Salting the Soil: Targeting the Microenvironment of Brain Metastases

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ABSTRACT

Paget’s “seed and soil” hypothesis of metastatic spread has acted as a foundation of the field for over a century, with continued evolution as mechanisms of the process have been elucidated. The central nervous system (CNS) presents a unique soil through this lens, relatively isolated from peripheral circulation and immune surveillance with distinct cellular and structural composition. Research in primary and metastatic brain tumors has demonstrated that this tumor microenvironment (TME) plays an essential role in the growth of CNS tumors. In each case, the cancerous cells develop complex and bidirectional relationships that reorganize the local TME and reprogram the CNS cells, including endothelial cells, pericytes, astrocytes, microglia, infiltrating monocytes, and lymphocytes. These interactions create a structurally and immunologically permissive TME with malignant processes promoting positive feedback loops and systemic consequences. Strategies to interrupt interactions with the native CNS components, on “salting the soil,” to create an inhospitable environment are promising in the preclinical setting. This review aims to examine the general and specific pathways thus far investigated in brain metastases and related work in glioma to identify targetable mechanisms that may have general application across the spectrum of intracranial tumors.

Introduction

Brain metastases represent the most common intracranial tumor and affect an estimated 10%–20% of all patients with cancer (1–3). The incidence of brain metastases continues to rise, likely due to increased detection with MRI and improved survival from continued progress in cancer management. Lung, breast, and melanoma are the most common primary tumors to metastasize to the brain. However, renal cell and colorectal brain metastases remain significant (4). There have been many recent advances in the multimodal management of brain metastases across surgery, radiotherapy, and systemic therapies; in particular, stereotactic radiosurgery and targeted therapies with greater intracranial penetration have altered the treatment paradigm in many cancers (5). Despite this, the presence of brain metastases continues to portend a poor prognosis as long-term survival rates remain unacceptably low (6, 7). Furthermore, neurologic symptoms such as headache, seizures, focal deficits, and cognitive impairment, as well as toxicity from treatment, can impair a patient’s quality of life and contribute to morbidity (8).

With this context, the origins of the classical “seed and soil” view of metastatic spread reach back to Paget’s work in 1889 (9). In the ensuing 130 years, a significant evolution in our understanding of these processes has, of course, taken place. However, some fundamental ideas remain true to this day. A greater biological understanding of brain metastases pathophysiology and the metastatic cascade is crucial to developing novel and improved therapeutic strategies. This review will focus on the soil itself, the central nervous system (CNS) tumor microenvironment (TME), and discuss the current state of knowledge regarding how brain-metastatic cells manipulate and restructure the native components and architecture to create an actively protumorigenic setting. Characterizing the changes within this “soil” and understanding the existing literature on preventing or reversing these processes will allow for the identification of common pathways shared across a range of primary tumor sources to pursue therapeutic strategies aimed toward creating an inhospitable CNS TME both before and after the establishment of macrometastatic lesions.

Physiologic Brain Microenvironment

It is necessary to appreciate the unique CNS microenvironment in nonpathologic conditions. The brain contains a dense microvascular network that circulates roughly 15%–20% of the total cardiac output, with outflow filtered into the dural sinuses, and eventually returned to the venous system (10). The CNS is isolated from peripheral circulation at the boundary of this vasculature by the blood–brain barrier (BBB). This highly selective filter regulates the passage of solutes into the extracellular fluid of the CNS (11). Beyond the BBB, the CNS’s cellular elements predominantly consist of neurons and supportive glial cells, including astrocytes, microglia, pericytes, and oligodendrocytes. The BBB itself comprises endothelial cells connected by tight junctions and supported through astrocyte projections with pericytes, similar to vascular smooth muscle cells, embedded in the basement membrane (12). The BBB permits the diffusion of hydrophobic molecules and small polar molecules in the physiologic state while restricting that of larger or hydrophilic solutes, relevantly including pathogens, antibodies, and many chemotherapeutic drugs.

Astrocytes within the CNS act as the primary support cell for neurons, with a range of functions that include regulation of nutrient and solute availability, neurotransmitter reuptake, blood flow, and the response to areas of inflammation or injury (13). Microglia are the primary effector cells of the innate immune system within the brain, the CNS equivalent of peripheral monocytes, whereas oligodendrocytes supply the myelin sheaths surrounding the axons of neurons in a manner analogous to peripheral Schwann cells (14, 15). Finally, the...
extracellular matrix of the CNS plays essential roles in physical and homeostatic support, from the pericyte-containing basement membrane of the BBB to the perineuronal and intraparenchymal matrices (16). Throughout the metastatic process, tumor cells manipulate and reorganize these cellular and extracellular components of the CNS through targetable mechanisms to create a pro-tumorigenic, therapy-resistant environment, as will be discussed in the following sections.

**Metastatic Cascade**

The metastatic spread, described as the “metastatic cascade” (17, 18), begins with local invasion at the primary tumor site, migration into blood vessels, extravasation at a distant site, the initial proliferation of micrometastases, and the eventual establishment of a macrometastatic lesion (19). The CNS setting is unique relative to other sites of metastasis for several reasons. First, circulating tumor cells must pass through the BBB to extravasation (depicted in Fig. 1A–C). Notably, alternative pathways that bypass the BBB have also been suggested, including traversal across the laminin-rich basement membrane of bridging vessels into the subarachnoid space in the case of leptomeningeal metastases of acute lymphoblastic leukemia or via functional lymphatic vessels lining the dural sinuses (20, 21). Regardless, the would-be metastatic cells then encounter a set of native cellular components and noncellular architecture with distinct immune parameters once within the CNS. In this setting, and before encountering it, a complex and bidirectional interplay occurs in which the metastatic cells manipulate the CNS TME to their advantage. Understanding the factors that set the stage for extravasation at the CNS rather than other locations and the subsequent changes within the microenvironment is critical to generating therapeutic strategies based on preventing or mitigating those factors.

