].

ICA injection is generally more suitable for studying BBB extravasation and early brain colonization, whereas left ventricular injection is more informative for investigating organ tropism and systemic distribution. Mechanisms identified with these models should be corroborated in spontaneous models and, where

possible, patient material before being generalized to human BCBM.

Intracranial injection models for studying brain growth and treatment response

Stereotactic intracranial injection places tumor cells directly in the brain parenchyma and produces lesions at a defined site and within a comparatively predictable interval [99]. It is useful for studying outgrowth after brain entry, local tumor-brain interactions, and treatment of an established intracranial mass [113]. The method bypasses primary-tumor dissemination, survival in blood, vascular adhesion, and BBB extravasation. It can therefore test intracranial growth but cannot demonstrate a role in natural brain metastasis or BBB crossing [117]. Needle injury may also trigger local inflammation and disrupt the BBB, and the resulting solitary lesion does not capture the multiplicity and spatial heterogeneity often seen clinically [119].

For therapeutic studies, treatment should preferably begin only after intracranial tumor formation has been confirmed by imaging. Tumor inhibition after systemic drug administration indicates activity against an intracranial lesion, but it does not by itself prove that the drug crosses an intact BBB. Local intracranial administration can establish a local pharmacological effect but cannot provide information about systemic exposure or BBB penetration.

Immunocompetent models

The route of tumor cell inoculation determines which stages of metastasis are retained, whereas the immune status of the host determines whether the model can address immunological questions. Immunocompetent syngeneic models can be combined with different inoculation routes according to the stage under investigation. A spontaneous 4T1/BALB/c model retains the steps from primary tumor dissemination to brain colonization and can be used to examine immune regulation across the metastatic cascade [126]. Injection of 4T1 cells through the carotid artery in BALB/c mice allows investigation of immune responses during vascular adhesion, extravasation, and early colonization [127]. Syngeneic intracranial models are more suitable for studying the immune microenvironment of established brain lesions

and their response to immunotherapy. One example is the preclinical evaluation of abemaciclib combined with programmed cell death protein 1 (PD-1) blockade [128].

Evans et al. integrated single-cell sequencing, immunocompetent mice, genetic models, humanized mice, and patient tissue. They found that microglia could restrict BCBM growth by promoting NK- and T-cell responses [31]. This study illustrates why immune mechanisms should be examined across several model systems and then compared with human tissue. In contrast, xenografts generated from human cell lines and most PDX models require immunodeficient mice. They cannot establish a T-cell-mediated mechanism or provide a complete assessment of immune checkpoint blockade. Humanized mice introduce some human immune components, but immune reconstitution remains incomplete and does not fully reproduce the immune environment of patients [129]. Findings from a single immunodeficient model should therefore not be presented as established immune mechanisms in human BCBM or as evidence of clinical immunotherapy efficacy.

PDX and organoid models

Unlike syngeneic models, which prioritize an intact host immune system, PDX and patient-derived tumor organoid platforms are designed to retain features of the human tumor. Contreras-Zárate et al. established eight brain metastasis-derived PDX (BM-PDX) models from surgically resected BCBM specimens representing different molecular subtypes. These models retained major markers, including ER, PR, and HER2, and preserved their ability to colonize the brain after intravascular inoculation [104]. The conclusions supported by a PDX model still depend on the inoculation route. A modified carotid artery method can be used to study extravasation and colonization by patient-derived tumor cells without directly disrupting the BBB [105]. In contrast, intracranial implantation can assess growth and treatment response within the brain but cannot reproduce the earlier stages of metastasis.

PDX models do not preserve every feature of the original tumor. Studies of PDX models derived from CNS metastases have shown that implantation and serial passage may expand

aggressive minor clones, thereby changing the clonal composition of the model [106]. In intracranial PDX models of HER2-positive BCBM, combined inhibition of phosphoinositide 3-kinase (PI3K) and mechanistic target of rapamycin complex 1 (mTORC1) produced durable regression in some models [107]. This finding demonstrates activity in a particular preclinical setting but does not establish that patients will derive the same benefit.

Patient-derived tumor organoids and human brain organoids should also be distinguished. Guillen et al. paired breast cancer PDXs with PDX-derived organoids for in vitro drug screening and subsequent in vivo testing, but the platform was not derived specifically from BCBM [108]. Wang et al. instead cocultured breast cancer cell lines with human embryonic stem cell-derived brain organoids to examine invasion and growth in brain-like tissue [109]. This approach models tumor-neural interactions but is not a patient-derived BCBM organoid.

Most organoid systems lack perfused vasculature, an intact BBB, and a systemic immune compartment. They cannot reproduce whole-body pharmacokinetics, establish that a systemically administered drug reaches the brain, or model the complete metastatic cascade. Findings from these platforms therefore require validation in complementary in vivo systems.

Targetable molecular pathways and emerging therapeutics in BCBM

Most systemic treatments used for BCBM were developed for extracranial breast cancer, and truly brain-directed pharmacological options remain limited. At the same time, molecular differences between primary tumors and brain metastases reveal dependencies that may be therapeutically exploitable. Because the incidence and biology of BCBM differ among HER2-positive, TNBC, and HR-positive/HER2-negative disease, mechanistic claims and treatment priorities must be interpreted by subtype [130]. **Figures 3 and 4**, together with **Table 3**, delineate pivotal signaling pathways implicated in BCBM alongside promising therapeutic targets [99, 112, 113, 116, 131-144].

