Tumor Immune Microenvironment of Brain Metastases: Toward Unlocking Antitumor Immunity

Matthew R. Strickland[1], Christopher Alvarez-Breckenridge[2], Justin F. Gainor[1], and Priscilla K. Brastianos[1]

ABSTRACT Brain metastasis (BrM) is a devastating complication of solid tumors associated with poor outcomes. Immune-checkpoint inhibitors (ICI) have revolutionized the treatment of cancer, but determinants of response are incompletely understood. Given the rising incidence of BrM, improved understanding of immunobiologic principles unique to the central nervous system (CNS) and dissection of those that govern the activity of ICIs are paramount toward unlocking BrM-specific antitumor immunity. In this review, we seek to discuss the current clinical landscape of ICI activity in the CNS and CNS immunobiology, and we focus, in particular, on the role of glial cells in the CNS immune response to BrM.

Significance: There is an urgent need to improve patient selection for and clinical activity of ICIs in patients with cancer with concomitant BrM. Increased understanding of the unique immunobiologic principles that govern response to ICIs in the CNS is critical toward identifying targets in the tumor microenvironment that may potentiate antitumor immunity.

INTRODUCTION

Brain metastasis (BrM) is an increasingly common complication from solid tumor malignancies owing to improved imaging techniques and increasing overall survival (OS) among patients with cancer secondary to improved systemic therapies (1–3). Although various tumor types can develop BrM, primary histologies that metastasize to the brain most frequently include lung cancer, melanoma, and breast cancer (Fig. 1A; refs. 4–7). Even histologies with comparatively lower frequencies of BrM, such as colorectal cancer and renal cell carcinomas, have more recently demonstrated increasing incidence (8, 9). Unfortunately, diagnosis of BrM is often associated with increased morbidity and decreased OS, with the majority of patients surviving less than 1 year after diagnosis (4, 10, 11). Current treatment approaches for BrM include whole-brain radiotherapy, stereotactic radiosurgery, and surgical resection. Conventional chemotherapy has traditionally shown limited efficacy in the treatment of BrM, which is largely attributed to poor penetration of the blood–brain barrier (BBB) and transmembrane efflux pumps (12, 13). Thus, there is an urgent need to improve therapeutic paradigms.

Although there are currently no approved central nervous system (CNS)–specific systemic therapies, evolving treatment paradigms such as targeted therapies and immune-checkpoint inhibition (ICI) offer new hope for the treatment of BrM. ICI has shown intracranial activity in a subset of patients with CNS disease (14–17); however, the determinants of response to ICI remain incompletely characterized, and existing data are conflicting as to whether the antitumor immune response is concordant versus discordant across central and peripheral compartments. To improve outcomes for patients with BrM, characterization of the determinants of antitumor immunity across these compartments is paramount. To date, efforts have been disproportionately directed toward study of the peripheral compartment, owing in part to technical difficulty and risk associated with CNS tissue sampling. In this review, we focus on the current knowledge and promising investigations underway toward inducing antitumor immunity against BrM, with a special focus on glial cell role and function (Fig. 1B). When applicable, we have used examples from the primary brain tumor literature but overall focus specifically on BrM, as the tumor immune microenvironment (TIME) of primary brain tumors has been expertly reviewed elsewhere (18, 19).

[1]Massachusetts General Hospital, Harvard Medical School, Boston, Massachusetts. [2]The University of Texas MD Anderson Cancer Center, Houston, Texas.

Corresponding Author: Priscilla K. Brastianos, Divisions of Hematology/Oncology and Neuro-Oncology, Department of Medicine, Massachusetts General Hospital, 55 Fruit Street, Yawkey 9E, Boston, MA 02114. Phone: 617-724-4000; E-mail: pbrastianos@mgh.harvard.edu

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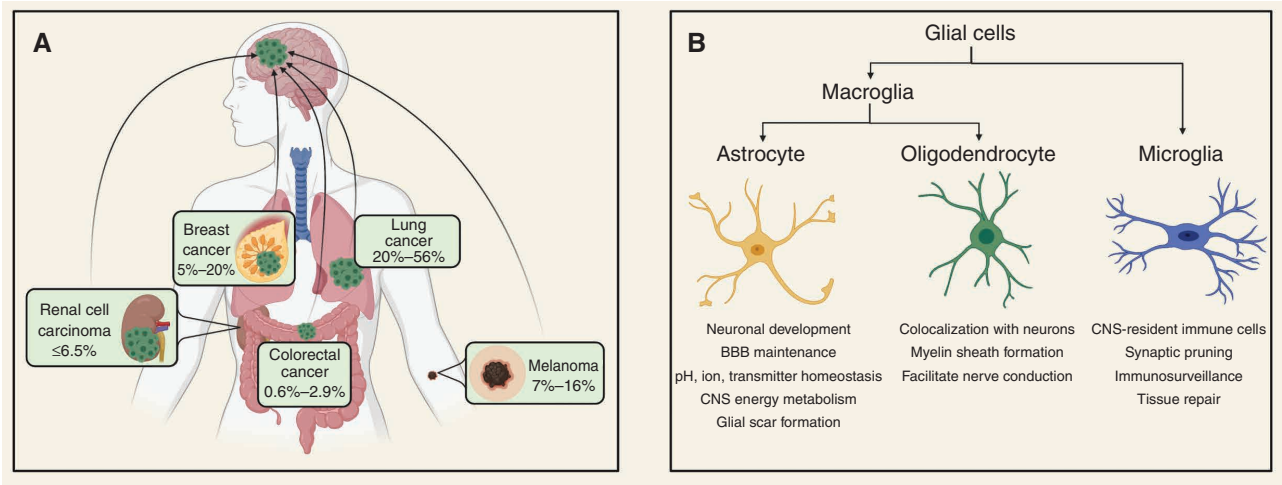

Figure 1. **A,** Most common primary tumor histologies associated with brain metastasis and frequency. **B,** Glial cells and their biological roles. Figure created with BioRender.com.

ICI CHALLENGES IN THE CNS: A CLINICAL SNAPSHOT

ICI has revolutionized cancer care: Programmed death 1 (PD-1) axis inhibitors are now approved in more than 19 different cancer types and have two tissue-agnostic indications (20). Depending on the underlying disease type, PD-1 pathway inhibitors are being used alone or in combination with chemotherapy, tyrosine kinase inhibitors, or other ICIs (e.g., CTLA4 inhibitors). Despite impressive survival benefits observed across cancer types, the extent of intracranial activity of ICI in patients with concomitant BrM remains understudied. Attempts to answer this question are hampered by several factors, including (i) patients with BrM have been historically excluded from clinical trials of ICIs; (ii) intracranial clinical activity is typically not a prespecified endpoint, and thus analyses are often limited to subgroups or are *post hoc* with limited power; (iii) patients with BrM that have participated in clinical trials are often highly selected for asymptomatic and/or treated disease; and (iv) for the small group of patients with symptomatic/progressing BrM that have participated in clinical trials, there is a high frequency of steroid dependence, which may mitigate ICI-induced antitumor immunity (21). Although the bulk of existing data may not fully reflect the immunobiologic landscape of CNS metastatic disease, prospective data for patients with untreated, symptomatic, or progressing BrM are growing—and form the foundation for our current understanding of translational challenges for evaluating ICI efficacy in this patient population.

A phase II trial by Tawbi and colleagues included patients with asymptomatic, untreated melanoma-derived BrM and demonstrated an impressive intracranial response rate (ICRR) of 54.5% using combined ipilimumab/nivolumab (Table 1; refs. 14–16, 22–26). In an updated analysis from the same trial focused solely on patients whose BrM were symptomatic (versus asymptomatic), an ICRR rate of 22.2% was observed, and all intracranial responders demonstrated concordant extracranial responses (22.2%; ref. 25). Median intracranial progression-free survival (IC-PFS) was 1.2 months, and OS was 8.7 months in these patients. By contrast, with a median follow-up of 20.6 months, median progression-free survival (PFS) and OS were not reached among asymptomatic participants. This landmark trial is perhaps the strongest evidence to date that ICI can induce clinically meaningful antitumor activity in the CNS and that the immune response is frequently concordant across central and peripheral compartments (50.5% of asymptomatic patients demonstrated a "global response"). Both of these intriguing observations directly challenge conventional CNS immune privilege dogma that would predict mitigated clinical activity in the CNS. In parallel, these data suggest impaired ICRR, PFS, and OS in patients with symptomatic versus asymptomatic BrM, and that clinical activity is diminished (though power is limited) in patients receiving corticosteroids, raising important questions regarding how the biology of a symptomatic lesion may differ compared with clinically asymptomatic disease. Intracranial activity of ICI leading to survival benefits in this population with historically increased morbidity and decreased survival may extend beyond melanoma as well, and thus these questions are in need of urgent exploration (27).

