Salting the Soil: Targeting the Microenvironment of Brain Metastases A C

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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 microvasculature 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

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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 at 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 VEGFA, 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 changes are mediated through disruption of tight junction proteins, including ZO-1, claudin 5, and occludin (30, 32). Soto and colleagues highlighted the importance of premetastatic conditioning in the CNS with their finding that brain vascular endothelial cells upregulate cell 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 β_4-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 S110A9. 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 $ITG\beta_3$ 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 vitro* 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 (CEMIP), enriched in brain-tropic breast and lung cancer tumor–derived exosomes. The group showed that CEMIP induces upregulated cytokine and chemokine production and angiogenesis in the brain, promoting metastatic colonization of the CNS. Furthermore, knocking out CEMIP reduced brain metastases by 70% *in vivo*, indicating that CEMIP is required for the early stages of metastatic colonization. These results were corroborated in clinical samples with the correlation of CEMIP 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.
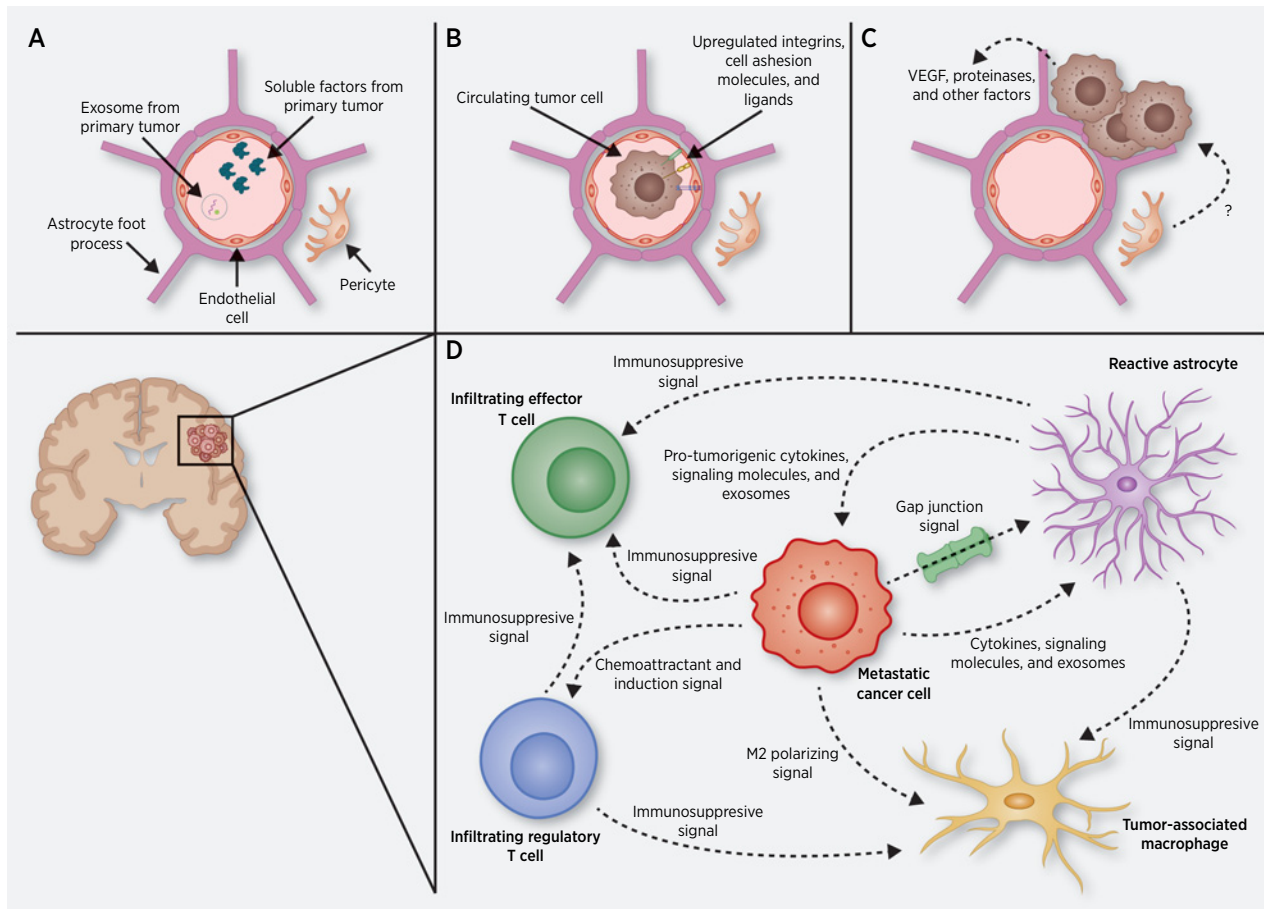
Figure 1.
Summary of interactions within the brain metastasis microenvironment: **A,** Priming of the BBB and establishment of the premetastatic niche through soluble factors and exosomes from the primary tumor site. **B,** Initial arrest of the circulating tumor cell within the brain microvasculature, mediated by a number of integrins, cell adhesion molecules, and secreted factors. **C,** Initial perivascular tumor niche with angioadaptive signaling. **D,** General case of interactions between the metastatic cancer cell and reactive astrocytes, tumor-associated macrophages, infiltrating effector T cells, and Tregs. Created with BioRender.com.

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 ITG$\alpha_v\beta_3$, 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, 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 MMP1, COX2, HBEGF, EREG, ST6GALNAC5, VEGF, and endothelial Rho-kinase, has been shown to significantly reduced incidence of brain metastases in preclinical studies [29, 50, 53, 57(p1), 58].

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 cele-coxib, 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 cells 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 $ITG\alpha_v\beta_3$, $ITG\beta_1$, and L1CAM) from both metastatic and CNS cells (63). Following perivascular 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 expression of neuronal-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. Ilhan-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 metastases 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 coopts 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× greater pericyte:endothelial cell ratio compared with other tissues, highlighting their importance in the early TME (92). Valdor 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 ITGβ$_1$ 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 are 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 survivorship. 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 IFNα and TNFα (96). Consequently, these inflammatory cytokines drive cell survival and chemoresistance mechanisms within the tumor cells via upregulation of STAT1, NFκB, GSTA5, BCL2L1, and TWIST1 (97). This interaction can be directly disrupted via BBB-penetrant gap-junction targeting drugs, including meclofenamate 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).

Priego 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 silibinin 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 ER$^+$ 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 ER$^+$ 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 CXCL-2, 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 cyclotraxin 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 chemo-resistance 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 TGFβ2 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.

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 depletion 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 iodothyronine 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 BrMs, 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.

