



From pre-clinical to translational brain metastasis research: current challenges and emerging opportunities

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Abstract

Brain metastasis, characterized by poor clinical outcomes, is a devastating disease. Despite significant mechanistic and therapeutic advances in recent years, pivotal improvements in clinical interventions have remained elusive. The heterogeneous nature of the primary tumor of origin, complications in drug delivery across the blood–brain barrier, and the distinct microenvironment collectively pose formidable clinical challenges in developing new treatments for patients with brain metastasis. Although current preclinical models have deepened our basic understanding of the disease, much of the existing research on brain metastasis has employed a reductionist approach. This approach, which often relies on either in vitro systems or in vivo injection models in young and treatment-naïve mouse models, does not give sufficient consideration to the clinical context. Given the translational importance of brain metastasis research, we advocate for the design of preclinical experimental models that take into account these unique clinical challenges and align more closely with current clinical practices. We anticipate that aligning and simulating real-world patient conditions will facilitate the development of more translatable treatment regimens. This brief review outlines the most pressing clinical challenges, the current state of research in addressing them, and offers perspectives on innovative metastasis models and tools aimed at identifying novel strategies for more effective management of clinical brain metastasis.

Keywords Brain · Central nervous system (CNS) · Cancer metastasis · Brain metastasis · Brain immunity · Pre-clinical brain metastasis models

Brain metastasis: a clinical challenge

Introduction

Brain metastases (BrMs) are generally considered secondary metastatic tumors, derived from the dissemination of a primary tumor. Brain metastatic relapse occurs in 20–30% of patients with advanced cancer. Most BrMs arise from lung (40–50%), breast (30%), melanoma (20%), colorectal

cancer (CRC, 1–2%), and renal cell carcinoma (RCC, 7–10%) [1–3]. Lung cancer BrM is the most common primary tumor of origin observed in male patients diagnosed with BrM, while breast cancer BrM accounts for the majority of cases in female patients [1]. BrMs, although derived from single primary tumors of origin, may still exhibit different molecular subtypes. Even in a single patient harboring one primary tumor, numerous distinct, clonally derived brain lesions may be present, each potentially differing from the others. All these factors together contribute to BrM heterogeneity. Furthermore, systemic and lifestyle factors, such as age and microbiome status, might also influence the disease trajectory [4, 5]. Despite poor clinical outcomes and patient survival, progress in advancing treatment options for BrM patients has been marginal. This is partly attributed to the low inclusion of BrM patients in clinical trials and limited funding for BrM research, compared to other CNS cancers [6].

In current clinical practice, surgery and radiotherapy, such as stereotaxic radiosurgery (SRS) and whole brain

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radiotherapy (WBRT), are primary treatment regimens for brain metastases (BrMs) (Table 1). WBRT is used in a subset of patients, despite its significant side effects, including impaired cognitive function, which can be slightly alleviated through hippocampal avoidance [7]. Systemic treatment regimens tailored to the specific molecular subtype of the primary tumor of origin are also used for treating/preventing brain metastasis. For example, HER2 + breast cancer BrM patients receive anti-HER2 targeted therapy such as trastuzumab, while more general systemic chemotherapies are indicated in triple negative breast cancer (TNBC) BrMs [8, 9]. A single-arm phase III clinical trial was concluded in 2020 for trastuzumab emtansine (T-DM1) for patients with HER2 + metastatic breast cancer (KAMILLA, NCT01702571). T-DM1 exhibited a low toxicity profile and showed reduction of BrM lesion size in 42.9% of patients [10]. Another commonly utilized molecular therapy involves treating asymptomatic BrM in lung cancer patients with anaplastic lymphoma kinase (ALK) rearrangement, using receptor tyrosine inhibitors (TKIs) such as the third generation ALK/ROS1 inhibitor lorlatinib. In one single-arm trial, lorlatinib treatment led to a measurable intracranial response in 64% of patients which was sustained for 12.4 months (median) [11].