Three additional trials have investigated the activity of ICI among patients with active or progressing parenchymal BrM. Margolin and colleagues conducted a phase II study in patients with melanoma-derived BrM who received ipilimumab monotherapy, which demonstrated an ICRR of 16% and 5% for asymptomatic and symptomatic patients, respectively—again possibly suggesting that corticosteroid dependence for symptomatic CNS disease may mitigate ICI activity in the CNS (24). Long and colleagues conducted a multicenter, randomized phase II clinical trial of nivolumab monotherapy versus combined ipilimumab/nivolumab in patients with asymptomatic, melanoma-derived BrM (15). Notably, they

Table 1. Key prospective investigations evaluating immune checkpoint blockade in patients with untreated and/or progressive BrM

Reference(s)	Study design	Primary histology	Total cohort size	Inclusion criteria and stratification	Stratum cohort size	Treatment regimen	Outcomes				
							ICRR (%)	ECRR (%)	Median IC-PFS (months)	Median EC-PFS (months)	Median OS (months)
Margolin et al. (24)	Phase II	Melanoma	72	Asymptomatic	51	Ipilimumab	16	14	1.9	3.3	7
				Symptomatic with stable corticosteroid dose	21		5	5	1.2	1.3	3.7
Goldberg et al. (14, 22)	Phase II	Melanoma	60	Asymptomatic, ≥1 untreated or progressive BrM, no steroid requirement	23	Pembrolizumab	26	47	2[a]	2[a]	17
Kluger et al. (23)		NSCLC			37		29.7	29.7	2.3	Not reported	9.9
Long et al. (15, 26)	Phase II	Melanoma	76	Asymptomatic, untreated	35	Ipilimumab + nivolumab	51	57	Not reached	13.8	Not reached
					25	Nivolumab	20	29	2.5	2.6	18.5
				Local failure, symptomatic and/or LMD	16	Nivolumab	6	25	2.3	2.6	5.1
Tawbi et al. (16, 25)	Phase II	Melanoma	119	Asymptomatic	101	Ipilimumab + nivolumab	54.5	48.5	Not reached	Not reached	Not reached
				Symptomatic, stable steroid dose	18		22.2	22.2	1.2	2.2	8.7
Brastianos et al. (17)	Phase II	Mixed	20	LMD	—	Pembrolizumab	0	0	2.6	3.6	3.6

Abbreviations: EC-PFS, extracranial progression-free survival; ECRR, extracranial response rate (complete response + partial response); IC-PFS, intracranial progression-free survival; ICRR, intracranial response rate (complete response + partial response); LMD, leptomeningeal disease; NSCLC, non–small cell lung cancer.

[a]PFS was not stratified by site, i.e., intracranial versus extracranial; therefore, median overall PFS (2 months) is indicated.

included an additional nivolumab monotherapy treatment arm for patients with progressing, symptomatic, or leptomeningeal lesions. Only one patient (6%, $N = 16$) demonstrated an intracranial response in this treatment arm. In the trial overall, there appeared to be higher ICRR with combined ipilimumab/nivolumab [46%; 95% confidence interval (CI), 29–63—which rose to 51% in an updated long-term analysis] versus nivolumab monotherapy (20%; 95% CI, 7–41; ref. 26). It remains unclear in what proportions the lower response rate observed in symptomatic patients was due to immunosuppression from corticosteroid use or perhaps a unique role may exist for combined PD-1 and CTLA4 inhibition in the setting of CNS metastases. Finally, Goldberg and colleagues conducted a phase II trial studying pembrolizumab in patients with non–small cell lung cancer (NSCLC) or melanoma with untreated or progressing BrM (14). In patients with NSCLC and PD-L1 ≥1%, 29.7% ($N = 11$; 95% CI, 15.9%–47%) demonstrated an intracranial response that was concordant with extracranial response. Similarly, 26% ($N = 6$) of patients with melanoma demonstrated an intracranial response—all of whom demonstrated an extracranial response (23). Data from these trials reinforce earlier findings that there is a higher than expected ICRR to ICI, responses are superior compared with dismal failures noted in several trials studying ICI for glioblastoma (NCT02617589, NCT02667587, and NCT02011717; ref. 28), responses tend to be concordant across intracranial and peripheral compartments, and patients with symptomatic BrM have decreased response rates and shorter relapse times compared with asymptomatic patients. However, these select trials are few in number with highly selected patient populations and do not include correlative work to dissect underlying biology at play. These trends should be interpreted with caution as the field awaits larger, randomized clinical trials across cancer types beyond melanoma and NSCLC with embedded translational plans focused on determinants of immune response.

Overall, there are few prospective clinical data and correlative studies assessing the determinants of ICI response across central and peripheral compartments. One retrospective series comprising 18 patients with lung cancer and BrM who received pembrolizumab or nivolumab showed that intracranial and peripheral responses were discordant. Of 11 patients (61%) who demonstrated partial response or stable disease extracranially, eight (72%) exhibited CNS progression (29). The remaining seven patients (39%) demonstrated concordant intracranial and extracranial progression of disease. In a separate cohort with available paired tissue specimens from primary and BrM lesions, Kim and colleagues showed that BrM specimens harbored statistically significant decreases in $PD1^+$ tumor-infiltrating lymphocytes (TIL). Although these data are retrospective, this study is notable because of available correlative data from paired specimens that suggest response rates and duration of response in the CNS may be lower compared with the extracranial compartment—and that the TIME could account for such discordance that stands in contrast to available clinical data. Given the historical view of an immune-privileged CNS and its distinct immunobiology, further investigation into the determinants of antitumor immunity in the CNS is necessary toward improved immunotherapy outcomes in patients with BrM.

ICI IN CORTICOSTEROID-DEPENDENT PATIENTS WITH BRM

Given poorer outcomes in patients with symptomatic brain metastasis receiving ICI, and the high frequency of corticosteroid dependence in this population, an examination of whether corticosteroids play a causal role in iatrogenic immunosuppression is critical. Conversely, it may be possible that symptomatic lesions tend to be larger and have a greater degree of peritumoral edema leading to decreased response. Additionally, corticosteroids are frequently used in patients to palliate symptoms such as poor performance status or low appetite—and a higher proportion of patients with BrM would be expected to be in this performance category. A recent meta-analysis reviewed 16 studies involving patients receiving ICI with available data for corticosteroid use (30). ICI treatment and concomitant corticosteroid use for any reason was associated with worse OS (HR = 1.54; 95% CI, 1.24–1.91; $P = 0.0001$). However, all studies were retrospective, and only three included patients with BrM. Interestingly, when stratified by corticosteroid indication on subgroup analysis, "supportive care" was associated with worse OS (HR = 2.51; 95% CI, 1.41–4.43; $P \leq 0.01$) versus BrM (HR = 1.51; 95% CI, 1.22–1.87; $P \leq 0.01$). These data suggest that although there may be a signal for reduced ICI efficacy in corticosteroid-dependent patients with BrM, this effect may not be as dominant as advanced disease with poor performance status requiring palliative corticosteroids. Jesserun and colleagues conducted a meta-analysis of 15 studies of human subjects with BrM who received ICI with available data for corticosteroid use (31). In pooled data, corticosteroid use was associated with worse OS (HR = 1.84; 95% CI, 1.22–2.77, $P = 0.007$) and worse extracranial (EC)-PFS (HR = 2.00; 95% CI, 1.37–2.91; $P = 0.007$), but not worse IC-PFS (HR = 1.31; 95% CI, 0.42–4.07; $P = 0.500$). No difference in ICRR was found between the corticosteroid and non-corticosteroid groups. Limitations to their analysis include significant heterogeneity across studies, conflicting data within the pooled analysis for IC-PFS, and heterogeneity in response criteria. Surprisingly, murine studies assessing the role of corticosteroids on anti–PD-1 responses found that intracranial anti–PD-1 tumor response was not abrogated by dexamethasone contrary to an observed immunosuppressive effect and impaired immune response against extracranial tumors (32). Prospective studies that include patients taking corticosteroids (most have excluded these patients to date) with prespecified steroid-use endpoints—including careful attention to dose and duration, ICI regimen, and local treatments—are needed to more definitively dissect the influence of corticosteroids on intracranial activity of ICI. However, data reviewed herein do suggest that corticosteroid use alone is insufficient to explain worse OS in patients with symptomatic BrM.

An interesting future direction would be investigation of intracranial activity of ICI combined with corticosteroid-sparing agents in patients with symptomatic BrM. For example, bevacizumab has shown promise in the treatment of

refractory BrM-associated edema, and ongoing clinical trials are studying this agent as an upfront steroid-sparing strategy in patients with BrM (NCT03175432; refs. 33–35). Promising prospective data exist for the steroid-sparing effect of other agents as well including corticorelin (peptide mimic of corticotropin-releasing factor), cediranib (VEGFR inhibitor), Boswellia serrata, and angiotensin II–converting enzyme inhibitors; however, most of these studies were done in patients with glioblastoma or primary brain tumors (36–40). Prospective trials with BrM-specific patients are needed to adequately elucidate steroid-sparing strategies in this patient population.

CNS IMMUNOBIOLOGY: UNIQUE FEATURES AND CONSIDERATIONS

CNS Immune Privilege versus Specialization

Immune privilege is defined as an inability to reject heterotypically transplanted tissue (18). CNS immune privilege achieved a conceptual stronghold in the early 20th century when Shirai and colleagues demonstrated that implanted rat sarcoma cells grow well in the brain but not in skin or muscle and later when Medawar and colleagues showed that the immune response against a skin homograft implanted into a rabbit brain was dependent on the presence of a concurrent skin homograft (41, 42). Ostensibly, the absence of CNS draining lymph nodes and consequent lack of an afferent immune arm was hypothesized to explain the missing immune response when a skin homograft was implanted into the CNS alone. However, recent evidence builds upon these data and supports an updated model better described as immune-specialized versus immune-privileged.