**The premetastatic niche**

Even before the arrival of circulating tumor cells at the distant site, the scene’s initial setting has occurred with creating the premetastatic niche. This phase of the cascade encompasses preparation of the colloquial “soil” in advance of colonization by circulating tumor cells. The process is mediated through the secretion of cytokines, chemokines, and angiogenic factors from the primary tumor site. Such mechanisms have been demonstrated in several primary and metastatic sites, with less direct investigation in brain metastases. In the general case of systemic metastases, implicated factors include VEGF, lysyl oxidase-like protein (LOXL2), C-C motif ligand 2 (CCL2), C-X-C motif chemokine ligand 17 (CXCL17), TNFα, TGFβ, vascular adhesion molecule 1 (VCAM-1), and C-X-C motif receptor 4 (CXCR4), among others (22–28).

Regarding brain metastases, several secreted factors have been shown to influence the permeability of the BBB including VEGFR, angiopoietin-2, matrix metalloproteinases (MMPs, specifically MMP-2 and MMP-9), and placental growth factor (PLGF; refs. 29–32). Feng and colleagues and Li and colleagues demonstrated that these adhesion molecules (CAMs) soon after the injection of metastatic cells into the peripheral circulation, including VCAM-1, ALCAM, ICAM-1, VLA-4, E-selectin, and β3-integrin, at the same time corresponding ligands are upregulated on circulating tumor cells. In consideration of potential therapeutic application, this group also demonstrated that neutralization of these CAMs through targeted mAbs significantly reduced tumor seeding within the brain (33). Another study by Liu and colleagues in mice found that before the development of brain metastases, the brains of mice bearing orthotopic breast tumors showed significant accumulation of bone marrow–derived CD11b+Gr1–myeloid cells expressing inflammatory chemokines S100A8 and S100A9. These inflammatory mediators attracted both the tumor cells and myeloid cells through Toll-like receptor-4 (TLR4), and treatment with both anti-Gr1 and COX2 inhibitors (as well as analogous knockout mouse models) reduced the infiltration of myeloid cells and subsequent formation of brain metastases (34).

Tumor-derived exosomes are another factor in conditioning the eventual metastatic site. These exosomes are extracellular vesicles containing tumor-produced factors, including proteins, lipids, and nucleic acids, released into circulation from the primary site. The exosomes then interact with resident cells at distant locations through extracellular signaling or fusion with subsequent intracellular cascades (35, 36). Studies in extracranial metastases of various primary tumors have demonstrated the role of tumor-derived exosomes in the induction of a protumorigenic premetastatic niche by modifying the inflammatory, immunologic, and angiogenic parameters of the eventual metastatic location. Some of the factors involved include PD-L1, miRNAs, intracellular signaling mediators, inflammatory cytokines, and various chemokines (37–41). Importantly, these exosomes have been shown to have site-specificity dependent on their integrin (ITG) profile, with ITGβ3 specific to the brain (42). These exosomes subsequently promote a site-specific local premetastatic niche, in part, through S100 gene regulation, and both knockdown and drug inhibition models aimed at target integrins have successfully blocked organ-specific tropism in vitro and in vivo (42). Another fascinating study made use of engineered nanoparticles to capture circulating breast cancer tumor–derived exosomes in vivo with significantly reduced rates of systemic metastases (43).

In applying these concepts to the CNS premetastatic niche, a recent study by Morad and colleagues demonstrated that such tumor-derived exosomes are capable of migrating through the BBB in vivo via transcytosis (44). Exosomes have been shown to contain miRNA that suppresses glucose uptake in astrocytes in vitro, through miR-122, which creates an environment favoring the proliferation of metastatic cells. The same study verified that the miR-122-containing tumor-derived exosomes increased brain metastases in vivo and that anti-miR-122 treatment reduced metastasis to both the brain and lungs (45). Exosomes containing the miRNA miR-181c in another brain-seeking breast cancer metastatic model were shown to promote the breakdown of the BBB in vivo and increase brain metastases in vivo. The group corroborated these findings with increased miR-181c in patient serum samples from those with brain metastases compared with those without (46). A study by Rodrigues and colleagues identified a particular protein, cell migration-inducing, and hyaluronan-binding protein (CEMP), enriched in brain-tropic breast and lung cancer–derived exosomes. The group showed that CEMP induces upregulated cytokine and chemokine production and angiogenesis in the brain, promoting metastatic colonization of the CNS. Furthermore, knocking out CEMP reduced brain metastases by 70% in vivo, indicating that CEMP is required for the early stages of metastatic colonization. These results were corroborated in clinical samples with the correlation of CEMP expression to brain metastases and survival (47). Extending these results and those identified in other distant sites to the CNS presents an opportunity to target tumor-derived exosomes and their associated pathways, with supportive preclinical data, for the prevention of brain metastases long before they become clinically relevant.
Therapeutic applications of targeting the premetastatic niche will need preclinical research strategies aimed at multiple sites. Reduced incidence of brain metastases was demonstrated with mAbs targeted toward upregulated CAMs and brain-specific ITGs, and treatment with anti-Gr1 and COX2 inhibitors, as well as with genetic knockdown and knockout studies of additional targets in the pathways. Such a strategy could prove immensely beneficial in actively preventing brain metastases rather than responding after the fact.

