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Metabolic Reprogramming in Brain Metastases: Integrating Spatial Omics with Non-Spatial Mechanistic Evidence

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Highlights

- Spatial multi-omics identifies candidate metabolic niches in brain metastases.
- Spatial studies associate inferred metabolic programs with regionally immunosuppressed niches.
- Spatial maps can prioritize candidate vulnerabilities for functional and clinical validation.

Abstract

Brain metastases (BrMs) develop within a metabolically specialized environment, where nutrient availability and interactions with neural, vascular and immune cells can influence tumor growth. Bulk and single-cell studies have identified metabolic alterations, while spatial approaches provide additional information on their regional and cellular context. This review article examines spatial transcriptomics, proteomics and mass spectrometry imaging alongside biochemical measurements and functional studies, focusing on central carbon metabolism, glutamate-glutamine metabolism, lipid

handling and oxidative phosphorylation. Available spatial studies describe regional differences in metabolism-associated molecular programs, lipid abundance and isotope labeling. Complementary experiments implicate tumor-cell synthesis, host-derived substrate supply and mitochondrial activity in supporting growth in the BrM models. These findings suggest recurring metabolic functions across tumor origins, although whether these functions depend on the same molecular pathways remains uncertain. Some BrM-associated features are also detectable in primary tumors, suggesting that these states may precede brain colonization. We therefore discuss how matched-site comparisons, tracing and cell-specific perturbation could help distinguish pre-existing features from local adaptations and clarify metabolic sources and requirements. Linking tissue findings to longitudinal imaging and liquid-biopsy measurements could support the evaluation of candidate targets and biomarkers while addressing limited opportunities for repeated tissue sampling.

Keywords:

brain metastases; spatial multi-omics; metabolic reprogramming; tumor microenvironment heterogeneity; metabolic-immune crosstalk; metabolic vulnerabilities; metabolic niches

1. Introduction

Brain metastases (BrMs) are a major clinical challenge and a frequent complication of advanced solid tumors, particularly lung cancer, breast cancer, and melanoma¹. Despite advances in surgery, radiotherapy, targeted therapy, and immunotherapy, outcomes remain poor². Disseminated tumor cells adapt to a specialized brain microenvironment shaped by the blood-brain barrier (BBB), restricted nutrient accessibility, and distinct neurovascular and neuroimmune interactions². Colonization therefore requires metabolic adaptation that sustains energy production, redox balance, and biosynthesis under conditions unlike those at the primary site³. These adaptations remain incompletely understood because conventional molecular approaches do not fully resolve the spatial and functional organization of the metastatic niche.

Bulk and single-cell omics have substantially advanced our understanding of cancer progression and metastatic adaptation, but each provides only a partial view of BrM biology. Bulk analyses average

signals across heterogeneous cell populations and anatomical regions, thereby obscuring localized metabolic programs and phenotypic gradients within the tumor microenvironment (TME)⁴. Single-cell and single-nucleus ribonucleic acid (RNA) sequencing improve cellular resolution, but the tissue dissociation required for these approaches disrupts the spatial relationships needed to interpret cell-cell and cell-niche interactions⁵. This limitation is particularly relevant for metabolism, which is strongly shaped by local oxygen tension, nutrient access, vascular proximity, and stromal crosstalk⁶. As a result, understanding metabolic adaptation in BrMs requires approaches that preserve tissue architecture while capturing molecular heterogeneity.

Spatially resolved technologies allow BrMs to be studied as anatomically organized ecosystems rather than uniform lesions. By preserving tissue context, they can localize metabolic programs relative to vascular or glial niches and investigate whether those programs coincide with hypoxic stress, immune suppression, or therapeutic vulnerability^{7,8}. Emerging studies implicate interactions with astrocytes, microglia, macrophages, endothelial cells, and other stromal elements^{9,10} and report regional heterogeneity associated with vascular, immune, and glial context¹¹⁻¹³. Most studies, however, infer metabolism from RNA or protein, include modest cohorts, and lack direct measurements of metabolite abundance or flux. Building on these spatial observations, further integration with direct metabolic measurements and functional perturbation would help clarify pathway activity and assess potential therapeutic vulnerabilities.

In this review, we summarize current knowledge of metabolic reprogramming in BrMs and examine how spatially resolved approaches refine its interpretation. We first outline the spatial technologies used to interrogate the BrM microenvironment and then assess evidence for central carbon metabolism, glutamate-glutamine utilization, lipid remodeling, and oxidative phosphorylation (OXPHOS). We conclude by considering translational implications, current limitations, and priorities for spatially informed metabolic research in BrMs.

1.1 Literature search and study selection

We conducted a focused narrative search of PubMed and Google Scholar, combining brain metastasis terms with spatial transcriptomics, spatial proteomics, mass spectrometry imaging, spatial

metabolomics, and metabolic terms including glycolysis, glutamine, lipid metabolism, oxidative phosphorylation and isotope tracing. Reference lists and citing articles of key reports were also screened. We prioritized human BrM spatial studies, matched-site comparisons and direct metabolic measurements, followed by BrM-specific functional studies. Evidence from other tumors was included for methodological or translational context and identified as indirect. Targeted reference and ClinicalTrials.gov checks were updated through 13 September 2026. This narrative review was not protocol-registered and does not claim systematic or exhaustive coverage.

2. The Spatial Toolkit: Decoding the Metabolic Microenvironment *In Situ*

A growing range of spatial technologies is being used to characterize metabolism-associated features in BrMs while preserving tissue architecture. These platforms differ in molecular coverage, spatial resolution, and the metabolic inferences they support, providing complementary views of the BrM microenvironment.

Spatial transcriptomics including array-based platforms such as 10x Visium and imaging-based approaches, e.g. multiplexed error-robust fluorescence in situ hybridization (MERFISH) and Xenium, maps gene-expression programs across tissue sections¹⁴⁻¹⁶. For example, in a melanoma BrM study, spatial profiling (Slide-seqV2) identified opposing glycolysis- and OXPHOS-associated RNA programs within one lesion, illustrating metabolic heterogeneity at the level of gene expression¹². These maps localize candidate metabolic programs, while post-transcriptional and post-translational regulation limits the inference of enzyme activity from RNA abundance¹⁷.

Multiplexed spatial proteomic technologies quantify proteins in defined tissue regions or cells. These include imaging mass cytometry (IMC)⁵, CO-Detection by indEXing (CODEX), and digital spatial profiling (DSP). Mapping metabolic enzymes and signaling mediators alongside cell-type markers connects candidate pathways to tumor, glial, vascular, and immune compartments. However, protein abundance does not necessarily reflect enzyme activity or metabolic flux. Integrating these maps with direct metabolic measurements would help clarify the functional significance of the observed spatial patterns.

Mass spectrometry imaging (MSI), particularly matrix-assisted laser desorption/ionization mass spectrometry imaging (MALDI-MSI), measures metabolite- and lipid-associated ion signals at defined locations across tissue sections, revealing spatial distributions that complement RNA and protein expression profiles^{18,19}. However, regional differences in signal intensity can reflect both metabolite abundance and local ionization effects, complicating quantitative comparisons²⁰. A recent workflow using isotope-labeled internal standards improved pixelwise normalization and relative quantification in brain and kidney tissue, illustrating how these effects can be reduced to support more reliable regional comparisons²¹. Whereas isotope-labeled internal standards improve abundance measurements, administering labeled nutrients before tissue collection allows MSI to trace their metabolic fate across tissue regions²². Samarah *et al.* combined stable-isotope tracing with MSI in mouse liver and intestine to visualize labeled-nutrient incorporation into downstream metabolites and identify regional differences in substrate utilization²². Initial isotope-imaging studies have already examined substrate labeling in BrM tissue, as discussed in Section 4.1. Extending these measurements across patients could clarify regional differences in substrate use.

Combining different spatial measurements allows gene and protein expression to be examined alongside metabolite distributions within a shared tissue context. Computational frameworks can align spatial transcriptomic, proteomic, and MSI data across adjacent or co-registered tissue sections²³⁻²⁵, while same-section workflows reduce uncertainty arising from anatomical differences between adjacent sections^{23,24,26,27}. Recent optical platforms extend this integration to cell-resolved chemical measurements. For example, Raman Enhanced Delineation of Cell Atlases in Tissues (REDCAT) combines Raman-based chemical imaging, optical redox readouts, and multiplexed immunofluorescence on the same section to associate lipid and redox-related features with specific cell types²⁸. Applying REDCAT in human lymphoid and liver tissues illustrates how this approach characterizes metabolic heterogeneity within defined cellular populations²⁸. Adapting such workflows to BrMs could help distinguish the metabolic features of tumor cells from those of neighboring glial, vascular, and immune cells.

Together, spatial RNA, protein, and chemical measurements connect lesion-level metabolic observations to local cellular neighborhoods. Integration with appropriately designed tracing and functional perturbation would help establish which localized programs are active and assess their potential as therapeutic vulnerabilities.

3. Spatial organization in BrMs

In BrMs, tumor cells interact with vascular, glial, and immune cells in spatially heterogeneous local environments⁹. Brain-specific vascular structures, including the neurovascular unit and the BBB or blood-tumor barrier (BTB), regulate access to the tissue, while astrocytes, microglia, and neurons contribute specialized metabolic functions². Their spatial arrangement creates cellular neighborhoods in which tumor-host interactions may influence colonization and treatment response⁵. Here, we focus on the microanatomical organization within and around parenchymal BrMs, considering perivascular regions, the lesion interior, and the brain-tumor interface with adjacent brain tissue before examining finer-scale neuronal contacts.

One major niche is the perivascular region, where metastatic cells interact with endothelial cells, pericytes, and astrocytic endfeet²⁹. Endothelial barrier properties, supported and regulated by pericytes and astrocytes, help control exchange between the circulation and surrounding tissue²⁹. In BrMs, BTB permeability varies within and between lesions, as demonstrated by permeability measurements and heterogeneous drug accumulation in mouse models of breast-cancer BrM³⁰. These observations raise a related metabolic question: whether this vascular heterogeneity is accompanied by differences in local nutrient availability and substrate utilization by tumor and neighboring cells.

The distinction between perivascular and perinecrotic regions is also reflected in lipid accumulation. Specifically, fluorescence imaging of human central nervous system (CNS) metastases originating from kidney cancer, melanoma, and lung cancer showed lipid-droplet-rich, carbonic anhydrase IX (CA-IX)-positive perinecrotic regions, contrasting with lipid-droplet-poor perivascular

regions³¹. This regional contrast illustrates how tissue architecture can coincide with differences in lipid storage within metastatic tissue.

At the boundary with adjacent brain tissue, the brain-tumor interface ranges from a well-demarcated border to an infiltrative margin. Histopathological analysis of human BrM autopsy specimens identified well-demarcated borders, perivascular extensions of tumor-cell clusters, and diffuse infiltration of individual tumor cells into adjacent brain tissue³². Along these margins, metastatic cells contact reactive astrocytes, microglia, and neurons^{10,33,34}. The contrast between circumscribed and infiltrative growth also extends to immune organization. For example, spatial profiling of human BrMs revealed a lymphocyte-rich band separating tumor from surrounding brain in minimally invasive lesions, while this organization was largely absent from highly invasive lesions³⁵. Thus, the interface differs across lesions in both the extent of tumor infiltration and the arrangement of neighboring host cells.

At a finer spatial scale, tumor cells can establish close contacts with neuronal structures. In breast-cancer BrM mouse models, tumor-cell protrusions were observed adjacent to excitatory synapses in pseudo-tripartite arrangements³⁶. Such observations extend the description of BrM organization from tissue compartments and cellular neighborhoods to subcellular contacts.

Together, the spatial complexity of BrMs and their interfaces with surrounding brain tissue raises the possibility that metabolic states and tumor-host interactions differ across local environments. This organization complicates the interpretation of lesion-level metabolic profiles, but also motivates spatially resolved studies that relate metabolic features to specific tissue regions and cellular neighborhoods. Against this spatial background, the following section examines metabolic adaptations in BrMs and considers how their interpretation depends on local context.

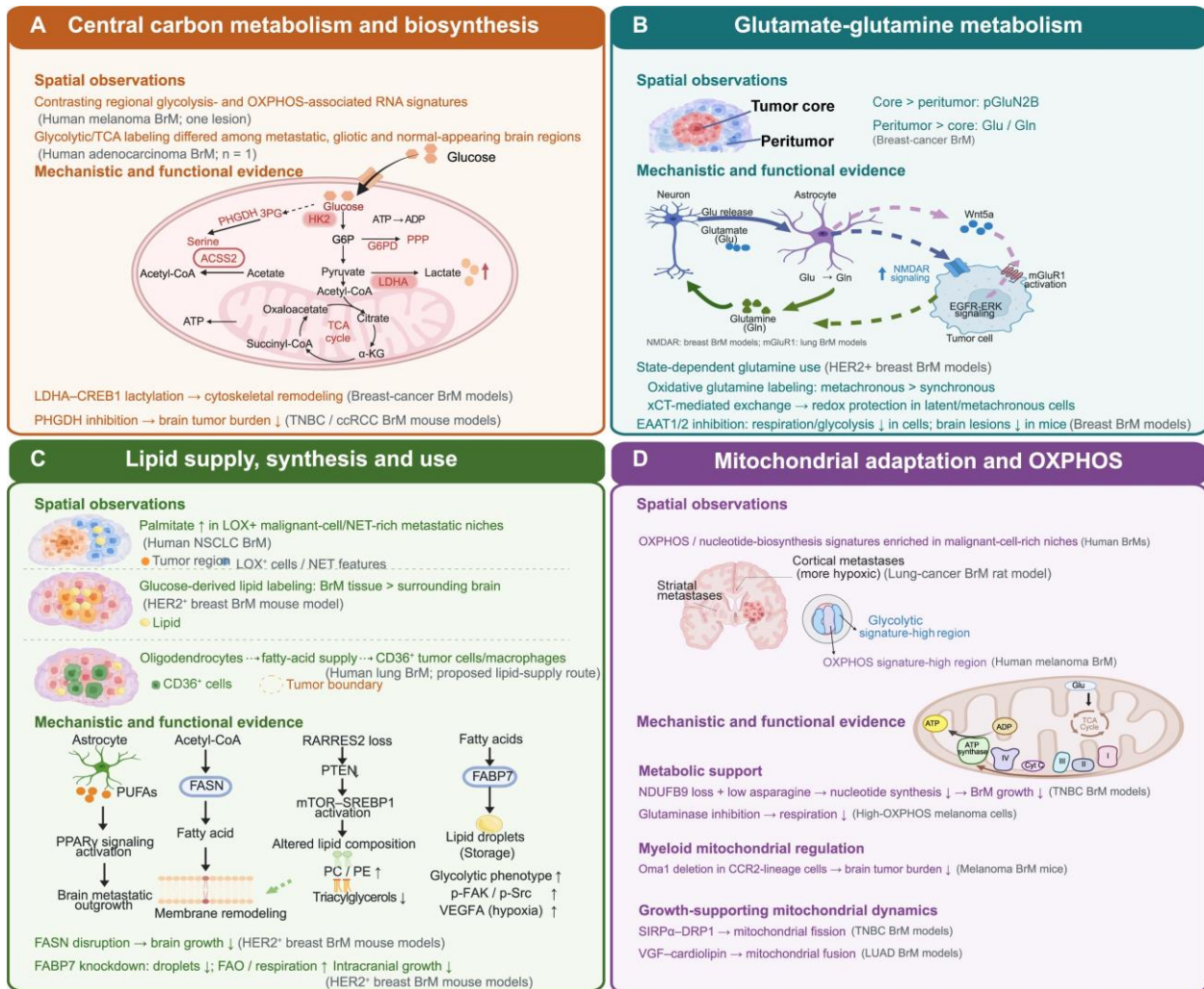


Figure 1. Spatial observations and complementary mechanisms of metabolic reprogramming in BrM. Each panel separates regional observations from mechanistic and functional findings obtained in the indicated tumor origins and experimental settings. (A) Central carbon metabolism and biosynthesis, linking regional glycolytic and TCA-associated measurements to pathways supporting carbon utilization and growth. (B) Glutamate-glutamine metabolism, distinguishing regional metabolite and receptor-phosphorylation patterns from neuronal and glial signaling, substrate transport and state-dependent glutamine use. (C) Lipid supply, synthesis and use, relating regional lipid enrichment and isotope labeling to tumor-cell synthesis, candidate glial supply, membrane composition and lipid storage. (D) Mitochondrial adaptation and OXPHOS, bringing together regional transcriptional and oxygenation differences with experimental findings on biosynthetic support, myeloid regulation and

mitochondrial dynamics. Findings from different studies are juxtaposed for comparison rather than combined into a single validated pathway. Arrows indicate the relationships or directions reported in the specified settings; dotted donor-recipient links denote a proposed supply route. Metabolite abundance, transcript enrichment and isotope labeling are distinct readouts and do not, by themselves, establish quantitative flux or a shared therapeutic dependency.

4. Metabolic reprogramming in BrMs: single-cell and spatial insights

Studies of metabolic reprogramming in BrMs implicate central carbon metabolism, glutamate-glutamine metabolism, lipid remodeling, and OXPHOS in tumor growth and tumor-host interactions. Direct spatial measurements of these processes remain limited, and current understanding draws largely on bulk and single-cell profiling, metabolic tracing, and functional studies. Here, we discuss these findings alongside spatial observations, examining the cellular and anatomical context of metabolic programs and their similarities and differences across primary-tumor origins. Figure 1 summarizes the major metabolic programs and their reported spatial context in BrMs.