Intervention	Patient population	Phase	Symptomatic	Prior local therapy	n	Intracranial response (%)	Median PFS (m)	References
Breast cancer								
HER2-targeted therapy								
Lapatinib	*HER2* positive, prior trastuzumab	II	No	Allowed	39	5	3.0	162
Lapatinib plus capecitabine	*HER2* positive, prior therapy allowed	II	Allowed	No	45	66	5.5	151
Neratinib	*HER2* positive, prior therapy allowed	II			40	8	1.9	163
Neratinib plus capecitabine	Cohort 3A: *HER2* positive, lapatinib-naïve	II	Allowed	Yes	37	49	5.5	152
	Cohort 3B: *HER2* positive, prior lapatinib		Allowed	Yes	12	33	3.1	
Trastuzumab deruxtecan	*HER2* positive, prior trastuzumab emtansine	II[a]	No	Yes	24	NR	18.1	164
Tucatinib, trastuzumab and capecitabine	*HER2* positive, prior trastuzumab, pertuzumab, trastuzumab emtansine	III[a]	No	Allowed	198	NR	7.6	154
Melanoma								
BRAF ± MEK TKI								
Dabrafenib	Cohort A: *BRAF* V600E mutation	II	No	No	74	39	3.7	165
	BRAF V600K mutation		No	No	15	7	1.9	
	Cohort B: *BRAF* V600E mutation		No	Yes	65	31	3.8	
	BRAF V600K mutation		No	Yes	18	22	3.7	
Dabrafenib plus trametinib	Cohort A: *BRAF* V600E mutation	II	No	No	76	58	5.6	154
	Cohort B: *BRAF* V600E mutation		No	Yes	16	56	7.2	
	Cohort C: *BRAF* V600D/K/R mutation		No	Allowed	16	44	4.2	
	Cohort D: *BRAF* V600D/K/R mutation		Yes	Allowed	17	59	5.5	
Vemurafenib	*BRAF* V600 mutation	II	Yes	Yes	24	37	4.4	166
Vemurafenib	Cohort 1: *BRAF* V600 mutation	II	Allowed	No	90	18	3.7	167
	Cohort 2: *BRAF* V600 mutation		Allowed	Yes	56	20	3.9	
Immunotherapy								
Ipilimumab	Cohort A: no prior immunotherapy	II	No	Allowed	51	25	1.9	168
	Cohort B: no prior immunotherapy		Yes	Allowed	21	10	1.2	
Ipilimumab plus nivolumab	No prior immunotherapy (unless given as adjuvant therapy)	II	No	No[b]	94	57	64%[c]	158
Ipilimumab plus nivolumab	Cohort A: no prior immunotherapy	II	No	No	25	44	50%[c]	159
Nivolumab	Cohort B: no prior immunotherapy	II	No	No	26	20	29%[c]	
	Cohort C: no prior immunotherapy		Yes	Yes	16	6	0%[c]	
Pembrolizumab	Prior immunotherapy allowed	II	No	Allowed	18	22	NR	160
NSCLC								
ALK TKI								
Alectinib	*ALK* rearranged, no prior TKI	III[a]	No	Allowed	64	59	NE	155
Alectinib	*ALK* rearranged, prior crizotinib	II[d]	No	Allowed	50	64	10.8	169
Brigatinib	*ALK* rearranged, no prior TKI	III[a]	No	Allowed	43	67	67%[e]	170
Ceritinib	*ALK* rearranged, no prior TKI	III[a]	No	Allowed	44	73	10.7	171
Ceritinib	Arm 1: *ALK* rearranged, prior crizotinib	II	No	Yes	42	39	9.2	156
	Arm 2: *ALK* rearranged, prior crizotinib		No	No	40	28	10.1	
	Arm 3: *ALK* rearranged, no prior TKI		No	Yes	12	29	NE	
	Arm 4: *ALK* rearranged, no prior TKI		No	No	44	52	7.5	
Crizotinib	*ALK* rearranged, no prior TKI	III[a]	No	Allowed	58	26	3.7	155
Crizotinib	*ALK* rearranged, no prior TKI	III[a]	No	Allowed	47	17	21%[e]	170
Lorlatinib	*ALK* rearranged, prior ALK TKI	II[a]	No	Allowed	81	63	14.5	172

(Continued on the following page)

Table 1. Pivotal clinical trials of targeted or immunotherapies in patients with brain metastases. (Cont'd)

Intervention	Patient population	Phase	Symptomatic	Prior local therapy	n	Intracranial response (%)	Median PFS (m)	References
EGFR TKI								
Gefitinib or erlotinib	*EGFR* mutation, no prior TKI	III[a]	No	No	67	43	71%[c]	157
Osimertinib	*EGFR* mutation, no prior TKI	III[a]	No	No	61	66	87%[c]	157
Immunotherapy								
Pembrolizumab	No prior immunotherapy	II	No	Allowed	18	33	NR	160

Abbreviations: N.E., not estimable; N.R., not reported; NSCLC, non–small cell lung cancer; O.S., overall survival; PFS, progression-free survival; TKI, tyrosine kinase inhibitor; WBRT, whole-brain radiotherapy.
[a]Prespecified subgroup of trial.
[b]Prior local therapy allowed to nontarget lesions.
[c]6-month PFS rate.
[d]Pooled analysis of two phase II trials.
[e]12-month PFS rate.

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, 53, 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

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References

1. Ostrom QT, Wright CH, Barnholtz-Sloan JS. Brain metastases: epidemiology. Handb Clin Neurol 2018;149:27–42.

2. Barnholtz-Sloan JS, Sloan AE, Davis FG, Vigneau FD, Lai P, Sawaya RE. Incidence proportions of brain metastases in patients diagnosed (1973 to 2001) in the Metropolitan Detroit Cancer Surveillance System. J Clin Oncol 2004;22: 2865–72.

3. Nayak L, Lee EQ, Wen PY. Epidemiology of brain metastases. Curr Oncol Rep 2012;14:48–54.

4. Berghoff AS, Schur S, Füreder LM, Gatterbauer B, Dieckmann K, Widhalm G, et al. Descriptive statistical analysis of a real life cohort of 2419 patients with brain metastases of solid cancers. ESMO Open 2016;1:e000024.

5. Fecci PE, Champion CD, Hoj J, McKernan CM, Goodwin CR, Kirkpatrick JP, et al. The evolving modern management of brain metastasis. Clin Cancer Res 2019;25:6570–80.

6. Hall WA, Djalilian HR, Nussbaum ES, Cho KH. Long-term survival with metastatic cancer to the brain. Med Oncol 2000;17:279–86.