Extravasation through the BBB and seeding of the CNS

The first step distinguishing brain metastases from other sites is the BBB transversal by circulating tumor cells, a process that is, as of yet, incompletely understood. The BBB is the basis of the "immune-privilege" designation of the CNS, though its immutability has been disproven with the identification of the CNS lymphatic network and mechanisms of infiltration by circulating immune cells, particularly in states of injury and inflammation (21, 48).

Various groups have suggested both paracellular and transcellular routes through the BBB, particularly with coopting existing pathways for leukocyte extravasation (49). Several surface molecules and soluble factors have been identified as essential factors in the process of BBB transmigration, including selectin ligands, integrins, cadherins, proteases, and various chemokines and cytokines. The range of these molecules is broad across primary tumor histologies, indicating multiple mechanisms with common factors. On the circulating tumor cells, specifically identified mediators include the expression of the adhesive membrane proteins ST6GALNAC5 and CD44, upregulation of COX2, CXCR4, HBEGF, EREG, and ITGαvβ3, increased secretion of VEGF, angiopoietin-2, PLGF, and S100A4 from tumor cells and brain endothelial cells, secretion of proteases including cathepsin S, matrix metalloproteinase (MMP) MMP-1, MMP-2, MMP-3, MMP-9, and ADAM-8, surface melanotransferrin expression on melanoma cells, rho kinase signaling in small-cell lung cancer (SCLC), and various other upregulated CAMs (29, 30, 38-56). These factors’ shared effect is to increase the permeability of and adherence to the BBB, permitting transmigration by the circulating tumor cells. Thus far, inhibition of a number of these factors, including cathepsin S, matrix metalloproteinase (MMP) MMP-1, MMP-2, MMP-3, MMP-9, and ADAM-8, surface melanotransferrin expression on melanoma cells, rho kinase signaling in small-cell lung cancer (SCLC), and various other upregulated CAMs (29, 30, 38, 50-56). These factors’ shared effect is to increase the permeability of and adherence to the BBB, permitting transmigration by the circulating tumor cells. Thus far, inhibition of a number of these factors, including cathepsin S, matrix metalloproteinase (MMP) MMP-1, MMP-2, MMP-3, MMP-9, and ADAM-8, surface melanotransferrin expression on melanoma cells, rho kinase signaling in small-cell lung cancer (SCLC), and various other upregulated CAMs (29, 30, 38, 50-56). These factors’ shared effect is to increase the permeability of and adherence to the BBB, permitting transmigration by the circulating tumor cells. Thus far, inhibition of a number of these factors, including cathepsin S, matrix metalloproteinase (MMP) MMP-1, MMP-2, MMP-3, MMP-9, and ADAM-8, surface melanotransferrin expression on melanoma cells, rho kinase signaling in small-cell lung cancer (SCLC), and various other upregulated CAMs (29, 30, 38, 50-56).

Many of these studies have used RNA knockdown or transgenic knockout experimental strategies. The existence of small-molecule inhibitors and mAbs against several targets presents an opportunity to disrupt essential pathways and protect the CNS from metastatic...
reprogramming, with supportive data for the COX2 inhibitor celecoxib, rho kinase inhibitor fasudil, anti-EREG mAb, and HBEGF inhibitors (34, 53, 59, 60). These results provide proof of concept for a strategy to prevent BrM by targeting the factors that mediate initial access to the CNS.

Initial tumor proliferation and colonization of the brain parenchyma

Single-cell in vivo studies have demonstrated that the vast majority of metastatic tumor cells fail to proliferate beyond the micrometastatic phase after initial transmigration through the BBB (61). For those that progress, the development of a complex and evolving TME begins as the metastatic cells interact with the resident CNS components. Initially, the metastatic cells remain near the extravasation site at the blood vessel’s abluminal surface, where the developing tumor is supplied with essential nutrients to facilitate its accelerating growth (61). As the metastatic cells proliferate, these needs multiply, and the tumor manipulates the local vasculature through cooption of existing vessels and induced angiogenesis (62). These vascular remodeling processes are thought to be directed through VEGF, integrins, and cell adhesion molecules (particularly ITGα6β1, ITGβ3, and L1CAM) from both metastatic and CNS cells (63). Following vascular migration, colonization of the brain parenchyma by metastatic tumors is dependent on the activation of diverse signaling networks that promote cross talk within the TME and the metastatic cell’s acquisition of neuronal phenotypes (5, 64, 65). Examples include cooption of γ-aminobutyric acid (GABA) as an oncometabolite and the activation of an AXL–ABL2–TAZ signaling axis to promote the export of metabolic-related factors in brain-metastasizing lung adenocarcinoma cells (65, 66). Among these factors is the neuronal CAM L1CAM, a target of TAZ-dependent transcription, which regulates vascular cooption and migration and tumor outgrowth (67, 68). Whereas there are no effective therapies to target L1CAM, pharmacologic inhibition of either ABL or AXL tyrosine kinases downregulates TAZ-driven L1CAM gene expression and decreases brain metastases in lung adenocarcinoma models (66).