Kinase signaling networks

SRC: SRC proto-oncogene, non-receptor tyrosine kinase (SRC), is activated in brain-metastasis.

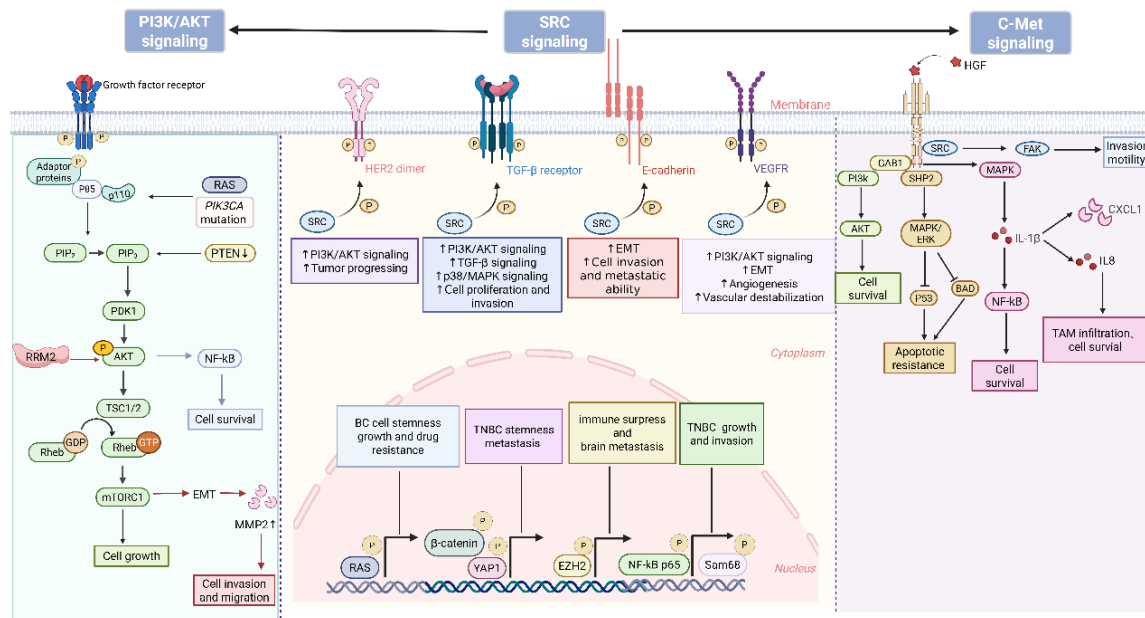


Figure 3. Kinase signaling networks driving BCBM progression. PI3K-AKT-mTOR signaling supports tumor-cell survival, invasion, and treatment resistance. SRC integrates HER2, TGF- β receptor, E-cadherin, and VEGFR inputs and promotes EMT, stemness, vascular destabilization, and immune suppression. c-MET signaling activates ERK/MAPK- and NF- κ B-linked programs that enhance survival, motility, angiogenesis, and inflammatory cell recruitment. Abbreviations: ERK, extracellular signal-regulated kinase; FAK, focal adhesion kinase; GAB1, GRB2-associated binding protein 1; HGF, hepatocyte growth factor; PDK1, 3-phosphoinositide-dependent protein kinase 1; PIP2, phosphatidylinositol 4,5-bisphosphate; PIP3, phosphatidylinositol 3,4,5-trisphosphate; RRM2, ribonucleotide reductase regulatory subunit M2; SAM68, SRC-associated in mitosis 68-kDa protein; SHP2, Src homology 2 domain-containing phosphatase 2; TSC1/2, tuberous sclerosis complexes 1 and 2; VEGFR, vascular endothelial growth factor receptor.

static breast cancer cells and contributes to both BBB extravasation and intracranial growth [145]. In preclinical models, SRC increased BBB permeability and facilitated the entry of tumor cells into the brain. Treatment with the SRC inhibitor saracatinib together with the EGFR/HER2 inhibitor lapatinib suppressed AKT signaling, increased apoptosis, and prolonged survival in mice bearing HER2-positive brain metastases [112]. SRC also links tumor-intrinsic signaling to the immune microenvironment. Zhang et al. showed that SRC phosphorylated EZH2 at tyrosine 696 (Y696) in brain-metastatic cells. Phosphorylated EZH2 acquired a methyltransferase-independent transcriptional function and increased granulocyte colony-stimulating factor (G-CSF) expression. G-CSF subsequently recruited immunosuppressive neutrophils and supported intracranial tumor growth [113]. The evidence for both mechanisms comes mainly from cell experiments and mouse models. Clinical benefit from SRC inhibition in patients with BCBM has not yet been demonstrated.

MET receptor tyrosine kinase: The mesenchymal-epithelial transition factor receptor, commonly referred to as c-MET, regulates interactions among brain-metastatic breast cancer cells, endothelial cells, and myeloid cells. Activation of c-MET induces an inflammatory program involving the mitogen-activated protein kinase (MAPK) pathway. This response increases interleukin-1 β (IL-1 β) expression and promotes the release of interleukin-8 (IL-8) and C-X-C motif chemokine ligand 1 (CXCL1). These mediators enhance tumor cell adhesion to brain endothelial cells, angiogenesis, BBB crossing, and brain colonization [132]. Breast cancer cells with high c-MET activity may also release CXCL1, CXCL2, G-CSF, and granulocyte-macrophage colony-stimulating factor (GM-CSF). The resulting cytokine network alters neutrophil behavior and creates conditions that favor BCBM growth [133]. Thus, c-MET signaling acts through both tumor-intrinsic and microenvironmental mechanisms. Gautam et al. evaluated c-MET as a therapeutic target in a preclinical HER2-positive BCBM