7. Cacho-Díaz B, Spínola-Maroño H, González-Aguilar A, Arrieta O. Factors associated with long-term survival in central nervous system metastases. J Neurooncol 2018;140:159–64.

8. Peters S, Bexelius C, Munk V, Leighl N. The impact of brain metastasis on quality of life, resource utilization and survival in patients with non-small-cell lung cancer. Cancer Treat Rev 2016;45: 139–62.

9. Paget S. The distribution of secondary growths in cancer of the breast. 1889. Cancer Metastasis Rev 1989;8:98–101.

10. Xing C-Y, Tarumi T, Liu J, Zhang Y, Turner M, Riley J, et al. Distribution of cardiac output to the brain across the adult lifespan. J Cereb Blood Flow Metab 2017;37:2848–56.

11. Obermeier B, Daneman R, Ransohoff RM. Development, maintenance and disruption of the blood-brain barrier. Nat Med 2013;19:1584–96.

12. Armulik A, Genové G, Betsholtz C. Pericytes: developmental, physiological, and pathological perspectives, problems, and promises. Dev Cell 2011;21: 193–215.

13. Khakh BS, Sofroniew MV. Diversity of astrocyte functions and phenotypes in neural circuits. Nat Neurosci 2015;18:942–52.

14. Saijo K, Glass CK. Microglial cell origin and phenotypes in health and disease. Nat Rev Immunol 2011;11:775–87.

15. Miller RH. Oligodendrocyte origins. Trends Neurosci 1996;19:92–6.

16. Lau LW, Cua R, Keough MB, Haylock-Jacobs S, Yong VW. Pathophysiology of the brain extracellular matrix: a new target for remyelination. Nat Rev Neurosci 2013;14:722–9.

17. Pantel K, Brakenhoff RH. Dissecting the metastatic cascade. Nat Rev Cancer 2004;4:448–56.

18. Mina LA, Sledge GW. Rethinking the metastatic cascade as a therapeutic target. Nat Rev Clin Oncol 2011;8:325–32.

19. Achrol AS, Rennert RC, Anders C, Soffietti R, Ahluwalia MS, Nayak L, et al. Brain metastases. Nat Rev Dis Primer 2019;5:1–26.

20. Yao H, Price TT, Cantelli G, Ngo B, Warner MJ, Olivere L, et al. Leukaemia hijacks a neural mechanism to invade the central nervous system. Nature 2018; 560:55–60.

21. Louveau A, Plog BA, Antila S, Alitalo K, Nedergaard M, Kipnis J. Understanding the functions and relationships of the glymphatic system and meningeal lymphatics. J Clin Invest 2017;127:3210–9.

22. Medeiros B, Allan AL. Molecular mechanisms of breast cancer metastasis to the lung: clinical and experimental perspectives. Int J Mol Sci 2019;20:2272.

23. Liu Y, Cao X. Characteristics and significance of the pre-metastatic niche. Cancer Cell 2016;30:668–81.

24. Wang D, Sun H, Wei J, Cen B, DuBois RN. CXCL1 is critical for premetastatic niche formation and metastasis in colorectal cancer. Cancer Res 2017;77: 3655–65.

25. Salvador F, Martin A, López-Menéndez C, Moreno-Bueno G, Santos V, Vázquez-Naharro A, et al. Lysyl oxidase-like protein LOXL2 promotes lung metastasis of breast cancer. Cancer Res 2017;77:5846–59.

26. Qian B-Z, Li J, Zhang H, Kitamura T, Zhang J, Campion LR, et al. CCL2 recruits inflammatory monocytes to facilitate breast tumor metastasis. Nature 2011;475:222–5.

27. Hsu Y-L, Yen M-C, Chang W-A, Tsai P-H, Pan Y-C, Liao S-H, et al. CXCL17-derived CD11b+Gr-1+ myeloid-derived suppressor cells contribute to lung metastasis of breast cancer through platelet-derived growth factor-BB. Breast Cancer Res 2019;21:23.

28. Safarzadeh E, Orangi M, Mohammadi H, Babaie F, Baradaran B. Myeloid-derived suppressor cells: important contributors to tumor progression and metastasis. J Cell Physiol 2018;233:3024–36.

29. Bohn KA, Adkins CE, Nounou MI, Lockman PR. Inhibition of VEGF and angiopoietin-2 to reduce brain metastases of breast cancer burden. Front Pharmacol 2017;8:193.

30. Li B, Wang C, Zhang Y, Zhao XY, Huang B, Wu PF, et al. Elevated PLGF contributes to small-cell lung cancer brain metastasis. Oncogene 2013;32: 2952–62.

31. Si M, Jiao X, Li Y, Chen H, He P, Jiang F. The role of cytokines and chemokines in the microenvironment of the blood–brain barrier in leukemia central nervous system metastasis. Cancer Manag Res 2018;10:305–13.

32. Feng S, Cen J, Huang Y, Shen H, Yao L, Wang Y, et al. Matrix metalloproteinase-2 and -9 secreted by leukemic cells increase the permeability of blood-brain barrier by disrupting tight junction proteins. PLoS One 2011;6:e20599.

33. Soto MS, Serres S, Anthony DC, Sibson NR. Functional role of endothelial adhesion molecules in the early stages of brain metastasis. Neuro Oncol 2014; 16:540–51.

34. Liu Y, Kosaka A, Ikeura M, Kohanbash G, Fellows-Mayle W, Snyder LA, et al. Premetastatic soil and prevention of breast cancer brain metastasis. Neuro Oncol 2013;15:891–903.

35. Théry C, Zitvogel L, Amigorena S. Exosomes: composition, biogenesis and function. Nat Rev Immunol 2002;2:569–79.

36. Chen G, Huang AC, Zhang W, Zhang G, Wu M, Xu W, et al. Exosomal PD-L1 contributes to immunosuppression and is associated with anti-PD-1 response. Nature 2018;560:382–6.

37. Pasquier J, Thawadi HA, Ghiabi P, Abu-Kaoud N, Maleki M, Guerrouahen BS, et al. Microparticles mediated cross-talk between tumoral and endothelial cells promote the constitution of a pro-metastatic vascular niche through Arf6 up regulation. Cancer Microenviron 2014;7:41–59.

38. Rolland Y, Demeule M, Fenart L, Béliveau R. Inhibition of melanoma brain metastasis by targeting melanotransferrin at the cell surface. Pigment Cell Melanoma Res 2009;22:86–98.

39. Czernek L, Düchler M. Functions of cancer-derived extracellular vesicles in immunosuppression. Arch Immunol Ther Exp 2017;65:311–23.