Bohn and colleagues demonstrated in their preclinical model that bevacizumab reduces brain metastases when administered 10 days after circulating tumor cell injection; however, whether this disrupted seeding or subsequent vascular remodeling is unclear from their design. İlhan-Mutlu and colleagues showed a potential preventative role for the therapy with the finding that administration of bevacizumab 24 hours after circulating tumor cell injection reduced single-cell, micro-, and macrometastases in the CNS at subclinical doses, along with prolonged overall survival and correlated clinical data from the AVAIL trial (69, 70). Furthermore, inhibition of PLGF has also shown success in slowing the growth of VEGF-resistant tumors as well as reducing the rate of metastasis and tumor-associated macrophage (TAM) M2 polarization, with promising phase I trial evidence supporting its safety (71–75).

Another implicated group throughout the initial phase of brain metastasis establishment are the MMPs. The strategy of interrupting MMP activity has been validated with RNA interference studies in CNS metastatic models of leukemia and breast cancer, and with MMP pharmacologic inhibitors in in vivo preclinical models (32, 58). The history of MMP inhibitors in clinical trials has been unfortunately unsuccessful. Although significant preclinical data supported their use, trials throughout the early 2000s showed few successes and significant musculoskeletal side effects. However, with the development of novel, specific MMP inhibitors, revisiting this strategy as a method to prevent tumor-driven reorganization of the CNS is a new opportunity for the defense of the CNS microenvironment. Specific targeting of MMP-9 in colorectal cancer has shown successes without the characteristic musculoskeletal side effects, and a similar strategy may be useful in preventing brain metastases (76). Notably, the earlier previous clinical trials were conducted on patients at all stages of progression, and a focus instead on preventing brain metastases may be the most promising avenue forward. Relevantly, in vivo administration of an MMP inhibitor (targeted at MMP-2, MMP-9, and MMP-13) 2 days after orthotopic breast cancer inoculation showed a significant reduction in tumor size and lung metastases. However, similar studies have not yet been performed in brain metastases (77). Interestingly, doxycycline is a multispecific MMP inhibitor with activity against MMP-9, and similar tetracyclines have been shown to prevent lung metastases from renal adenocarcinoma and bone metastases of breast cancer in combination with a COX2 inhibitor, as well as inhibiting glioma growth (78–82).

Targeting this initial phase of metastatic propagation could significantly improve the effectiveness of the early antitumor response. The studies above showed success in reducing brain metastases by blocking the influence of key tumor-initiated signaling pathways in the early phases of vascular remodeling and parenchymal invasion. The existence of current targeted drugs for these purposes presents an opportunity to further explore brain metastases treatment before the development of clinically significant lesions.

Cellular Interactions

While the metastatic lesion grows, its interactions with the surrounding TME form an evolving relationship with distinct temporal profiles. The initial response is a frequently effective antitumor program initiated by activated astrocytes termed reactive astrocytes, which successful metastatic cells evade through the plasminogen-activator inhibiting protein neuroserpin (61, 68). From there, the metastatic cells quickly begin to influence the native CNS components towards a supportive and accelerative growth milieu. The key cellular actors in the CNS include the reactive astrocytes, endothelium, pericytes, neurons, microglia, and bone marrow–derived macrophages (together called TAMs), and tumor-infiltrating lymphocytes (TILs). Although more significant work has been done in glioma, the current literature suggests several potentially targetable interactions in the brain metastases TME. Thus far, most investigation has focused on communication between metastatic cells, reactive astrocytes, and TAMs, and a summary of these interactions is depicted in Fig. 1D.

Endothelium and the perivascular niche

The initial perivascular niche remains an important tumor development site and interaction with the vascular architecture, endothelial cells, and pericytes. However, most work in the CNS has been completed in gliomas, and characterization of these interactions in brain metastases growth should be considered extrapolation. Notably, in glioma, the perivascular niche is an essential location for cancer stem cells, a population within the tumor defined by its ability to sustain growth and angiogenesis with particular resistance to radio- and chemotherapy, in part, mediated through Akt signaling pathways (83, 84). These cancer stem cells have even been shown in glioma to transform into vascular endothelial cells, pericytes, and mural cells, directly driving the essential vascular reorganization of the CNS (85–87). While similar processes have not yet been explored in brain metastases, interactions with endothelial cells are essential to the initial extravasation of the metastatic cells. This interaction continues within the perivascular niche as the tumor cooperates and manipulates the local vasculature (88, 89). This signal has also been proven to be
Bidirectional in glioma, as brain endothelial cells drive glioma growth through direct interactions with the cancer stem cells, potentially through nitric oxide (NO) signaling (90, 91).

Notably, cerebral microvessels have also been found to have a 10-30 x greater pericyte:endothelial cell ratio compared with other tissues, highlighting their importance in the early TME (92). Valdar and colleagues demonstrated that pericytes promote glioblastoma (GBM), and contribute to local immunosuppression through a GBM cell-induced secretion of anti-inflammatory cytokines (IL10 and TGFβ) with corresponding decreases in proinflammatory cytokines (93). Within the perivascular niche, even the extracellular components of the vascular basement membrane, largely collagen type IV and laminins, are central to the initial metastatic cell proliferation through interactions with the surface ITGβ3 subunit (94). In total, the perivascular niche is an early and essential site of interactions between metastatic cells and the native CNS components.