Tissue graft rejection in the CNS does occur but is site-specific: Rejection has been observed when tissue is implanted into cerebral ventricles but not parenchyma (43, 44). Activated T cells can pass through the BBB and patrol in the absence of neuroinflammation—a process called "immunosurveillance" (45, 46). Importantly, new insights on CNS routes of lymphatic drainage have also come into focus. In 2012, a fluid exchanger system responsible for moving unwanted byproducts from the parenchymal interstitial fluid into the draining cerebrospinal fluid (CSF) that exits via a network of meningeal lymphatic vessels (MLV) was described and termed the "glymphatic system" (47, 48). Alitalo and colleagues characterized MLVs in the dura mater of the murine brain that drain out of the skull via the foramina alongside arteries, veins, and cranial nerves (49). Using injection tracer experiments, they confirmed that these MLVs absorb both interstitial fluid of the brain parenchyma and CSF from the subarachnoid space for transport into deep cervical lymph nodes (dCLN). In separate work, Kipnis and colleagues similarly found murine lymphatic vessels that line the dural sinuses and drain preferentially to the dCLNs (50). Interestingly, they showed that resection of the dCLNs resulted in an increased number of meningeal T cells attributed to an inability of T cells to drain from the meningeal space. More recently, their group demonstrated that preferential CSF drainage and stromal-mediated immune cell recruitment results in an immune–CNS interface located specifically at the dural sinuses in the murine brain, allowing peripheral surveillance of CNS antigens (51). With these new discoveries, the conceptual CNS immune-privilege model has appropriately been revised to one of "CNS immune specialization," and preclinical work toward induction of CNS antitumor immunity has commenced.

Hu and colleagues investigated the roles of MLVs in mouse models of glioma and melanoma and found that intracranial tumors induced extensive remodeling of dorsal MLVs and that disruption of these MLVs attenuated the efficacy of combined PD-1/CTLA4 blockade (52). Lymphangiogenesis of MLVs was mediated by VEGF-C. Indeed, VEGF-C–overexpressing mice receiving combined PD-1/CTLA4 blockade showed improved OS due to a potentiated ICI response. These mice were noted to have increased $CD8^+$ T-cell/regulatory T cell (Treg) ratios within tumors and dCLNs. Finally, antibody-mediated blockade of the chemokine-ligand 21 (CCL21)/C–C chemokine receptor 7 (CCR7) pathway abrogated the efficacy of combined ICI, suggesting that VEGF-C potentiation of ICI-induced antitumor immunity is dependent on CCL21/CCR7 axis signaling. The CCL21/CCR7 axis has been shown to have dual roles in various cancer models, supporting antitumor immune responses in immune cells yet promoting tumor cell propagation (53). These results are consistent with separate work that demonstrated therapeutic delivery of VEGF-C potentiated ICI activity in a murine glioblastoma model (54). Further studies and development of CCL21/CCR7 axis inhibitors must proceed cautiously, but, more broadly, future development of therapeutic strategies to increase lymphangiogenesis of MLVs and/or optimize lymphatic drainage to dCLNs via VEGF-C signaling is attractive toward achieving improved intracranial immune response to ICI.

CNS Antitumor Immunity: The Role of Extracranial Disease

Despite these exciting new discoveries regarding CNS immune specialization, seminal work by Taggart and colleagues (55) using a murine B16 melanoma tumor transplantation model with extracranial (subcutaneous) plus intracranial tumors (which models the majority of patients who have concurrent disease intracranially and extracranially) suggests that Shirai's and Medawar's model of immune privilege, as it pertains to BrM, remains insightful. Extracranial tumors were requisite for induction of intracranial tumor response by combined PD-1/CTLA4 blockade, and response correlated with (i) increased infiltration of $CD8^+$ T cells, peripheral macrophages, and microglia and (ii) gene expression changes associated with activation of T cells, natural killer cells, and macrophages/microglia. However, flow cytometry analysis for T-cell activation markers confirmed that increased T-cell activation gene expression was due to an increased intratumoral percentage of $CD8^+$ T cells. Thus, increased trafficking after peripheral expansion explains the observed T-cell activation flux as opposed to activation of already centrally located T cells. Finally, and of particular translational importance, they showed via gene pathway analysis and immunofluorescence that increased $CD8^+$ T-cell trafficking after combined ICI may occur via

upregulation of T-cell entry receptors on tumor vasculature (ICAM1/VCAM1). Inhibitors of ICAM1/VCAM1 signaling in humans, mostly monoclonal antibody–based treatments, have been studied in inflammatory and autoimmune contexts but await validation in antitumor applications. Interestingly, chimeric antigen receptor (CAR) T cells targeting ICAM1 have been developed and have shown success in murine and patient-derived xenograft models of anaplastic thyroid and gastric cancers (56, 57). The role for inhibitors of ICAM1/VCAM1 in the treatment of BrM and primary brain tumors remains unexplored but represents an attractive target for future investigation.

Clinical data supporting the importance of extracranial disease burden for effective intracranial response secondary to ICI are supported by a retrospective analysis conducted by Rauschenberg and colleagues studying the impact of radiation and systemic therapy (including PD-1/CTLA4 blockade) on survival in patients with melanoma-derived BrM (58). They found that the presence of extracranial metastatic disease correlated with improved OS on both univariate (HR = 0.1; 95% CI, 0.1–0.2; $P < 0.001$) and multivariate analyses (HR = 0.1; 95% CI, 0.01–0.35; $P < 0.001$). Taken together with work performed by Taggart and colleagues, preclinical and clinical evidence suggests that burden of extracranial disease may be a determinant of intracranial response of BrM to ICI. Therefore, a prospective clinical trial design should account for this variable and ensure precise baseline and serial characterization of extracranial disease response as it pertains to assessment of intracranial treatment response. Additionally, the critical role of the extracranial compartment in orchestrating the priming, activation, and trafficking of peripheral T cells to the CNS suggests consideration of delivery to the extracranial space for the future development of CNS-specific T-cell therapies or personalized cancer vaccines.

The BBB and Blood–Tumor Barrier

The BBB is composed of nonfenestrated endothelial cells, pericytes, a basal lamina layer, and astrocytic endfeet that form a layer known as the astrocytic glia limitans. This tightly regulated neurovascular bundle serves to maintain CNS homeostasis and protects against unregulated transport of potentially harmful molecules or substances into the CNS (18, 59). Similarly, the BBB restricts antigen presentation and immune cell infiltration in the normal resting state. In order to gain entry into the CNS parenchymal space in the setting of inflammation, T cells must first pass through the endothelial layer followed by passage through the glia limitans (60). In the context of BrM, vascular structures lose integrity such that otherwise restricted entry by peripheral immune cells may be facilitated (61). However, a more modern conceptual framework is that the BBB does not break down per se but forms a "blood–tumor barrier" (BTB) in which lymphocytes can traverse the intact BBB via chemokine axes and multistep adhesion processes (62–64). Indeed, the BTB was shown to have heterogeneous permeability (regulated by reactive astrocytes; ref. 65), which is a likely a driver of a variable immune cell infiltrate. The BTB (structure, function, pharmacokinetics, and complementary *in vitro/in vivo* data) has been expertly reviewed elsewhere (59, 64). Broadly speaking, however, the role of the BTB in modulation of the therapeutic immune response to BrM is largely unknown, including patterns of infiltration, immune cell subsets involved, spatial and temporal dynamics, interactions with CNS resident populations, and downstream functional consequences.

Recently, thromboinflammation studied in murine models of acute stroke has offered intriguing insight toward possible overlapping biology with respect to BrM and the immune interface of the BBB/BTB. Thromboinflammation results from the pathologic interplay between platelets and T cells in response to CNS tissue insult resulting in exacerbation of underlying tissue injury (66). Work by Feinauer and colleagues has established an early role in BrM initiation and outgrowth dependent on thromboinflammation (67). Using multiphoton laser scanning microscopy to study the brain metastatic cascade in murine models, their group showed that clot formation occurs in brain microvessels preferentially at sites with intravascularly arrested tumor cells—and that cancer cells embedded in a clot had a higher success rate in extravasation and formation of a macrometastasis. Although an intriguing etiologic connection between thromboinflammation and BrM initiation/propagation may exist, future examination of the inflammatory component is needed (such as immune cell subsets, cytokine and chemokine signaling, and multicellular interaction at the BBB/BTB interface) before therapeutic strategies targeting thromboinflammation can be developed.

Translational strategies involving the development of BBB/BTB disruption methods, permeability-altering receptor agonists, radiosensitizing nanoparticles, and novel delivery platforms have rarely progressed past phase I clinical trials to date. Cellular approaches that leverage the high CNS tumor tropism of neural stem cells and mesenchymal stem cells are attractive options for the development of therapeutic carriers (68). Cell-mediated delivery of immune-supporting products (i.e., cytokines and chemokines) or oncolytic adenovirus are examples (59, 69). Future studies focusing on further characterization of structure/function, improved model systems representative of (and compared with) human tissue studies, patterns of permeability (both innate and induced secondary to therapeutic modulation), and drug distribution are imperative to bring actionable understanding of the BTB to light. These areas of focus for future investigations are needed not only to increase understanding of the BBB/BTB proper but also to specifically characterize the role of BBB/BTB as it pertains to modulating CNS antitumor immunity.