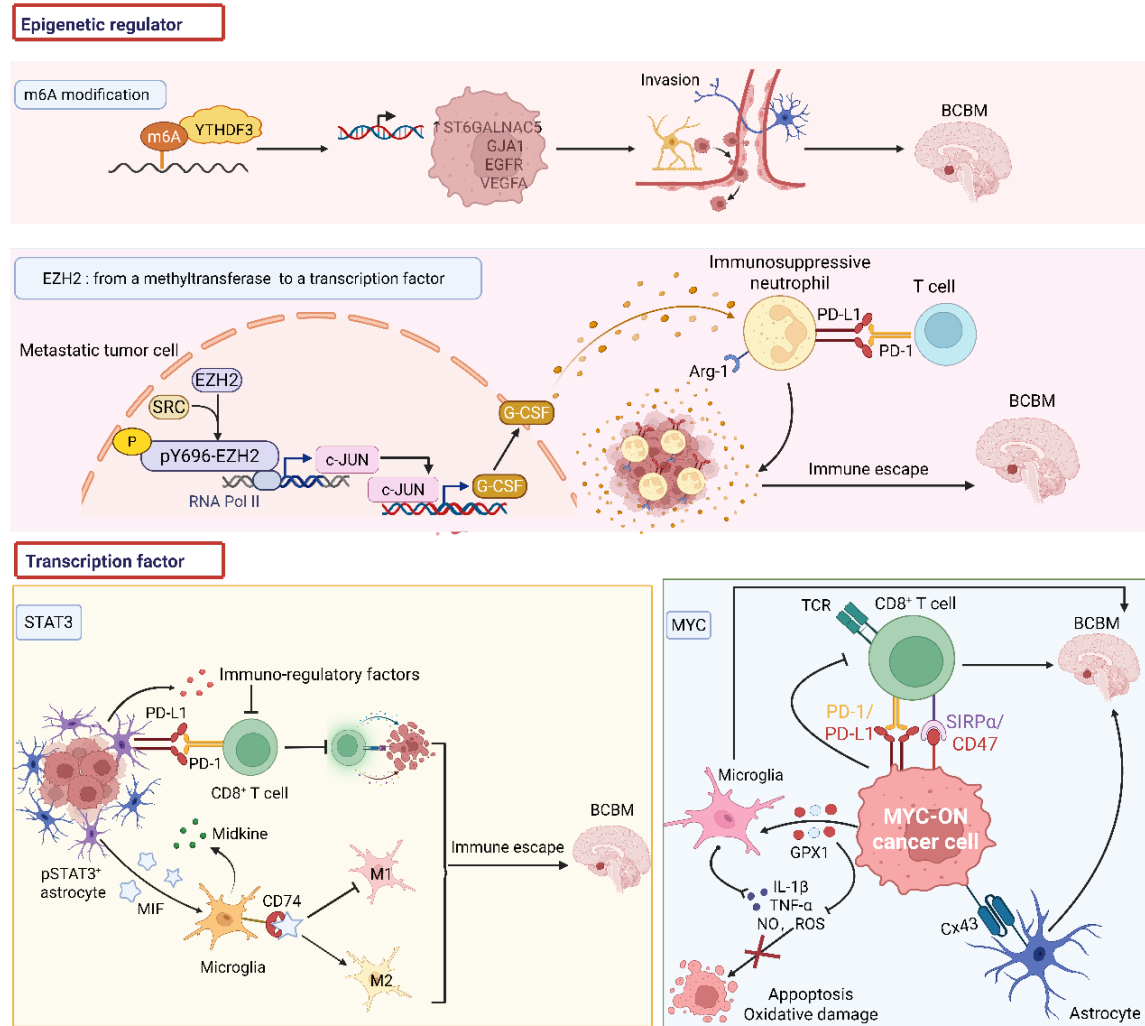


Figure 4. Epitranscriptomic, epigenetic, and transcriptional regulation of BCBM. YTHDF3-mediated m6A recognition enhances the translation of brain metastasis-associated transcripts, facilitating blood-brain barrier invasion and brain colonization. SRC-dependent phosphorylation of EZH2 activates c-JUN/G-CSF signaling and recruits immunosuppressive neutrophils. STAT3-activated astrocytes and MYC-driven tumor programs further promote immune escape and resistance to microglia-induced oxidative stress. Abbreviations: ARG1, arginase 1; c-JUN, cellular Jun; Cx43, connexin 43; EGFR, epidermal growth factor receptor; EZH2, enhancer of zeste homolog 2; G-CSF, granulocyte colony-stimulating factor; GJA1, gap junction protein alpha 1; GPX1, glutathione peroxidase 1; MIF, macrophage migration inhibitory factor; NO, nitric oxide; pY696-EZH2, EZH2 phosphorylated at tyrosine 696; RNA Pol II, RNA polymerase II; SIRPα, signal regulatory protein alpha; SRC, SRC proto-oncogene non-receptor tyrosine kinase; TCR, T-cell receptor; YTHDF3, YTH domain family member 3.

model. Neratinib was combined with cabozantinib, a multikinase inhibitor with activity against c-MET. The combination reduced extracellular signal-regulated kinase (ERK) activation downstream of the c-MET-EGFR axis and inhibited brain metastasis formation [134].