Currently, there is little data on strategies aimed to disrupt the perivascular niche of brain metastases. However, with the importance of cancer stem cells in driving tumor growth and recurrence, it may prove a particularly relevant context in promoting long-term survival. Extending glioma models to brain metastases will elucidate whether these interactions are conserved and identify targets that could be specific or general in intracranial tumors.

**Reactive astrocytes**

The relationship between the metastatic cells and reactive astrocytes may be the most intimate connection within the TME. Release of inflammatory mediators by reactive astrocytes, including TNFα, IL6, and IL1β, are induced by lung cancer brain metastases (95). Furthermore, the development of connexin-43 (Cx43)-based gap junctions between metastatic cells and reactive astrocytes has been identified in preclinical models of breast and lung cancer brain metastases. Through this mechanism, the metastatic cell initiates a cGAMP-mediated paracrine signaling loop that promotes reactive astrocytes’ release of inflammatory mediators, including IFNγ and TNFα (96). Consequently, these inflammatory cytokines drive cell survival and chemoresistance mechanisms within the tumor cells via upregulation of STAT1, NFκB, GATA3, BCL2L1, and TWIST1 (97). This interaction can be directly disrupted via BBB-penetrant gap-junction targeting drugs, including melofenamate and tonabersat, which were both shown to inhibit brain metastases in vivo (96). In addition, reactive astrocytes release miR-19a-containing exosomes that inhibit the expression of tumor suppressor PTEN in metastatic cells, consequently increasing tumor chemokine secretion as well as recruiting protumorigenic brain-derived myeloid cells into the TME (98).

Prieo and colleagues identified STAT3 as the essential driver within these protumorigenic reactive astrocytes, further promoting protumorigenic TAMs, and showed that inhibition of STAT3 activation through the BBB-penetrant drug silbinbin disrupted astrocyte activation, reduced brain metastases, and showed efficacy against established brain metastases. The same group administered the STAT3 inhibitor to 18 patients with treatment-failed lung cancer brain metastases and found significantly improved overall survival to a matched historical control, regardless of driver mutation status (99). Furthermore, a multispecific tyrosine kinase inhibitor, pazopanib, that targets several mediators of angiogenesis has been demonstrated to reduce the population of metastasis-associated reactive astrocytes in a metastatic breast cancer model and significantly inhibit brain metastases (100, 101).

Several other tumor-promoting paracrine loops have been identified between reactive astrocytes and metastatic cells. Estrogen-dependent signaling in breast brain metastases has been identified to stimulate ERβ reactive astrocytes toward protumorigenic chemokine secretion through a pathway mediated by S100A4, BDNF, and tropomyosin kinase receptor B (TrKB; refs. 102, 103). Sartorius and colleagues demonstrated that disruption of S100A4 activity through shRNA knockdown prevented the protumorigenic reactive astrocytes’ activity and identified a potential role for antiestrogen therapies and aromatase inhibitors in brain metastases (102). Contreras-Zarate and colleagues supported the efficacy of letrozole, an aromatase inhibitor, in preventing brain metastases of triple-negative brain-seeking breast cancer cells injected intravascularly with improved overall survival, mediated through a pathway involving ERα reactive astrocytes (103). TrKB knockdown and inhibition also reduced the incidence of brain metastases, and together these results provide a rationale for implementing antiestrogenic therapies even in the setting of triple-negative breast cancer. Furthermore, Xing and colleagues showed that breast brain metastases could create a positive feedback loop in which upregulation of c-Met increases HGF-dependent tumor cell secretion of protumorigenic IL1β, IL8, and CXCL2, which subsequently increase HGF secretion by local reactive astrocytes (104). The same group demonstrated that inhibition of the c-Met pathway by BBB-penetrant pterostilbene significantly blocked brain metastases development in vivo and extended survival (104).

IL1β has also been demonstrated to drive reactive astrocyte-mediated activation of protumorigenic Notch signaling in cancer stem-like cells of breast brain metastases (105). Jandial and colleagues demonstrated that reactive astrocytes upregulate protumorigenic Reelin signaling in HER2+ breast brain metastases, while Choy and colleagues found that reactive astrocyte-produced bone-derived neurotrophic factor (BDNF) is essential to the proliferation of HER2+ breast brain metastases via TrKB signaling, with targeted inhibition of BDNF and HER2 by cyclotaxin B and lapatinib, respectively, significantly inhibiting tumor cell growth in vivo (106, 107). Kim and colleagues showed that IL6 and IL8 production by breast brain metastases increased endothelin production by reactive astrocytes and upregulated endothelin receptors on metastatic cells, subsequently promoting a range of tumor proliferative and chemoresistance signaling pathways (108). Reactive astrocytes can also be induced by metastatic melanoma cells to express IL23 via MMP2, which increases parenchymal invasion that can be blocked by IL23 inhibition (109). Another study highlighted the role of TGFB2 from reactive astrocytes in upregulating ANGPTL4 in triple-negative breast cancer brain metastases, a gene involved in tumor progression through an unknown mechanism (110). This interaction is stimulated through metastatic cell release of IL1β and TNFα. Reactive astrocytes have also been shown to contribute to local immunosuppression via the induced STAT3-dependent expression profile that inhibits CD8+ T-cell activation and polarizes TAMS to the anti-inflammatory M2 profile (111). Although a comprehensive model of the interactions between metastatic cells and reactive astrocytes has yet to be developed, the findings above highlight several common mediators and their roles and relationships in brain metastases progression that can potentially be interrupted pharmacologically. Researchers above have validated methods of disrupting specific intercellular signaling pathways and intracellular pathways within the reactive astrocytes themselves, with promising preclinical results. Given existing safety data for some candidate therapies, translation into clinical use may be closer than typically feasible.
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Tumor-associated macrophages