CNS Adaptive Immunity and T Cells: A Deeper Dive

Infiltration of brain tumors by $CD8^+$ and $CD4^+$ T cells has been observed (70), the patterns of which are variable and dependent on primary tumor histology (71). In a retrospective study, Berghoff and colleagues evaluated 116 BrM resection specimens from various cancer types for TIL density and subset patterns (72). Tumors showed frequent tumor penetration by TILs, and TIL density was highest in the tumor stroma and tumor–parenchyma border, whereas solid tumor areas were sparsely populated. Interestingly, no

associations were found between preoperative corticosteroid treatment and any differences in TIL subset. Peritumoral edema was inversely associated with TIL density. Higher TIL, CD8$^+$ T-cell, and CD45RO$^+$ T-cell density was associated with improved OS. In a separate study, Berghoff and colleagues showed that BrM in patients with small cell lung cancer similarly showed frequent TIL rates across cell subtypes, and that improved OS was associated with higher density of CD45RO$^+$ TILs (73). Thus, there is heterogeneous spatial and immune cell subtype distribution in BrM across multiple cancer types, and further characterization of these patterns is required, especially in patient-matched intra- and extracranial specimens.

One study performed immune gene expression profiling on paired intra- and extracranial samples from 39 patients with NSCLC and found that BrM samples demonstrated reduced T-cell infiltration and clonal expansion compared with extracranial samples, but that T-cell receptor repertoires were largely shared (74). Interestingly, another study also looking at paired samples in a smaller NSCLC patient cohort found that T-cell clonality was largely nonoverlapping across paired samples and that BrM harbored contracted T-cell diversity (75). Surprisingly, TMB was higher in the BrM samples compared with their respective extracranial samples, but this did not correspond to a statistically higher predicted neoantigen load. Proposed reasons for the discrepant results were that the former study focused selectively on abundant clones and that there were largely shared somatic hotspot mutations between samples (74). Further study in larger cohorts is needed, but it is possible that divergent underlying genetics between extracranial and intracranial lesions as well as across distinct intracranial lesions could result in nonoverlapping T-cell repertoires (76–78). Collectively, these data suggest that there could be spatiotemporal heterogeneity in the immune response across CNS lesions in the same patient.

CD4$^+$ T Cells

CD4$^+$ T cells have diverse and context-dependent "helper" roles in mediating the antitumor immune response as well as immunosuppression via Treg function (79). Thus far, immuno-oncology has disproportionately focused on CD8$^+$ T-cell biology. However, there is a growing body of preclinical and clinical evidence that CD4$^+$ T cells play a critical role in supporting antitumor immunity outside of the brain (reviewed elsewhere by Tay and colleagues; ref. 79). However, the antitumor role of CD4$^+$ T cells in BrM remains to be defined.

In contrast to the antitumor role of CD4$^+$ T cells, CD4$^+$CD25$^+$FOXP3$^+$ Tregs may play protumor roles acting as drivers of immunosuppression in the BrM TIME (80). Kim and colleagues (81) performed a comprehensive single-cell transcriptomic characterization of 208,506 cells obtained from normal, intracranial, and extracranial tumors from 44 patients with NSCLC (29,060 cells were from BrM) and showed that, at all stages of progression, a shift toward a protumoral and an immunosuppressive tumor microenvironment marked by replacement of myeloid populations with monocyte-derived macrophages and increased T-cell exhaustion was observed. On the whole, further characterization of the BrM-specific role of CD4$^+$ T-cell subtypes should be prioritized going forward and is likely to yield novel opportunities to enhance the efficacy of ICI as well as further develop cancer vaccine approaches. One preclinical example of modulating CD4$^+$ T-cell activity in brain tumors to enhance antitumor immunity comes from the work of Bunse and colleagues (82). They studied the role of the oncometabolite R-2-hydroxyglutarate (R-2-HG) accumulation in gliomas and its influence on T cells and the tumor microenvironment (82). They demonstrated that R-2-HG impairs antigen-specific T-cell activation and that CD4$^+$ T cells specifically are more susceptible to R-2-HG–mediated inhibition. They also validated in IDH1-mutant glioma murine models that R-2-HG impairs antitumor immunity induced by IDH1-specific vaccination, adoptive T-cell transfer, and checkpoint inhibition. Their study showcases that T cell–specific therapies can be developed toward primary brain tumors and underscores the need to further dissect CD4$^+$ T-cell biology in the treatment of BrM.

CNS Antitumor Response

The main driver for BrM-specific, activated CD8$^+$ T cells remains to be defined. One possibility is that BrM outgrowth results in tissue injury leading to release of endogenous peptides known as danger-associated molecular patterns (DAMP) or alarmins, which are recognized by pattern-recognition receptors (PRR) on microglia, neurons, and astrocytes and lead to innate immune system activation (83). Subsequent cytokine release results in an inflammatory cascade and immune cell infiltrate including both innate and adaptive immune cells (84–86). However, if DAMP/alarmin release was the sole determinant of activated T cells with receptor specificity toward newly liberated CNS antigens, we might expect to see evidence of CNS-specific autoimmunity in patients who develop BrM—a very uncommon scenario. Rather, it seems more probable that BrM harbor tumor neoantigen profiles that have both overlapping and nonoverlapping components when compared with the periphery.

A unifying hypothesis is that local tissue injury secondary to BrM outgrowth leads to DAMP/alarmin release and PRR activation, which drives, in part, a T cell–mediated adaptive immune response via two mechanisms (Fig. 2). First, T cells with receptor specificity for a peripheral neoantigen shared with a BrM lesion will subsequently traffic from the periphery to the CNS to execute an effector response. Second, BrM-specific neoantigens could drain to peripheral lymph nodes, resulting in T cells with BrM-unique receptor specificity that then subsequently traffic to the CNS. However, many questions remain pertaining to what proportion these T-cell activation patterns might play out, anatomical considerations such as how T cells might overcome an immunosuppressive milieu on arrival, and the role of the BBB/BTB in adaptive antitumor immunity.

CNS Innate Immunity

Neuroinflammation that results from CNS injury (such as tumor outgrowth), infection, or neurodegenerative disease activates the innate immune system via a complex interplay of CNS-resident cells, centrally recruited peripheral immune cells, cytokine signaling, and complement