Immunotherapy, particularly immune checkpoint therapy (ICT) targeting the PD-1/PD-L1 and CTLA-4 axes, shows promise as a cancer treatment for certain patient subsets with

metastatic melanoma, non-small cell lung cancer, RCC, and head and neck cancers. ICT modalities generally have better side effect profiles compared to traditional chemotherapies [12]. Thus far, immunotherapies have only been used in a subset of BrM patients such as a recent phase II study utilizing combinatorial ICT in asymptomatic BrMs derived from melanoma. Although the treatment regimen achieved efficacy in 57% of melanoma patients in some studies, ranging from stable disease for 6 months to complete response, such targeted approaches have been ineffective in treating other BrMs [13–17].

Aside from direct actions on the tumor cells, radiotherapy is known to induce immunogenic cell death (ICD) and abscopal effects, a phenomenon whereby radiotherapy treatment also affects lesions outside the radiation field [18, 19]. Consequently, recent efforts have been directed towards exploring combinatorial radioimmunotherapy in clinical settings. Melanoma patients who were treated with immune checkpoint therapy (ICT) ipilimumab in combination with WBRT achieved longer median survival compared to standard of care WBRT (21.3 versus 4.9 months) and an increased 2-year OS (47.2% versus 19.7%). In addition, SRS plus ipilimumab has also been shown to yield an increased OS (18.3 versus 5.3 months) in favor of the ICT + WBRT combination [20]. While promising, the current clinical practice of combining ICT with radiotherapy requires more informative guidance

Table 1 Summary of standard of care treatments for brain metastases grouped by tumor of origin

Primary tumor of origin	Surgery (S)	Radiotherapy (R)	Chemotherapy (C)	Targeted/molecular therapy (T)	Immunotherapy (I)	Combination therapy
Non-small-cell lung cancer (NSCLC) [76]	Yes (1–3* BrMs)	SRS, WBRT	Not common**	Osimertinib-EGFR TKI	Pembrolizumab	EGFR+: S/R + T
Small-cell lung cancer (SCLC) [77]	No	PCI, SRS, WBRT	Teniposide	Not common	Not common	R alone, or R + C
Breast cancer—HER2+ [8, 80, 81]	Yes (1 BrM)	SRS, WBRT	Emtansine	T-DM1, Neratinib, T-DXd	Not common	S/R + T, or S/R + T + C
Breast cancer—TNBC [9]	Yes (1–3 BrMs)	SRS, WBRT	Capecitabine, eribulin, carboplatin	Iniparib PARP inhibitor, Bevacizumab	Not common	S/R, or S/R + T + C
Melanoma [78]	Yes (1 BrM)	SRS, WBRT	Not common	Dabrafenib, Trametinib-BRAF V600E/K	Nivolumab, Ipilimumab	T + I → S/R
Renal cell carcinoma (RCC) [79]	Yes	SRS, WBRT	Not common	Sunitinib, Cabozantinib-TKI inhibitor	Nivolumab, Ipilimumab	T + I, or S/R + I
Colorectal cancer (CRC) [3]	Yes (1 BrM)	SRS, WBRT	Oxaliplatin, irinotecan	Not common	Not common	S/R, or S/R + C

SRS stereotactic radiosurgery, WBRT whole brain radiation therapy, PCI prophylactic cranial irradiation

*Number of individual brain metastasis lesions in the brain; **Not common for metastatic disease, could be indicated for primary tumor of origin

regarding treatment timing and dosing schedules. As the ideal schedule of each treatment modality is likely to vary between different BrM subtypes, there is a critical need for preclinical models to identify the optimal regimen with respect to immunotherapy and primary tumor/sub-type of origin.