Less thoroughly investigated is the interaction between metastatic cells and TAMs, a group consisting of microglia and infiltrating bone marrow–derived macrophages. Bone marrow–derived macrophages are infiltrating monocytes from the peripheral circulation. These two cell populations are indistinguishable by current experimental techniques. However, murine models and clinical samples show that up to 30% of the total tumor mass consists of TAMs (51). Classically, two polarizations have been described with M1 considered a proinflammatory profile and M2 anti-inflammatory. However, the validity of this distinction has been debated (14, 112). The protumorigenic M2 TAMs demonstrate inhibited cytotoxic activity and secrete factors involved in local immunosuppression, tumor growth, and ECM remodeling (113). Andreou and colleagues showed that selective inhibition of M2 TAMs significantly reduced brain metastases in a metastatic breast cancer model (114). Induction of this TAM profile is regulated by WNT, CXCR4, and PI3K pathway signaling, with targeted inhibition of each leading to reduced TAM-associated parenchymal infiltration (115–117). Breast brain metastases have been shown to secrete neurotrophin-3 to reduce TAM cytotoxicity and drive a broad shift toward the M2 polarization profile (118). Xing and colleagues demonstrated that downregulation of X-inactive specific transcript (XIST) in breast brain metastases promotes metastatic growth through increased secretion of miR-503 from metastatic cells, which suppresses microglial cytokine progression and, subsequently, T-cell proliferation (119). The group found that a drug targeting XIST-low breast metastatic cells blocked brain metastases in vivo and correlated these findings with XIST quantification in patient tumor samples (119). In glioma, TAMs have been shown to additionally produce VEGF, driving angiogenesis, and express IL10 and TGFβ, which stimulate Tregs and perpetuate the immunosuppressive environment (120).

Notably, the polarization of TAMs is known to exist on a reversible spectrum, dependent on dynamic extracellular or intracellular cues (121). While tumors manipulate this fluidity to their advantage, targeting the opposite is another potential therapeutic approach as treatment with the PI3K inhibitor buparlisib in a breast cancer model inhibited metastatic tumor growth and specifically drove TAMs toward the more classically activated phenotype (115). Significant work remains to be done in characterizing the role of TAMs in the progression of brain metastases and identifying potentially additional targetable interactions between the metastatic cells and TAMs.

Infiltrating immune cells

After the initial seeding and development of the metastatic niche, an additional element of the CNS TME arrives in the form of TILs. CD4+ T cells, CD8+ T cells, and Tregs infiltrate significantly in both preclinical models, and clinical specimens of NSCLC and melanoma brain metastases (122). Similar findings have been reported in glioma with more extensive research into mechanisms and implications (123). In nonpathologic states, Tregs function to resolve inflammation; however, in the TME, this action encourages further proliferation of the metastatic tumor (124). As such, these cells present an attempted immune response and another route of local immune suppression. Glioma research demonstrated chemokines’ role, including C-C motif ligand 2 (CCL2), and local induction as the cause for the enriched Treg population within glioma [125, p2]. Similar experiments have not yet been conducted in metastatic models to confirm an analogous pathway. However, direct extrapolation from glioma should be viewed with some skepticism, as recent work highlighted significant differences between the TME of the two. Study of multiple tumor subtypes demonstrated that, in general, brain metastases contain significantly greater populations of T cells and neutrophils compared with the immunologically cold glioma, with relevant differences in their genomic and proteomic profiles. These findings highlight a contrast that could be particularly relevant to the future of immunotherapeutics in the CNS (126, 127).

In consideration of differential treatment responsiveness, studies across brain metastases from various primary tumors have also noted differences in the profile of TILs in the metastatic lesions, with lung cancer metastases showing more significantly upregulated immune checkpoint expression, including programmed death-ligand 1 (PD-L1), PD-L2, and liothyronine deiodinase 1 (IOD1), compared with breast and colorectal cancer (128). In comparison with the primary tumor site, the NSCLC brain metastases show fewer TILs in total with more anti-inflammatory TAMs, presenting a uniquely immunosuppressed local environment that supports tumor proliferation (129). Notably, Berghoff and colleagues examined the density and distribution of infiltrating immune cells in clinical brain metastases samples and found no correlation with overall TIL or Treg density and survival (62). Recent work investigating the mechanisms of local and systemic immunosuppression associated with intracranial tumors, including the sequestration of functional T cells in the bone marrow, is also relevant to understanding the presence and function of these TILs (130–132). Thus far, significant effort has been invested in attempts to reverse the immunosuppressive environment and permit infiltrating immune cells to actively engage with metastatic lesions, with the most relevant clinical studies involving immunotherapies and targeted strategies summarized in Table 1.

Leptomeningeal metastases

Metastatic spread to leptomeninges, either focally or diffusely, and with or without BrM, is seen in 8% of cancer patients in autopsy studies and also seems to be increasing as patients with cancer live longer (133). Hematologic, melanoma, lung, and breast cancer are common causes of such spread. Leptomeningeal tumors usually elicit an inflammatory response, even without malignant cells in the cerebrospinal fluid (CSF), often called carcinomatous meningitis (133). The preclinical study by Boire and colleagues suggests that C3 expression in primary tumors is predictive of leptomeningeal relapse. Pharmacologic manipulation with C3 signaling was shown to suppress leptomeningeal metastasis in preclinical models (134). Considering strategies to intervene in these processes may also potentially prevent access to the CNS and present another avenue for further research.