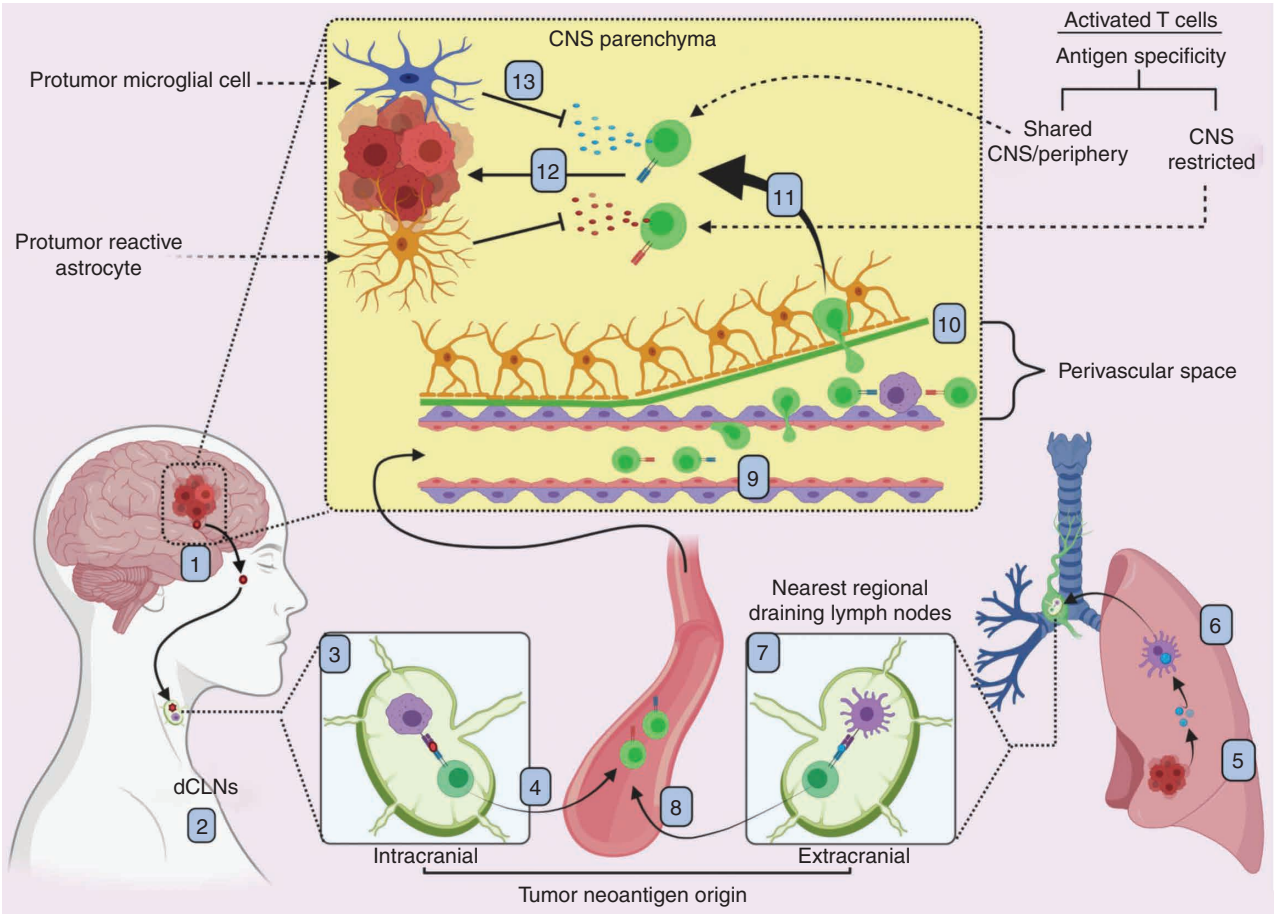

Figure 2. A model for T cell–mediated adaptive immune response toward CNS-specific and centrally/peripherally shared tumor neoantigens: 1. CNS-specific tumor neoantigen (red) is shed from BrM tumor cells. 2. Lymphatic drainage to the nearest regional draining lymph nodes, i.e., deep cervical lymph nodes. 3. CNS-specific tumor neoantigen is phagocytosed and presented to a naïve T cell by an antigen-presenting cell. 4. The T cell is primed and activated entering the bloodstream en route to the brain. 5. Centrally/peripherally shared tumor neoantigen is shed from an extracranial metastatic tumor. 6. Patrolling dendritic cell recognizes and phagocytoses the tumor neoantigen, followed by trafficking to the nearest draining lymph node (afferent immunity). 7. In the lymph node, the dendritic cell presents the centrally/peripherally shared tumor neoantigen to a naïve T cell. 8. The T cell is primed and activated entering the bloodstream en route to the brain. 9. Activated T cells slow, roll, and crawl as they begin extravasation into the perivascular space. 10. Activated T cells encounter perivascular macrophages that induce restimulation of the T cells. 11. Extravasation across the glia limitans (basal lamina and astrocyte endfeet layers) into the CNS parenchyma is completed. 12. Activated T cells encounter respective target tumor cells and execute a cytotoxic attack. 13. Protumor glial cells in the tumor microenvironment drive immunosuppression and mitigate T-cell attack. Figure created with BioRender.com.

(87). Neuroinflammation in the short term is considered neuroprotective, whereas prolonged neuroinflammation can have deleterious consequences such as support/promotion of underlying pathophysiology. Complement was initially thought to be an absent component of neuroinflammation, but we now understand that neuronal and glial cells have complement receptors and can produce complement (87). Further, cancer cells may hijack complement signaling, resulting in outgrowth in the CSF in the setting of leptomeningeal disease (88). The presence of functional dendritic cells within the CNS has been a topic of debate. Historically, lack of evidence for dendritic cells exhibiting antigen uptake and processing, cell-surface display via MHC class II, afferent lymph node trafficking, and naïve T-cell activation has formed the cellular basis of immune privilege. However, dendritic cells can be found in the human brain, and increases in dendritic cell infiltration in the setting of neuroinflammation have been observed (although it is unclear to what degree these are peripheral in origin given perceived BBB disruption; ref. 89). Dendritic cell role and function in the CNS remain incompletely characterized, especially in the context of BrM. Finally, glial cells (Fig. 1B) act as CNS-resident innate immune cells, yet their diverse functions extend far beyond innate immunity.

Next, we choose to focus in depth on glial cells, such as astrocytes and microglia, given their multilateral roles in BrM propagation in addition to innate and adaptive immune signaling. For detailed examinations of nonglial cells and their role in the TIME of brain tumors, see expert work by Quail and colleagues, Doron and colleagues, and Klemm and colleagues (19, 90, 91).

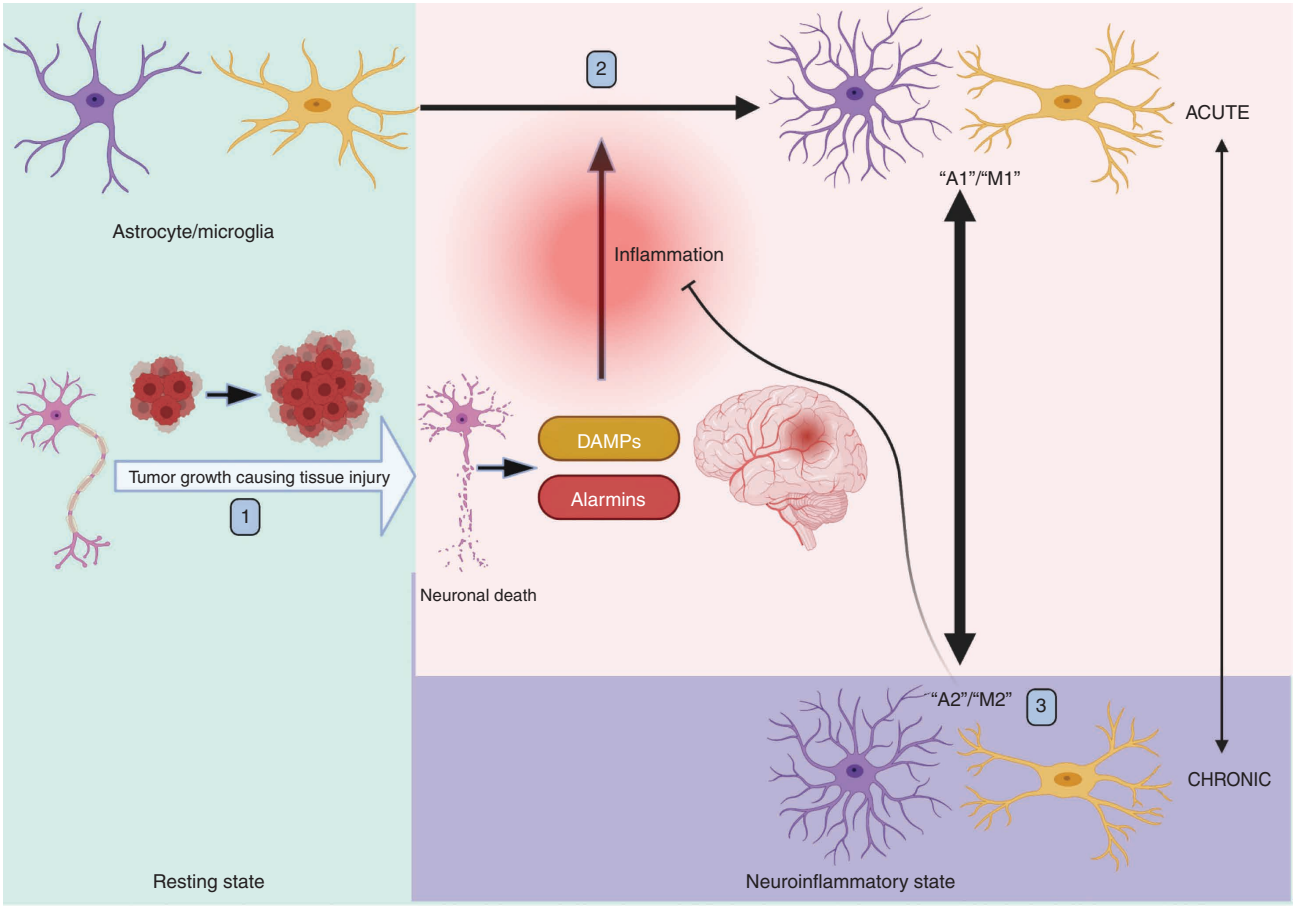

Figure 3. Phenotypic and functional continuum for astrocytes and microglia in the setting of BrM-mediated neuroinflammation. 1. Outgrowth of brain metastasis results in local tissue injury resulting in DAMP and alarmin release, which drives a neuroinflammation cascade. 2. Glial cells in resting state adopt phenotypic and functional changes resulting in a reactive state in the setting of acute inflammation. 3. In the chronic setting of neuroinflammation, glial cells adopt a suppressed phenotype thought to be neuroprotective and limit damage from an acute insult and resulting immune response. Figure created with BioRender.com.

GLIAL CELLS: CNS-RESIDENT IMMUNE CELLS AND BEYOND

Astrocytes

Astrocytes, the most abundant cell type in the CNS, participate in a wide variety of homeostatic functions in the normal brain, including maintenance of the BBB, immune signaling, modulation of neuronal networks, and maintenance of ion, pH, and transmitter balance in synaptic, interstitial fluid (refs. 92–95; Fig. 1B). Given the wide-ranging role of astrocytes, it is unsurprising that significant functional and phenotypic heterogeneity exists for the resident sentinels of the CNS. Classically, the astrocyte functional phenotype has been defined within the confines of binary polarization states: the neuroinflammatory and antitumor "A1" state versus the neuroprotective and tumor supportive "A2" state (96, 97). It is increasingly recognized, however, that these functional states exist on a dynamic continuum and that these states are temporally and contextually determined (98–100). In the presence of a tissue insult such as a tumor or infection, astrocytes activate into "reactive astrocytes" undergoing morphologic and phenotypic changes—a process known as astrogliosis that culminates in the formation of a glial scar (Fig. 3; ref. 93).