Tropomyosin receptor kinase: Tropomyosin receptor kinase (Trk) signaling can support the survival and brain colonization of breast cancer cells through cooperation with other receptors.

Choy et al. found that HER2 and TrkB formed heterodimers that provided tumor cells with a survival advantage in the brain. Simultaneous inhibition of HER2 and TrkB reduced intracranial tumor growth in preclinical models [146]. The precursor of nerve growth factor (proNGF) can also facilitate BBB crossing through a signaling complex formed by TrkA and Eph receptor A2 (EphA2). Combined inhibition of TrkA and EphA2 reduced brain metastasis formation in TNBC models [135]. In a separate mech-

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Table 3. The summary of targetable molecular pathways in BCBM

Key therapeutic pathways	Therapeutic targets	Mechanism	Subtype association	Clinical/Experimental outcome	References
Kinase signaling networks	PI3K/AKT/mTOR	Modulates metastasis-promoting macrophages/microglia, reducing immunosuppressive gene expression (PD-L1, CTLA4, CSF1, CSF1R).	HER2+, TNBC	Buparlisib attenuated microglial metastasis-facilitating phenotype in vivo.	[131]
	PI3K/mTOR	Combined PI3K and mTOR blockade decreased mTORC1 effector 4EBP1 phosphorylation; treatment resistance correlated with genomic instability.	HER2+	Significant therapeutic benefit observed in HER2+ PDX models.	[107]
	SRC	Activation of SRC facilitates extravasation of breast cancer cells into brain parenchyma by increasing BBB permeability.	HER2+, TNBC	Combined SRC and EGFR/HER2 inhibition (e.g., lapatinib) improved survival in BCBM mouse models by inducing apoptosis.	[112]
	SRC-EZH2 axis	SRC phosphorylates EZH2 at Y696, upregulating G-CSF and recruiting immunosuppressive neutrophils that promote BCBM progression.	HER2+, TNBC	Targeting SRC-EZH2-G-CSF axis reduced neutrophil recruitment and brain colonization.	[113]
	c-Met	c-Met activation triggers IL1 β -MAPK-IL8/CXCL1 inflammatory cascade enhancing adhesion, angiogenesis, and BBB penetration.	HER2+	c-Met inhibition disrupted tumor-stroma inflammatory signaling and reduced BCBM.	[132, 133]
	c-Met, HER2	Dual inhibition blocks ERK signaling downstream of c-Met/ERBB1, preventing HER2+ BCBM development.	HER2+	Combination therapy effectively prevented HER2-positive BCBM in vivo.	[134]
	TrkA/EphA2	NGF precursor activates TrkA/EphA2 complex to mediate BBB traversal of TNBC cells.	TNBC	Combined inhibition of TrkA/EphA2 significantly reduced BM formation.	[135]
	Estradiol-BDNF/TrkB	Estradiol activates BDNF/TrkB signaling, promoting TNBC brain metastasis.	TNBC	Blocking TrkB reduced estradiol-driven metastatic growth.	[136]
Epigenetic Modulators	YTHDF3	Promotes translation of ST6GALNAC5 and GJA1, enhancing tumor-endothelial and tumor-astrocyte interactions and angiogenesis.	-	High YTHDF3 correlates with poor prognosis and increased BBB extravasation.	[99]
	CECR2	Acetyl-lysine reader that activates NF- κ B-mediated transcription of metastasis-promoting genes, inducing M2 macrophage polarization.	-	CECR2 depletion inhibited M2 polarization and metastatic dissemination including BM.	[137]
	BET	Upregulated BET family members reduce vinorelbine sensitivity by suppressing TUBB3; BET inhibitors synergize with radiotherapy.	-	BET inhibitors combined with radiotherapy extended survival in BCBM models.	[138]
	EZH2	SRC-mediated EZH2 phosphorylation elevates c-JUN expression in a methyltransferase-independent manner, enhancing pro-inflammatory cytokines and neutrophil recruitment.	HER2+, TNBC	Inhibition of this axis mitigated pro-tumor neutrophil infiltration and BCBM formation.	[113]
Transcriptional Regulators	MYC	MYC overexpression reprograms metabolism toward glycolysis and glutaminolysis, promoting tumor growth and survival in the brain microenvironment.	TNBC, HER2+	MYC inhibition impaired metabolic adaptation and suppressed brain colonization in BCBM models.	[116, 139]
Non-coding RNAs	miR-509	Suppresses RhoC and TNF- α signaling to inhibit transmigration across BBB.	HER2+, TNBC	Overexpression of miR-509 reduced brain metastatic colonization in vivo.	[144]
	circBCBM1	Acts as a miR-125a sponge to activate BRD4, promoting migration, invasion and intracranial growth.	HER2+, TNBC	circBCBM1 knockdown impaired brain metastasis formation.	[143]

Abbreviations: AKT, protein kinase B; BDNF, brain-derived neurotrophic factor; BET, bromodomain and extraterminal domain; BRD4, bromodomain-containing protein 4; c-JUN, cellular Jun; c-MET, mesenchymal-epithelial transition factor receptor; CECR2, cat eye syndrome chromosome region candidate 2; EGFR, epidermal growth factor receptor; EphA2, Eph receptor A2; ERK, extracellular signal-regulated kinase; EZH2, enhancer of zeste homolog 2; G-CSF, granulocyte colony-stimulating factor; GJA1, gap junction protein alpha 1; GPX1, glutathione peroxidase 1; IL, interleukin; MAPK, mitogen-activated protein kinase; MMP9, matrix metalloproteinase 9; mTOR, mechanistic target of rapamycin; NF- κ B, nuclear factor kappa B; NGF, nerve growth factor; PD-L1, programmed death-ligand 1; PI3K, phosphoinositide 3-kinase; RhoC, Ras homolog family member C; SLC7A11, solute carrier family 7 member 11; SRC, SRC proto-oncogene non-receptor tyrosine kinase; TIMP1, tissue inhibitor of metalloproteinases 1; TNBC, triple-negative breast cancer; TNF- α , tumor necrosis factor alpha; Trk, tropomyosin receptor kinase; TUBB3, class III beta-tubulin; VEGF-A, vascular endothelial growth factor A; YTHDF3, YTH domain family member 3.