Therapeutic Challenges and Opportunities

Until recently, surgical resection followed by radiotherapy was the main therapy strategy for patients with brain metastases, with laser interstitial thermal therapy (LITT) use rising for patients with recurrent disease (5). Tailoring of radiotherapeutic doses, schedule, and techniques has advanced significantly to improve efficacy and limit toxicities. These include stereotactic radiosurgery (SRS) alone instead of whole-brain radiotherapy (WBRT) and the use of hippocampal sparing strategies with memantine administration in patients requiring WBRT (135, 136). Traditionally, BrMs have been notably resistant to both radio- and chemotherapy. In particular, melanoma and renal cell carcinoma metastases are known to be radioresistant, though SRS does extend survival in these patients as well (137). Choi and colleagues demonstrated that TopBP1 and Claspin genes are increased in such radioresistant cells and their targeted depletion enhances sensitivity, as...
Table 1. Pivotal clinical trials of targeted or immunotherapies in patients with brain metastases.

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<tr>
<th>Intervention</th>
<th>Patient population</th>
<th>Phase</th>
<th>Symptomatic</th>
<th>Prior local therapy</th>
<th>n</th>
<th>Intracranial response (%)</th>
<th>Median PFS (m)</th>
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<tr>
<td>Lapatinib</td>
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<td>Lapatinib plus capcitabine</td>
<td>HER2 positive, prior therapy allowed</td>
<td>II</td>
<td>Allowed</td>
<td>No</td>
<td>45</td>
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<td></td>
<td>40</td>
<td>8</td>
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<td>163</td>
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<td>Neratinib plus capcitabine</td>
<td>Cohort 3A: HER2 positive, lapatinib-naive</td>
<td>II</td>
<td>Allowed</td>
<td>Yes</td>
<td>37</td>
<td>49</td>
<td>5.5</td>
<td>152</td>
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<td></td>
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<td>12</td>
<td>33</td>
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<td>II*</td>
<td>No</td>
<td>Allowed</td>
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<td>18.1</td>
<td>164</td>
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<td>Allowed</td>
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<td>II</td>
<td>No</td>
<td>No</td>
<td>74</td>
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<td>No</td>
<td>15</td>
<td>15</td>
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<td>Yes</td>
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<td>II</td>
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<td>Cohort C: BRAF V600D/K/R mutation</td>
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<td>Allowed</td>
<td>No</td>
<td>16</td>
<td>44</td>
<td>4.2</td>
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<td>Yes</td>
<td>Allowed</td>
<td>Yes</td>
<td>17</td>
<td>59</td>
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<td>BRAF V600 mutation</td>
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<td>Yes</td>
<td>Yes</td>
<td>24</td>
<td>37</td>
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<td>II</td>
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<td>Yes</td>
<td>90</td>
<td>18</td>
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<td>Yes</td>
<td>56</td>
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<td>II</td>
<td>No</td>
<td>Allowed</td>
<td>51</td>
<td>25</td>
<td>1.9</td>
<td>168</td>
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<td></td>
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<td>Yes</td>
<td>Allowed</td>
<td>Alloweed</td>
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<td>10</td>
<td>1.2</td>
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<td>No prior immunotherapy (unless given as adjuvant therapy)</td>
<td>II</td>
<td>No</td>
<td>No*</td>
<td>94</td>
<td>57</td>
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<td>II</td>
<td>No</td>
<td>No</td>
<td>25</td>
<td>44</td>
<td>50%*</td>
<td>159</td>
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<td></td>
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<td>No</td>
<td>No</td>
<td>26</td>
<td>20</td>
<td>29%*</td>
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<td>Yes</td>
<td>Yes</td>
<td>16</td>
<td>6</td>
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<td>Allowed</td>
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<td>III*</td>
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<td>Allowed</td>
<td>64</td>
<td>59</td>
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<td>67%*</td>
<td>170</td>
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<td>III*</td>
<td>No</td>
<td>Allowed</td>
<td>44</td>
<td>73</td>
<td>10.7</td>
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<td>Yes</td>
<td>42</td>
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<td>40</td>
<td>28</td>
<td>10.1</td>
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<td>Yes</td>
<td>12</td>
<td>29</td>
<td>NE</td>
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<td>26</td>
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<td>47</td>
<td>17</td>
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<td>No</td>
<td>Allowed</td>
<td>81</td>
<td>63</td>
<td>14.5</td>
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</table>

(Continued on the following page)
does interruption of DNA damage checkpoint pathways (138, 139). Several studies have also investigated various radiation protocols for optimization against such radioresistant tumors, with success in fractionated and hypofractionated SRS plans (137, 140, 141). Many conventional chemotherapy and targeted drugs lack effective penetration of the BBB and are actively extruded from the brain, and encounter several further resistance mechanisms once within the CNS parenchyma (142–144). Some of this hurdle is directly linked to the interplay between metastatic cells and the native CNS components, as demonstrated with the findings that reactive astrocytes actively enhance chemoresistance through calcium sequestration and the upregulation of survival genes in tumor cells (97, 145). Niessner and colleagues similarly found that interactions between melanoma metastases and brain-derived factors lead to therapy resistance mediated by AKT hyperactivation and PTEN loss (146). Specific signals from the brain microenvironment upregulating the PI3K–AKT–mTOR pathway has also been implicated in treatment-resistant breast cancer brain metastases (147, 148). This protective role of reactive astrocytes in treatment resistance has been shown to be conducted through direct cellular contact and supported across a number of primary tumor sources (97, 145, 149). The structural and functional heterogeneity within the brain microenvironment and the tumor must also be considered, both within lesions and across tumor subtypes (150). The development of improved biomarkers of drug penetrability and delivery will allow for greater evaluation of the efficacy of novel therapeutic strategies for brain metastases.