40. Grange C, Tapparo M, Collino F, Vitillo L, Damasco C, Deregibus MC, et al. Microvesicles released from human renal cancer stem cells stimulate angiogenesis and formation of lung premetastatic niche. Cancer Res 2011; 71:5346–56.

41. Peinado H, Alečković M, Lavotshkin S, Matei I, Costa-Silva B, Moreno-Bueno G, et al. Melanoma exosomes educate bone marrow progenitor cells toward a pro-metastatic phenotype through MET. Nat Med 2012;18:883–91.

42. Hoshino A, Costa-Silva B, Shen T-L, Rodrigues G, Hashimoto A, Mark MT, et al. Tumour exosome integrins determine organotropic metastasis. Nature 2015;527:329–35.

43. Ye H, Wang K, Lu Q, Zhao J, Wang K, Kan Q, et al. Nanosponges of circulating tumor-derived exosomes for breast cancer metastasis inhibition. Biomaterials 2020;242:119932.

44. Morad G, Carman CV, Hagedorn EJ, Perlin JR, Zon LI, Mustafaoglu N, et al. Tumor-derived extracellular vesicles breach the intact blood-brain barrier via transcytosis. ACS Nano 2019;13:13853–65.

45. Fong MY, Zhou W, Liu L, Alontaga AY, Chandra M, Ashby J, et al. Breast-cancer-secreted miR-122 reprograms glucose metabolism in premetastatic niche to promote metastasis. Nat Cell Biol 2015;17:183–94.

46. Tominaga N, Kosaka N, Ono M, Katsuda T, Yoshioka Y, Tamura K, et al. Brain metastatic cancer cells release microRNA-181c-containing extracellular vesicles capable of destructing blood–brain barrier. Nat Commun 2015;6:6716.

47. Rodrigues G, Hoshino A, Kenific CM, Matei IR, Steiner L, Freitas D, et al. Tumour exosomal CEMIP protein promotes cancer cell colonization in brain metastasis. Nat Cell Biol 2019;21:1403–12.

48. Wilson EH, Weninger W, Hunter CA. Trafficking of immune cells in the central nervous system. J Clin Invest 2010;120:1368–79.

49. Wilhelm I, Molnár J, Fazakas C, Haskó J, Krizbai IA. Role of the blood-brain barrier in the formation of brain metastases. Int J Mol Sci 2013;14:1383–411.

50. Bos PD, Zhang XH-F, Nadal C, Shu W, Gomis RR, Nguyen DX, et al. Genes that mediate breast cancer metastasis to the brain. Nature 2009; 459:1005–9.

51. Sevenich L, Bowman RL, Mason SD, Quail DF, Rapaport F, Elie BT, et al. Analysis of tumour- and stroma-supplied proteolytic networks reveals a brain-metastasis-promoting role for cathepsin S. Nat Cell Biol 2014;16:876–88.

52. Lee T-H, Avraham HK, Jiang S, Avraham S. Vascular endothelial growth factor modulates the transendothelial migration of MDA-MB-231 breast cancer cells through regulation of brain microvascular endothelial cell permeability. J Biol Chem 2003;278:5277–84.

53. Yang X, Di J, Zhang Y, Zhang S, Lu J, Liu J, et al. The Rho-kinase inhibitor inhibits proliferation and metastasis of small cell lung cancer. Biomed Pharmacother 2012;66:221–7.

54. Li B, Zhao W-D, Tan Z-M, Fang W-G, Zhu L, Chen Y-H. Involvement of Rho/ROCK signalling in small cell lung cancer migration through human brain microvascular endothelial cells. FEBS Lett 2006;580:4252–60.

55. Herwig N, Belter B, Pietzsch J. Extracellular S100A4 affects endothelial cell integrity and stimulates transmigration of A375 melanoma cells. Biochem Biophys Res Commun 2016;477:963–9.

56. Lee B-C, Lee T-H, Avraham S, Avraham HK. Involvement of the chemokine receptor CXCR4 and its ligand stromal cell-derived factor 1alpha in breast cancer cell migration through human brain microvascular endothelial cells. Mol Cancer Res 2004;2:327–38.

57. Liu H, Kato Y, Erzinger SA, Kiriakova GM, Qian Y, Palmieri D, et al. The role of MMP-1 in breast cancer growth and metastasis to the brain in a xenograft model. BMC Cancer 2012;12:583.

58. Wu K, Fukuda K, Xing F, Zhang Y, Sharma S, Liu Y, et al. Roles of the cyclooxygenase 2 matrix metalloproteinase 1 pathway in brain metastasis of breast cancer. J Biol Chem 2015;290:9842–54.

59. Zhang J, Iwanaga K, Choi KC, Wislez M, Raso MG, Wei W, et al. Intratumoral epiregulin is a marker of advanced disease in non–small cell lung cancer patients and confers invasive properties on EGFR-mutant cells. Cancer Prev Res 2008;1:201–7.

60. Sanui A, Yotsumoto F, Tsujioka H, Fukami T, Horiuchi S, Shirota K, et al. HB-EGF inhibition in combination with various anticancer agents enhances its antitumor effects in gastric cancer. Anticancer Res 2010;30:3143–9.

61. Kienast Y, von Baumgarten L, Fuhrmann M, Klinkert WEF, Goldbrunner R, Herms J, et al. Real-time imaging reveals the single steps of brain metastasis formation. Nat Med 2010;16:116–22.

62. Berghoff AS, Rajky O, Winkler F, Bartsch R, Furtner J, Hainfellner JA, et al. Invasion patterns in brain metastases of solid cancers. Neuro Oncol 2013;15: 1664–72.

63. Lorger M, Krueger JS, O'Neal M, Staflin K, Felding-Habermann B. Activation of tumor cell integrin alphavbeta3 controls angiogenesis and metastatic growth in the brain. Proc Natl Acad Sci U S A 2009;106:10666–71.

64. Neman J, Termini J, Wilczynski S, Vaidehi N, Choy C, Kowolik CM, et al. Human breast cancer metastases to the brain display GABAergic properties in the neural niche. Proc Natl Acad Sci U S A 2014;111:984–9.

65. Zeng Q, Michael IP, Zhang P, Saghafinia S, Knott G, Jiao W, et al. Synaptic proximity enables NMDAR signalling to promote brain metastasis. Nature 2019;573:526–31.

66. Hoj JP, Mayro B, Pendergast AM. A TAZ-AXL-ABL2 feed-forward signaling axis promotes lung adenocarcinoma brain metastasis. Cell Rep 2019;29: 3421–34.

67. Er EE, Valiente M, Ganesh K, Zou Y, Agrawal S, Hu J, et al. Pericyte-like spreading by disseminated cancer cells activates YAP and MRTF for metastatic colonization. Nat Cell Biol 2018;20:966–78.