anism, estradiol activated brain-derived neurotrophic factor (BDNF)-TrkB signaling and promoted brain metastatic progression in TNBC [136].

Epigenetic modulators

YTH domain family member 3 (YTHDF3), a reader of N6-methyladenosine (m6A), is upregulated in BCBM and has been associated with poor prognosis. YTHDF3 increases the translation of brain metastasis-related transcripts, including ST6GALNAC5 and gap junction protein alpha 1 (GJA1, also known as connexin 43). This promotes interactions between metastatic cells, brain endothelial cells, and astrocytes and supports BBB extravasation, angiogenesis, and intracranial outgrowth [99]. Cat eye syndrome chromosome region candidate 2 (CECR2) is an acetyl-lysine reader that activates nuclear factor- κ B (NF- κ B)-dependent transcription. In preclinical breast cancer models, CECR2 increased the expression of metastasis-related genes and promoted M2-like macrophage polarization, thereby supporting metastatic dissemination, including spread to the brain [137]. Bromodomain and extraterminal domain (BET) proteins have also been implicated in BCBM. BET activity was associated with reduced sensitivity to vinorelbine through regulation of class III β -tubulin (TUBB3). In mouse models, combining BET inhibition with radiotherapy prolonged survival [138]. EZH2 has a noncanonical role in BCBM that is independent of its methyltransferase activity. Following phosphorylation by SRC, EZH2 increases cellular Jun (c-JUN)-dependent transcription. c-JUN then promotes the production of inflammatory cytokines that recruit immunosuppressive neutrophils to the brain [113].

Transcriptional regulators

Transcriptional programs in both metastatic cells and cells of the brain microenvironment contribute to immune escape and intracranial adaptation. A subset of reactive astrocytes surrounding brain metastatic lesions shows sustained STAT3 activation [37]. These astrocytes suppress cytotoxic T-cell activity by increasing programmed death-ligand 1 (PD-L1), vascular endothelial growth factor A (VEGF-A), lipocalin 2, and tissue inhibitor of metalloproteinases 1 (TIMP1) [37]. Macrophage migration inhibitory factor (MIF) binds cluster of differentiation 74

(CD74) and has been associated with M2-like microglial polarization and reduced interferon- γ (IFN- γ) production [147]. MYC supports the adaptation of metastatic cells to oxidative and immune stress in the brain. MYC amplification has been detected in brain-tropic breast cancer-derived CTCs. Activated microglia increase reactive oxygen species (ROS) in nearby tumor cells, whereas MYC increases glutathione peroxidase 1 (GPX1) expression and reduces oxidative damage and apoptosis [116]. Lee et al. further showed that MYC promoted BCBM by increasing tumor cell invasion, macrophage infiltration, and gap junction formation between metastatic cells and astrocytes [139].

Regulated cell death pathways

Autophagy: Autophagy has a context-dependent role in breast cancer. It may limit cellular damage, but it can also help metastatic cells survive nutrient deprivation, oxidative stress, and anticancer treatment. Patient tissue and preclinical studies suggest that increased autophagy-related activity is associated with BCBM progression and poor outcome [125, 140, 148]. A retrospective study analyzed brain metastasis specimens from 44 patients with breast cancer, primary breast tumors, and 16 matched primary-metastatic pairs. Microtubule-associated protein 1 light chain 3 beta (LC3B) puncta and FAK family-interacting protein of 200 kDa (FIP200/ATG17) were detected in more than 90% of the brain metastases and were generally more abundant than in primary tumors [140]. Among patients with TNBC, the median survival after diagnosis of brain metastasis was 26.8 months in those with a low proportion of LC3-positive tumor cells and 3.9 months in those with a high proportion [140]. Independent tissue analyses have similarly reported higher levels of autophagy-related proteins in brain metastases than in primary breast tumors [149]. Experimental studies provide possible explanations for this observation. Mitochondrial complex I activity can regulate AKT-mTORC1-dependent autophagy and affect the spread of breast cancer cells to distant organs, including the brain [150]. Astrocytes can also activate a prosurvival autophagy program in metastatic cells through the C-X-C motif chemokine ligand 12 (CXCL12)-miR-345-kisspeptin 1 (KISS1) axis [151]. Preclinical studies, including work from our group, further

indicate that autophagy and mitophagy can protect breast cancer cells from treatment-induced stress. Genetic or pharmacological interference with these pathways enhanced the activity of selected anticancer treatments in breast cancer and brain metastasis models [125, 148]. These findings support autophagy as a possible treatment-sensitization target. However, clinical benefit from autophagy inhibition has not been demonstrated in patients with BCBM.