4.1 Central carbon metabolism and associated biosynthetic pathways

Central carbon metabolism connects glucose breakdown and tricarboxylic acid (TCA) cycle oxidation with pathways that supply biosynthetic precursors. BrM studies implicate glycolysis, acetate utilization, and connected biosynthetic pathways, but whether glycolytic or TCA-cycle activity is consistently increased across primary-tumor origins remains unclear⁹. Spatial profiling and isotope imaging provide complementary information on regional metabolic programs and substrate use, while functional studies examine how these processes support BrM growth.

Spatial transcriptomics provides a starting point for examining how central carbon metabolism varies within a lesion. In the treatment-naïve melanoma cohort of Biermann *et al.*, Slide-seqV2 analyzed 16 sections, including 11 from BrMs and five from extracranial metastases. In one malignant-cell-rich BrM, glycolysis- and OXPHOS-associated transcriptional signatures showed contrasting regional distributions¹². Alongside regional variation within BrMs, spatial profiling can reveal metabolic programs shared with the primary tumor. In three patients with triple-negative breast cancer (TNBC), Visium profiling of matched residual primary tumors and BrMs identified a glycolysis-related gene

module common to both sites³⁷. Thus, a metabolic program detected in BrMs may be retained from the primary tumor rather than acquired in the brain. Together, these studies describe regional variation within BrMs and metabolic gene-expression programs shared with primary tumors, while direct metabolic measurements are needed to clarify local pathway activity.

Isotope imaging adds a spatial readout of glucose-derived carbon incorporation to the transcriptional patterns described above. In a study of two patients with glioblastoma and one with an adenocarcinoma BrM, intraoperative [U-¹³C]glucose infusion was followed by desorption electrospray ionization mass spectrometry imaging (DESI-MSI) of resected tissue. Analysis of seven regions from the BrM at a pixel size of 65 μ m identified differences in labeled glycolytic and TCA-associated metabolites between metastatic tissue, gliotic tissue, and normal-appearing brain³⁸. This single-patient observation provides an initial view of regional glucose metabolism in human BrM, while the quantitative relationship between these labeling patterns and local pathway flux remains unclear.

The spatial observations above reveal regional metabolic differences, but leave open which enzymes contribute to tumor growth. Cell and animal experiments address this question through perturbation of glycolytic enzymes and their regulators in BrM models. Hexokinase 2 (HK2) catalyzes glucose phosphorylation at the entry to glycolysis. In 123 surgically resected human breast-cancer BrMs, high HK2 immunoreactivity was associated with shorter post-craniotomy survival, while HK2 knockdown reduced proliferation of 231-BR cells under glucose limitation³⁹. Another glycolytic enzyme, phosphoglycerate mutase 1 (PGAM1), has been implicated in non-small cell lung cancer (NSCLC) BrM growth through its stabilization by SEC61 translocon subunit gamma (SEC61G), an endoplasmic-reticulum translocon component⁴⁰. Experiments in brain-selected lung-cancer sublines and intracardiac mouse models linked SEC61G-mediated PGAM1 stabilization to increased glycolysis and brain metastatic growth. Single-cell profiling and patient-tissue immunohistochemistry provided complementary information on SEC61G expression⁴⁰. These findings suggest that glycolytic enzymes and their regulators can support growth in the breast- and lung-cancer BrM models examined, while their contribution within the spatially mapped regions remains to be tested.

Studies of lactate production and protein lactylation further link glycolysis to tumor-cell behavior and immune regulation. Lactate dehydrogenase A (LDHA) converts pyruvate, the end product of glycolysis, to lactate. In breast cancer, LDHA staining in primary TNBC specimens and serum lactate dehydrogenase (LDH) measurements were associated with subsequent brain relapse⁴¹. Mechanistic studies in breast-cancer models linked LDHA-mediated cyclic adenosine monophosphate response element-binding protein 1 (CREB1) lactylation to cytoskeletal remodeling, while LDHA knockdown reduced brain tumor burden in mice⁴². Lung-cancer studies examined myeloid-cell responses: an intracranial Lewis lung carcinoma (LLC) model showed lactate accumulation, and murine macrophage experiments associated Rac family small GTPase 2 (RAC2) lactylation with altered polarization. Human observations were limited to histone H3 lysine 18 lactylation (H3K18la) staining in two primary-BrM pairs and two non-BrM primary tumors⁴³. These findings motivate spatial studies asking whether lactate-rich regions coincide with tumor-cell lactylation or distinct macrophage states, and whether these relationships vary across primary-tumor origins.

Glucose metabolism also branches into the pentose phosphate pathway (PPP), which supplies reduced nicotinamide adenine dinucleotide phosphate (NADPH) and nucleotide precursors⁴⁴. In a tissue-microarray study of 126 breast-cancer metastases, including 36 brain lesions, positivity for the pathway enzyme glucose-6-phosphate dehydrogenase (G6PD) was most frequent in BrMs, although molecular subtype composition differed across metastatic sites⁴⁴.

Beyond glucose, acetate can supply TCA-cycle metabolism in human BrMs. Proteomic comparisons of breast-cancer brain- and bone-metastatic variants identified differences in central carbon enzymes⁴⁵. Intraoperative [1,2-¹³C]acetate infusion in four brain-tumor patients, including one breast-cancer BrM and one NSCLC BrM, yielded labeling, detected by nuclear magnetic resonance (NMR), consistent with acetate oxidation⁴⁶. The regional distribution of this substrate use within BrMs remains unclear.

Acetyl-CoA synthetase 2 (ACSS2) supplies acetyl-coenzyme A (acetyl-CoA) from acetate, linking alternative carbon use to biosynthesis. Brain-penetrant ACSS2 inhibitors reduced breast-cancer BrM growth in cell, brain-slice, and mouse systems⁴⁷. Further perturbation studies linked ACSS2 to

ferroptosis suppression and reduced brain metastatic growth after inhibition; human observations comprised phosphorylated ACSS2 staining in 43 BrMs of mixed origin⁴⁸. These preclinical findings motivate spatial investigation of acetate-related vulnerabilities, although ACSS2 requirements for acetate oxidation in patient BrMs remain unclear.

Phosphoglycerate dehydrogenase (PHGDH) initiates serine biosynthesis from the glycolytic intermediate 3-phosphoglycerate. Transcript profiling of patient tissue showed serine-pathway enrichment in melanoma BrMs versus matched extracranial metastases, while immunohistochemistry detected high PHGDH expression in 27 of 30 breast-cancer BrMs⁴⁹. Glucose tracing and PHGDH inhibition in BrM-derived or brain-selected systems linked serine synthesis to growth under serine/glycine limitation. PH-755 reduced intracranial tumor burden after tumor formation in TNBC and clear-cell renal cell carcinoma (ccRCC) models; melanoma inhibitor testing was limited to culture⁴⁹. Whether spatial differences in nutrient availability influence this sensitivity within patient BrMs remains unclear.

Branched-chain amino-acid aminotransferase 1 (BCAT1) uses the TCA intermediate α -ketoglutarate (α -KG) in branched-chain amino-acid transamination. Staining was higher in NSCLC BrMs than in largely unpaired primary lung adenocarcinoma (LUAD) specimens⁵⁰. A549/H1299 cell and intracranial H1299 xenograft experiments linked BCAT1-dependent α -KG depletion to altered AlkB homolog 5 (ALKBH5) activity and N⁶-methyladenosine (m⁶A) regulation⁵⁰. Unlike the biosynthetic role of serine synthesis, this mechanism links central carbon metabolism to RNA regulation.

Glucose-sensitive and pyruvate-based imaging add spatial information on central carbon metabolism at the scale of lesions and brain regions. Dynamic glucose-enhanced chemical exchange saturation transfer (DGE-CEST) signals were elevated relative to contralateral brain in two of four BrMs, although barrier leakage complicates interpretation as glucose uptake⁵¹. In 18 patients with 44 BrMs, pretreatment hyperpolarized [1-¹³C]pyruvate magnetic resonance imaging (MRI) detected a higher lactate-to-bicarbonate signal ratio in lesions that subsequently progressed after stereotactic radiosurgery⁵². The association links a regional metabolic readout to treatment outcome, but the cohort was small and spatial resolution was 1.5 cm. By contrast, a separate study of 12 patients and 46

controls found higher lactate-to-pyruvate and bicarbonate-to-pyruvate ratios in normal-appearing brain, excluding tumor-containing regions and regions abnormal on T2-weighted fluid-attenuated inversion recovery (T2-FLAIR) images⁵³. Thus, these studies provide spatially localized observations in different compartments: lesion metabolism and changes in surrounding or remote brain should be distinguished when interpreting regional metabolic variation.

Central carbon metabolism in BrMs encompasses glucose oxidation, lactate-related regulation, acetate use, and biosynthetic pathways. Spatial transcriptomics and isotope imaging reveal regional gene-expression and labeling patterns, whereas genetic and pharmacological perturbations identify candidate functional requirements in specific cancer models. Whether these requirements are shared across primary-tumor origins remains unclear. Integrating spatial information with functional testing could clarify whether regions with distinct substrate use depend on the same enzymes or harbor different metabolic vulnerabilities.

4.2 Glutamate-glutamine metabolism

Glutamate-related adaptations in BrMs involve receptor signaling as well as substrate use. In breast-cancer BrM models, microscopy localized tumor-cell protrusions beside neuronal synapses, while neuronal co-cultures and GRIN2B perturbation in intracardiac mouse models linked glutamate-responsive N-methyl-D-aspartate receptor (NMDAR) signaling to metastatic growth³⁶. Paired human primary-tumor and BrM specimens provided complementary observations of phosphorylated GluN2B (pGluN2B), the NMDAR subunit encoded by GRIN2B³⁶. These anatomical observations raise the question of whether glutamate availability and receptor activation are greatest in the same regions.

Regional measurements in breast-cancer BrMs suggest that receptor phosphorylation and metabolite abundance need not be greatest in the same compartment. Biochemical analysis of manually delineated human tissue regions found higher glutamate, glutamine, and their combined pool (Glx) in peritumoral tissue, whereas immunohistochemistry localized stronger pGluN2B staining to the tumor core⁵⁴. This regional contrast suggests that tissue metabolite abundance alone may not predict the intensity of receptor signaling. At the lesion scale, glutamine positron emission tomography (PET) in the same study detected higher tracer uptake in brain lesions than in paired mammary tumors in six

mice; clinical imaging was limited to one woman with a breast-cancer BrM⁵⁴. These tissue and imaging measurements provide regional context, although metabolite abundance and tracer uptake are not direct measures of cell-specific metabolic flux.

Functional studies help explain how glial signals can alter tumor-cell responses to local glutamate. In human lung-cancer cells cultured with mouse-derived mixed glia and corresponding mouse BrM models, astrocyte-derived Wnt5a induced metabotropic glutamate receptor 1 (mGluR1) through prickly planar cell polarity protein 1 (PRICKLE1) and RE1-silencing transcription factor (REST), enabling glutamate-dependent epidermal growth factor receptor (EGFR) stabilization and extracellular signal-regulated kinase (ERK) activation⁵⁵. Glial regulation of receptor signaling may therefore influence tumor-cell responses to local glutamate, alongside the mechanisms controlling substrate supply and uptake discussed below.

Changes in host-cell metabolite handling and tumor-cell transport could contribute to regional differences in substrate availability. In breast-cancer systems, cell-culture, mouse brain-slice, and brain-interstitial analyses linked extracellular-vesicle-associated microRNA-199b-5p (miR-199b-5p) to suppression of neuronal and astrocytic transporters and greater extracellular availability of glutamate, glutamine, and lactate. Circulating miR-199b-5p was also associated with BrMs in 42 women with stage-IV breast cancer⁵⁶. Complementary experiments in brain-tropic breast-cancer cells linked nuclear factor κ B (NF- κ B) signaling to increased glutamate uptake mediated by excitatory amino-acid transporters 1 and 2 (EAAT1/EAAT2). Transporter inhibition reduced respiration and glycolytic readouts in culture and brain-lesion formation in mice⁵⁷. Together, these experiments suggest that altered substrate handling by host cells and increased uptake by tumor cells could both favor access to extracellular metabolites. Whether these mechanisms account for the spatial distributions observed in human BrMs remains to be tested.

Once substrates are available to tumor cells, their metabolic use may also depend on tumor-cell state. In human epidermal growth factor receptor 2 (HER2)-positive breast-cancer models derived from HCC1954 and SKBR3 cells, phenotypic selection generated synchronous, latent, and metachronous states. In vivo [U-¹³C₅]glutamine tracing showed greater oxidative labeling in

metachronous than synchronous lesions, whereas comparisons involving latent cells were performed in vitro. Latent and metachronous cells also relied on cystine-glutamate exchange mediated by the xCT antiporter for redox control, showing that glutamate handling contributes to antioxidant protection alongside the oxidative use of glutamine⁵⁸. Immunohistochemistry assessed xCT expression in seven matched HER2-positive primary and metachronous metastatic cases, although its contribution to redox control in patient lesions remains unclear⁵⁸. Together, these findings suggest that glutamine use and glutamate-linked redox regulation may vary with tumor-cell state, and how these experimentally selected states relate to clinical latency or spatial niches warrants further investigation.

Glutamate metabolism also connects to γ -aminobutyric acid (GABA) production through glutamate decarboxylase 1 (GAD1). Comparative transcriptomic analysis found enrichment of a GABA-related gene set in brain tumors across models of four primary-tumor origins, suggesting a potentially shared transcriptional response to the brain environment. Functional experiments in breast-cancer and melanoma systems linked glia-induced GAD1 expression to glutamine utilization and redox regulation; GAD1 knockdown reduced brain metastases in mice⁵⁹. The overlap across models supports investigating a recurring GABA-related adaptation, while the breadth of the transcriptional finding exceeds that of the functional testing.

Within this GABA-related response, accumulation and metabolic utilization may support growth through different mechanisms. In NSCLC brain-tropic cells and mouse models, reduced forkhead box A2 (FOXA2) and 4-aminobutyrate aminotransferase (ABAT) expression was linked to GABA accumulation, NF- κ B signaling, and astrocyte activation; patient analyses also found higher circulating GABA in those with BrMs⁶⁰. A smoking-linked lung-cancer study instead connected nicotine-induced microglial microRNA-32-3p (miR-32-3p) signaling to neuronal GABA release and tumor-cell GABA-shunt use in ex vivo and in vivo models. Patient tissue associated smoking history with perineural invasion, providing anatomical context, and GABA-uptake inhibitors reduced brain-metastatic progression in mice⁶¹. These findings distinguish GABA accumulation from substrate use rather than implying uniformly increased GABA catabolism. Their local balance and therapeutic relevance in patient BrMs remain open questions.

Together, these studies connect glutamate-glutamine metabolism to neural signaling, extracellular substrate handling, and state-dependent metabolic requirements. GABA-related findings recur across several cancer origins, but their functional importance may differ by tumor state and microenvironment. Spatial microscopy, regional biochemistry, and imaging provide complementary information on cellular contacts and metabolite distribution. Integrating these observations with local substrate-use measurements and perturbation could clarify where receptor signaling, oxidative metabolism, and redox protection contribute to BrM growth.

4.3 Lipid metabolism and membrane remodeling

Although the brain is lipid-rich, the availability of these lipids to metastatic cells may vary across local environments⁶². Cancer cells can acquire fatty acids through de novo synthesis or uptake and incorporate them into membrane lipids or store them in lipid droplets^{63,64}. The relative importance of these routes may depend on both local lipid supply and tumor-cell regulation. This distinction matters when interpreting spatial lipid maps, because enrichment within a lesion may reflect differences in lipid production, uptake, or subsequent use. Spatial measurements can locate lipid enrichment and isotope labeling, while complementary functional studies help assess which aspects of lipid metabolism support growth.

In HER2-amplified breast cancer BrM models, spatial isotope imaging and functional experiments suggest that tumor growth in the brain is particularly sensitive to disruption of fatty-acid synthesis. Fatty acid synthase (FASN) uses acetyl-CoA and malonyl-CoA, together with NADPH, to produce palmitate, a 16-carbon saturated fatty acid⁶³. Tracing glucose-derived carbon into this product showed greater palmitate labeling in brain tumors than in mammary-fat-pad tumors. MALDI-MSI added regional context by localizing greater labeling of palmitate and other lipids to BT474 brain tumors than to surrounding brain tissue⁶³. The labeling supports incorporation of glucose-derived carbon into tumor lipids, although interpreting it as an absolute synthesis rate would require further kinetic measurements. To test whether this synthetic activity supported growth, FASN was disrupted using clustered regularly interspaced short palindromic repeats (CRISPR) and CRISPR-associated protein 9 (Cas9). This reduced tumor growth in the brain, with little effect in the mammary fat pad or liver, and

pharmacological FASN inhibition also reduced intracranial growth, suggesting a site-dependent contribution of fatty-acid synthesis in these models⁶³. Mechanistic experiments in NSCLC cells provide a possible explanation for how tumor cells sustain FASN expression. Insulin-like growth factor 2 messenger RNA-binding protein 3 (IGF2BP3) stabilized FASN messenger RNA (mRNA) and supported lipid accumulation, while intracranial implantation experiments linked this regulatory axis to brain tumor growth⁶⁵. These results suggest that maintaining FASN expression may support lipid-dependent growth in the brain beyond the HER2-amplified breast cancer models, although the NSCLC experiments did not map this mechanism spatially⁶⁵.