68. Valiente M, Obenauf AC, Jin X, Chen Q, Zhang XH-F, Lee DJ, et al. Serpins promote cancer cell survival and vascular co-option in brain metastasis. Cell 2014;156:1002–16.

69. Ilhan-Mutlu A, Osswald M, Liao Y, Gömmel M, Reck M, Miles D, et al. Bevacizumab prevents brain metastases formation in lung adenocarcinoma. Mol Cancer Ther 2016;15:702–10.

70. Reck M, von Pawel J, Zatloukal P, Ramlau R, Gorbounova V, Hirsh V, et al. Overall survival with cisplatin–gemcitabine and bevacizumab or placebo as first-line therapy for nonsquamous non-small-cell lung cancer: results from a randomised phase III trial (AVAiL). Ann Oncol 2010;21:1804–9.

71. Lassen U, Nielsen D, Sørensen M, Rønnengart E, Eldrup K, Bentzon K, et al. Abstract A111: a phase I, dose escalation study of TB-403, a monoclonal antibody directed against PlGF, in patients with solid tumors. Mol Cancer Ther 2009;8:A111.

72. Vandewynckel Y-P, Laukens D, Devisscher L, Bogaerts E, Paridaens A, Van den Bussche A, et al. Placental growth factor inhibition modulates the interplay between hypoxia and unfolded protein response in hepatocellular carcinoma. BMC Cancer 2016;16:9.

73. Fischer C, Jonckx B, Mazzone M, Zacchigna S, Loges S, Pattarini L, et al. Anti-PlGF inhibits growth of VEGF(R)-inhibitor-resistant tumors without affecting healthy vessels. Cell 2007;131:463–75.

74. Bu J, Bu X, Liu B, Chen F, Chen P. Inhibition of metastasis of oral squamous cell carcinoma by anti-PLGF treatment. Tumor Biol 2015;36:2695–701.

75. Rolny C, Mazzone M, Tugues S, Laoui D, Johansson I, Coulon C, et al. HRG inhibits tumor growth and metastasis by inducing macrophage polarization and vessel normalization through downregulation of PlGF. Cancer Cell 2011; 19:31–44.

76. Marshall DC, Lyman SK, McCauley S, Kovalenko M, Spangler R, Liu C, et al. Selective allosteric inhibition of MMP9 is efficacious in preclinical models of ulcerative colitis and colorectal cancer. PLoS One 2015;10:e0127063.

77. Winer A, Janosky M, Harrison B, Zhong J, Moussai D, Siyah P, et al. Inhibition of breast cancer metastasis by presurgical treatment with an oral matrix metalloproteinase inhibitor: a preclinical proof-of-principle study. Mol Cancer Ther 2016;15:2370–7.

78. Lee CZ, Yao JS, Huang Y, Zhai W, Liu W, Guglielmo BJ, et al. Dose–response effect of tetracyclines on cerebral matrix metalloproteinase-9 after vascular endothelial growth factor hyperstimulation. J Cereb Blood Flow Metab 2006;26: 1157–64.

79. Masumori N, Tsukamoto T, Miyao N, Kumamoto Y, Saiki I, Yoneda J. Inhibitory effect of minocycline on in vitro invasion and experimental metastasis of mouse renal adenocarcinoma. J Urol 1994;151:1400–4.

80. Markovic DS, Vinnakota K, van Rooijen N, Kiwit J, Synowitz M, Glass R, et al. Minocycline reduces glioma expansion and invasion by attenuating microglial MT1-MMP expression. Brain Behav Immun 2011;25:624–8.

81. Niu G, Liao Z, Cai L, Wei R, Sun L. The combined effects of celecoxib and minocycline hydrochloride on inhibiting the osseous metastasis of breast cancer in nude mice. Cancer Biother Radiopharm 2008;23:469–76.

82. Lokeshwar BL, Selzer MG, Zhu B-Q, Block NL, Golub LM. Inhibition of cell proliferation, invasion, tumor growth and metastasis by an oral

non-antimicrobial tetracycline analog (COL-3) in a metastatic prostate cancer model. Int J Cancer 2002;98:297–309.

83. Eyler CE, Foo W-C, LaFiura KM, McLendon RE, Hjelmeland AB, Rich JN. Brain cancer stem cells display preferential sensitivity to Akt inhibition. Stem Cells Dayt Ohio 2008;26:3027–36.

84. Wang X, Prager BC, Wu Q, Kim LJY, Gimple RC, Shi Y, et al. Reciprocal signaling between glioblastoma stem cells and differentiated tumor cells promotes malignant progression. Cell Stem Cell 2018;22:514–28.

85. Ricci-Vitiani L, Pallini R, Biffoni M, Todaro M, Invernici G, Cenci T, et al. Tumour vascularization via endothelial differentiation of glioblastoma stem-like cells. Nature 2010;468:824–8.

86. Scully S, Francescone R, Faibish M, Bentley B, Taylor SL, Oh D, et al. Transdifferentiation of glioblastoma stem-like cells into mural cells drives vasculogenic mimicry in glioblastomas. J Neurosci 2012;32:12950–60.

87. Cheng L, Huang Z, Zhou W, Wu Q, Donnola S, Liu JK, et al. Glioblastoma stem cells generate vascular pericytes to support vessel function and tumor growth. Cell 2013;153:139–52.

88. Haskó J, Fazakas C, Molnár K, Mészáros Á, Patai R, Szabó G, et al. Response of the neurovascular unit to brain metastatic breast cancer cells. Acta Neuropathol Commun 2019;7:133.

89. Fidler IJ, Yano S, Zhang R, Fujimaki T, Bucana CD. The seed and soil hypothesis: vascularisation and brain metastases. Lancet Oncol 2002;3:53–7.

90. Calabrese C, Poppleton H, Kocak M, Hogg TL, Fuller C, Hamner B, et al. A perivascular niche for brain tumor stem cells. Cancer Cell 2007;11:69–82.

91. Charles N, Ozawa T, Squatrito M, Bleau A-M, Brennan CW, Hambardzumyan D, et al. Perivascular nitric oxide activates notch signaling and promotes stem-like character in PDGF-induced glioma cells. Cell Stem Cell 2010;6:141–52.

92. Shepro D, Morel NM. Pericyte physiology. FASEB J 1993;7:1031–8.

93. Valdor R, García-Bernal D, Bueno C, Ródenas M, Moraleda JM, Macian F, et al. Glioblastoma progression is assisted by induction of immunosuppressive function of pericytes through interaction with tumor cells. Oncotarget 2017; 8:68614–26.