Recent advances in targeted and immunotherapies have stimulated the development of clinical trials specific to patients with brain metastases (Table 1). Small-molecule inhibitors and targeted antibodies have demonstrated varying efficacy in the treatment of brain metastases in patients with oncogene driven cancers such as HER-2, ALK, EGFR, AXL, ABL, and BRAF-driven tumors (151–157). Immune checkpoint inhibitors, having transformed the landscape in melanoma, lung cancer, and many other solid tumors, have also shown encouraging efficacy in patients with brain metastases (158–160). Overall prognosis of patients with brain metastases remains poor, as therapy responses are often short-lived. Many brain metastases tumor types are neither driven by targetable oncogenes nor responsive to immune checkpoint blockade. The local immunosuppressive environment-induced, as described above, presents an additional challenge to the broad application of immunotherapeutics in the CNS (161). Brain metastases, therefore, remains an active area of unmet clinical need, and further research is needed in order to exploit their molecular and immunologic vulnerabilities. Harnessing our growing understanding of the metastatic cascade and pursuing a strategy that targets the surrounding TME is one path forward that may have a role in future clinical practice.

### Targeting the Microenvironment

The review above characterized and highlighted the range of complex interactions that occur between brain metastases and the native CNS components. Considering the therapeutic value of these investigations requires a broad view of the shared and specific implicated pathways and an understanding of analogous mechanisms in more thoroughly studied primary and systemically metastatic cancers. Even before metastatic cells have gained a foothold in the CNS, there are opportunities to disrupt and evade their influence, as with the destruction of circulating tumor-derived exosomes through novel nanoparticles, mAb-directed blockade of essential endothelial adhesion mediators, inhibitors of essential chemotactic mediators, and targeted disruption of the BBB-transversal pathway (33, 34, 42, 43, 59, 60). Once within the CNS, various groups have shown the efficacy of interrupting specific signaling pathways between the metastatic cells and surrounding cellular components, such as blocking the formation of gap junctions or estrogen-dependent signaling in all subtypes of breast cancer, or BDNF in HER2+ breast cancer. Other groups have shown the potential for disrupting the intracellular cascades within reactive astrocytes or TAMs, as with pharmacologic STAT3, cMET, and PI3K inhibition.

Furthermore, common factors appear at various stages throughout the metastatic process, such as VEGF and MMPs. Potential avenues for their inhibition and the existing preclinical data are discussed previously, with promising directions for future therapeutic opportunities. Continued research into halting CNS invasion mechanisms and the reprogramming of native CNS components through preemptive or reactive pharmacologic intervention presents a new strategy to reduce and treat brain metastases. The findings discussed throughout this review emphasize the numerous potential targets therein.

### Conclusion

Brain metastases present a clinical problem with limited therapeutic answers thus far. The CNS is a unique environment for metastatic spread due to its relative isolation from the rest of the body and distinct immune and cellular milieu. The development of the metastatic TME begins likely long before circulating tumor cells cross the BBB, with the initial setting of the premetastatic niche by secreted factors from the primary site. As the TME evolves with selective pressures from the metastatic cells, the growth of the lesion becomes dependent on...
the nitial metastatic spread. Current research points to some shared pathways across primary tumor sources but indicates a vast range of diversity within the brain metastases TME. Investing in research that explores how brain metastases induce change in the surrounding native CNS is a promising avenue to progress in a dire clinical context.

**Authors’ Disclosures**

A.C. Tan reports personal fees from Amgen and Thermo Fisher Scientific outside the submitted work. C.K. Anders reports other from PUMA, Lilly, Merck, Seattle Genetics, Nektar, Tesaro, G1 Therapeutics, ZION, Novartis, Pfizer, Genentech, Eisai, IPSEN, Astra Zeneca, Immunomedics, and other from Elucida outside the submitted work. A.M. Pendergast has a patent for US 9,931,342 B2 issued; and is a consultant and scientific advisory board member for the Pew Charitable Trusts. D.M. Ashley reports personal fees from Ixerari and Diverse Biopharma outside the submitted work. M. Khasraw reports grants from AbbVie, grants from BMS, personal fees from Pfizer, personal fees from Specialised Therapeutics, personal fees from Jackson Lab, and personal fees from Roche outside the submitted work. No disclosures were reported by the other authors.

**Acknowledgments**

C.K. Anders is a supported Translating Duke Health Scholar. A.M. Pendergast is supported by grants R01-CA195549 and the Emerson Collective. M. Khasraw is supported by the following grants: P50-CA199091 (SPORE), P01-CA225622, and R01-CA235612.

**References**


Molecular Cancer Therapeutics

Salting the Soil: Targeting the Microenvironment of Brain Metastases


Mol Cancer Ther 2021;20:455-466. Published OnlineFirst January 5, 2021.

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