Retinoic acid receptor responder 2 (RARRES2) has also been linked to lipid regulation in breast cancer cells and reduced tumor growth in experimental BrMs. In mouse models using 4T1 breast cancer cells, RARRES2 overexpression reduced tumor growth after intracranial implantation and brain metastatic burden after intracardiac injection⁶⁶. Mechanistic experiments in MDA-MB-231 cells linked RARRES2 overexpression to increased phosphatase and tensin homolog (PTEN) and reduced signaling through mechanistic target of rapamycin (mTOR) and sterol regulatory element-binding protein 1 (SREBP1)⁶⁶. SREBP1 is a transcription factor that regulates lipogenic enzyme expression, providing a connection between this signaling pathway and lipid synthesis. Conversely, RARRES2 knockdown increased phosphatidylcholine and phosphatidylethanolamine, major membrane phospholipids, while reducing triacylglycerols used for lipid storage⁶⁶. Together, these cell experiments suggest that RARRES2 influences both lipogenic signaling and lipid composition, but whether these changes mediate its effects on BrM growth remains unresolved.

Spatial lipid measurements also reveal regional differences in lipid accumulation that may have several metabolic explanations. In a spontaneous breast cancer BrM mouse model, MALDI-MSI localized long-chain acylcarnitines and selected phospholipids to a macrometastatic lesion⁶⁷. Although acylcarnitines are involved in fatty-acid handling, their accumulation does not necessarily indicate increased oxidation. The study instead proposed an association with ischemia or hypoxia⁶⁷. A different spatial pattern emerged from integrated spatial transcriptomics and air-flow-assisted desorption electrospray ionization mass spectrometry imaging (AFADESI-MSI) of human primary NSCLC and

BrM tissue, which associated palmitate-rich regions with lysyl oxidase (LOX)-positive malignant cells, neutrophil-rich niches, and programs related to neutrophil extracellular traps (NETs) and epithelial-mesenchymal transition (EMT)⁶⁸. Palmitate exposure promoted EMT-associated changes in NSCLC cells, providing a possible functional explanation for the spatial association⁶⁸. However, these cell experiments did not establish the source of palmitate or its contribution to EMT within the human tissue regions. Lipid storage and oxidation offer another distinction when interpreting such maps. In HER2-positive breast cancer BrM models, knockdown of the intracellular fatty-acid-binding protein 7 (FABP7) reduced glycolytic readouts and lipid-droplet accumulation while increasing fatty-acid oxidation (FAO) and mitochondrial respiration in cell assays. In these cells, FABP7 knockdown also reduced phosphorylation of focal adhesion kinase (FAK) and Src and attenuated hypoxia-induced accumulation of hypoxia-inducible factor 1 α (HIF-1 α) and expression of vascular endothelial growth factor A (VEGFA) mRNA. The same intervention also limited intracranial growth⁶⁴, suggesting that greater oxidative activity need not accompany greater tumor growth. These findings help explain why regional lipid enrichment should be interpreted alongside measurements of lipid use, rather than as evidence of a particular metabolic dependency.

Relating tumor regions to neighboring cells can help identify potential sources of the fatty acids used by BrM cells. In lung-cancer BrMs, human single-cell and spatial transcriptomics, together with lipidomics and functional experiments, implicated oligodendrocytes as a potential source⁶⁹. Spatial data placed oligodendrocytes mainly at tumor margins and showed enrichment of transcripts encoding the fatty-acid uptake protein cluster of differentiation 36 (CD36) in tumor regions⁶⁹. These observations motivated functional tests of lipid synthesis in the proposed donor cells and uptake in recipient cells. In co-culture, FASN knockdown in MO3.13 oligodendrocytes or CD36 knockdown in recipient tumor or microglial cells attenuated tumor proliferation or the induction of immunosuppression-associated markers. Co-injection of FASN-silenced MO3.13 cells with H2030-BM cells also reduced brain metastatic burden⁶⁹. Taken together, the spatial localization and perturbation results support a model in which oligodendrocyte lipid synthesis and recipient-cell uptake contribute to tumor-immune

interactions. Direct tracing of lipid transfer in vivo would be needed to establish this donor-recipient relationship more firmly.

Experiments using astrocyte-conditioned medium and genetic perturbations suggest that tumor genotype can influence how BrM cells use host-derived fatty acids. In breast cancer BrM models, p53 inactivation increased stearoyl-CoA desaturase 1 (SCD1), which converts saturated fatty acyl-CoAs into monounsaturated forms. SCD1 knockout counteracted the growth-promoting effects of p53 loss, linking this genotype to a requirement for fatty-acid desaturation⁷⁰. Astrocyte-conditioned medium also promoted the proliferation of p53-deficient breast cancer cells, and CD36-mediated fatty-acid uptake contributed to this proliferative effect⁷⁰. These results suggest that the response to astrocyte-derived lipids may involve both uptake and intracellular processing. In addition to serving as substrates, astrocyte-derived fatty acids can influence tumor growth through signaling. In breast cancer and melanoma BrM models, arachidonic and mead acids released by astrocytes activated the lipid-responsive transcription factor peroxisome proliferator-activated receptor γ (PPAR γ) and promoted tumor-cell proliferation. PPAR γ inhibition reduced brain metastatic burden, supporting a role for this signaling pathway in those models⁷¹. Thus, astrocyte-associated lipid metabolism may support growth through distinct processes, although their spatial distribution and relative contributions within human BrMs remain to be determined.

Together, these studies suggest that lipid metabolism supports BrM growth through several processes rather than simply through lipid accumulation. FASN-dependent synthesis contributes to brain tumor growth in HER2-amplified breast cancer and NSCLC models, while studies of astrocyte-derived fatty acids implicate uptake and lipid-responsive signaling in additional growth-supporting mechanisms. The contrasting effects of FABP7 knockdown on lipid storage, oxidation, and intracranial growth further indicate that these metabolic responses should not be treated as interchangeable. Spatial imaging reveals regional lipid enrichment and isotope incorporation, and integrated spatial and functional analyses implicate oligodendrocytes as potential local lipid suppliers. What remains unresolved is how tumor-cell synthesis, host-derived supply, and intracellular lipid use are coordinated within individual human BrMs, and whether the resulting dependencies recur across tumor lineages.

4.4 Mitochondrial adaptation and oxidative phosphorylation

Mitochondrial adaptation in BrMs involves respiratory metabolism, biosynthetic support, and interactions with host cells^{72,73}. These functions vary across the models examined⁹. Spatial studies help resolve where mitochondrial programs occur, while complementary functional experiments examine how they contribute to growth.

The regional glycolytic-OXPHOS contrast described in Section 4.1 provides an initial example of spatial variation in mitochondrial-associated programs within BrM¹². In a larger proteogenomic atlas, spatial transcriptomics of 12 BrMs and four matched primary tumors identified OXPHOS and nucleotide-biosynthesis enrichment in two malignant-cell-rich molecular niches, MN4 and MN9⁷⁴. These findings associate oxidative transcriptional programs with regional cell composition. Spatial proteomics from one patient with melanoma further showed higher mitochondrial and glycolytic protein abundance in one primary-tumor region than another, with related features in lung and brain metastases⁷⁵. Thus, some mitochondrial-associated states may already occur within the primary tumor, although this retrospective comparison does not establish their clonal origin or respiratory activity.

Regional oxygen availability provides a physiological context for these molecular maps. In nude rats with stereotactically implanted H2030-BrM3 lung-cancer lesions, cortical tumors were larger and showed stronger hypoxia- and oxidative-stress-associated signals than striatal tumors, assessed by proteomics, histology, and copper-64-labeled copper(II) diacetyl-bis(N⁴-methylthiosemicarbazone) ([⁶⁴Cu]Cu-ATSM) PET⁷⁶. Related work combined CA-IX staining in human NSCLC BrMs with rat imaging and documented regional oxygen heterogeneity in the model⁷⁷. Oxygen supply could therefore influence the expression and function of respiratory pathways, but the accompanying size difference prevents attribution to brain region alone. These measurements provide oxygenation context, not regional OXPHOS flux.

Cellular composition is also important when assigning mitochondrial programs to a tissue region. GeoMx profiling of 44 NSCLC patients distinguished tumor, immune, and adjacent-brain compartments with different immune and fibrotic programs¹¹. Although this study did not measure regional respiration, functional work shows why neighboring myeloid cells merit metabolic investigation.

In experimental and human BrMs, tissue staining localized the macrophage migration inhibitory factor (MIF) receptor cluster of differentiation 74 (CD74) to microglia/macrophages, and experiments implicated tumor-derived MIF in their reprogramming. Bulk RNA sequencing of sorted mouse CD74-positive cells showed OXPHOS enrichment, and conditional deletion of the mitochondrial quality-control protease Oma1 in C-C chemokine receptor 2 (CCR2)-lineage cells reduced brain burden in a melanoma BrM mouse model without reducing CD74-positive cell numbers⁷⁸. These results connect myeloid mitochondrial regulation to tumor support, rather than simply to myeloid-cell accumulation.

Spatial and functional studies also connect mitochondrial remodeling in tumor cells to their interactions with the brain microenvironment. In TNBC mouse tumors, DSP identified immune changes associated with signal regulatory protein α (SIRP α)-regulated fibronectin. Complementary experiments linked fibronectin to microglial tolerance and tumor-cell SIRP α signaling through SH2 domain-containing protein tyrosine phosphatase 2 (SHP2) and ERK to mitochondrial fission mediated by dynamin-related protein 1 (DRP1), a regulator of mitochondrial division⁷⁹. In LUAD BrM models, a separate study linked the neuropeptide VGF to mitochondrial fusion. VGF limited abhydrolase domain-containing protein 12B (ABHD12B)-mediated degradation of cardiolipin, a phospholipid that supports mitochondrial membrane organization, favoring fusion and oxidative metabolism. Deleting the VGF N-terminal domain impaired these processes and reduced brain colonization in mice⁸⁰. Together, these studies suggest that both mitochondrial fission and fusion can support brain metastatic growth in the respective TNBC and LUAD models. Whether the difference between these models reflects tumor origin, genotype, or experimental conditions remains unclear.

Functional studies in TNBC BrM models suggest that nutrient availability shapes how mitochondrial metabolism supports tumor growth. CRISPR screening, isotope tracing, and respiratory assays identified the complex I subunit NADH:ubiquinone oxidoreductase subunit B9 (NDUFB9) as important under low-asparagine conditions. NDUFB9 knockout reduced intracellular aspartate production, while low asparagine increased asparagine synthetase expression, drawing on the remaining aspartate pool to sustain asparagine synthesis. Together, these effects limited aspartate availability for nucleotide biosynthesis and restricted brain tumor outgrowth⁸¹. These findings link

respiratory-chain function to the supply of biosynthetic precursors, rather than adenosine triphosphate (ATP) production alone, and raise the question of whether regional differences in asparagine availability influence sensitivity to NDUFB9 loss within BrMs.

Broader cohort comparisons place these local observations in context. Bulk multi-omics across breast, lung, and renal cancers showed higher OXPHOS gene-set scores in BrMs than in matched primary or extracranial specimens⁸². Earlier breast-cancer analyses found enrichment versus unmatched primary tumors but not extracranial metastases; separate oligomycin experiments were confined to conventional MDA-MB-231 cell assays⁸³. The comparison tissue therefore matters when interpreting apparent brain-associated enrichment. An OXPHOS-enriched epithelial transcriptional state projected onto primary-lung spatial sections⁸⁴ and a dissociated pan-cancer BrM atlas⁸⁵ provide complementary comparisons, but neither supplies regional measurements within BrM tissue.

In melanoma, dependence on glutamine can make oxidative tumor states susceptible to metabolic inhibition. RNA sequencing of 88 resected BrMs identified a High-OXPHOS subgroup associated with shorter post-craniotomy survival. The glutaminase inhibitor CB-839 reduced oxygen consumption in High-OXPHOS cells and prolonged survival in mice bearing mitogen-activated protein kinase (MAPK)-inhibitor-resistant intracranial xenografts, linking glutamine metabolism to respiratory support⁸⁶. In a separate B16-F10 intracranial model, metformin plus anti-programmed cell death protein 1 (PD-1) therapy prolonged survival versus control and metformin alone, but not significantly versus anti-PD-1 alone⁸⁶. A 2025 bioRxiv preprint provided genetic support through peroxisome proliferator-activated receptor γ coactivator 1 α (PGC1 α), a coactivator of mitochondrial biogenesis, and NADH:ubiquinone oxidoreductase subunit S4 (NDUFS4), a complex I subunit. PGC1 α deletion reduced lung and brain metastatic incidence in an autochthonous melanoma model, whereas NDUFS4 deletion reduced metastatic burden in transplantation models⁸⁷. These preclinical results implicate substrate use and mitochondrial machinery at different steps of metastatic growth.

In lung cancer, mitochondrial-associated states can also accompany changes in the immune environment. Bulk and single-cell multi-omics associated BrMs with mitochondrial-pathway enrichment and reduced immune programs. Gamitrinib, which targets mitochondrial heat shock

protein 90 (Hsp90)-dependent protein homeostasis, showed activity in 20 BrM-derived organoid models. Combined with anti-PD-1, it prolonged survival versus control in an LLC-Luc mouse BrM model, but not significantly versus either monotherapy⁸⁸. This supports further testing of mitochondrial targeting without establishing an advantage over monotherapy. Nor are lung-cancer BrMs metabolically uniform. Transcriptomic comparisons found relatively greater glycolytic programs in hepatocyte growth factor receptor (MET)-amplified LUAD BrMs and oxidative programs in MET-wild-type BrMs⁸⁹, suggesting that molecular subtype may help interpret the mitochondrial states identified by spatial profiling.

Together, these studies suggest that mitochondrial adaptation supports BrM growth through context-dependent functions in both tumor and host cells. Spatial analyses locate oxidative transcriptional programs and mitochondrial-associated protein patterns, while functional studies implicate glutamine-supported respiration, aspartate-dependent biosynthesis, and mitochondrial remodeling. Their convergence is on mitochondrial involvement, not on one shared respiratory dependency. Linking these functions to defined cells and regions could clarify how nutrient supply, tumor genotype, and immune composition influence mitochondrial vulnerabilities within individual BrMs.

4.5 Shared, lineage-associated, and brain-site-associated programs

Across breast-cancer, lung-cancer, and melanoma BrMs, recurring features include altered substrate use, lipid handling, and mitochondrial support for growth, but their regulation varies. For example, fatty-acid synthesis can support growth in both breast-cancer and NSCLC BrM models^{63,65}. Pan-cancer profiling also identifies molecular states that extend across primary-tumor origins⁷⁴, suggesting that lineage alone does not explain BrM metabolic heterogeneity. These findings support convergence on related metabolic functions, rather than identical dependencies. Conversely, a mechanism studied in only one lineage cannot yet be considered specific to that lineage.

Brain-site adaptation is more convincingly supported when functional dependence differs across anatomical sites. Greater sensitivity to FASN disruption in brain than mammary-fat-pad or liver tumors provides such support in HER2-amplified breast-cancer models⁶³. Yet a metabolic state detected in

BrMs need not have arisen in the brain. In the melanoma case discussed in Section 4.4, related glycolytic and mitochondrial protein features were already detectable in the primary tumor⁷⁵. Such pre-existing variation, together with selection, local induction, and treatment, may shape the metastatic state. Matched primary and extracranial comparisons help distinguish these possibilities, but functional or temporal evidence is needed to determine how they arise. Therapeutic prioritization should therefore consider tumor origin together with molecular state and local metabolic context.

Table 1. Candidate metabolism-directed interventions relevant to BrMs: evidence base, clinical status, and limitations.

Inhibitor/Intervention	Target/Mechanism of Action	Metabolic Axis	Evidence and limitations	Development status
Telaglenastat (CB-839)	GLS1 inhibition	Glutamine-supported anaplerosis and respiration	CB-839 reduced respiration in high-OXPHOS melanoma cells and prolonged survival in established A375-R1 and MEL624 intracranial xenografts ⁸⁶ . Systemic trials assessed GLS1 inhibition and tolerability ^{90,91} .	Phase I monotherapy completed. The nivolumab combination trial stopped for insufficient efficacy (NCT02771626); no dedicated BrM efficacy cohort.
GABA uptake inhibitors Tiagabine; NO-711	GABA transporter inhibition	GABA-shunt substrate uptake	Both agents suppressed brain metastatic progression in nicotine-associated lung-cancer mouse models, supporting GABA uptake as an experimental target ⁶¹ .	Tiagabine is approved as adjunctive therapy for partial seizures, not BrMs. NO-711 is a research compound.
FASN inhibitors BI99179; TVB-2640 (denifanstat)	FASN inhibition	De novo fatty-acid synthesis	BI99179 inhibited HER2-positive breast tumor growth in brain, but not mammary fat pad ⁶³ . TVB-2640 reduced TNBC BrM-related cell outgrowth/invasion and synergized with free SN-38 in vitro ⁹² . The NSCLC spatial/NET study did not establish intracranial TVB-2640 efficacy because its A549 model lacked clear BrMs ⁶⁸ .	BI99179 remains preclinical. Denifanstat phase II trials in astrocytoma and HER2-positive metastatic breast cancer are completed (NCT03032484; NCT03179904), with results reported but no dedicated BrM efficacy evaluation.
SCD1 inhibitor SW-203668	SCD1 inhibition	Fatty-acid desaturation	In human breast-BrM organotypic cultures, SW203668 increased apoptosis (n = 3); reduced Ki67 was a nonsignificant trend (n = 5). These are ex vivo responses, not patient efficacy ⁷⁰ .	Experimental compound; clinical BrM efficacy and tolerability unestablished.