Ferroptosis: Ferroptosis is an iron-dependent form of regulated cell death caused by excessive lipid peroxidation. It influences tumor growth, metastatic survival, and treatment response in breast cancer and other malignancies [152]. In matched samples from patients with HER2-positive breast cancer, the cystine/glutamate antiporter light chain xCT, encoded by SLC7A11, was more highly expressed in metachronous brain metastases than in the corresponding primary tumors [142]. xCT maintains cysteine availability and redox balance, allowing metastatic cells to tolerate oxidative stress and persist under innate immune surveillance. Inhibition of xCT disrupts this protection and increases susceptibility to ferroptosis [142]. These findings identify ferroptosis resistance as a possible therapeutic vulnerability in HER2-positive BCBM. The functional evidence remains preclinical, and it is not yet clear whether the same strategy will be effective in TNBC or HR+/HER2-negative disease.

Non-coding RNAs

A myriad of non-coding RNAs have emerged as significant players in BCBM [153]. Xing et al. revealed that miR-509 expression is downregulated in brain metastatic lesions of breast cancer, exerting inhibitory effects on BCBM through the regulation of Ras Homolog Gene Family Member C (RhoC) and Tumor Necrosis Factor- α (TNF- α) [144]. Circular RNA circBCBM1 has been identified as a promoter of BCBM, modulating the miR-125a/Bromodomain Containing 4 (BRD4) axis. circBCBM1 suppresses miR-125a activity, leading to upregulation of BRD4 and subsequent Matrix Metalloproteinase 9 (MMP9) overexpression through the Sonic Hedgehog signaling pathway, thereby facilitating in vitro migration and intracerebral growth of brain metastatic cells [143].

Additionally, other non-coding RNAs such as microRNA-211, Lnc-BM and Lnc BMOR have been implicated in promoting BCBM through immune modulation, BBB penetration, and other mechanisms [154].

Treatment strategies

Management of BCBM begins with determining whether the intracranial disease requires immediate local control. Systemic treatment is then selected according to molecular subtype, previous therapy, and the pattern of intracranial and extracranial progression [155]. Surgery or radiotherapy should not be delayed in patients with progressive neurological symptoms, a large lesion causing mass effect, obstructive hydrocephalus, intracranial hemorrhage, or an uncertain diagnosis [156, 157]. In patients with small, asymptomatic lesions without substantial mass effect, deferral of local treatment may be considered only when a systemic regimen has prospective evidence of CNS activity. Such a decision requires multidisciplinary review and close MRI surveillance [155, 156].

Treatment after local therapy depends partly on whether intracranial and extracranial disease are progressing together. Leptomeningeal metastasis requires separate assessment based on neurological symptoms, brain and spinal MRI, CSF cytology, and molecular testing. Evidence derived from parenchymal brain metastases cannot be applied directly to leptomeningeal disease [158].

HER2-positive BCBM

HER2-positive BCBM has the strongest clinical evidence for CNS-active systemic treatment. Management therefore depends not only on which HER2-directed agents are available, but also on whether local treatment is immediately required and how previous therapy has controlled intracranial and extracranial disease.

Local treatment: Surgery should be considered first for patients with a symptomatic lesion, substantial mass effect, or a need for pathological confirmation. In addition to relieving intracranial pressure, resection provides metastatic tissue for reassessment of ER, PR, and HER2 status [156, 157]. Postoperative radiation is generally required. In a randomized trial, postoperative cavity SRS caused less cognitive

deterioration than WBRT and did not compromise overall survival, although WBRT provided better control of disease elsewhere in the brain [159]. Cavity SRS is therefore appropriate after complete or near-complete resection when the surgical cavity can be treated safely.

SRS is also the principal local treatment for patients with a limited intracranial tumor burden who do not require surgery. Selection should take into account the total tumor volume, lesion size and location, performance status, and expected survival rather than lesion number alone [156, 157]. WBRT remains an option for diffuse disease or for patients who are unsuitable for SRS. When there is no disease in the hippocampal region and the patient's condition permits, hippocampal avoidance and memantine should be considered to reduce cognitive toxicity [160]. If only a small number of intracranial lesions progress after local therapy while extracranial disease remains controlled, repeat SRS may allow the existing HER2-directed regimen to be continued. Repeated intracranial failure or concurrent intracranial and extracranial progression favors a switch to a systemic treatment with greater CNS activity [161].

Systemic treatments with established CNS activity: HER2CLIMB enrolled patients with both stable and active brain metastases. Adding tucatinib to trastuzumab and capecitabine improved intracranial disease control and overall survival compared with trastuzumab and capecitabine alone [162-164]. This trial provides direct randomized evidence for the tucatinib regimen in patients with active or repeatedly progressing brain metastases. Guidelines from the American Society of Clinical Oncology (ASCO) also allow selected patients with asymptomatic lesions and no substantial mass effect to receive this regimen before local therapy, provided that the decision is made by a multidisciplinary team and the patient undergoes close intracranial monitoring [161]. HER2CLIMB was conducted before widespread use of trastuzumab deruxtecan (T-DXd), however, so the efficacy of the tucatinib regimen after T-DXd is supported mainly by nonrandomized and real-world evidence.

Evidence for T-DXd comes from several study settings. In the brain metastasis subgroup of the randomized phase III DESTINY-Breast03

trial, T-DXd produced better intracranial outcomes than trastuzumab emtansine (T-DM1) in patients with stable brain metastases [165]. The phase II TUXEDO-1 trial subsequently reported intracranial responses in patients with active brain metastases, although only 15 patients were enrolled [166]. DESTINY-Breast12, a larger nonrandomized study, included 263 patients with stable or active brain metastases and confirmed that T-DXd has clinically relevant intracranial activity [167]. T-DXd is therefore an important option when both intracranial and extracranial disease require treatment, particularly in patients who have not previously received the drug. Careful monitoring for interstitial lung disease and pneumonitis is required.