Brain metastases of unknown origin

Although BrMs are usually identified in patients later during the clinical treatment course of the diagnosed primary tumor, a subset of patients develop BrMs as the first symptom of previously undiagnosed cancer [1, 21]. A recent estimate is that up to 15% of all BrM patients present as BrMs with cancer of unknown primary (BM-CUP) and these patients are often treated with SRS. The diagnosis procedure for BM-CUP includes a physical examination, blood and biochemical tests, immunohistochemistry (IHC), mammography (for breast cancer), whole body computed tomography (CT), and/or magnetic resonance imaging (MRI). If a primary tumor is still not identified through these efforts, immunohistochemistry (IHC) on BrM biopsies using common primary tumor markers could provide clues on the BrM tissue of origin [22]. Despite these efforts, the primary tumor of origin can often remain elusive even after autopsy due to the lack of specific prognostic or predictive tumor markers and complete lack of molecular mechanistic insight into the disease [22]. Next generation sequencing (NGS) is the most valuable and feasible tool to guide identification of molecular targets in BM-CUP, in a similar fashion to primary tumors. A 2018 NGS study of formalin-fixed paraffin embedded (FFPE) tissue sections of 21 BM-CUP patients noted 76 gene variants, some of which are targetable. The commonly detected genomic variants in BM-CUP were *TP53*, *KRAS*, *MET*, and *MYC* [23]. Beyond correlative genetic studies, the enigmatic manifestation of BM-CUP, characterized by early metastatic spread to the brain and simultaneous regression of the primary tumor site, is difficult to model pre-clinically. Recently, a study proposed “agnospheres”, stem-like cells originating from human CUPs, as a novel disease model that recapitulates both early cell dissemination and multi-organ metastasis [24]. This study showed that agnosphere properties, namely proliferation and self-renewal while maintaining stemness, rely on the constitutive activation of the MAPK pathway and the *MYC* oncogene. Targeted inhibition of the MAPK pathway component MEK by trametinib caused cancer cell death and necrosis. The gene expression signature responsive to trametinib is a feature of most patient CUPs, suggesting that MAPK amplification is a common mechanism, potentially contributing to BM-CUPs [24].

Unique challenges in drug delivery to brain metastases

Separate from the features of originating primary tumor stroma, the brain environment poses unique obstacles for the delivery of treatments. The central nervous system (CNS) is protected by two main functional neurovascular barriers: (1) the blood–brain barrier (BBB), and (2) the blood–cerebrospinal fluid barrier [1]. The BBB consists of endothelial cells with high rates of efflux that are connected by tight junctions as well as pericytes and astrocytic end-feet (basement membrane). When intact, the BBB is mostly permeable to only small uncharged compounds that can diffuse passively. Although the mechanisms are not fully understood, egress of cells such as macrophages and disseminated tumor cells into the CNS occurs during BrM, while most chemotherapy agents and targeted- or immuno-therapies, such as ICT cross the BBB poorly with reduced therapeutic effects [1, 25]. The blood-cerebrospinal fluid barrier on the other hand is formed by choroid plexus epithelial cells. The breaching of this barrier is thought to be permissive for leptomeningeal metastasis [1, 26].

There is now both pre-clinical and clinical evidence that the BBB becomes more permeable in the later outgrowth stages of BrM, owing to the formation of the blood tumor barrier (BTB) [1, 27]. The BTB however is only partially permeable, and the degree of permeability can vary from lesion to lesion within the same patient [1, 25, 27, 28]. There are currently many clinical trials underway investigating various (molecular, biological, and physical) approaches to increase BBB permeability for brain cancers, as well as recent pre-clinical advances in non-invasive approaches [29]. Recent studies in mice and rat models have shown that a combination of low-intensity focused ultrasound (FUS) pulses and circulating microbubbles provide a physical/mechanical approach to transiently disrupt and permeabilize the BBB [30] and to deliver doxorubicin for the treatment of glioblastoma [31]. A more recent 2023 preclinical study aimed specifically at BrM examined FUS combined with paclitaxel and Doxil in metastatic TNBC. They showed that the FUS + chemo combinatorial regimen increased median survival and led to the slowest brain tumor burden progression compared to other groups [32]. More pre-clinical and translational studies are needed to assess the efficacy of such approaches in treating BrM.