Inhibitor/Intervention	Target/Mechanism of Action	Metabolic Axis	Evidence and limitations	Development status
ACSS2 inhibitors AD-5584; AD-8007	ACSS2 inhibition	Acetate-to-acetyl-CoA conversion	Both compounds showed mouse brain exposure and activity in ex vivo breast-BrM slices. AD-8007 reduced intracranial burden and prolonged survival; AD-5584 suppressed BrM growth through a ferroptosis-associated mechanism ^{47,48} .	Preclinical leads; human dosing and efficacy remain unestablished.
PHGDH inhibitors PH-719; PH-755	PHGDH inhibition	Serine/glycine synthesis	Serine/glycine restriction sensitized TNBC, melanoma and ccRCC BrM cells in culture. PH-755 reduced established TNBC/ccRCC intracranial tumors and prolonged survival in a separate preventive TNBC experiment. Glucose tracing and hyperpolarized pyruvate imaging provided metabolic readouts ⁴⁹ .	Preclinical BrM studies; no established clinical use.
IACS-010759	Complex I inhibition	OXPHOS	Treatment prolonged survival in intracranial melanoma xenografts and reduced BrM incidence in a spontaneous model ⁹³ . In the breast-cancer experiment, dosing began before inoculation, supporting prevention rather than established-lesion treatment ⁸² .	Phase I development was limited by elevated lactate and neurotoxicity; no recommended phase II dose was established ⁹⁴ (NCT03291938).
Metformin	Pleiotropic biguanide; indirect complex I inhibition	Respiration and immune signaling	In intracranial B16-F10 melanoma tumors, metformin increased immune-activation transcripts. Metformin plus anti-PD-1 improved survival versus control and metformin alone, but not significantly versus anti-PD-1 alone ⁸⁶ .	Approved for type 2 diabetes; BrM treatment remains investigational.
DRP1 depletion / mdivi-1	Genetic DRP1 depletion; pharmacological tool	Mitochondrial dynamics and FAO	DRP1 depletion and mdivi-1 reduced intracranial burden in HER2-positive breast-cancer models ⁹⁵ . Genetic evidence supports DRP1 involvement, but mdivi-1 also inhibits complex I independently of DRP1 ⁹⁶ .	Preclinical BrM evidence; mdivi-1 has limited target specificity.
Gamitrinib	Mitochondrial Hsp90/TRAP1 inhibition	Mitochondrial protein homeostasis	Induced apoptosis in lung-BrM patient-derived organoids and prolonged mouse survival as monotherapy. Adding anti-PD-1 was not significantly superior to either monotherapy ⁸⁸ .	Phase I in advanced cancers, registry-listed as recruiting (NCT04827810); no reported BrM-specific clinical efficacy.
L-arginine	NO-linked metabolic modulation; radiosensitization hypothesis	Arginine/NO and lactate metabolism	A 63-patient randomized BrM study reported improved response and CNS control with oral L-arginine plus twice-daily whole-brain radiotherapy and a boost ⁹⁷ . An intravenous study (n = 20) showed acute MRS lactate/creatine changes, not efficacy or an optimal dose ⁹⁸ .	Investigational radio-sensitization; the small trial and radiation schedule limit generalization. Early phase I BrM trial registry-listed as recruiting (NCT06328686).

Inhibitor/Intervention	Target/Mechanism of Action	Metabolic Axis	Evidence and limitations	Development status
Ketogenic diet	Carbohydrate restriction to induce ketosis	Systemic glucose/ketone availability	The cited NSCLC-BrM case began dietary treatment after chemoradiotherapy and later stopped because of dyslipidemia; benefit cannot be attributed to diet alone ⁹⁹ .	Investigational. NCT05428852 is suspended; NCT05564949 is registry-listed as recruiting. Neither has posted results.
MCT1 inhibitors AR-C117977; AZD3965	MCT1 inhibition	Lactate/pyruvate transport	AR-C117977 reduced glioblastoma stem-like cell viability in vitro; no BrM model or tumor-stromal lactate-transfer experiment was included ¹⁰⁰ .	AZD3965, a different compound, completed phase I in advanced cancers (NCT01791595); no dedicated BrM efficacy analysis.
JHU-083	Prodrug of glutamine antagonist DON	Glutamine-dependent biosynthesis	Oral JHU-083 produced measurable brain exposure to active DON in mice; antitumor activity was reported in medulloblastoma and orthotopic glioma, not BrMs ¹⁰¹⁻¹⁰³ .	Preclinical evidence from primary CNS and extracranial tumors; no clinical BrM data.
Etomoxir	CPT1-directed tool compound	Fatty-acid oxidation	Glioma cultures and orthotopic models showed growth inhibition ^{104,105} . High concentrations (including 100 μ M) and off-target effects limit attribution to CPT1-dependent FAO ¹⁰⁶ .	Laboratory probe; no established BrM application.
Devimistat (CPI-613)	Lipoate analogue affecting PDH and α -KGDH	TCA-cycle metabolism	TCA-cycle perturbation was demonstrated in non-BrM carcinoma cells and xenografts; direct BrM efficacy and brain exposure were not reported ¹⁰⁷ .	No BrM trial. Phase III AVENGER 500 did not improve survival in pancreatic cancer ¹⁰⁸ (NCT03504423).

Evidence is specific to the compound, tumor origin and experimental setting shown; the final four entries provide indirect evidence from non-BrM models. Intracranial implantation tests brain tumor growth but bypasses metastatic dissemination. Clinical status refers to the named compound and indication, not proven BrM efficacy; registry status was checked on 13 September 2026 and may lag actual recruitment.

5. Translational implications of spatially resolved metabolism

5.1 From spatial vulnerability to therapeutic prioritization

Spatially resolved metabolism can help prioritize therapeutic hypotheses by showing where candidate vulnerabilities occur within BrMs. Vascular, fibrotic, glial, and immune regions contain distinct malignant and microenvironmental programs¹¹, providing a tissue context for the metabolic mechanisms discussed in Section 4. Relating these states to tumor origin and treatment history may

inform target selection and the development of tissue or imaging assays^{9,109}. Candidate interventions targeting glycolysis, glutamine metabolism, lipid synthesis, and OXPHOS are summarized in Table 1.

The path to translation depends on whether a spatial finding is being developed as a therapeutic target or a biomarker. For a target, regional RNA or protein enrichment should be linked to metabolic function and tested for selective dependence using genetic or pharmacological perturbation, with attention to drug exposure in the brain and effects on normal cells (Figure 2). A biomarker need not itself cause the phenotype it predicts. Instead, its validation should begin with a defined diagnostic, monitoring, or treatment-selection use, followed by analytical reliability and independent clinical validation in the intended population¹¹⁰. If it is to guide treatment, evidence is also needed that acting on the result improves decisions or outcomes beyond existing clinical assessment. Spatial discovery can inform both routes, but it does not by itself establish clinical utility.

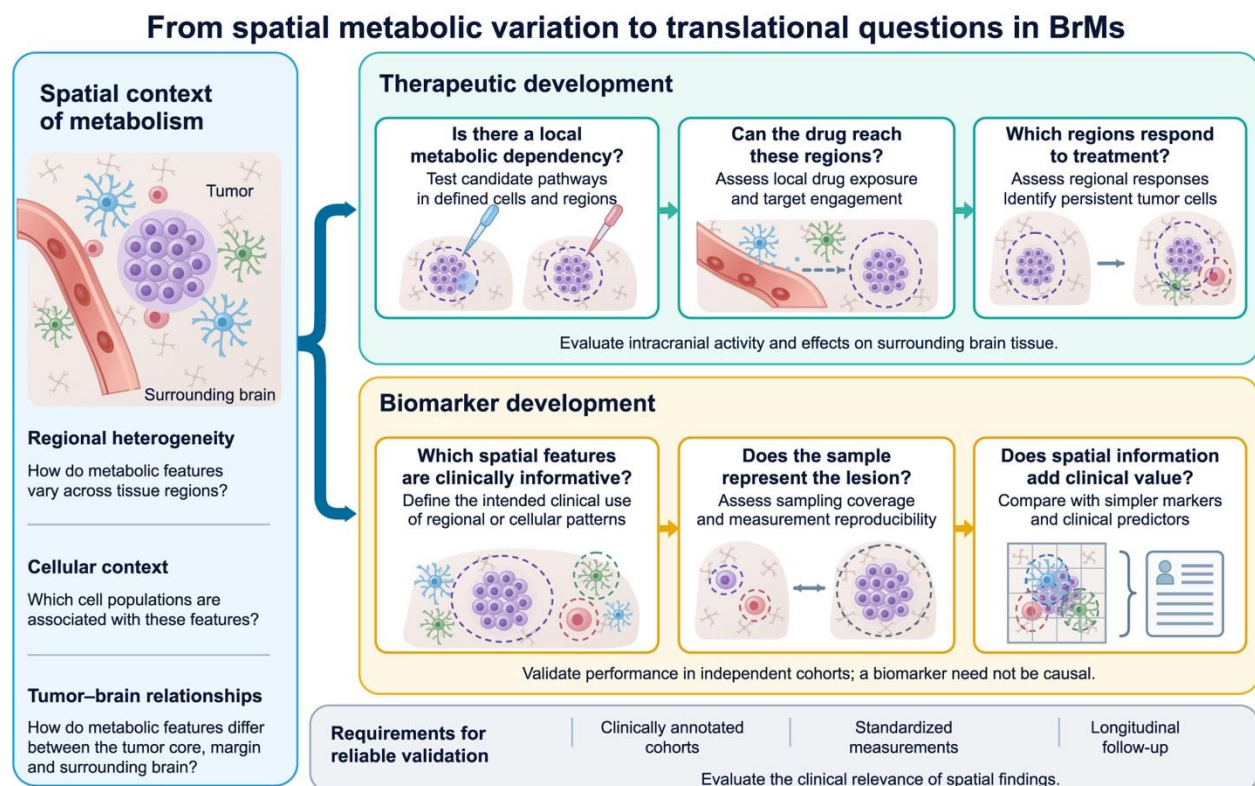


Figure 2. Spatial metabolic heterogeneity and translational development in BrMs. Spatial findings are interpreted within their regional and cellular contexts, including the tumor core, margin and surrounding brain. The upper pathway outlines therapeutic development through tests of local metabolic

dependency, drug exposure and target engagement, followed by evaluation of regional treatment responses and effects on surrounding brain tissue. The lower pathway outlines biomarker development, emphasizing the intended clinical use, sampling coverage, measurement reproducibility and added value over simpler markers and clinical predictors. Biomarkers require validation in independent cohorts but need not be causal. Clinically annotated cohorts, standardized measurements and longitudinal follow-up support both routes. The schematic presents questions for investigation rather than established clinical applications. BrMs, brain metastases.

5.2 Niche-linked metabolic targeting and the bridge to imaging

Mitochondrial inhibition illustrates how recurrent metabolic features can nominate treatments without yet defining a patient-selection biomarker. In lung-cancer BrMs, multi-omic analysis linked a mitochondrial-high state to preclinical gamitrinib sensitivity⁸⁸, while melanoma and lung-cancer studies connected OXPHOS-related programs to aggressive behavior or sensitivity to mitochondrial inhibition in experimental models^{9,86,88}. Together with the regional oxidative programs discussed in Section 4.4, these results motivate testing whether the mapped states identify drug-sensitive tumor regions. They do not yet show that an OXPHOS-high region in a patient is selectively dependent on that pathway.

Fatty-acid synthesis and host-derived lipid signaling provide a second set of therapeutic hypotheses. Breast-cancer BrM models support both FASN-dependent synthesis and growth responses to astrocyte-derived polyunsaturated fatty acids^{63,71}. In TNBC brain-tropic and BrM-derived cell lines, TVB-2640 reduced spheroid outgrowth, migration, and invasion and showed synergy with SN-38 in three-dimensional assays, without an in vivo BrM efficacy test⁹². In human NSCLC BrM tissue, integrated spatial analyses associated palmitate enrichment with neutrophil-rich regions and EMT-related programs⁶⁸. These observations, together with separate FASN-inhibition experiments, motivate further investigation of this pathway, although the NSCLC animal model did not establish intracranial efficacy. Across these studies, regional lipid enrichment has not been validated as a predictor of response to FASN inhibition.

Serine biosynthesis and glutamate-centered neurometabolic coupling offer additional preclinical targets^{49,55,56}. Because these dependencies were largely established through tracing, cell-based

assays, or animal experiments, spatial analysis could help determine where they are relevant in human lesions and whether their distribution varies with tumor origin or treatment. Such comparisons could inform future studies of treatment stratification without assuming that a mechanism observed in one model is shared by all BrMs.

Clinical evaluation of these strategies remains at an early stage. Gamitinib has entered a phase I trial in advanced cancers (NCT04827810), whereas TVB-2640 (denifanstat) was evaluated with bevacizumab in recurrent high-grade astrocytoma, not specifically in BrMs (NCT03032484). Registered ketogenic-diet studies include patients with BrMs (NCT05428852; NCT05564949), and address feasibility or clinical outcomes rather than selection by a spatial metabolic signature. The next translational step is to connect a defined intervention to a measurable patient state and an appropriate intracranial endpoint.

Imaging offers a complementary route to translation by following lesion features that cannot be sampled repeatedly. In lung-derived BrM models, oxygen-enhanced imaging localized hypoxia, and hypoxia image-guided radiotherapy improved tumor control and animal survival⁷⁷. This provides a preclinical example of a regional physiological measurement informing treatment. In patients, amino-acid PET has a more developed framework for acquisition and response assessment through the joint guideline from the European Association of Nuclear Medicine (EANM), European Association of Neuro-Oncology (EANO), Response Assessment in Neuro-Oncology (RANO) working group, and Society of Nuclear Medicine and Molecular Imaging (SNMMI) and PET RANO BM 1.0 criteria^{111,112}. Serial uptake measurements can complement MRI assessment¹¹¹, as illustrated by longitudinal O-(2-[¹⁸F]fluoroethyl)-L-tyrosine (¹⁸F-FET) PET studies in melanoma- and lung-cancer BrMs¹¹³. The PURSUE study further addressed the specific problem of distinguishing recurrence from treatment-related change after radiotherapy. Among 23 patients with MRI-indeterminate lesions and histopathological verification, an ¹⁸F-fluciclovine maximum standardized uptake value (SUVmax) threshold derived in the study yielded 80% sensitivity and 85% specificity¹¹⁴. *The threshold* requires independent validation beyond this small, surgically selected cohort; the study did not establish that PET-guided management improves outcomes.

Delayed glucose-tracer imaging illustrates both the potential and the limitations of an additional diagnostic readout. In a retrospective series of 34 irradiated BrM patients, a delayed 2-deoxy-2-[¹⁸F]fluoro-D-glucose (¹⁸F-FDG) tumor-to-background SUVmax ratio threshold yielded 94.1% specificity but 58.8% sensitivity for recurrent disease¹¹⁵. No individual PET metric outperformed MRI overall, only ten patients had histopathological verification, and the threshold was derived within the cohort. Delayed FDG-PET may therefore provide supplementary information in selected indeterminate cases, rather than replace MRI or establish a tissue-level metabolic dependency.

Magnetic resonance spectroscopy (MRS) can address a different translational question by measuring a localized metabolic response to an intervention. In a randomized 20-patient pharmacodynamic study, serial single-voxel ¹H-MRS showed a decrease in the lactate-to-creatine peak-area ratio after intravenous L-arginine compared with saline. The largest descriptive median reduction occurred in the 30-g group, but baseline imbalance limited dose comparisons, and five patients per group with single-dose follow-up supported feasibility rather than therapeutic efficacy⁹⁸. Such measurements could provide pharmacodynamic readouts alongside tissue-derived hypotheses, although a change in a metabolite ratio is not a direct measure of flux.

A longitudinal study could connect tissue discovery to these clinical readouts at the first clinically indicated BrM resection. Co-registering a spatial map with MRI and, where available, amino-acid PET would allow candidate tissue states to be related to imaging features and cerebrospinal fluid (CSF) or plasma analytes. Serial imaging and liquid biopsy, with CSF obtained when clinically indicated, could then follow treatment; repeat tissue would be collected only at clinically indicated re-resection or autopsy. Existing liquid-biopsy studies show why the sampling compartment matters. In 92 patients with newly diagnosed NSCLC BrMs, an early fall in CSF circulating tumor DNA (ctDNA) tracked intracranial response more closely than plasma, whereas plasma ctDNA was undetectable in all 13 patients with intracranial-only melanoma in a separate cohort^{116,117}. These findings support CNS-aware monitoring but have not yet connected serial ctDNA changes to specific spatial metabolic states. That link, and the added value of CSF or plasma metabolomics, would need to be tested prospectively.

5.3 Spatial niche states as candidate biomarkers

Spatial immune features and metabolism-associated tissue states offer different kinds of biomarker candidates. Some are associated with treatment response or survival, whereas others primarily identify regions in which a metabolic mechanism could be investigated. Distinguishing these uses helps avoid treating an immune neighborhood, an enzyme-expression signal, and a validated metabolic readout as equivalent.

Clinical studies provide emerging support for considering immune-cell arrangement alongside phenotype. Human BrM imaging and tissue analyses linked highly invasive, immune-cold lesions to chitinase-3-like protein 1 (CHI3L1), with complementary experiments supporting a role in the immune phenotype³⁵. In melanoma BrMs, clustered T-cell infiltration was associated with immune checkpoint blockade response and survival¹¹⁸. These findings suggest that the arrangement of immune cells and the invasive interface may carry clinically relevant information, although neither is, by itself, a metabolic biomarker.

Macrophage studies further show why cell identity and location should be considered together. IMC in treatment-naïve breast-cancer BrMs identified a cluster of differentiation 163 (CD163)-positive macrophage-exhausted T-cell neighborhood associated with immune escape¹¹⁹. By contrast, spatial and single-nucleus profiling across lung-, breast-, and melanoma-derived BrMs associated tumor-contacting human leukocyte antigen DR (HLA-DR)-positive inflammatory macrophages with treatment responsiveness and longer survival, while macrophages associated with extracellular matrix (ECM) and transforming growth factor β 1 (TGF β 1) programs tracked with poorer outcomes¹²⁰. These studies support evaluating specific macrophage states rather than interpreting macrophage abundance uniformly. Their different populations and endpoints do not establish a common metabolic biomarker across tumor origins.