Other clinically studied options: When T-DXd and tucatinib-based therapy are unavailable, not tolerated, or no longer effective, other HER2-directed regimens may be considered. Exploratory KAMILLA data showed activity of trastuzumab emtansine (T-DM1) in some patients with brain metastases [168]. Neratinib plus capecitabine (TBCRC 022) and pyrotinib plus capecitabine (PERMEATE) produced intracranial responses in phase II studies, although neratinib requires proactive diarrhea management [169, 170]. In PATRICIA, pertuzumab plus high-dose trastuzumab achieved a confirmed intracranial response rate of 11% [171]. These nonrandomized data support selected use but do not place the regimens at the same evidence level as T-DXd or tucatinib, trastuzumab, and capecitabine.

Investigational strategies: HER2CLIMB-04 reported activity with tucatinib plus T-DXd in a single-arm phase II study that included patients with and without brain metastases [172]. Without a T-DXd-only control, the study cannot determine whether tucatinib adds benefit. The combination should remain investigational pending randomized comparative evidence.

HR-positive/HER2-negative BCBM

HR-positive/HER2-negative tumors usually reach the brain later than HER2-positive or triple-negative disease. Systemic treatment is shaped by endocrine sensitivity, prior cyclin-dependent kinase 4/6 (CDK4/6) inhibition, the pace of intracranial progression, and actionable alterations in PIK3CA, AKT1, PTEN, or ESR1.

Local treatment: The indications for surgery, SRS, and WBRT follow the general principles described above [156, 157]. Because endocrine and targeted therapies have not consistently produced rapid intracranial responses, symptomatic lesions or lesions causing mass effect should be controlled locally before systemic treatment is adjusted.

Endocrine and molecularly selected treatment: For patients whose intracranial disease is stable after local therapy, whose tumors remain endocrine sensitive, and who do not have rapidly progressive visceral disease, endocrine therapy combined with a CDK4/6 inhibitor may remain the basis of systemic treatment [173]. This approach is supported primarily by survival benefits observed in the broader population with HR-positive/HER2-negative metastatic breast cancer. It should not be presented as having established activity against untreated or progressing brain metastases.

After progression on endocrine therapy, treatment can be selected according to tumor genotype. Alpelisib combined with fulvestrant is an option for PIK3CA-mutated disease [174]. Capivasertib plus fulvestrant may be considered for tumors with alterations in PIK3CA, AKT1, or PTEN [175]. Elacestrant, an oral selective estrogen receptor degrader (SERD), is an option for endocrine-dependent tumors with an acquired ESR1 mutation [176]. Each of these strategies is supported by randomized trials in metastatic breast cancer, but their intracranial efficacy in patients with active BCBM has not been established.

Treatments with potential CNS activity: Abemaciclib is the CDK4/6 inhibitor that has undergone prospective evaluation specifically in HR-positive/HER2-negative BCBM. In a nonrandomized phase II study, abemaciclib and its active metabolites reached concentrations in resected brain metastases that were sufficient to inhibit CDK4/6. Tissue penetration, however, did not translate into a high intracranial response rate [177]. Drug detection in tumor tissue should therefore not be interpreted as proof of substantial clinical efficacy.

T-DXd is also relevant to HR-positive/HER2-negative BCBM when the metastatic tumor is HER2-low, defined as immunohistochemistry (IHC) 1+ or IHC 2+ with negative in situ hybrid-

ization (ISH). DESTINY-Breast04 and DESTINY-Breast06 established the systemic efficacy of T-DXd in previously treated HER2-low and trial-defined HER2-ultralow metastatic breast cancer, respectively [178, 179]. These trials were not designed primarily to evaluate active brain metastases.

More direct evidence comes from the single-arm phase II DEBBRAH study. Among 12 patients with HER2-low active brain metastases, the intracranial objective response rate was 50.0% in six patients with untreated, asymptomatic lesions and 33.3% in six patients whose brain metastases had progressed after local therapy [180]. The antibody-drug conjugate (ADC) sacituzumab govitecan improved survival in heavily pretreated HR-positive/HER2-negative metastatic breast cancer in TROPICS-02 [181]. Active brain metastases were excluded, and the trial did not include an intracranial efficacy endpoint. A preoperative window study detected its active payload, SN-38, in resected brain metastasis tissue [182]. This finding confirms drug delivery but not clinical benefit. In a later retrospective study of 33 patients with HER2-negative CNS metastases, the overall CNS objective response rate was 13%; no confirmed intracranial response was observed among the seven patients with active brain metastases [183]. Sacituzumab govitecan may therefore be used according to its systemic indication, but it cannot yet be classified as a preferred CNS-active treatment.

TNBC

TNBC brain metastases often progress rapidly, and no systemic regimen has yet demonstrated BCBM-specific efficacy in a large prospective randomized trial.

Local treatment: Because reliable systemic control of active TNBC brain metastases remains limited, symptomatic, rapidly progressing, or space-occupying lesions generally require early local treatment [156, 157]. Surgery or SRS may be used for accessible lesions and a limited intracranial burden. WBRT may be required for diffuse disease or in selected patients with extensive symptomatic leptomeningeal involvement who are unsuitable for focal treatment [184-186].