Role for Astrocytes in BrM

Initiation of Micrometastasis

In the early development of BrM, sometimes referred to as micrometastatic initiation, astrocytes appear hostile to BrM-initiating cells (Fig. 4A). Valiente and colleagues demonstrated in lung and breast cancer models that reactive astrocytes are a major source of plasminogen activator (PA) leading to the production of plasmin, which plays a role in stromal response to injury (101). Plasmin accumulation inhibited the development of BrM by triggering FAS ligand–dependent apoptosis in BrM-initiating cells. To overcome this, breast and lung cancer cells (human and murine) were found to secrete serpins with inhibitory activity against PA, thereby promoting survival against tumor-inhibiting effects of plasmin (101). In contrast, Lorger and colleagues demonstrated in a murine breast cancer model that astrocytes preferentially colocalized

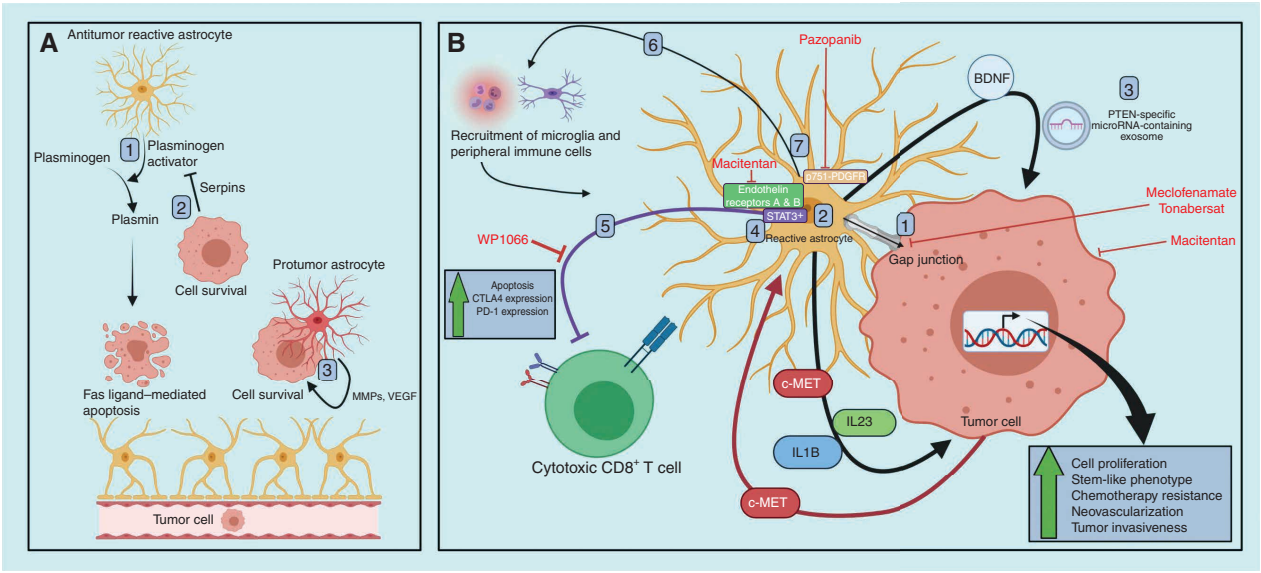

Figure 4. Astrocyte role in brain metastasis initiation and propagation including protumor immunosuppression. **A,** Astrocytes have been shown to be both hostile and supportive to invading BrM-initiating cells: 1. Astrocytes secrete PA, resulting in plasmin accumulation that can induce FAS ligand–dependent apoptosis of tumor cells. 2. Tumor cells can secrete serpins, which inhibit astrocyte-secreted PA resulting in tumor cell survival. 3. Reactive astrocytes can adopt protumor roles associated with colocalization with tumor cells and secretion of protumor factors such as matrix metalloproteinases (MMP), VEGF, and others. **B,** 1. Tumor cells benefit from formation of gap junctions with tumor cells in which astrocyte-secreted metabolites lead to downstream expression of tumor survival genes conferring resistance to chemotherapy. Direct targeting of gap junctions (with inhibitors such as meclofenamate and tonabersat) as well as dual inhibition of endothelin receptors with macitentan (approved for the treatment of renal cell carcinoma and soft-tissue sarcoma) have been shown to decrease astrocyte-mediated survival gene expression in tumor cells and reduce outgrowth. 2. Astrocytes have diverse secretory programs including BDNF, c-MET, JAG1, IL23, and IL1B, resulting in protumor proliferation, adoption of stem-like features, vascular reprogramming, and increased invasiveness. 3. Astrocytes also secrete PTEN-specific microRNA-containing exosomes, which result in decreased tumor cell expression of PTEN shown to be unique to BrM tumors compared with paired extracranial lesions. 4. STAT3$^+$ astrocytes colocalize with and shield tumor cells from immune attack by mitigating cytotoxic attack by T cells. WP1066 is one example of a STAT3 pathway inhibitor that has shown preclinical efficacy in treating BrM. 5. Astrocytes oppose the adaptive immune response via direct induction of apoptosis and upregulation of exhaustion markers in T cells. 6. Astrocytes appear to harbor the ability to recruit peripheral immune cells as well as macrophages/microglia to the local tumor environment; however, it is unclear in what proportion this recruitment results in an anti- versus pro-BrM effect. 7. Platelet-derived growth factor receptor phosphorylated at residue 751 (p751-PDGF) was found to be a marker of a protumor astrocyte subpopulation, and inhibition using pazopanib (which is approved for use in renal cell carcinoma and soft-tissue sarcoma) results in decreased BrM outgrowth. Figure created with BioRender.com.

with invading tumor cells followed by activation and secretion of proangiogenic and growth-promoting matrix metalloproteinase 9 (MMP9), suggesting a supportive role for astrocytes in the early BrM initiation phase (102). Doron and colleagues showed in murine models of melanoma-derived BrM that astrocyte-secreted CXCL10 resulted in chemoattraction of CNS-tropic melanoma cells expressing CXCR3 (CXCL10 receptor), and that targeted inhibition of CXCR3 decreased BrM formation (103). Taken together, available data conflict on whether astrocytes aid or impede BrM initiation despite detailed mechanistic work underscoring the overall complexity of the functional role of astrocytes and BrM. Further, whether astrocytes assume reactive functional states due to cancer cells directly or indirectly as a result of tissue injury associated with BrM outgrowth is unknown (104); however, Chen and colleagues demonstrated that the former is highly likely (105). In coculture and BrM murine experiments, they showed that BrM cells containing cytosolic DNA and cyclic GMP engage astrocytes in Cx43-based gap junctions, resulting in the co-option of astrocyte-produced cytokines that support cancer cell growth and survival. Thus, future characterization of the role of astrocytes in early BrM formation should focus on precise timing of BrM initiator cell entry into the CNS microenvironment.

Propagation/Outgrowth of Macrometastasis

Astrocytes have been shown to surround BrM and promote propagation and outgrowth via co-option of astrocyte gene expression programs resulting in tumor-supportive programming (101, 102, 105–108). For example, coculture of astrocytes with tumor cells of breast, lung, and skin origin led to astrocyte-induced upregulation of survival genes in tumor cells, resulting in protection from various chemotherapeutic agents (107, 108). Underscoring this mechanism, work by Kim and colleagues demonstrated in a murine BrM model that dual targeting of endothelin receptors resulted in decreased survival gene expression in tumor cells and resensitization to antineoplastic therapy (109).

Astrocytes also have many protumor secretory functions. Astrocytes were found to secrete brain-derived neurotrophic factor (BDNF) leading to heterodimerization of tumor cell tropomyosin-related kinase B (Trkb) and human epidermal growth factor receptor 2 (HER2) in breast cancer cells, resulting in increased cell proliferation (110). An autoparacrine feedback loop was discovered in which tumor cells with

mesenchymal–epithelial transition factor (c-MET) expression led to protumor vascular reprogramming and interleukin 1 beta (IL1B) expression, which induced tumor-associated astrocytes to secrete additional c-MET ligand, thus supporting tumor neovascularization (110). In a separate study, Xing and colleagues found that breast cancer cells secreted IL1B, which was found to induce astrocyte production of Jagged 1 (JAG1; ref. 111). JAG1 is a transmembrane protein that facilitates Notch signaling, and its production led to an increase of a stem-like phenotype of cancer cells via Notch–Hes5 (111). In a melanoma BrM model, Klein and colleagues demonstrated that tumor cells induced IL23 secretion by astrocytes, which resulted in increased MMP2-mediated tumor invasion (112). Lastly, in a notable study by Zhang and colleagues, the relationship between tumor microenvironment across anatomic compartments was explored (113). Downregulation of PTEN expression was found to be unique to murine and human breast cancer–derived BrM specimens compared with paired primary and other extracranial metastases in a process mediated by microRNA-containing exosomes secreted by astrocytes. Collectively, these results across multiple BrM tumor models suggest that astrocytes modulate the tumor microenvironment through various tumor-supportive secretory functions that promote tumor initiation as well as outgrowth.

Astrocytes and CNS Antitumor Immunity

Astrocytes play a role in modulating the innate and adaptive immunity. Priego and colleagues identified a subpopulation of signal transducer and activator of transcription 3-positive (STAT3$^+$)–reactive astrocytes that surround BrM and elegantly demonstrated that not only was outgrowth of human and murine lung-, breast-, and melanoma-derived BrM dependent on this reactive astrocyte subpopulation, but these cells directly influenced components of innate and adaptive immunity toward favoring tumor survival (Fig. 4B; ref. 114). In the same study, Priego and colleagues treated patients with metastatic lung cancer with BrM ($N = 18$) with oral legasil, an available silibinin-containing nutraceutical with STAT3-inhibitory activity, in combination with varying palliative chemotherapy regimens and demonstrated intracranial clinical responses with an overall response rate of 75%. Their work nominated STAT3$^+$ reactive astrocytes as putative targets for achieving CNS-specific therapeutic activity. Further, their demonstration that culture media from STAT3$^+$ astrocytes are sufficient alone to abrogate CD8$^+$ T-cell antitumor activity further underscores that this astrocyte subtype may be a promising target that could sensitize BrM to concurrent treatment with ICI.