In experimental BrMs, spatial profiling can also be paired with perturbation to test immune mechanisms. In a breast-cancer BrM mouse model, spatial transcriptomics localized arginase 1 (ARG1)-positive macrophages to the invasive edge; tumor-cell interleukin 34 (IL34) deletion prevented cerebellar tumor establishment, and colony-stimulating factor 1 receptor (CSF1R) blockade reduced

tumor growth, while human tissue supported IL34 expression¹²¹. This links a spatially localized macrophage state to a targetable signaling pathway in the model, but does not establish regional arginine flux or validate IL34/ARG1 as a clinical response assay.

Metabolism-associated immune states remain less directly connected to clinical decisions. In LUAD BrMs, integrated multi-omic analysis linked glycolytic signatures and lactate-related remodeling to reduced cytotoxic immune features and immunosuppressive programs⁴³. This provides a rationale for testing whether a glycolytic-immune state is associated with treatment response.

Tryptophan-catabolic programs illustrate how spatial data can refine such a hypothesis. GeoMx profiling of NSCLC primary tumors and BrMs identified reduced antigen-presentation and lymphocyte programs in the brain metastatic microenvironment¹¹. Within tumor-adjacent brain, interleukin-4-induced protein 1 (IL4I1) expression was higher than in control brain, while indoleamine 2,3-dioxygenases 1 and 2 (IDO1/IDO2) and tryptophan 2,3-dioxygenase 2 (TDO2) were higher in nonfibrotic than highly fibrotic regions. Given the immunoregulatory role of tryptophan-derived aryl hydrocarbon receptor (AHR) ligands^{11,122}, these RNA-level differences nominate a locally variable mechanism rather than demonstrate increased pathway activity. The same NSCLC analysis associated highly fibrotic tumor-adjacent regions with greater inferred M2-macrophage abundance and hypoxia-related programs, whereas nonfibrotic regions had greater inferred cluster of differentiation 8 (CD8)-positive T-cell abundance¹¹. Fibrosis may therefore help define the cellular context in which tryptophan-catabolic signals are interpreted, but neither the enzyme-expression pattern nor the immune-fibrotic state was validated as a treatment-response biomarker.

Vascular organization may provide another context for metabolism-associated immune variation. In gastric-cancer BrMs, angiogenic and vessel-co-opting regions differed in immune composition and features related to oxidized nicotinamide adenine dinucleotide (NAD⁺), including nicotinamide phosphoribosyltransferase (NAMPT) expression¹³. This associates a candidate metabolic signal with vascular and cellular context, without measuring regional NAD⁺ turnover. Similarly, Visium profiling of mouse BrM sections identified enrichment of translation, ribosomal, and selenoamino-acid-metabolism pathways in thalamic S1 regions, defined as tumor-surrounding spots within

approximately 105 μm of the tumor border¹²³. Such maps can nominate regional features for biomarker evaluation, although their associations with treatment response or patient outcomes require further investigation.

Clinical development should therefore start with the decision a candidate marker is intended to inform. An assay proposed to predict treatment benefit should distinguish response to a specified therapy from general prognosis. A monitoring assay should add useful information to serial MRI and established response assessment, while a recurrence classifier should be tested against pathology where available and rigorous follow-up. Single markers and composite spatial signatures are both legitimate candidates; the latter should demonstrate added value over simpler assays, tumor origin, treatment history, and other relevant clinical predictors. Independent validation in the intended setting, followed by evaluation of how the result changes management, is more informative than requiring every biomarker to describe an entire niche.

6. Challenges and future directions

Clinical access determines which BrMs are available for study, while tissue handling and preservation influence how accurately measurements reflect their metabolic state. Most specimens come from surgically accessible, clinically advanced, symptomatic, or treatment-exposed lesions, leaving early colonization, micrometastatic and dormant states underrepresented^{12,74,85}. Cold ischemia, handling and preservation can also alter labile metabolites¹²⁴, while interactions with metabolically responsive host cells add to the biological complexity¹⁰. Differences between tissue maps may therefore reflect disease stage and sample processing as well as tumor biology.

Combining complementary tissue sources could help address this sampling bias, while imaging and clinically indicated CSF profiling could provide longitudinal follow-up. The first clinically indicated BrM resection can provide a useful sampling point, although it is not necessarily early in colonization or before treatment. Matched primary, extracranial-metastasis and BrM tissue can inform evolutionary relationships and site-associated programs, while rapid-autopsy collections can capture microscopic foci; these cross-sectional designs do not follow occult colonization or dormancy in the same patient^{82,125,126}. For example, rapid-autopsy tissue from two patients with TNBC and two with HER2-

positive breast cancer contained microscopic brain metastases with perivascular or spheroidal growth patterns, extending anatomical observations beyond surgically resected macrometastases¹²⁷. Combining surgical and autopsy collections across centers could broaden the disease states represented, with treatment histories helping to interpret differences between samples. Serial imaging or clinically indicated CSF profiling can extend follow-up to unresected lesions, without research-only biopsy of small, deep or eloquent-region lesions.

When repeat tissue sampling is clinically indicated, spatial analysis can add a temporal dimension to these comparisons. A metastatic TNBC cohort of extracranial disease illustrates the value of comparing primary, pretreatment metastatic and on-treatment specimens¹²⁸. In BrMs, opportunities are more restricted and should be connected to the imaging and liquid-biopsy follow-up outlined in Section 5.2. Such follow-up can track disease change between tissue collections, but does not reconstruct the intervening spatial metabolic states.

The central technical challenge is to connect a regional molecular pattern to metabolic function. Enzyme regulation can separate transcript abundance from activity^{17,129}, and protein localization does not remove this distinction^{130,131}. Metabolite imaging and single-cell metabolomics provide complementary chemical measurements¹³², with increasing molecular coverage¹³³, but static abundance still cannot establish pathway activity^{9,133}. A claim of metabolic dependency needs selective perturbation linked to a relevant functional outcome, with controls for off-target effects. Flux estimation requires tracer design and quantitative interpretation appropriate to the pathway, including consideration of tracer availability, labeling time and metabolite pool sizes; isotope labeling alone does not establish a quantitative flux¹³⁴.

The studies reviewed in Section 4 provide a starting point for this validation. Bulk metabolite profiles of BrMs⁷⁴, spatial lipid maps^{67,68}, melanoma substrate-tracing experiments⁹³, and complementary tracing or perturbation studies in human brain tumors and BrM models of breast cancer and melanoma address different parts of the problem^{46,63,87}. Isotope-resolved imaging and tissue-slice tracing in other tumors offer additional experimental approaches^{135,136}. Notably, intraoperative glucose tracing with DESI-MSI resolved regional labeling in one human BrM³⁸. The next

priority is to reproduce such measurements across patients while accounting for tissue composition and tracer delivery. Model studies can then help test the functional significance of substrate use, as illustrated by the respiratory and biosynthetic role of NDUFB9⁸¹, without assuming that the same dependency has already been demonstrated in the mapped human regions.

Assigning metabolic activity to its cellular source is particularly important in the brain, where tumor cells coexist with metabolically active neural, glial and vascular cells. A key question is whether metabolites detected in tumor regions are synthesized by tumor cells or taken up from the surrounding environment. Isotope tracing can help assess substrate contributions and metabolic fate¹³⁴ and could be combined with cell-type-resolved localization and selective perturbation in experimental models to investigate these alternatives. For a proposed donor-recipient interaction, disrupting synthesis in the candidate donor and uptake in the recipient, while measuring labeled metabolites and recipient function, would help distinguish local production from supply. This design would test the proposed exchange rather than infer it from proximity.

Future technologies should be selected to resolve these biological questions. Co-registering molecular measurements with histopathology can relate inferred pathways to measured metabolites and tissue composition^{23,26,137,138}. Harmonized protocols and matched primary, extracranial and BrM collections, analyzed by tumor origin, genotype and treatment exposure, would make site-associated differences more interpretable^{25,27,74}. Regional biochemical and imaging measurements can help select questions for model-based tracing and perturbation^{54,58}. Three-dimensional reconstruction or artificial intelligence (AI)-assisted alignment may improve integration, but their accuracy and uncertainty need evaluation in held-out samples. Subsequent clinical validation should follow the intended use of a target or biomarker, as discussed in Section 5^{109,112}. The immediate priorities are therefore comparable sampling, regional functional testing and independent replication, rather than adding platforms without a defined biological or clinical question¹³⁹.

Cost and access will influence whether these designs can be reproduced beyond specialist centers. Discovery-scale spatial profiling remains expensive and computationally demanding, while protocol and analysis differences impede cross-center comparison¹³⁹. Shared cores could support

discovery, with targeted panels or multiplex imaging used for replication when they retain the relevant biological information. Common reference materials, open analysis pipelines and cross-center benchmarking would help broaden participation without requiring every center to operate the full discovery workflow.

7. Conclusion

Spatial omics changes the unit of analysis in BrMs from the average lesion to the local niche. Read alongside functional studies, these maps point to recurring roles for substrate use, lipid handling and mitochondrial support of growth, rather than a single metabolic program shared by all BrMs. Similar functions can involve different molecular routes, with tumor genotype, neighboring cells and local supply influencing their contribution. Spatial context therefore helps distinguish where a metabolite accumulates from which cells may produce, use or respond to it. Nor does a state observed in BrMs necessarily originate in the brain; pre-existing tumor features, selection during metastasis and local or treatment-induced changes may all contribute. The next step is to test which candidate metabolic processes sustain growth or immune suppression in the nominated regions, and in which biological settings. For clinical translation, corresponding tissue or imaging readouts will also need independent validation for a defined purpose, such as treatment stratification or response monitoring. By locating candidate metabolic vulnerabilities within specific cellular and tissue contexts, spatial omics can help prioritize interventions for testing in the BrM settings most likely to depend on those processes.

List of Abbreviations

3PG: 3-Phosphoglycerate
ABAT: 4-Aminobutyrate aminotransferase
ABHD12B: Abhydrolase domain-containing protein 12B
Acetyl-CoA: Acetyl-coenzyme A
ACSS2: Acetyl-CoA synthetase 2 (acyl-CoA synthetase short-chain family member 2)
ADP: Adenosine diphosphate
AFADESI-MSI: Air-flow-assisted desorption electrospray ionization mass spectrometry imaging
AHR: Aryl hydrocarbon receptor
AI: Artificial intelligence
ALKBH5: AlkB homolog 5
ARG1: Arginase 1
ATP: Adenosine triphosphate
BBB: Blood-brain barrier
BCAT1: Branched-chain amino-acid aminotransferase 1
BrM; Brain metastasis
BrMs: brain metastases
BTB: Blood-tumor barrier
CA-IX: Carbonic anhydrase IX
Cas9: CRISPR-associated protein 9
CCR2: C-C chemokine receptor 2
ccRCC: Clear-cell renal cell carcinoma
CD163: Cluster of differentiation 163
CD36: Cluster of differentiation 36
CD74: Cluster of differentiation 74
CD8: Cluster of differentiation 8
CHI3L1: Chitinase-3-like protein 1
CNS: Central nervous system
CoA: Coenzyme A
CODEX: CO-Detection by indEXing
CPT1: Carnitine palmitoyltransferase 1
CREB1: Cyclic adenosine monophosphate response element-binding protein 1
CRISPR: Clustered regularly interspaced short palindromic repeats
CSF: Cerebrospinal fluid
CSF1R: Colony-stimulating factor 1 receptor
ctDNA: Circulating tumor DNA

Cu-ATSM: Copper(II) diacetyl-bis(N⁴-methylthiosemicarbazone)
 Cyt c: Cytochrome c
 DESI-MSI: Desorption electrospray ionization mass spectrometry imaging
 DGE-CEST: Dynamic glucose-enhanced chemical exchange saturation transfer
 DON: 6-Diazo-5-oxo-L-norleucine
 DRP1: Dynamin-related protein 1
 DSP: Digital spatial profiling
 EAAT1; EAAT2: Excitatory amino-acid transporters 1 and 2
 EANM: European Association of Nuclear Medicine
 EANO: European Association of Neuro-Oncology
 ECM: Extracellular matrix
 EGFR: Epidermal growth factor receptor
 EMT: Epithelial-mesenchymal transition
 ERK: Extracellular signal-regulated kinase
 FABP7: Fatty-acid-binding protein 7
 FAK: Focal adhesion kinase
 FAO: Fatty-acid oxidation
 FASN: Fatty acid synthase
 FDG: 2-Deoxy-2-fluoro-D-glucose (¹⁸F-labeled in PET)
 FET: O-(2-Fluoroethyl)-L-tyrosine (¹⁸F-labeled in PET)
 FOXA2: Forkhead box A2
 G6P: Glucose-6-phosphate
 G6PD: Glucose-6-phosphate dehydrogenase (also termed G6PDH)
 GABA: γ-Aminobutyric acid
 GAD1: Glutamate decarboxylase 1
 Gln: Glutamine
 Glu: Glutamate
 GLS1: Glutaminase 1
 Glx: Combined glutamate-glutamine pool
 GluN2B: GluN2B subunit of the NMDAR, encoded by GRIN2B
 GRIN2B: Glutamate ionotropic receptor NMDA type subunit 2B (gene encoding GluN2B)
 H3K18la: Histone H3 lysine 18 lactylation
 HER2: Human epidermal growth factor receptor 2
 HIF-1α: Hypoxia-inducible factor 1α
 HK2: Hexokinase 2
 HLA-DR: Human leukocyte antigen DR, a major histocompatibility complex class II molecule

Hsp90: Heat shock protein 90
 IDO1; IDO2: Indoleamine 2,3-dioxygenases 1 and 2
 IGF2BP3: Insulin-like growth factor 2 messenger RNA-binding protein 3
 IL34: Interleukin 34
 IL4I1: Interleukin-4-induced protein 1
 IMC: Imaging mass cytometry
 LDH: Lactate dehydrogenase
 LDHA: Lactate dehydrogenase A
 LLC: Lewis lung carcinoma
 LOX: Lysyl oxidase
 LUAD: Lung adenocarcinoma
 MALDI-MSI: Matrix-assisted laser desorption/ionization mass spectrometry imaging
 MAPK: Mitogen-activated protein kinase
 MCT1: Monocarboxylate transporter 1
 MERFISH: Multiplexed error-robust fluorescence in situ hybridization
 MIF: Macrophage migration inhibitory factor
 miR-199b-5p: MicroRNA-199b-5p
 MET: MET receptor tyrosine kinase (hepatocyte growth factor receptor)
 mGluR1: Metabotropic glutamate receptor 1
 miR-32-3p: MicroRNA-32-3p
 MN: Molecular niche (MN4 and MN9 are study-defined niche labels)
 MRI: Magnetic resonance imaging
 mRNA: Messenger RNA
 MRS: Magnetic resonance spectroscopy
 MSI: Mass spectrometry imaging
 mTOR: Mechanistic target of rapamycin
 m⁶A: N⁶-Methyladenosine
 NAD⁺: Oxidized nicotinamide adenine dinucleotide
 NADH: Reduced nicotinamide adenine dinucleotide
 NADPH: Reduced nicotinamide adenine dinucleotide phosphate
 NAMPT: Nicotinamide phosphoribosyltransferase
 NDUFB9: NADH:ubiquinone oxidoreductase subunit B9
 NDUF54: NADH:ubiquinone oxidoreductase subunit S4
 NET; NETs: Neutrophil extracellular trap; neutrophil extracellular traps
 NF-κB: Nuclear factor κB
 NMDAR: N-Methyl-D-aspartate receptor

NMR: Nuclear magnetic resonance
 NO: Nitric oxide
 NSCLC: Non-small cell lung cancer
 Oma1: Mouse gene encoding the mitochondrial metalloprotease OMA1
 OXPHOS: Oxidative phosphorylation
 p53: Tumor protein p53
 PC: Phosphatidylcholine
 PD-1: Programmed cell death protein 1
 PDH: Pyruvate dehydrogenase
 PE: Phosphatidylethanolamine
 PET: Positron emission tomography
 PGAM1: Phosphoglycerate mutase 1
 PGC1 α : Peroxisome proliferator-activated receptor γ coactivator 1 α
 pGluN2B: Phosphorylated GluN2B
 PHGDH: Phosphoglycerate dehydrogenase
 PPP: Pentose phosphate pathway
 PPAR γ : Peroxisome proliferator-activated receptor γ
 PRICKLE1: Prickle planar cell polarity protein 1
 PTEN: Phosphatase and tensin homolog
 PUFA; PUFAs: Polyunsaturated fatty acid; polyunsaturated fatty acids
 RAC2: Rac family small GTPase 2
 RANO: Response Assessment in Neuro-Oncology
 RARRES2: Retinoic acid receptor responder 2 (gene encoding chemerin)
 SEC61G: SEC61 translocon subunit gamma
 REDCAT: Raman Enhanced Delineation of Cell Atlases in Tissues
 REST: RE1-silencing transcription factor
 RNA: Ribonucleic acid
 S1: Study-defined tumor-surrounding region comprising spatial spots within approximately 105 μ m of the tumor border
 SCD1: Stearoyl-CoA desaturase 1
 SHP2: SH2 domain-containing protein tyrosine phosphatase 2
 SIRP α : Signal regulatory protein α
 SNMMI: Society of Nuclear Medicine and Molecular Imaging
 Src: Proto-oncogene tyrosine-protein kinase Src
 SREBP1: Sterol regulatory element-binding protein 1
 SUVmax: Maximum standardized uptake value

T2-FLAIR: T2-weighted fluid-attenuated inversion recovery
TCA: Tricarboxylic acid
TDO2: Tryptophan 2,3-dioxygenase 2
TGF β 1: Transforming growth factor β 1
TME: Tumor microenvironment
TNBC: Triple-negative breast cancer
TRAP1: Tumor necrosis factor receptor-associated protein 1
VEGFA: Vascular endothelial growth factor A
VGF: VGF nerve growth factor inducible (neuropeptide precursor)
Wnt5a: Wnt family member 5A
xCT: Cystine-glutamate antiporter
 α -KG: α -Ketoglutarate
 α -KGDH: α -Ketoglutarate dehydrogenase

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H. Lan: Conceptualization; Methodology; Investigation (lead); Writing – original draft (lead); Visualization and Writing – review & editing. X. Wang: Visualization and writing – review & editing. Z. Zeng: Investigation and supervision. All authors have read and agreed to the submitted version of the manuscript.