Mechanisms of brain metastasis: an update

Our current understanding of key brain metastasis drivers from primary tumors of various origins has been extensively reviewed by Campbell et al. [33] and Achrol et al. [1]. Here,

we only highlight some of the most recent advances in our understanding of BrM.

Novel insights gained from pre-clinical models

Cancer cell dormancy, therapy resistance, and metabolic alterations

Most disseminated tumor cells (DTCs) do not survive the process of extravasation. Among the surviving DTC seeds, some may enter an extended period of dormancy, characterized by a slow or non-cell-cycling state and activation of quiescence effectors downstream of p38 [34]. These cells can persist for long periods of time and later reactivate the cell cycle to induce overt metastases [35]. Moreover, due to their slow cycling state and high stemness/phenotypic plasticity, dormant cells that later lead to disease relapse are often therapy resistant [36], highlighting the importance of successful identification and eradication of these rare cells. General molecular drivers of dormancy along with current strategies for treating latent disease have been reviewed in detail [34] and several dormancy markers have been identified specifically in glioblastoma: *H2BK*, *IFGBP5*, and *EPHA5*. Despite clinical evidence that these markers are present in certain BrM patient populations [35], detailed mechanistic studies in pre-clinical models have not been performed to assess their functional relevance and equivalence in BrM. Recent work in a pre-clinical mouse model of TNBC BrM, however, demonstrated that astrocyte-deposited laminin-211 drives DTC quiescence within the perivascular niche. Stripping of astrocyte end feet is required for activation of nuclear YAP-mediated signaling and BrM outgrowth [37]. This study not only identified a brain-specific mechanism of DTC dormancy, the rate limiting step of BrM outgrowth, but also highlighted the importance of conducting more studies exploring both the cancer cell intrinsic and extrinsic mechanisms governing entry and exit out of quiescence.

Therapy resistance in the context of brain metastasis is closely linked to resistance to radiotherapy and targeted therapy. Although radiotherapy is the primary treatment for most patients, the molecular mechanisms underlying resistance acquisition have been insufficiently studied. Melanoma and renal cell carcinoma (RCC) BrMs are largely resistant to radiotherapy [38]. Thus, there is critical need to better understand underlying drivers of resistance. A recent study employed radiotherapy (WBRT)-resistant experimental models of lung adenocarcinoma and breast cancer BrMs to delineate the molecular characteristics of resistance. They treated oncospheres derived from lung and breast cancer cell lines with radiation and conducted RNA sequencing to identify transcriptional differences between radioresistant and radiosensitive oncospheres. This study revealed that the S100A9-RAGE-NF- κ B-JunB pathway mediates

radioresistance in these models and established a correlation between S100A9 expression levels in BrM patient datasets and clinical responses to WBRT. These findings not only elucidated a mechanism of resistance acquisition but also point to a potential biomarker for assessing WBRT efficacy in clinical settings [39]. Elucidating the molecular mechanisms of resistance to targeted therapies presents a separate challenge, as these therapies are specific to different primary tumor subtypes. Recent studies in lung cancer mouse models have investigated brain lesions resistant to the EGFR inhibitor osimertinib. One such study linked S100A9 overexpression, via a distinct pathway involving retinoic acid signaling, to BrM relapse following osimertinib treatment [40]. Another study identified a RhoA/SRF signaling axis that activates gene programs associated with drug resistance. Importantly, combining RhoA inhibition with osimertinib resulted in a substantial reduction of BrM burden in this preclinical model [41].

The role of altered cellular metabolism in cancer in a wider context is well established, yet metabolic reprogramming in BrM has been less extensively examined and is an emerging area of exploration [42, 43]. Studies by Parida et al. demonstrated that metabolic diversity and flexibility within BrM cells is critical for successful BrM outgrowth in a HER2+ breast cancer model. Moreover, latent BrM cells utilize dynamin-related protein 1 (DRP1) to sustain fatty acid oxidation and survival, thus pharmacological inhibition, or genetic depletion of DRP1 reduces BrM burden [44, 45].