Astrocytes appear to harbor the ability to recruit immune cells locally from the periphery in response to tissue damage (Fig. 4B). In a murine model to simulate intracranial tissue damage and inflammation, IL1B was injected intracranially, and it was demonstrated that astrocyte-shed extracellular vesicles are responsible for recruiting peripheral leukocytes into the CNS (115). However, astrocyte-mediated recruitment patterns may be dependent on astrocyte subtype and location. Juxtavascular astrocytes, for example, were shown to negatively regulate the invasion of peripheral monocytes at the vascular interface in murine models (116). Although the interactions between astrocytes and other immune cells such as microglia/macrophages remain underexplored (70), Priego and colleagues showed that an increased number of CD74$^+$ microglia/macrophages surrounding BrM was astrocyte-dependent (114).

Astrocytes and T cells appear to have bidirectional mechanisms for suppressive signaling. For example, astrocytes are targeted by Tregs, resulting in attenuated signals that otherwise threaten neuronal viability, a mechanism to limit further tissue damage in the setting of an existing inflammatory insult (70). However, astrocytes also harbor some ability to induce FAS ligand–mediated apoptosis of T cells in the setting of neuroinflammation, similarly thought to be a neuroprotective mechanism (117). Other work has shown that astrocytes in coculture with T cells induce CTLA4 and PD-1 upregulation on activated T cells, resulting in attenuated CD8$^+$ T-cell activity suggestive of a driving role in CNS immune escape (Fig. 4B; refs. 118, 119). Thus, there is strong preclinical and clinical evidence that astrocytes play a direct role in modulating the adaptive immune response in the setting of BrM. However, astrocyte subtype, location, and interactions with other immune cells form a complex network that must be characterized before a clear target that could enhance antitumor immunity in patients receiving ICI can be identified.

Astrocytes as Therapeutic Targets

Given growing evidence for multifaceted roles of some astrocyte subpopulations in supporting BrM initiation and outgrowth, these cells are attractive therapeutic targets (Fig. 4B). Sarmiento and colleagues showed in rat models of BrM that STAT3$^+$-reactive astrocytes drove cerebral vascular dysfunction, which was reversible by treatment with WP1066, a STAT3 pathway small-molecule inhibitor (120). Macitentan, an approved drug for treating pulmonary hypertension, targets endothelin receptors that, when combined with paclitaxel in murine lung and breast cancer–derived BrM models, showed a reduced tumor cell division, increased apoptosis in both tumor and endothelial cells, and increased OS in treated mice (121). Given preclinical evidence of efficacy, a phase I clinical trial (NCT01499251) was conducted to determine the tolerability of combined macitentan and temozolomide in recurrent glioblastoma and/or gliosarcoma but was terminated early due to lack of efficacy. It remains unclear whether endothelin receptors may offer a viable astrocyte-specific target in the treatment of BrM, and therefore BrM-specific clinical trials are needed.

A murine breast-derived BrM model was used to identify a previously unidentified subpopulation of astrocytes characterized by phosphorylated tyrosine 751 platelet-derived growth factor receptor (p751-PDGFR) that preferentially colocalized with BrM lesions in the perivascular space (65). Gril and colleagues validated this association in human BrM samples and demonstrated in their murine model that pazopanib, a multikinase inhibitor with activity against PDGFR, depleted the p751-PDGFR astrocytes, which was associated with decreased BrM outgrowth (65, 122). Validation of targeting p751-PDGFR in humans with BrM from breast cancer and other histologies is needed, but it is encouraging that there is growing clinical experience with pazopanib in the

treatment of renal cell carcinoma and advanced soft-tissue sarcoma (123, 124). Further, there are case reports of renal cell carcinoma–derived BrM demonstrating an intracranial response to pazopanib treatment (125, 126).

Reeducation of astrocytes by tumor cells into tumor-supportive phenotypes via established gap junctions has led to interest in targeting these cell-to-cell signaling structures. Chen and colleagues demonstrated in murine models of BrM that protocadherin 7 (PCDH7) promotes assembly of tumor cell–astrocyte gap junctions (105). They demonstrated that meclofenamate and tonabersat, two approved oral drugs targeting gap junctions, are able to effectively break the tumor-supportive paracrine loop, resulting in the inhibition of BrM outgrowth. Results from an ongoing single-arm, phase II clinical trial (NCT02429570) studying meclofenamate in patients with recurrent or progressive BrM from solid tumors are eagerly awaited.

Microglia/Macrophages

Microglia are myeloid cells of the CNS parenchyma derived from the yolk sac, which colonize the CNS during early embryonic development, relying on local self-renewal thereafter (127, 128). In steady state, microglia have long processes that execute constant monitoring of healthy neural tissue and the local microenvironment—a process called "immuno-surveillance" (129, 130). To maintain a resting, surveillant state, microglia are thought to be repressed by healthy neurons through diverse mechanisms (131). The dependent relationship microglia have on neurons is logical for cells whose fate is to spring into action at the first signs of local neuronal injury and/or death, which would result in loss of neuron-mediated inhibitory inputs. Additionally, this mechanism suggests that microglial activation may be induced by removal of neuronal inhibition—an attractive guiding principle for the discovery of potential modulatory targets. In certain disease states, such as stroke, infection, or malignancy, microglial activation triggers morphologic and cell-surface marker changes, resulting in the ability to secrete inflammatory signals, phagocytose, and participate in both oxidative burst and antigen presentation (Fig. 5; ref. 127). Although microglia have been shown to be activated via CX3CR1 (132) and plasma-derived fibrinogen (133), much work remains to gain an understanding of the activation and effector states of these cells due to their highly heterogeneous and dynamic transcriptional programs (134, 135).

It has been proposed that microglia—despite their competence as antigen-presenting cells—cannot leave the CNS, thus barring their participation in afferent and consequently efferent immunity (127). Rather, it is believed that non-parenchymal macrophages (choroid plexus, meninges, and perivascular) are responsible for T-cell restimulation upon their arrival (Fig. 2, part 10; refs. 127, 136). Although work remains to differentiate and functionally characterize CNS-native microglia and macrophages, another dimension of complexity is added by infiltrating monocytes/macrophages that traffic to the CNS from the circulation in the setting of neuroinflammation (137). The challenge of differentiating microglia and resident CNS macrophages from peripheral, monocyte-derived, CNS-infiltrating macrophages has been vast owing to substantial phenotypic and cell-surface marker overlap. Only recently have markers such as *TMEM119*, *MS4A7*, *Gpr56,* and *CD49d* have been shown to differentiate between microglia and bone marrow–derived macrophages (BMDM)—a critical distinction when trying to study the heterogeneous mix of CNS-native microglia/macrophages and bone marrow–derived myeloid cells that have infiltrated the CNS in the disease state (91, 138, 139).

Role for Microglia/Macrophages in BrM

In vitro data suggest that invasion of BrM-initiating cells can be rapidly sensed by microglia, and the presence of a single tumor cell is sufficient to activate and recruit microglia (102, 140). Both microglia and BMDMs have been shown to infiltrate and persist within malignant brain lesions and undergo unique cell-specific tumor education that results in a tumor-supportive phenotype (Fig. 5; ref. 139). Interestingly, most tumor-associated macrophages (TAM) in brain metastases appear to be derived from peripheral monocytes and not from resident microglia (139, 141). Microglia/macrophages appear to polarize in a highly context-dependent manner along a continuum between two extremes (141). One extreme, commonly referred to as "M1," is defined as a proinflammatory phenotype characterized by increased levels of inflammatory cytokines and the ability to elicit a T cell–mediated antitumor response. Another extreme, commonly referred to as "M2," is defined as an anti-inflammatory phenotype that promotes angiogenesis and tumor growth (141). Work by Andreou and colleagues has shown inducible nitric oxide (iNOS) and cyclooxygenase 2 (COX2) to be surface markers for a microglial proinflammatory state, and mannose receptor c-type 1 (MRC1) and arginase 1 (ARG1) to denote an anti-inflammatory state in a murine model of breast cancer–derived BrM (141). Recent work by Gulder and colleagues used single-cell profiling technologies to reveal that CNS-native myeloid cells appear to support BrM outgrowth by driving an immunosuppressive TME via CXCL10 signaling, and that this contribution dominates compared with that of BMDMs (142). Other mechanisms that promote microglia/BMDM-mediated tumor growth include secretion of immunosuppressive factors, decreased cytotoxic activity, tumor necrosis factor (TNF), and iNOS expression (143).