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Conflicts of Interest

The authors declare no conflicts of interest.

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References

1. Bhambhani, H.P., Granucci, M., Rodrigues, A., Kakusa, B.W., and Hayden Gephart, M. (2020). The primary sites leading to brain metastases: Shifting trends at a tertiary care center. *Journal of Clinical Neuroscience* 80, 121-124. <https://doi.org/10.1016/j.jocn.2020.08.006>.
2. Lu, Y., Huang, Y., Zhu, C., Li, Z., Zhang, B., Sheng, H., Li, H., Liu, X., Xu, Z., Wen, Y., et al. (2025). Cancer brain metastasis: molecular mechanisms and therapeutic strategies. *Molecular Biomedicine* 6, 12. 10.1186/s43556-025-00251-0.
3. Pinto, M., Violante, S., Cascão, R., and Faria, C.C. (2025). Unlocking the Role of Metabolic Pathways in Brain Metastatic Disease. *Cells* 14, 707.
4. Hsieh, W.-C., Budiarto, B.R., Wang, Y.-F., Lin, C.-Y., Gwo, M.-C., So, D.K., Tzeng, Y.-S., and Chen, S.-Y. (2022). Spatial multi-omics analyses of the tumor immune microenvironment. *Journal of Biomedical Science* 29, 96. 10.1186/s12929-022-00879-y.
5. Karimi, E., Yu, M.W., Maritan, S.M., Perus, L.J.M., Rezanejad, M., Sorin, M., Dankner, M., Fallah, P., Doré, S., Zuo, D., et al. (2023). Single-cell spatial immune landscapes of primary and metastatic brain tumours. *Nature* 614, 555-563. 10.1038/s41586-022-05680-3.
6. Du, Y., Ding, X., and Ye, Y. (2024). The spatial multi-omics revolution in cancer therapy: Precision redefined. *Cell Reports Medicine* 5. 10.1016/j.xcrm.2024.101740.
7. Kunachowicz, D., Tomecka, P., Sędzik, M., Kalinin, J., Kuźnicki, J., and Rembiałkowska, N. (2025). Influence of Hypoxia on Tumor Heterogeneity, DNA Repair, and Cancer Therapy: From Molecular Insights to Therapeutic Strategies. *Cells* 14, 1057.
8. Wu, C., Xu, T., Zhang, H., Hu, Y., Jiao, J., Qiu, K., Gu, J., Li, W., and Sun, L. (2025). Hypoxia and immunometabolism in the tumor microenvironment: insights into mechanisms and therapeutic potential. *Cancer Letters* 631, 217913. <https://doi.org/10.1016/j.canlet.2025.217913>.
9. Parida, P.K., and Malladi, S. (2025). Metabolic adaptations of brain metastasis. *Nature Reviews Cancer* 25, 723-739. 10.1038/s41568-025-00848-1.
10. Zhou, T., Yan, Y., Lin, M., Zeng, C., Mao, X., Zhu, Y., Han, J., Li, D.-D., and Zhang, J. (2025). Astrocyte involvement in brain metastasis: from biological mechanisms to therapeutic strategies. *Cancer and Metastasis Reviews* 44, 60. 10.1007/s10555-025-10276-0.
11. Zhang, Q., Abdo, R., Iosef, C., Kaneko, T., Cecchini, M., Han, V.K., and Li, S.S.-C. (2022). The spatial transcriptomic landscape of non-small cell lung cancer brain metastasis. *Nature Communications* 13, 5983. 10.1038/s41467-022-33365-y.

12. Biermann, J., Melms, J.C., Amin, A.D., Wang, Y., Caprio, L.A., Karz, A., Tagore, S., Barrera, I., Ibarra-Arellano, M.A., Andreatta, M., et al. (2022). Dissecting the treatment-naïve ecosystem of human melanoma brain metastasis. *Cell* **185**, 2591-2608.e2530. <https://doi.org/10.1016/j.cell.2022.06.007>.
13. Liu, K., Wang, Y., Wang, C., Guo, C., Zhang, D., Zhong, Y., Yin, L., Lu, Y., Liu, F., Zhang, Y., and Zhang, D. (2024). Spatial transcriptomics of gastric cancer brain metastasis reveals atypical vasculature strategies with supportive immune profiles. *Gastroenterol Rep (Oxf)* **12**, goae067. 10.1093/gastro/goae067.
14. Cheng, M., Jiang, Y., Xu, J., Mentis, A.-F.A., Wang, S., Zheng, H., Sahu, S.K., Liu, L., and Xu, X. (2023). Spatially resolved transcriptomics: a comprehensive review of their technological advances, applications, and challenges. *Journal of Genetics and Genomics* **50**, 625-640. <https://doi.org/10.1016/j.jgg.2023.03.011>.
15. Marx, V. (2021). Method of the Year: spatially resolved transcriptomics. *Nature Methods* **18**, 9-14. 10.1038/s41592-020-01033-y.
16. Ozirmak Lermi, N., Molina Ayala, M., Hernandez, S., Lu, W., Khan, K., Serrano, A., Lubo, I., Hamana, L., Tomczak, K., Barnes, S., et al. (2025). Comparison of imaging based single-cell resolution spatial transcriptomics profiling platforms using formalin-fixed paraffin-embedded tumor samples. *Nature Communications* **16**, 8499. 10.1038/s41467-025-63414-1.
17. Sawant Dessai, A., Kalhotra, P., Novickis, A.T., and Dasgupta, S. (2023). Regulation of tumor metabolism by post translational modifications on metabolic enzymes. *Cancer Gene Therapy* **30**, 548-558. 10.1038/s41417-022-00521-x.
18. Duncan, K.D., Pětrošová, H., Lum, J.J., and Goodlett, D.R. (2024). Mass spectrometry imaging methods for visualizing tumor heterogeneity. *Current Opinion in Biotechnology* **86**, 103068. <https://doi.org/10.1016/j.copbio.2024.103068>.
19. Petrova, B., and Guler, A.T. (2025). Recent Developments in Single-Cell Metabolomics by Mass Spectrometry—A Perspective. *Journal of Proteome Research* **24**, 1493-1518. <https://doi.org/10.1021/acs.jproteome.4c00646>.
20. Unsihuay, D., Mesa Sanchez, D., and Laskin, J. (2021). Quantitative Mass Spectrometry Imaging of Biological Systems. *Annu Rev Phys Chem* **72**, 307-329. 10.1146/annurev-physchem-061020-053416.
21. Wang, G., van den Berg, B.M., Kostidis, S., Pinkham, K., Jacobs, M.E., Liesz, A., Giera, M., and Rabelink, T.J. (2025). Spatial quantitative metabolomics enables identification of remote and sustained ipsilateral cortical metabolic reprogramming after stroke. *Nature Metabolism* **7**, 1791-1800. 10.1038/s42255-025-01340-8.
22. Samarah, L.Z., Zheng, C., Xing, X., Lee, W.D., Afriat, A., Chitra, U., MacArthur, M.R., Lu, W., Jankowski, C.S.R., Ma, C., et al. (2025). Spatial metabolic gradients in the liver and small intestine. *Nature* **648**, 182-190. 10.1038/s41586-025-09616-5.
23. Tian, R., Xue, Z., Chen, Y., Qi, Y., Zhang, J., Yuan, J., Ruan, D., Lin, J., Liu, J., Wang, D., et al. (2025). Integrating cross-sample and cross-modal data for spatial transcriptomics and metabolomics with SpatialMETA. *Nature Communications* **16**, 8855. 10.1038/s41467-025-63915-z.

24. Liu, X., Peng, T., Xu, M., Lin, S., Hu, B., Chu, T., Liu, B., Xu, Y., Ding, W., Li, L., et al. (2024). Spatial multi-omics: deciphering technological landscape of integration of multi-omics and its applications. *Journal of Hematology & Oncology* *17*, 72. 10.1186/s13045-024-01596-9.
25. Wess, M., Andersen, M.K., Midtbust, E., Guillem, J.C.C., Viset, T., Størkersen, Ø., Krossa, S., Rye, M.B., and Tessem, M.-B. (2025). Spatial integration of multi-omics data from serial sections using the novel Multi-Omics Imaging Integration Toolset. *GigaScience* *14*. 10.1093/gigascience/giaf035.
26. Kim, D.Y., Lee, J., Choi, J., Shin, H., Lee, J.S., and Kim, E.J. (2026). Spatial multi-omics in precision medicine: Integrating biological insights through multidisciplinary collaboration. *Seminars in Cancer Biology* *119*, 24-37. <https://doi.org/10.1016/j.semcancer.2025.12.009>.
27. Vicari, M., Mirzazadeh, R., Nilsson, A., Shariatgorji, R., Bjärterot, P., Larsson, L., Lee, H., Nilsson, M., Foyer, J., Ekvall, M., et al. (2024). Spatial multimodal analysis of transcriptomes and metabolomes in tissues. *Nature Biotechnology* *42*, 1046-1050. 10.1038/s41587-023-01937-y.
28. Li, Y., Zhang, Z., Enninfu, A., Farzad, N., Rajbhandari, P., Tian, H., Nam, J., Qin, X., Villazon, J., Fung, A.A., et al. (2026). All-optical multimodal mapping of single-cell-type-specific metabolic activities via REDCAT. *Nature Methods* *23*, 1840-1853. 10.1038/s41592-026-03180-0.
29. Tobar, L.E., Farnsworth, R.H., and Stacker, S.A. (2022). Brain Vascular Microenvironments in Cancer Metastasis. *Biomolecules* *12*, 401.
30. Lockman, P.R., Mittapalli, R.K., Taskar, K.S., Rudraraju, V., Gril, B., Bohn, K.A., Adkins, C.E., Roberts, A., Thorsheim, H.R., Gaasch, J.A., et al. (2010). Heterogeneous Blood–Tumor Barrier Permeability Determines Drug Efficacy in Experimental Brain Metastases of Breast Cancer. *Clinical Cancer Research* *16*, 5664-5678. 10.1158/1078-0432.Ccr-10-1564.
31. Bång-Rudenstam, A., Cerezo-Magaña, M., Horvath, M., Talbot, H., Gustafsson, E., Jonathan, S., Chakraborty, C., Nissen, I., Gonçalves de Oliveira, K., Boukredine, A., et al. (2026). Tumour acidosis remodels the glycocalyx to control lipid scavenging and ferroptosis. *Nature Cell Biology* *28*, 567-580. 10.1038/s41556-026-01879-y.
32. Berghoff, A.S., Rajky, O., Winkler, F., Bartsch, R., Furtner, J., Hainfellner, J.A., Goodman, S.L., Weller, M., Schittenhelm, J., and Preusser, M. (2013). Invasion patterns in brain metastases of solid cancers. *Neuro Oncol* *15*, 1664-1672. 10.1093/neuonc/not112.
33. Garzia, L. (2024). Astrocytes are enablers of brain metastases. *Nature Cell Biology* *26*, 1632-1633. 10.1038/s41556-024-01516-6.
34. Chen, P., Zuo, Y., Wang, M., Liu, R., Li, Y., Wang, Z., Huang, H., Luo, X., Wang, W., Wu, Y., and Yu, Z. (2025). Lung cancer cells upregulate stearyl-CoA desaturase 1 in microglia by activating the STAT3 pathway to change microglial inflammatory response in lung-to-brain metastases. *Cell Death Dis* *16*, 702. 10.1038/s41419-025-08003-2.
35. Maritan, S.M., Karimi, E., Dankner, M., Hernandez-Corchado, A., Yu, M.W., Annis, M.G., Aghazadeh Habashi, Y., Rezanejad, M., Liu, B., Koudieh, N., et al.

- (2026). Distinct immune landscapes characterize highly versus minimally invasive brain metastases. *JCI Insight* 11. 10.1172/jci.insight.199498.
36. Zeng, Q., Michael, I.P., Zhang, P., Saghafeina, S., Knott, G., Jiao, W., McCabe, B.D., Galván, J.A., Robinson, H.P.C., Zlobec, I., et al. (2019). Synaptic proximity enables NMDAR signalling to promote brain metastasis. *Nature* 573, 526-531. 10.1038/s41586-019-1576-6.
 37. Yoo, J., Park, I., Kim, H.J., Park, H.H., Lee, S., Kim, J.H., and Cha, Y.J. (2026). Spatial Transcriptomic Landscape of Brain Metastases from Triple-Negative Breast Cancer: Comparison of Primary Tumor and Brain Metastases Using Spatial Analysis. *Cancer Res Treat* 58, 182-197. 10.4143/crt.2025.033.
 38. Tsyben, A., Dannhorn, A., Hamm, G., Pitoulis, M., Couturier, D.-L., Sawle, A., Briggs, M., Wright, A.J., Brodie, C., Mendil, L., et al. (2025). Cell-intrinsic metabolic phenotypes identified in patients with glioblastoma, using mass spectrometry imaging of ¹³C-labelled glucose metabolism. *Nature metabolism* 7, 928-939. 10.1038/s42255-025-01293-y.
 39. Palmieri, D., Fitzgerald, D., Shreeve, S.M., Hua, E., Bronder, J.L., Weil, R.J., Davis, S., Stark, A.M., Merino, M.J., Kurek, R., et al. (2009). Analyses of resected human brain metastases of breast cancer reveal the association between up-regulation of hexokinase 2 and poor prognosis. *Mol Cancer Res* 7, 1438-1445. 10.1158/1541-7786.Mcr-09-0234.
 40. Zhou, C., Yang, Y., Cui, H., Li, S., Wang, Z., Chen, L., Feng, M., Li, D., Chen, X., Hao, B., et al. (2025). SEC61G Facilitates Brain Metastases via Antagonizing PGAM1 Ubiquitination and Immune Microenvironment Remodeling in Non-Small Cell Lung Cancer. *Int J Biol Sci* 21, 1436-1458. 10.7150/ijbs.109187.
 41. Dong, T., Liu, Z., Xuan, Q., Wang, Z., Ma, W., and Zhang, Q. (2017). Tumor LDH-A expression and serum LDH status are two metabolic predictors for triple negative breast cancer brain metastasis. *Scientific Reports* 7, 6069. 10.1038/s41598-017-06378-7.
 42. Liu, S., Bai, Z., Qiao, Y., Ge, Y., Qu, F., Li, M., Wang, J., Shi, X., Ma, Y., Li, J., et al. (2026). Lactate dehydrogenase A-mediated CREB1 lactylation promotes breast cancer brain metastasis by transcriptionally activating cytoskeletal remodeling. *Neuro-Oncology* 28, 1970-1985. 10.1093/neuonc/noag114.
 43. Yi, Y., Xu, W., Yu, H., Luo, Y., Zeng, F., Luo, D., Zeng, Z., Xiong, L., Huang, L., Cai, J., and Liu, A. (2025). Integrated multi-omics reveals glycolytic gene signatures of lung adenocarcinoma brain metastasis and the impact of Rac2 lactylation on immunosuppressive microenvironment. *J Transl Med* 23, 1193. 10.1186/s12967-025-07207-6.
 44. Cha, Y.J., Jung, W.H., and Koo, J.S. (2017). Differential Site-Based Expression of Pentose Phosphate Pathway-Related Proteins among Breast Cancer Metastases. *Dis Markers* 2017, 7062517. 10.1155/2017/7062517.
 45. Chen, E.I., Hewel, J., Krueger, J.S., Tiraby, C., Weber, M.R., Kralli, A., Becker, K., Yates, J.R., III, and Felding-Habermann, B. (2007). Adaptation of Energy Metabolism in Breast Cancer Brain Metastases. *Cancer Research* 67, 1472-1486. 10.1158/0008-5472.Can-06-3137.
 46. Mashimo, T., Pichumani, K., Vemireddy, V., Hatanpaa, K.J., Singh, D.K., Sirasanagandla, S., Nannepaga, S., Piccirillo, S.G., Kovacs, Z., Foong, C., et al.