94. Carbonell WS, Ansorge O, Sibson N, Muschel R. The vascular basement membrane as "soil" in brain metastasis. PLoS One 2009;4:e5857.

95. Seike T, Fujita K, Yamakawa Y, Kido MA, Takiguchi S, Teramoto N, et al. Interaction between lung cancer cells and astrocytes via specific inflammatory cytokines in the microenvironment of brain metastasis. Clin Exp Metastasis 2011;28:13–25.

96. Chen Q, Boire A, Jin X, Valiente M, Er EE, Lopez-Soto A, et al. Carcinoma–astrocyte gap junctions promote brain metastasis by cGAMP transfer. Nature 2016;533:493–8.

97. Kim S-J, Kim J-S, Park ES, Lee JS, Lin Q, Langley RR, et al. Astrocytes upregulate survival genes in tumor cells and induce protection from chemotherapy. Neoplasia 2011;13:286–98.

98. Zhang L, Zhang S, Yao J, Lowery FJ, Zhang Q, Huang W-C, et al. Microenvironment-induced PTEN loss by exosomal microRNA primes brain metastasis outgrowth. Nature 2015;527:100–4.

99. Priego N, Zhu L, Monteiro C, Mulders M, Wasilewski D, Bindeman W, et al. STAT3 labels a subpopulation of reactive astrocytes required for brain metastasis. Nat Med 2018;24:1024–35.

100. Gril B, Palmieri D, Qian Y, Anwar T, Liewehr DJ, Steinberg SM, et al. Pazopanib inhibits the activation of PDGFRβ-expressing astrocytes in the brain metastatic microenvironment of breast cancer cells. Am J Pathol 2013;182:2368–79.

101. Gril B, Palmieri D, Qian Y, Smart DD, Ileva L, Liewehr DJ, et al. Pazopanib reveals a role for tumor cell B-Raf in the prevention of HER2+ breast cancer brain metastasis. Clin Cancer Res 2011;17:142–53.

102. Sartorius CA, Hanna CT, Gril B, Cruz H, Serkova NJ, Huber KM, et al. Estrogen promotes the brain metastatic colonization of triple negative breast cancer cells via an astrocyte-mediated paracrine mechanism. Oncogene 2016;35:2881–92.

103. Contreras-Zárate MJ, Day NL, Ormond DR, Borges VF, Tobet S, Gril B, et al. Estradiol induces BDNF/TrkB signaling in triple-negative breast cancer to promote brain metastases. Oncogene 2019;38:4685–99.

104. Xing F, Liu Y, Sharma S, Wu K, Chan MD, Lo H-W, et al. Activation of the c-Met pathway mobilizes an inflammatory network in the brain microenvironment to promote brain metastasis of breast cancer. Cancer Res 2016;76:4970–80.

105. Xing F, Kobayashi A, Okuda H, Watabe M, Pai SK, Pandey PR, et al. Reactive astrocytes promote the metastatic growth of breast cancer stem-like cells by activating Notch signalling in brain. EMBO Mol Med 2013;5:384–96.

106. Choy C, Ansari KI, Neman J, Hsu S, Duenas MJ, Li H, et al. Cooperation of neurotrophin receptor TrkB and Her2 in breast cancer cells facilitates brain metastases. Breast Cancer Res 2017;19:51.

107. Jandial R, Choy C, Levy DM, Chen MY, Ansari KI. Astrocyte-induced Reelin expression drives proliferation of Her2+ breast cancer metastases. Clin Exp Metastasis 2017;34:185–96.

108. Kim SW, Choi HJ, Lee H-J, He J, Wu Q, Langley RR, et al. Role of the endothelin axis in astrocyte- and endothelial cell-mediated chemoprotection of cancer cells. Neuro Oncol 2014;16:1585–98.

109. Klein A, Schwartz H, Sagi-Assif O, Meshel T, Izraely S, Ben Menachem S, et al. Astrocytes facilitate melanoma brain metastasis via secretion of IL-23. J Pathol 2015;236:116–27.

110. Gong X, Hou Z, Endsley MP, Gronseth EI, Rarick KR, Jorns JM, et al. Interaction of tumor cells and astrocytes promotes breast cancer brain metastases through TGF-β2/ANGPTL4 axes. NPJ Precis Oncol 2019;3:1–9.

111. McFarland BC, Benveniste EN. Reactive astrocytes foster brain metastases via STAT3 signaling. Ann Transl Med 2019;7:S83.

112. Ransohoff RM. A polarizing question: do M1 and M2 microglia exist? Nat Neurosci 2016;19:987–91.

113. Soto MS, Sibson NR. The multifarious role of microglia in brain metastasis. Front Cell Neurosci 2018;12:414.

114. Andreou KE, Soto MS, Allen D, Economopoulos V, de Bernardi A, Larkin JR, et al. Anti-inflammatory microglia/macrophages as a potential therapeutic target in brain metastasis. Front Oncol 2017;7:251.

115. Blazquez R, Wlochowitz D, Wolff A, Seitz S, Wachter A, Perera-Bel J, et al. PI3K: a master regulator of brain metastasis-promoting macrophages/microglia. Glia 2018;66:2438–55.

116. Pukrop T, Dehghani F, Chuang H-N, Lohaus R, Bayanga K, Heermann S, et al. Microglia promote colonization of brain tissue by breast cancer cells in a Wnt-dependent way. Glia 2010;58:1477–89.

117. Chuang H-N, van Rossum D, Sieger D, Siam L, Klemm F, Bleckmann A, et al. Carcinoma cells misuse the host tissue damage response to invade the brain. Glia 2013;61:1331–46.

118. Louie E, Chen XF, Coomes A, Ji K, Tsirka S, Chen EI. Neurotrophin-3 modulates breast cancer cells and the microenvironment to promote the growth of breast cancer brain metastasis. Oncogene 2013;32:4064–77.

119. Xing F, Liu Y, Wu S-Y, Wu K, Sharma S, Mo Y-Y, et al. Loss of XIST in breast cancer activates MSN-c-Met and reprograms microglia via exosomal micro-RNA to promote brain metastasis. Cancer Res 2018;78:4316–30.

120. Wagner S, Czub S, Greif M, Vince GH, Süss N, Kerkau S, et al. Microglial/macrophage expression of interleukin 10 in human glioblastomas. Int J Cancer 1999;82:12–6.

121. Chhor V, Le Charpentier T, Lebon S, Oré M-V, Celador IL, Josserand J, et al. Characterization of phenotype markers and neurotoxic potential of polarised primary microglia in vitro. Brain Behav Immun 2013;32:70–85.