Systemic treatment: For metastatic TNBC with a PD-L1 combined positive score (CPS) of at least 10, KEYNOTE-355 established pembrolizumab plus chemotherapy as an effective first-line systemic treatment [187]. ASCENT-04/KEYNOTE-D19 subsequently showed that first-line sacituzumab govitecan plus pembrolizumab prolonged progression-free survival compared with chemotherapy plus pembrolizumab in PD-L1-positive advanced TNBC [188]. Neither trial was designed to establish treatment efficacy in active BCBM, and intracranial response was not the primary endpoint. These results therefore do not justify postponing local treatment for symptomatic brain metastases.

For patients with a pathogenic germline BRCA1/2 variant and HER2-negative metastatic breast cancer, OlympiAD and EMBRACA support the systemic use of the poly(ADP-ribose) polymerase (PARP) inhibitors olaparib and talazoparib, respectively [189, 190]. Only a limited number of patients with brain metastases were included, and most had previously treated, stable lesions. PARP inhibitors should therefore be selected according to germline BRCA status and the overall pattern of disease rather than being described as treatments with established BCBM-specific activity.

For patients who are not candidates for immunotherapy, have no actionable alteration, or experience progression after targeted treatment, systemic therapy may include capecitabine, platinum-based chemotherapy, or another agent selected according to prior exposure, organ function, and the pace of progression [191, 192]. The evidence for these agents primarily concerns extracranial TNBC, and their ability to control active intracranial disease remains uncertain.

Intracranial activity of ADCs: In the subgroup of patients with stable brain metastases enrolled in ASCENT, sacituzumab govitecan prolonged median progression-free survival relative to treatment of the physician's choice, but the objective response rate was low and no clear overall survival improvement was observed [193]. Detection of SN-38 in resected BCBM tissue in the preoperative window study shows that the payload can reach an intracranial tumor, but pharmacological delivery cannot substitute for evidence of clinical response [182]. Retrospective studies have likewise sug-

gested some CNS activity, although confirmed responses among patients with active brain metastases remain uncommon [183]. Sacituzumab govitecan is an important systemic treatment for TNBC, but the available evidence does not support describing it as a BCBM-specific therapy with established intracranial efficacy.

T-DXd may be considered when a TNBC brain metastasis is confirmed to be HER2-low. DESTINY-Breast04 demonstrated systemic efficacy in the overall HER2-low population, but both the HR-negative subgroup and the number of patients with brain metastases were limited [178]. DEBBRAH reported intracranial responses in a small group of patients with HER2-low active brain metastases, but the study was not restricted to TNBC [180]. T-DXd is therefore a systemic option for HER2-low TNBC, but current evidence does not support using it in place of indicated local treatment for all patients with active brain metastases.

Investigational strategies: Current studies are examining the intracranial activity of ADCs and methods for improving drug delivery. TUXEDO-4 is prospectively evaluating T-DXd in patients with HER2-low active brain metastases [194]. Low-intensity focused ultrasound combined with microbubbles can temporarily increase blood-tumor barrier permeability. Human proof-of-concept evidence in BCBM has been obtained mainly in patients with HER2-positive disease, whereas evidence for therapeutic benefit in TNBC is still largely preclinical [195, 196]. This technique should therefore remain classified as experimental until prospective studies show that improved drug delivery leads to better clinical outcomes.

Conclusions and future perspectives

The evidence discussed in this review indicates that BCBM is not the passive endpoint of systemic dissemination. It develops through successive selective pressures imposed by the cerebral vasculature, brain-resident cells, immune surveillance, and the metabolic conditions of the brain. These pressures also differ among HER2-positive, TNBC, and HR-positive/HER2-negative disease. This brain-specific and subtype-dependent biology provides a common framework for interpreting mechanisms,

biomarkers, experimental models, and treatment.

Important biological gaps remain. The cell states that permit successful BBB passage are incompletely defined, and it is unclear why most disseminated cells disappear while a minority persists and later forms detectable lesions. Matched primary tumors, extracranial metastases, and brain lesions collected longitudinally could distinguish transient adaptation from stable selection. Single-cell and spatial analyses will be most informative when anchored to such paired samples and to treatment history.

Monitoring intracranial evolution poses a separate challenge. MRI measures lesion burden but offers little direct molecular information; repeated brain biopsy is rarely feasible, and plasma biomarkers may be dominated by extracranial tumors. Serial CSF circulating tumor DNA could complement MRI and CSF cytology when intracranial and systemic disease diverge, but collection, processing, sequencing, and reporting must be standardized before molecular changes can guide care.

The interpretation of experimental findings depends equally on model selection. Spontaneous models retain the complete metastatic cascade but are inefficient and variable. Intravascular models are useful for examining vascular arrest, BBB extravasation, and early colonization, whereas intracranial models are suited to established tumor growth and treatment response. The latter cannot demonstrate natural dissemination or drug passage across an intact BBB. Mechanistic and therapeutic conclusions will be more reliable when they are reproduced across complementary models and supported by patient-derived material.

Clinical trial design remains a major limitation. Patients with active brain metastases or leptomeningeal disease are still frequently excluded, even when the investigational treatment may have CNS activity. Future trials need separate intracranial endpoints, prospective assessment of neurological symptoms, and stratification by molecular subtype, intracranial burden, previous local treatment, and extracranial disease control. Greater attention is also required to patients whose intracranial and extracranial disease follow different clinical

courses. Connecting these trial designs with longitudinal biomarkers and question-appropriate models may allow brain-specific mechanisms to inform treatment rather than remain isolated preclinical observations.

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