- (2014). Acetate is a bioenergetic substrate for human glioblastoma and brain metastases. *Cell* 159, 1603-1614. 10.1016/j.cell.2014.11.025.
47. Esquea, E.M., Ciraku, L., Young, R.G., Merzy, J., Talarico, A.N., Ahmed, N.N., Karuppiah, M., Ramesh, A., Chatoff, A., Crispim, C.V., et al. (2024). Selective and brain-penetrant ACSS2 inhibitors target breast cancer brain metastatic cells. *Front Pharmacol* 15, 1394685. 10.3389/fphar.2024.1394685.
 48. Young, R.G., Esquea, E.M., Ciraku, L., Merzy, J., Ahmed, N.N., Talarico, A.N., Karuppiah, M., Medori, S.M., Warade, R.P., Gocal, W., et al. (2026). ACSS2 Suppresses Ferroptosis to Drive Breast Cancer Brain Metastasis. *Cancer Research*. 10.1158/0008-5472.Can-25-3006.
 49. Ngo, B., Kim, E., Osorio-Vasquez, V., Doll, S., Bustraen, S., Liang, R.J., Luengo, A., Davidson, S.M., Ali, A., Ferraro, G.B., et al. (2020). Limited Environmental Serine and Glycine Confer Brain Metastasis Sensitivity to PHGDH Inhibition. *Cancer Discov* 10, 1352-1373. 10.1158/2159-8290.Cd-19-1228.
 50. Mao, L., Wang, L., Lyu, Y., Zhuang, Q., Li, Z., Zhang, J., Gu, Z., Lu, S., Wang, X., Guan, Y., et al. (2024). Branch Chain Amino Acid Metabolism Promotes Brain Metastasis of NSCLC through EMT Occurrence by Regulating ALKBH5 activity. *Int J Biol Sci* 20, 3285-3301. 10.7150/ijbs.85672.
 51. Wu, Y., Derks, S., Wood, T.C., de Blois, E., van der Veldt, A.A.M., Smits, M., and Warnert, E.A.H. (2023). Improved postprocessing of dynamic glucose-enhanced CEST MRI for imaging brain metastases at 3 T. *Eur Radiol Exp* 7, 78. 10.1186/s41747-023-00390-5.
 52. Cappelletto, N.I.C., Soliman, H., Uthayakumar, B., Sahgal, A., Bragagnolo, N.D., Chen, A.P., Endre, R., Ma, N., Perks, W.J., Detsky, J.S., et al. (2025). Hyperpolarized (13)C lactate-to-bicarbonate signal ratio predicts brain metastases response to stereotactic radiosurgery. *Neurooncol Adv* 7, vdaf121. 10.1093/noajnl/vdaf121.
 53. Cappelletto, N.I.C., Soliman, H., Uthayakumar, B., Sahgal, A., Chen, A.P., Endre, R., Ma, N., Perks, W.J., Detsky, J.S., Heyn, C., and Cunningham, C.H. (2026). Widespread elevated hyperpolarized 13C-lactate and 13C-bicarbonate signal in the brain of patients with brain metastases undergoing treatment. *Scientific Reports*. 10.1038/s41598-026-67063-2.
 54. Tang, J., Zhao, Y., Chen, L., Liu, Y., Xie, H., Wu, Z., Zhang, L., Fan, D., Xu, X., Ai, L., et al. (2025). Distribution of glutamate and glutamine in tumor microenvironments of brain metastasis: Insights from biochemical analysis and molecular imaging. *iScience* 28. 10.1016/j.isci.2025.113371.
 55. Ishibashi, K., Ichinose, T., Kadokawa, R., Mizutani, R., Iwabuchi, S., Togi, S., Ura, H., Tange, S., Shinjo, K., Nakayama, J., et al. (2024). Astrocyte-induced mGluR1 activates human lung cancer brain metastasis via glutamate-dependent stabilization of EGFR. *Developmental Cell* 59, 579-594.e576. 10.1016/j.devcel.2024.01.010.
 56. Ruan, X., Yan, W., Cao, M., Daza, R.A.M., Fong, M.Y., Yang, K., Wu, J., Liu, X., Palomares, M., Wu, X., et al. (2024). Breast cancer cell-secreted miR-199b-5p hijacks neurometabolic coupling to promote brain metastasis. *Nature Communications* 15, 4549. 10.1038/s41467-024-48740-0.

57. Di Russo, S., Borsatti, G.E., Bouzidi, A., Liberati, F.R., Riva, A., Tripodi, F., Rolfi, L.R., Spizzichino, S., Tramutola, A., Perluigi, M., et al. (2025). NF- κ B-mediated cytokine secretion and glutamate metabolic reprogramming converge in breast cancer brain tropism. *Cancer Letters* 630, 217907. 10.1016/j.canlet.2025.217907.
58. Parida, P.K., Marquez-Palencia, M., Nair, V., Kaushik, A.K., Kim, K., Sudderth, J., Quesada-Diaz, E., Cajigas, A., Vemireddy, V., Gonzalez-Ericsson, P.I., et al. (2022). Metabolic diversity within breast cancer brain-tropic cells determines metastatic fitness. *Cell Metabolism* 34, 90-105.e107. 10.1016/j.cmet.2021.12.001.
59. Schnepf, P.M., Lee, D.D., Guldner, I.H., O'Tighearnagh, T.K., Howe, E.N., Palakurthi, B., Eckert, K.E., Toni, T.A., Ashfeld, B.L., and Zhang, S. (2017). GAD1 Upregulation Programs Aggressive Features of Cancer Cell Metabolism in the Brain Metastatic Microenvironment. *Cancer Research* 77, 2844-2856. 10.1158/0008-5472.CAN-16-2289.
60. Xie, M., Qin, H., Liu, L., Wu, J., Zhao, Z., Zhao, Y., Fang, Y., Yu, X., and Su, C. (2025). GABA regulates metabolic reprogramming to mediate the development of brain metastasis in non-small cell lung cancer. *Journal of Experimental & Clinical Cancer Research* 44, 61. 10.1186/s13046-025-03315-9.
61. Wu, S.-Y., Tyagi, A., Smith, E.C., Lee, J.S., Perng, P.S., Kuo, Y.-M., Wang, T.-F., Chen, H.-F., Deshpande, R.P., Kim, J.W., et al. (2026). GABA shunt metabolism drives smoking-related peri-neuronal brain metastasis by hijacking synaptic signaling. *Neuro-Oncology*. 10.1093/neuonc/noag201.
62. Tyagi, A., Wu, S.Y., and Watabe, K. (2022). Metabolism in the progression and metastasis of brain tumors. *Cancer Lett* 539, 215713. 10.1016/j.canlet.2022.215713.
63. Ferraro, G.B., Ali, A., Luengo, A., Kodack, D.P., Deik, A., Abbott, K.L., Bezwada, D., Blanc, L., Prideaux, B., Jin, X., et al. (2021). FATTY ACID SYNTHESIS IS REQUIRED FOR BREAST CANCER BRAIN METASTASIS. *Nat Cancer* 2, 414-428. 10.1038/s43018-021-00183-y.
64. Cordero, A., Kanojia, D., Miska, J., Panek, W.K., Xiao, A., Han, Y., Bonamici, N., Zhou, W., Xiao, T., Wu, M., et al. (2019). FABP7 is a key metabolic regulator in HER2+ breast cancer brain metastasis. *Oncogene* 38, 6445-6460. 10.1038/s41388-019-0893-4.
65. Li, J., Ouyang, S., Lin, Z., Peng, K., Yan, J., Lu, M., Ding, W., Mo, J., Su, Y., Wang, L., et al. (2025). The IGF2BP3–FASN axis drives lipid metabolic reprogramming to promote brain colonization in non-small cell lung cancer. *Cell Death & Disease* 16, 684. 10.1038/s41419-025-08006-z.
66. Li, Y.-Q., Sun, F.-Z., Li, C.-X., Mo, H.-N., Zhou, Y.-T., Lv, D., Zhai, J.-T., Qian, H.-L., and Ma, F. (2023). RARRES2 regulates lipid metabolic reprogramming to mediate the development of brain metastasis in triple negative breast cancer. *Military Medical Research* 10, 34. 10.1186/s40779-023-00470-y.
67. Roux, A., Winnard, P.T., Van Voss, M.H., Muller, L., Jackson, S.N., Hoffer, B., Woods, A.S., and Raman, V. (2023). MALDI-MSI of lipids in a model of breast cancer brain metastasis provides a surrogate measure of ischemia/hypoxia.

- Molecular and Cellular Biochemistry 478, 2567-2580. 10.1007/s11010-023-04685-4.
68. Chen, B., Kiang, K.M., Liu, F., Li, C., Li, X., Weiwei, C., Fu, X., Xiao, G., Sun, J., Da, E., et al. (2026). Neutrophil Extracellular Trap Reprograms Cancer Metabolism to Form a Metastatic Niche Promoting Non-Small Cell Lung Cancer Brain Metastasis. *Adv Sci (Weinh)* 13, e08478. 10.1002/adv.202508478.
 69. Yang, Y., Gao, Y., Qi, Z., Li, S., Wang, Z., Li, D., Chen, L., Zhou, C., Feng, M., Chen, X., et al. (2026). Single-cell and spatial transcriptomes reveal oligodendrocyte remodeling fatty acid metabolism microenvironment of lung cancer brain metastases. *Journal of Translational Medicine* 24. 10.1186/s12967-026-08447-w.
 70. Laue, K., Pozzi, S., Zerbib, J., Bertolio, R., Eliezer, Y., Cohen-Sharir, Y., Winkler, T., Caputo, M., Ricci, A.A., Adler, L., et al. (2026). p53 inactivation drives breast cancer metastasis to the brain through SCD1 upregulation and increased fatty acid metabolism. *Nature Genetics* 58, 116-131. 10.1038/s41588-025-02446-1.
 71. Zou, Y., Watters, A., Cheng, N., Perry, C.E., Xu, K., Alicea, G.M., Parris, J.L.D., Baraban, E., Ray, P., Nayak, A., et al. (2019). Polyunsaturated Fatty Acids from Astrocytes Activate PPAR γ Signaling in Cancer Cells to Promote Brain Metastasis. *Cancer Discovery* 9, 1720-1735. 10.1158/2159-8290.Cd-19-0270.
 72. Greene, J., Segaran, A., and Lord, S. (2022). Targeting OXPHOS and the electron transport chain in cancer; Molecular and therapeutic implications. (Elsevier), pp. 851-859.
 73. Porporato, P.E., Filigheddu, N., Pedro, J.M.B.-S., Kroemer, G., and Galluzzi, L. (2018). Mitochondrial metabolism and cancer. *Cell Research* 28, 265-280. 10.1038/cr.2017.155.
 74. Yang, Z., Wei, S., Duan, H., Wang, X., Zhang, D., Kiang, K.M.-Y., Deng, Y., Yang, Y., Ku, Y., Hu, W., et al. (2026). A proteogenomic atlas of 1032 brain metastases identifies molecular subtypes, immune landscapes, and therapeutic vulnerabilities. *Nature Communications* 17, 2038. 10.1038/s41467-026-68748-y.
 75. Guedes, J., Woldmar, N., Baltas, E., Kriston, A., Migh, E., Kovács, F., Oskolás, H., Marko-Varga, M., Appelqvist, R., Wieslander, E., et al. (2026). AI-based digital pathology and spatial proteomics enable precision oncology: A case report of recurrent melanoma in a young patient. *npj Precision Oncology* 10. 10.1038/s41698-026-01569-w.
 76. Fantin, J., Toutain, J., Pérès, E.A., Bernay, B., Mehani, S.M., Helaine, C., Bourgeois, M., Brunaud, C., Chazalviel, L., Pontin, J., et al. (2023). Assessment of hypoxia and oxidative-related changes in a lung-derived brain metastasis model by [(64)Cu][Cu(ATSM)] PET and proteomic studies. *EJNMMI Res* 13, 102. 10.1186/s13550-023-01052-8.
 77. Corroyer-Dulmont, A., Valable, S., Fantin, J., Chatre, L., Toutain, J., Teulier, S., Bazille, C., Letissier, E., Levallet, J., Divoux, D., et al. (2021). Multimodal evaluation of hypoxia in brain metastases of lung cancer and interest of hypoxia image-guided radiotherapy. *Scientific Reports* 11, 11239. 10.1038/s41598-021-90662-0.
 78. Alvaro-Espinosa, L., Marquez-Galera, A., Priego, N., García-Calvo, V., Perea-García, M., Hernandez-Oliver, C., Retana, D., Sanchez, O., de Pablos-

- Aragoneses, A., García-Gómez, P., et al. (2026). MIF-Induced CD74+ Microglia and Macrophages Promote Progression of Brain Metastasis and Are Clinically Relevant across Central Nervous System Disorders. *Cancer Res* 86, 3249-3269. 10.1158/0008-5472.Can-25-4018.
79. Tsai, Y.-T., Mackert, J.D., Wilson, A., Kooshki, M., Payne, V., Sagastume, J.J., Szymonski, A., Westwood, B., Miller, L.D., Triozzi, P.L., et al. (2026). Cancer-intrinsic SIRP α Signaling Triggers Mitochondrial Fission and Immune Tolerance to Promote TNBC Breast-to-Brain Metastasis. *Neuro-Oncology*. 10.1093/neuonc/noag202.
 80. Wang, Y., Sheng, X., Yang, Y., Liu, C., Wang, S., Guo, R., Gu, Y., Chen, M., Zhang, H., Du, J., et al. (2026). VGF-Mediated Mitochondrial Remodeling Fuels Lung Adenocarcinoma Brain Metastasis. *Cancer Research* 86, 2104-2125. 10.1158/0008-5472.Can-25-2712.
 81. Lin, M., Wen, Z., Zeng, C., Jin, Y., Zhou, T., Yan, Y., Huang, S., Hu, X., Guan, X., Hu, X., and Zhang, J. (2026). Complex I protein NDUFB9 is a metabolic vulnerability in triple negative breast cancer brain metastases. *Nature Communications* 17. 10.1038/s41467-026-72927-2.
 82. Fukumura, K., Malgulwar, P.B., Fischer, G.M., Hu, X., Mao, X., Song, X., Hernandez, S.D., Zhang, X.H., Zhang, J., Parra, E.R., et al. (2021). Multi-omic molecular profiling reveals potentially targetable abnormalities shared across multiple histologies of brain metastasis. *Acta Neuropathol* 141, 303-321. 10.1007/s00401-020-02256-1.
 83. Xiao, L., Zhou, J., Liu, H., Zhou, Y., Chen, W., Cui, W., and Zhao, Y. (2021). RNA Sequence Profiling Reveals Unique Immune and Metabolic Features of Breast Cancer Brain Metastases. *Front Oncol* 11, 679262. 10.3389/fonc.2021.679262.
 84. Xiao, Y., Hu, F., and Chi, Q. (2024). Single-cell RNA sequencing and spatial transcriptome reveal potential molecular mechanisms of lung cancer brain metastasis. *International Immunopharmacology* 140, 112804. <https://doi.org/10.1016/j.intimp.2024.112804>.
 85. Xing, X., Zhong, J., Biermann, J., Duan, H., Zhang, X., Shi, Y., Gao, Y., He, K., Zhai, D., Luo, F., et al. (2025). Pan-cancer human brain metastases atlas at single-cell resolution. *Cancer Cell* 43, 1242-1260.e1249. 10.1016/j.ccell.2025.03.025.
 86. Fischer, G.M., Guerrieri, R.A., Hu, Q., Joon, A.Y., Kumar, S., Haydu, L.E., McQuade, J.L., Vashisht Gopal, Y.N., Knighton, B., Deng, W., et al. (2021). Clinical, molecular, metabolic, and immune features associated with oxidative phosphorylation in melanoma brain metastases. *Neurooncol Adv* 3, vdaa177. 10.1093/noajnl/vdaa177.
 87. Guerrieri, R.A., Fischer, G.M., Kircher, D.A., Joon, A.Y., Cortez, J.R., Grossman, A.H., Hudgens, C.W., Ledesma, D.A., Lazcano, R., Onana, C.Y., et al. (2025). Oxidative Phosphorylation (OXPHOS) Promotes the Formation and Growth of Melanoma Lung and Brain Metastases. *bioRxiv*, 2025.2001.2023.633049. 10.1101/2025.01.23.633049.
 88. Duan, H., Ren, J., Wei, S., Yang, Z., Li, C., Wang, Z., Li, M., Wei, Z., Liu, Y., Wang, X., et al. (2024). Integrated analyses of multi-omic data derived from paired primary lung cancer and brain metastasis reveal the metabolic

- vulnerability as a novel therapeutic target. *Genome Medicine* 16, 138. 10.1186/s13073-024-01410-8.
89. Burns, T.F., Dacic, S., Chakka, A., Miller, E., Velez, M.A., Somasundaram, A., Bhattacharya, S., Gaither-Davis, A., Devadassan, P., and Jin, J. (2026). MET alterations are enriched in lung adenocarcinoma brain metastases, defining a distinct biologic subtype. *The Journal of Clinical Investigation* 136.
 90. Harding, J.J., Telli, M., Munster, P., Voss, M.H., Infante, J.R., DeMichele, A., Dunphy, M., Le, M.H., Molineaux, C., Orford, K., et al. (2021). A Phase I Dose-Escalation and Expansion Study of Telaglenastat in Patients with Advanced or Metastatic Solid Tumors. *Clinical Cancer Research* 27, 4994-5003. 10.1158/1078-0432.Ccr-21-1204.
 91. Gouda, M.A., Voss, M.H., Tawbi, H., Gordon, M., Tykodi, S.S., Lam, E.T., Vaishampayan, U., Tannir, N.M., Chaves, J., Nikolinakos, P., et al. (2025). A phase I/II study of the safety and efficacy of telaglenastat (CB-839) in combination with nivolumab in patients with metastatic melanoma, renal cell carcinoma, and non-small-cell lung cancer. *ESMO Open* 10, 104536. 10.1016/j.esmoop.2025.104536.
 92. Serhan, H.A., Bao, L., Cheng, X., Qin, Z., Liu, C.-J., Heth, J.A., Udager, A.M., Soellner, M.B., Merajver, S.D., Morikawa, A., and Merrill, N.M. (2024). Targeting fatty acid synthase in preclinical models of TNBC brain metastases synergizes with SN-38 and impairs invasion. *npj Breast Cancer* 10, 43. 10.1038/s41523-024-00656-0.
 93. Fischer, G.M., Jalali, A., Kircher, D.A., Lee, W.-C., McQuade, J.L., Haydu, L.E., Joon, A.Y., Reuben, A., de Macedo, M.P., Carapeto, F.C.L., et al. (2019). Molecular Profiling Reveals Unique Immune and Metabolic Features of Melanoma Brain Metastases. *Cancer Discovery* 9, 628-645. 10.1158/2159-8290.cd-18-1489.
 94. Yap, T.A., Daver, N., Mahendra, M., Zhang, J., Kamiya-Matsuoka, C., Meric-Bernstam, F., Kantarjian, H.M., Ravandi, F., Collins, M.E., Francesco, M.E.D., et al. (2023). Complex I inhibitor of oxidative phosphorylation in advanced solid tumors and acute myeloid leukemia: phase I trials. *Nature Medicine* 29, 115-126. 10.1038/s41591-022-02103-8.
 95. Parida, P.K., Marquez-Palencia, M., Ghosh, S., Khandelwal, N., Kim, K., Nair, V., Liu, X.-Z., Vu, H.S., Zacharias, L.G., Gonzalez-Ericsson, P.I., et al. (2023). Limiting mitochondrial plasticity by targeting DRP1 induces metabolic reprogramming and reduces breast cancer brain metastases. *Nature Cancer* 4, 893-907. 10.1038/s43018-023-00563-6.
 96. Bordt, E.A., Clerc, P., Roelofs, B.A., Saladino, A.J., Tretter, L., Adam-Vizi, V., Cherok, E., Khalil, A., Yadava, N., Ge, S.X., et al. (2017). The Putative Drp1 Inhibitor mdivi-1 Is a Reversible Mitochondrial Complex I Inhibitor that Modulates Reactive Oxygen Species. *Developmental Cell* 40, 583-594.e586. 10.1016/j.devcel.2017.02.020.
 97. Marullo, R., Castro, M., Yomtoubian, S., Calvo-Vidal, M.N., Revuelta, M.V., Krumsiek, J., Cho, A., Morgado, P.C., Yang, S., Medina, V., et al. (2021). The metabolic adaptation evoked by arginine enhances the effect of radiation in brain metastases. *Science Advances* 7, eabg1964. 10.1126/sciadv.abg1964.