122. Berghoff AS, Fuchs E, Ricken G, Mlecnik B, Bindea G, Spanberger T, et al. Density of tumor-infiltrating lymphocytes correlates with extent of brain edema and overall survival time in patients with brain metastases. Oncoimmunology 2015;5:e1057388.

123. Distribution of tumor-infiltrating immune cells in glioblastoma | CNS Oncology; 2020. Available from: https://www.futuremedicine.com/doi/full/10.2217/cns-2017-0037

124. Sharma A, Rudra D. Emerging functions of regulatory T cells in tissue homeostasis. Front Immunol 2018;9:883.

125. Chang AL, Miska J, Wainwright DA, Dey M, Rivetta CV, Yu D, et al. CCL2 produced by the glioma microenvironment is essential for the recruitment of regulatory T cells and myeloid-derived suppressor cells. Cancer Res 2016;76: 5671–82.

126. Klemm F, Maas RR, Bowman RL, Kornete M, Soukup K, Nassiri S, et al. Interrogation of the microenvironmental landscape in brain tumors reveals disease-specific alterations of immune cells. Cell 2020;181:1643–60.

127. Friebel E, Kapolou K, Unger S, Núñez NG, Utz S, Rushing EJ, et al. Single-cell mapping of human brain cancer reveals tumor-specific instruction of tissue-invading leukocytes. Cell 2020;181:1626–42.

128. Harter PN, Bernatz S, Scholz A, Zeiner PS, Zinke J, Kiyose M, et al. Distribution and prognostic relevance of tumor-infiltrating lymphocytes (TILs) and PD-1/PD-L1 immune checkpoints in human brain metastases. Oncotarget 2015;6:40836–49.

129. Kudo Y, Haymaker C, Zhang J, Reuben A, Duose DY, Fujimoto J, et al. Suppressed immune microenvironment and repertoire in brain metastases from patients with resected non-small-cell lung cancer. Ann Oncol 2019;30:1521–30.

130. Chongsathidkiet P, Jackson C, Koyama S, Loebel F, Cui X, Farber SH, et al. Sequestration of T cells in bone marrow in the setting of glioblastoma and other intracranial tumors. Nat Med 2018;24:1459–68.

131. Lowery FJ, Yu D. Brain metastasis: unique challenges and open opportunities. Biochim Biophys Acta 2017;1867:49–57.

132. Sampson JH, Gunn MD, Fecci PE, Ashley DM. Brain immunology and immunotherapy in brain tumours. Nat Rev Cancer 2020;20:12–25.

133. Khasraw M, Posner JB. Neurological complications of systemic cancer. Lancet Neurol 2010;9:1214–27.

134. Boire A, Zou Y, Shieh J, Macalinao DG, Pentsova E, Massagué J. Complement component 3 adapts the cerebrospinal fluid for leptomeningeal metastasis. Cell 2017;168:1101–13.

135. Brown PD, Gondi V, Pugh S, Tome WA, Wefel JS, Armstrong TS, et al. Hippocampal avoidance during whole-brain radiotherapy plus memantine for patients with brain metastases: Phase III Trial NRG Oncology CC001. J Clin Oncol 2020;38:1019–29.

136. Kocher M, Soffietti R, Abacioglu U, Villà S, Fauchon F, Baumert BG, et al. Adjuvant whole-brain radiotherapy versus observation after radiosurgery or surgical resection of one to three cerebral metastases: results of the EORTC 22952-26001 study. J Clin Oncol 2011;29:134–41.

137. Brown PD, Brown CA, Pollock BE, Gorman DA, Foote RL. Stereotactic radiosurgery for patients with "radioresistant" brain metastases. Neurosurgery 2002;51:656–67.

138. Choi SH, Yang H, Lee SH, Ki J-H, Nam D-H, Yoo HY. TopBP1 and Claspin contribute to the radioresistance of lung cancer brain metastases. Mol Cancer 2014;13:211.

139. Yang H, Yoon SJ, Jin J, Choi SH, Seol HJ, Lee J-I, et al. Inhibition of checkpoint kinase 1 sensitizes lung cancer brain metastases to radiotherapy. Biochem Biophys Res Commun 2011;406:53–8.

140. Ahmed KA, Freilich JM, Abuodeh Y, Figura N, Patel N, Sarangkasiri S, et al. Fractionated stereotactic radiotherapy to the post-operative cavity for radioresistant and radiosensitive brain metastases. J Neurooncol 2014;118:179–86.

141. Scorsetti M, Facoetti A, Navarria P, Bignardi M, De Santis M, Ninone SA, et al. Hypofractionated stereotactic radiotherapy and radiosurgery for the treatment of patients with radioresistant brain metastases. Anticancer Res 2009;29:4259–63.

142. Okimoto T, Tsubata Y, Hotta T, Hamaguchi M, Nakao M, Hamaguchi S-I, et al. A low crizotinib concentration in the cerebrospinal fluid causes ineffective treatment of anaplastic lymphoma kinase-positive non-small cell lung cancer with carcinomatous meningitis. Intern Med 2019;58:703–5.

143. Agarwal S, Sane R, Gallardo JL, Ohlfest JR, Elmquist WF. Distribution of gefitinib to the brain is limited by P-glycoprotein (ABCB1) and breast cancer resistance protein (ABCG2)-mediated active efflux. J Pharmacol Exp Ther 2010;334:147–55.

144. Lagas JS, van Waterschoot RAB, Sparidans RW, Wagenaar E, Beijnen JH, Schinkel AH. Breast cancer resistance protein and P-glycoprotein limit sorafenib brain accumulation. Mol Cancer Ther 2010;9:319–26.

145. Lin Q, Balasubramanian K, Fan D, Kim S-J, Guo L, Wang H, et al. Reactive astrocytes protect melanoma cells from chemotherapy by sequestering intracellular calcium through gap junction communication channels. Neoplasia 2010;12:748–54.

146. Niessner H, Forschner A, Klumpp B, Honegger JB, Witte M, Bornemann A, et al. Targeting hyperactivation of the AKT survival pathway to overcome therapy resistance of melanoma brain metastases. Cancer Med 2013;2:76–85.

147. Kabraji S, Ni J, Lin NU, Xie S, Winer EP, Zhao JJ. Drug resistance in HER2-positive breast cancer brain metastases: blame the barrier or the brain? Clin Cancer Res 2018;24:1795–804.

148. Kodack DP, Askoxylakis V, Ferraro GB, Sheng Q, Badeaux M, Goel S, et al. The brain microenvironment mediates resistance in luminal breast cancer to PI3K inhibition through HER3 activation. Sci Transl Med 2017;9:eaal4682.