Microglia as Therapeutic Targets

Colony-stimulating factor 1 receptor and its cognate ligand (CSF1/CSF1R) have been shown to regulate macrophage survival, proliferation, differentiation, and chemotaxis, and signaling through this axis appears to mediate TAM recruitment and survival (144). Pyonteck and colleagues demonstrated that inhibition of macrophage/microglia via CSF1R inhibition with BLZ945 in a murine model of glioblastoma resulted in increased survival and tumor regression (Fig. 5). Interestingly, inhibition led to microglial—but not macrophage—depletion (145). Further, CSF1R inhibition led to a loss of M2 marker expression suggesting at least partial repolarization toward the proinflammatory, antitumor M1 state. In a murine melanoma orthotopic BrM model, CSF1R inhibition with PLX3397 was shown to decrease BrM initiation and lead to reduced tumor burden (140). A phase I/II

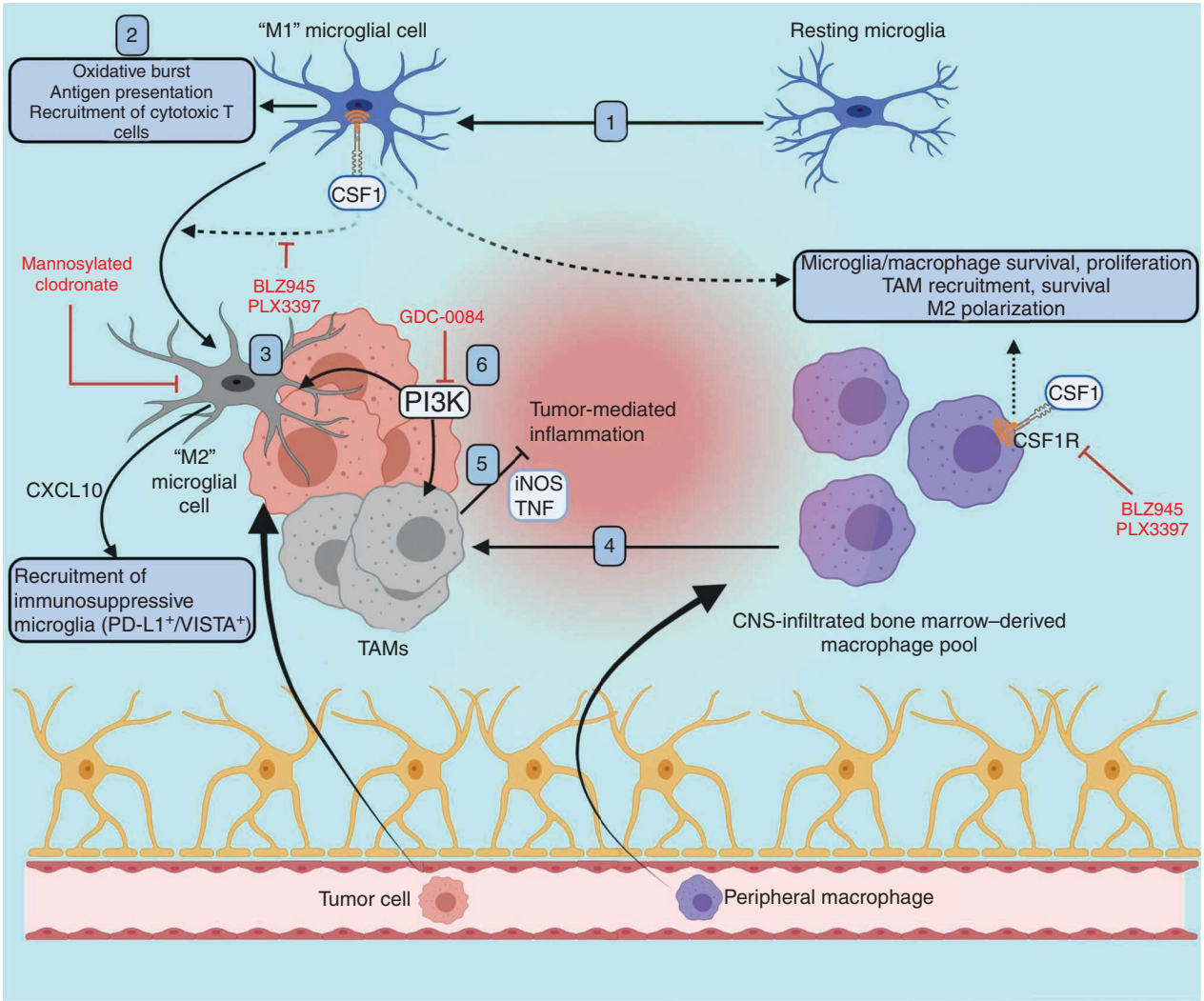

Figure 5. Role of microglia and bone marrow–derived macrophages in BrM. 1. Presence of invading tumor cells in the CNS parenchyma is sufficient to activate microglia from their resting state. 2. Activated or "M1-like" microglia play a role in the immune response to neuroinflammation and are capable of oxidative burst and antigen presentation and recruiting cytotoxic T cells. 3. Tumor cells can co-opt the functional state of nearby microglia, resulting in an anti-inflammatory phenotype ("M2") that promotes angiogenesis, tumor growth, and immunosuppression by recruitment of additional PD-L1$^+$/VISTA$^+$ microglia. Selective targeting for this functional state has been demonstrated experimentally with mannosylated clodronate, for example. 4. Bone marrow–derived macrophages infiltrate the neuroinflamed CNS and, similar to microglia, adopt a functional continuum ranging from antitumor to prosurvival tumor-associated macrophages (TAM). 5. TAMs protect tumor cells by secretion of immunosuppressive factors such as inducible nitric oxide (iNOS) and tumor necrosis factor (TNF), which mitigates the overall immune response. Colony-stimulating factor 1 (CSF1) axis signaling drives additional TAM recruitment and M2 polarization, resulting in further support and protection of tumor cells. In mice, CSF1 axis inhibitors such as BLZ945 and PLX3397 have demonstrated reduced tumor outgrowth. 6. BrM have been shown to have enriched PI3K signaling activity and that PI3K is a master regulator of metastasis-promoting microglia/macrophages. PI3K is an attractive therapeutic target with a growing list of CNS-penetrant inhibitors available such as GDC-0084. Figure created with BioRender.com.

clinical trial (NCT02452424) investigating the combination of PLX3397 and pembrolizumab in a cohort of patients with metastatic melanoma and other solids tumors was unfortunately terminated early due to lack of efficacy underscoring the challenge of translating findings from murine models to humans.

The fractalkine receptor (CX3CR1) has been shown to be unique to microglia and modulates microglial response to neuronal injury (132). Guldner and colleagues found that CNS-myeloid cells in BrM uniquely downregulated CX3CR1, leading to increased CXCL10 resulting in recruitment of PD-1– and V-domain Ig suppressor of T-cell activation (VISTA)–expressing cells and a shift toward an immunosuppressive program (142). Targeting of PD-1 and VISTA resulted in impaired BrM outgrowth, paving the way for potential future therapeutic strategies that may be translatable clinically.

Blazquez and colleagues found a cohort of human breast cancer–derived BrM to be enriched in PI3K signaling activity and that PI3K was a master regulator of metastasis-promoting microglia/macrophages (146). BrM outgrowth in mice was impaired when using a CNS-penetrant

small-molecule inhibitor of PI3K. The promise of targeting PI3K in the CNS is further supported by the high frequency of PI3K alterations observed in human BrM and the availability of CNS-penetrant PI3K inhibitors (76, 147). Clinical trials investigating PI3K as a putative target in patients with BrM are ongoing (NCT03994796 and NCT04192981). Finally, Andreou and colleagues found in a murine breast cancer orthotopic BrM model that selectively depleting anti-inflammatory/M2 microglial cells with mannosylated clodronate liposomes resulted in decreased BrM burden. This finding suggests that targeting microglia polarized to a tumor-supportive state may be a viable therapeutic strategy (141). Further work should focus on identifying specific targets of the anti-inflammatory microglial phenotype and corresponding high-affinity small-molecule inhibitors.

CONCLUSION

BrM is a devastating and increasingly common complication in patients with cancer. Although ICI has revolutionized the treatment of various cancers, the determinants of response remain incompletely understood, especially within the CNS. Increased understanding of the unique anatomical, cellular, and immune architecture of the CNS is paramount in developing novel CNS-directed immunotherapy strategies. Critical roles for astrocytes and microglia have emerged in helping drive the growth of BrM as well as interacting with the innate and adaptive immune system. There is substantial preclinical evidence that these glial cells may be rational targets for treating BrM and/or enhancing antitumor immunity in patients treated with ICI.

Translation of promising therapeutic strategies based on preclinical *in vitro* and *in vivo* work remains a major challenge (148). Specifically, the identification of therapeutic targets that may sensitize a host to ICI depends on an intact immune system. Therefore, data from syngeneic murine models are difficult to generalize to humans given interspecies differences in innate and adaptive immune responses. To this end, patient-derived organotypic spheroid models that allow for *ex vivo* studies with an intact immune infiltrate are an exciting tool currently in use (149). Development of such a model with viable astrocytes and microglia would offer an opportunity to rapidly translate understanding of glial cell, BrM, and TIME interactions into early-phase clinical trials. Another model system that may be advantageous is immunodeficient mice engrafted with human immune cells or tissues, that is, "human immune system (HIS) mice," for which development is ongoing (150) Additionally, a significant portion of the current preclinical evidence for targeting glial cells comes with the use of already approved agents, thus providing immediate opportunity to develop clinical trials studying agents with astrocyte-specific activity. With implementation of clinical trials investigating therapies with potential CNS activity, ongoing refinement of existing model systems and increasingly available tools such as single-cell RNA sequencing to combat tissue heterogeneity, the future is promising for therapies that will effectively unlock antitumor immunity in the CNS.

Authors' Disclosures

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