98. Liu, X., Du, G., Mu, N., and He, L. (2026). A pharmacodynamic study of rapid lactate metabolic modulation by intravenous L-arginine in brain metastases. *Frontiers in Oncology* 16. 10.3389/fonc.2026.1845249.
99. Evangelidou, A.E., Spilioti, M.G., Vassilakou, D., Goutsaridou, F., Seyfried, T.N., Spilioti, M., and Vasilakou, D. (2022). Restricted ketogenic diet therapy for primary lung cancer with metastasis to the brain: a case report. *Cureus* 14.
100. Takada, T., Takata, K., and Ashihara, E. (2016). Inhibition of monocarboxylate transporter 1 suppresses the proliferation of glioblastoma stem cells. *The Journal of Physiological Sciences* 66, 387-396. <https://doi.org/10.1007/s12576-016-0435-6>.
101. Hanaford, A.R., Alt, J., Rais, R., Wang, S.Z., Kaur, H., Thorek, D.L.J., Eberhart, C.G., Slusher, B.S., Martin, A.M., and Raabe, E.H. (2019). Orally bioavailable glutamine antagonist prodrug JHU-083 penetrates mouse brain and suppresses the growth of MYC-driven medulloblastoma. *Transl Oncol* 12, 1314-1322. 10.1016/j.tranon.2019.05.013.
102. Yamashita, A.S., da Costa Rosa, M., Stumpo, V., Rais, R., Slusher, B.S., and Riggins, G.J. (2021). The glutamine antagonist prodrug JHU-083 slows malignant glioma growth and disrupts mTOR signaling. *Neuro-Oncology Advances* 3. 10.1093/oaajnl/vdaa149.
103. Leone, R.D., Zhao, L., Englert, J.M., Sun, I.-M., Oh, M.-H., Sun, I.-H., Arwood, M.L., Bettencourt, I.A., Patel, C.H., Wen, J., et al. (2019). Glutamine blockade induces divergent metabolic programs to overcome tumor immune evasion. *Science* 366, 1013-1021. doi:10.1126/science.aav2588.
104. Lin, H., Patel, S., Affleck, V.S., Wilson, I., Turnbull, D.M., Joshi, A.R., Maxwell, R., and Stoll, E.A. (2017). Fatty acid oxidation is required for the respiration and proliferation of malignant glioma cells. *Neuro Oncol* 19, 43-54. 10.1093/neuonc/now128.
105. Shim, J.-K., Choi, S., Yoon, S.-J., Choi, R.J., Park, J., Lee, E.H., Cho, H.J., Lee, S., Teo, W.-Y., Moon, J.H., et al. (2022). Etomoxir, a carnitine palmitoyltransferase 1 inhibitor, combined with temozolomide reduces stemness and invasiveness in patient-derived glioblastoma tumorspheres. *Cancer Cell International* 22, 309. 10.1186/s12935-022-02731-7.
106. Divakaruni, A.S., Hsieh, W.Y., Minarrieta, L., Duong, T.N., Kim, K.K.O., Desousa, B.R., Andreyev, A.Y., Bowman, C.E., Caradonna, K., Dranka, B.P., et al. (2018). Etomoxir Inhibits Macrophage Polarization by Disrupting CoA Homeostasis. *Cell Metabolism* 28, 490-503.e497. 10.1016/j.cmet.2018.06.001.
107. Guardado Rivas, M.O., Stuart, S.D., Thach, D., Dahan, M., Shorr, R., Zachar, Z., and Bingham, P.M. (2022). Evidence for a novel, effective approach to targeting carcinoma catabolism exploiting the first-in-class, anti-cancer mitochondrial drug, CPI-613. *PLoS One* 17, e0269620. 10.1371/journal.pone.0269620.
108. Philip, P.A., Sahai, V., Bahary, N., Mahipal, A., Kasi, A., Rocha Lima, C.M.S., Alistar, A.T., Oberstein, P.E., Golan, T., Metges, J.-P., et al. (2024). Devimistat (CPI-613) With Modified Fluorouracil, Oxaliplatin, Irinotecan, and Leucovorin (FFX) Versus FFX for Patients With Metastatic Adenocarcinoma of the Pancreas: The Phase III AVENGER 500 Study. *Journal of Clinical Oncology* 42, 3692-3701. 10.1200/JCO.23.02659.

109. Walker, C., and Angelo, M. (2024). Toward clinical applications of spatial-omics in cancer research. *Nature Cancer* 5, 1771-1773. 10.1038/s43018-024-00868-0.
110. FDA-NIH Biomarker Working Group (2020). Validation. BEST (Biomarkers, EndpointS, and other Tools) Resource. <https://www.ncbi.nlm.nih.gov/books/NBK464453/>.
111. Albert, N.L., Galldiks, N., Ellingson, B.M., van den Bent, M.J., Chang, S.M., Cicone, F., Koh, E.-S., Law, I., Le Rhun, E., Mair, M.J., et al. (2025). RANO criteria for response assessment of brain metastases based on amino acid PET imaging. *Nature Medicine* 31, 1424-1430. 10.1038/s41591-025-03633-7.
112. Verger, A., Tolboom, N., Cicone, F., Chang, S.M., Furtner, J., Galldiks, N., Gempt, J., Guedj, E., Huang, R.Y., Johnson, D.R., et al. (2025). Joint EANM/EANO/RANO/SNMMI practice guideline/procedure standard for PET imaging of brain metastases: version 1.0. *European Journal of Nuclear Medicine and Molecular Imaging* 52, 1822-1839. 10.1007/s00259-024-07038-5.
113. Galldiks, N., Abdulla, D.S.Y., Scheffler, M., Wolpert, F., Werner, J.M., Hüllner, M., Stoffels, G., Schweinsberg, V., Schlaak, M., Kreuzberg, N., et al. (2021). Treatment Monitoring of Immunotherapy and Targeted Therapy Using (18)F-FET PET in Patients with Melanoma and Lung Cancer Brain Metastases: Initial Experiences. *J Nucl Med* 62, 464-470. 10.2967/jnumed.120.248278.
114. Kotecha, R., Chiang, V., Tom, M.C., Parent, E., Nabavizadeh, A., Siegel, B.A., Huang, J., Zan, E., Peddi, S., Brem, S., et al. (2026). 18F-Fluciclovine for Detection of Recurrent Brain Metastases After Radiation Therapy: Image Interpretation Criteria and Diagnostic Performance From the PURSUE Study. *International Journal of Radiation Oncology*Biophysics* 125, 1410-1420. 10.1016/j.ijrobp.2026.02.239.
115. Ciavarra, B., Krayyem, A., Ansari, S., Shindell, E., Gule-Monroe, M.K., Patel, C.B., and Johnson, J.M. (2026). Delayed 18F-FDG PET for the evaluation of suspected recurrent disease in brain metastases treated with radiation. *Clinical Radiology* 99, 107390. 10.1016/j.crad.2026.107390.
116. Li, M., Chen, J., Zhang, B., Yu, J., Wang, N., Li, D., Shao, Y., Zhu, D., Liang, C., Ma, Y., et al. (2022). Dynamic monitoring of cerebrospinal fluid circulating tumor DNA to identify unique genetic profiles of brain metastatic tumors and better predict intracranial tumor responses in non-small cell lung cancer patients with brain metastases: a prospective cohort study (GASTO 1028). *BMC Medicine* 20. 10.1186/s12916-022-02595-8.
117. Lee, J.H., Menzies, A.M., Carlino, M.S., McEvoy, A.C., Sandhu, S., Weppler, A.M., Diefenbach, R.J., Dawson, S.-J., Kefford, R.F., Millward, M.J., et al. (2020). Longitudinal Monitoring of ctDNA in Patients with Melanoma and Brain Metastases Treated with Immune Checkpoint Inhibitors. *Clinical Cancer Research* 26, 4064-4071. 10.1158/1078-0432.ccr-19-3926.
118. Voglis, S., Schulz, D., Eling, N., De Souza, N., Regli, L., Neidert, M.C., Czabanka, M., Weller, M., Le Rhun, E., Mihic-Probst, D., et al. (2026). Spatially-resolved single-cell imaging of melanoma brain metastases identifies localized immune patterns predictive of immune checkpoint blockade response. *Neuro-Oncology* 28, 2211-2223. 10.1093/neuonc/noag128.

119. Zhu, J., Ye, J., Ma, Y., Lin, Z., Wen, Y., Sheng, J., and Yang, M. (2026). Spatial immune atlas of breast cancer brain metastasis reveals CD163+ macrophage reprogramming associated with immune escape. *Journal for ImmunoTherapy of Cancer* 14, e014421. 10.1136/jitc-2025-014421.
120. Khatri, A., McKernan, C.M., Van Swearingen, A.E.D., Jain, V., Thrash, H.L., Towne, A., Gu, J.J., Gregory, S.G., Anders, C.K., and Pendergast, A.M. (2026). Spatially resolved transcriptomics in human brain metastases identifies macrophage-tumor interactions associated with survival. *iScience* 29, 116517. 10.1016/j.isci.2026.116517.
121. Cheng, X., Patel, K.K., Zhou, B., Fu, Y., Cleary, R.T., Highkin, M., Hsia, J., Jin, X., Kohn, B., Prior, J.L., et al. (2026). Interleukin-34–Induced Arg1+ Macrophages Play a Key Role in Breast Cancer Brain Metastasis. *Cancer Research Communications* 6, 1388-1404. 10.1158/2767-9764.crc-25-0639.
122. Sadik, A., Somarribas Patterson, L.F., Öztürk, S., Mohapatra, S.R., Panitz, V., Secker, P.F., Pfänder, P., Loth, S., Salem, H., Prentzell, M.T., et al. (2020). IL4I1 Is a Metabolic Immune Checkpoint that Activates the AHR and Promotes Tumor Progression. *Cell* 182, 1252-1270.e1234. 10.1016/j.cell.2020.07.038.
123. Ahn, J.Y., Dong, W., Puri, A., Vasquez, M., Raghunathan, R., Yang, L., Sheng, J., Zhao, H., and Wong, S.T.C. (2025). Spatial Crosstalk Modeling of the Tumor Microenvironment Identifies CCR5-Mediated Glia-to-Glia Signaling as a Key Regulator of Brain Metastatic Progression. *Cancer Research*. 10.1158/0008-5472.Can-25-0237.
124. Su, R., Ma, X., Guo, X., Huang, S., Liu, R., Song, Z., Gao, H., Zhao, K., Li, Y., and Liang, K. (2025). Impact of Cold Ischemia on the Proteome and Metabolome of Tissue Samples and Preanalytical Quality Control Using Mass Spectrometry Imaging. *J Proteome Res* 24, 2968-2980. 10.1021/acs.jproteome.5c00157.
125. Brastianos, P.K., Carter, S.L., Santagata, S., Cahill, D.P., Taylor-Weiner, A., Jones, R.T., Van Allen, E.M., Lawrence, M.S., Horowitz, P.M., Cibulskis, K., et al. (2015). Genomic Characterization of Brain Metastases Reveals Branched Evolution and Potential Therapeutic Targets. *Cancer Discovery* 5, 1164-1177. 10.1158/2159-8290.cd-15-0369.
126. Valiente, M., Aydoğan, L.E., Feigin, N., Gahn, C., Hernandez-Oliver, C., Milling, F., Oktem, E., Ortega-Sabater, C., Priego, N., Ricci, A.A., et al. (2026). Patient-derived resources for decoding and targeting brain metastases ecosystems. *EMBO Molecular Medicine* 18, 3017-3027. 10.1038/s44321-026-00472-y.
127. Gan, S., Macalinao, D.G., Shahoei, S.H., Tian, L., Jin, X., Basnet, H., Bibby, C., Muller, J.T., Atri, P., Seffar, E., et al. (2024). Distinct tumor architectures and microenvironments for the initiation of breast cancer metastasis in the brain. *Cancer Cell* 42, 1693-1712.e1624. 10.1016/j.ccell.2024.08.015.
128. Greenwald, N.F., Nederlof, I., Sowers, C., Ding, D.Y., Park, S., Kong, A., Houlahan, K.E., Varra, S.R., de Graaf, M., Geurts, V., et al. (2026). Temporal and spatial composition of the tumor microenvironment predicts response to immune checkpoint inhibition in metastatic TNBC. *Nature Cancer* 7, 435-450. 10.1038/s43018-026-01114-5.
129. Li, M., Wang, T., Zhang, Z., and Dongye, Y. (2025). Metabolic reprogramming in the post-metastatic tumor microenvironment: multi-omics insights into

- determinants of immunotherapy response. *Front Immunol* **16**, 1742855. 10.3389/fimmu.2025.1742855.
130. Zemaitis, K.J., and Paša-Tolić, L. (2024). Challenges in Spatial Metabolomics and Proteomics for Functional Tissue Unit and Single-Cell Resolution. *Semin Nephrol* **44**, 151583. 10.1016/j.semnephrol.2025.151583.
 131. Haque, S.Y., Shatabda, S., Islam, S., Dipu, S.A., and Azim, R. (2026). The good, the bad, and the ugly: opportunities, challenges, and pitfalls in spatial proteomics modeling. *Briefings in Bioinformatics* **27**. 10.1093/bib/bbag169.
 132. Saunders, K.D.G., Lewis, H.-M., Beste, D.J.V., Cexus, O., and Bailey, M.J. (2023). Spatial single cell metabolomics: Current challenges and future developments. *Current Opinion in Chemical Biology* **75**, 102327. <https://doi.org/10.1016/j.cbpa.2023.102327>.
 133. Luo, M., Kou, T., Yin, Y., Zhou, S., Zhu, X., Zeng, X., Hu, J., and Zhu, Z.-J. (2026). Deep-coverage single-cell metabolomics enabled by ion mobility-resolved mass cytometry. *Nature Methods* **23**, 585-595. 10.1038/s41592-025-02970-2.
 134. Jang, C., Chen, L., and Rabinowitz, J.D. (2018). Metabolomics and Isotope Tracing. *Cell* **173**, 822-837. 10.1016/j.cell.2018.03.055.
 135. Schwaiger-Haber, M., Stancliffe, E., Anbukumar, D.S., Sells, B., Yi, J., Cho, K., Adkins-Travis, K., Chheda, M.G., Shriver, L.P., and Patti, G.J. (2023). Using mass spectrometry imaging to map fluxes quantitatively in the tumor ecosystem. *Nature Communications* **14**. 10.1038/s41467-023-38403-x.
 136. Fan, T., Lane, A., and Higashi, R. (2016). Stable Isotope Resolved Metabolomics Studies in ex vivo Tissue Slices. *BIO-PROTOCOL* **6**. 10.21769/bioprotoc.1730.
 137. Zhou, Y., Xiao, X., Dong, L., Tang, C., Xiao, G., and Xu, L. (2025). Cooperative integration of spatially resolved multi-omics data with COSMOS. *Nature Communications* **16**, 27. 10.1038/s41467-024-55204-y.
 138. Liu, Y., Wang, C., Wang, Z., Chen, L., Li, Z., Song, J., Zou, Q., Gao, R., Qian, B.-Z., Feng, X., et al. (2026). High-parameter spatial multi-omics through histology-anchored integration. *Nature Methods* **23**, 373-386. 10.1038/s41592-025-02926-6.
 139. Roy, S., Nassor, M., Zahin, F., Chaudhry, S., Odei, K.P.K., Nkrumah-Boateng, P.A., and Wireko, A.A. (2026). Leveraging single-cell and spatial omics for brain tumour insights to improve therapeutic strategies. *Molecular Brain* **19**. 10.1186/s13041-026-01319-w.