149. Fidler IJ. The role of the organ microenvironment in brain metastasis. Semin Cancer Biol 2011;21:107–12.

150. Arvanitis CD, Ferraro GB, Jain RK. The blood-brain barrier and blood-tumour barrier in brain tumours and metastases. Nat Rev Cancer 2020;20:26–41.

151. Bachelot T, Romieu G, Campone M, Diéras V, Cropet C, Dalenc F, et al. Lapatinib plus capecitabine in patients with previously untreated brain metastases from HER2-positive metastatic breast cancer (LANDSCAPE): a single-group phase 2 study. Lancet Oncol 2013;14:64–71.

152. Freedman RA, Gelman RS, Anders CK, Melisko ME, Parsons HA, Cropp AM, et al. TBCRC 022: a phase II trial of neratinib and capecitabine for patients with human epidermal growth factor receptor 2-positive breast cancer and brain metastases. J Clin Oncol 2019;37:1081–9.

153. Murthy RK, Loi S, Okines A, Paplomata E, Hamilton E, Hurvitz SA, et al. Tucatinib, trastuzumab, and capecitabine for HER2-positive metastatic breast cancer. N Engl J Med 2020;382:597–609.

154. Davies MA, Saiag P, Robert C, Grob J-J, Flaherty KT, Arance A, et al. Dabrafenib plus trametinib in patients with BRAFV600-mutant melanoma brain metastases (COMBI-MB): a multicentre, multicohort, open-label, phase 2 trial. Lancet Oncol 2017;18:863–73.

155. Peters S, Camidge DR, Shaw AT, Gadgeel S, Ahn JS, Kim DW, et al. Alectinib versus crizotinib in untreated ALK-positive non–small-cell lung cancer. N Engl J Med 2017;377:829–38.

156. Chow LQ, Barlesi F, Bertino EM, van den Bent MJ, Wakelee H, Wen PY, et al. Results of the ASCEND-7 phase II study evaluating ALK inhibitor (ALKi) ceritinib in patients (pts) with ALK+ non-small cell lung cancer (NSCLC) metastatic to the brain. Ann Oncol 2019;30:v602–3.

157. Reungwetwattana T, Nakagawa K, Cho BC, Cobo M, Cho EK, Bertolini A, et al. CNS response to osimertinib versus standard epidermal growth factor receptor tyrosine kinase inhibitors in patients with untreated EGFR-mutated advanced non-small-cell lung cancer. J Clin Oncol 2018;36:3290–97.

158. Tawbi HA, Forsyth PA, Algazi A, Hamid O, Hodi FS, Moschos SJ, et al. Combined nivolumab and ipilimumab in melanoma metastatic to the brain. N Engl J Med 2018;379:722–30.

159. Long GV, Atkinson V, Menzies AM, Lo S, Guminski AD, Brown MP, et al. A randomized phase 2 study of nivolumab and nivolumab combined with ipilimumab in patients (pts) with melanoma brain metastases: the Anti-PD1 Brain Collaboration (ABC Study). ASCO Meet Abstr 2016;34:TPS9591.

160. Goldberg SB, Gettinger SN, Mahajan A, Chiang AC, Herbst RS, Sznol M, et al. Pembrolizumab for patients with melanoma or non-small-cell lung cancer and untreated brain metastases: early analysis of a non-randomised, open-label, phase 2 trial. Lancet Oncol 2016;17:976–83.

161. Leibold AT, Monaco GN, Dey M. The role of the immune system in brain metastasis. Curr Neurobiol 2019;10:33–48.

162. Lin NU, Carey LA, Liu MC, Younger J, Come SE, Ewend M, et al. Phase II trial of lapatinib for brain metastases in patients with human epidermal growth factor receptor 2-positive breast cancer. J Clin Oncol 2008;26:1993–9.

163. Freedman RA, Gelman RS, Wefel JS, Melisko ME, Hess KR, Connolly RM, et al. Translational breast cancer research consortium (TBCRC) 022: a phase II trial of neratinib for patients with human epidermal growth factor receptor 2-positive breast cancer and brain metastases. J Clin Oncol 2016;34:945–52.

164. Modi S, Saura C, Yamashita T, Park YH, Kim S-B, Tamura K, et al. Trastuzumab deruxtecan in previously treated HER2-positive breast cancer. N Engl J Med 2020;382:610–21.

165. Long GV, Trefzer U, Davies MA, Kefford RF, Ascierto PA, Chapman PB, et al. Dabrafenib in patients with Val600Glu or Val600Lys BRAF-mutant melanoma metastatic to the brain (BREAK-MB): a multicentre, open-label, phase 2 trial. Lancet Oncol 2012;13:1087–95.

166. Dummer R, Goldinger SM, Turtschi CP, Eggmann NB, Michielin O, Mitchell L, et al. Vemurafenib in patients with BRAF(V600) mutation-positive melanoma with symptomatic brain metastases: final results of an open-label pilot study. Eur J Cancer 2014;50:611–21.

167. McArthur GA, Maio M, Arance A, Nathan P, Blank C, Avril M-F, et al. Vemurafenib in metastatic melanoma patients with brain metastases: an open-label, single-arm, phase 2, multicentre study. Ann Oncol 2017;28:634–41.

168. Margolin K, Ernstoff MS, Hamid O, Lawrence D, McDermott D, Puzanov I, et al. Ipilimumab in patients with melanoma and brain metastases: an open-label, phase 2 trial. Lancet Oncol 2012;13:459–65.

169. Gadgeel SM, Shaw AT, Govindan R, Gandhi L, Socinski MA, Camidge DR, et al. Pooled analysis of CNS response to alectinib in two studies of pretreated patients with ALK-positive non-small-cell lung cancer. J Clin Oncol 2016;34:4079–85.

170. Camidge DR, Kim HR, Ahn M-J, Chih-Hsin Yang J, Han J-Y, Leeet J-S, et al. Brigatinib versus crizotinib in ALK-positive non-small-cell lung cancer. N Engl J Med 2018;379:2027–39.

171. Soria J-C, Tan DSW, Chiari R, Wu Y-L, Paz-Ares L, Wolf J, et al. First-line ceritinib versus platinum-based chemotherapy in advanced ALK-rearranged non-small-cell lung cancer (ASCEND-4): a randomised, open-label, phase 3 study. Lancet 2017;389:917–29.

172. Solomon BJ, Besse B, Bauer TM, Felip E, Soo RA, Camidge DR, et al. Lorlatinib in patients with ALK-positive non-small-cell lung cancer: results from a global phase 2 study. Lancet Oncol 2018;19:1654–67.