The CNS cellular microenvironment

Aside from the BBB, resident brain cells engage in dynamic crosstalk and together shape normal CNS function at steady state. Neurons, oligodendrocytes, astrocytes, microglia, and pericytes comprise the CNS-native cellular compartment which can additionally interact with peripheral immune cells [46].

CNS-Native cells During the evolution of BrM, the brain environment undergoes dramatic remodeling [46]. It has been clinically observed that activated microglia and astrocytes encircle BrM lesions in a process called gliosis [47, 48]. There have been numerous pre-clinical studies in mouse models investigating the roles of microglia and astrocytes in BrM. It is now well established that astrocytes play pro- and anti-metastatic roles depending on the stage and specific astrocyte subtype involved. Astrocytes can directly induce apoptotic cell death in cancer cells via plasminogen secretion, while a subtype of reactive astrocytes marked with pSTAT3 can deliver cGAS and miRNAs to promote BrM progression [49, 50]. Moreover, astrocytes have been shown to activate proliferator-activated receptor γ (PPAR γ) in cancer cells, inducing their proliferation and outgrowth [51].

Microglia, the main CNS-native immune macrophage, have similarly dichotomous roles in BrM. Recent work has highlighted the tumor-promoting roles of this cell population. In our recent study, Guldner et al. utilized scRNA-seq in a BrM mouse model and showed that microglia downregulate homeostatic genes such as *Cx3cr1* in favor of a pro-inflammatory, interferon driven gene program. Excessive Cxcl10 release cultivated the microglia-dependent immunosuppressive niche. Moreover, microglial expressed a high level of Vista, a novel checkpoint molecule that can be targeted in combination with ICT for BrM treatment [52].

Peripheral immune cell infiltration Immune cells, such as cytotoxic CD8+T lymphocytes, are present in brain metastases (BrM), and immune surveillance occurs via the meningeal lymphatic system. However, the tumor-induced reprogramming of the microenvironment favors the accumulation of anti-inflammatory regulatory T cells (Tregs) [20, 46]. Currently, therapies such as anti-PD-1/PD-L1 and anti-CTLA-4, including nivolumab and ipilimumab, are clinically administered to treat melanoma and non-small cell lung cancer (NSCLC) derived BrMs but are not standard for BrMs from other primary tumors [20].

In our recent work, we detailed the role of the enhancer of zeste homolog 2 (EZH2), a well-documented histone methyltransferase, in the advancement of BrM. We discovered an upregulation of *EZH2* in human BrM specimens which facilitates the recruitment of immunosuppressive neutrophils, characterized by high arginase1 (Arg1) and PD-L1 expression, into the brain metastatic niche, thereby aiding BrM proliferation. Pharmacological interventions that impede neutrophil recruitment, ICT, and the inhibition of EZH2 phosphorylation through Src inhibitors have shown significant reductions in BrM across various mouse models [53]. More recently, research has indicated that persistent type I interferon (IFN) signaling in astrocytes and cancer cells instigates the secretion of C–C motif chemokine receptor 2 (CCR2) by astrocytes, which in turn attracts pro-tumor monocytes from the periphery. Both genetic and pharmacological disruption of the CCR2/CCL2 pathway consequently decreased the BrM load in breast and melanoma mouse models [54].

Novel insights from profiling clinical brain metastases

Despite extensive research efforts, current experimental preclinical models of brain metastasis have been limited by the utilization of largely homogeneous cancer cell lines and immunocompromised mouse hosts when using human-derived tumor cells. Thus, the translational potential of these discoveries has varied. Recent omics profiling efforts, however, have been extremely valuable in building a more

comprehensive unbiased view of the patient brain metastasis ecosystem containing heterogenous populations of cancer and stromal cells.

Genomic profiling

A 2022 study led by Nguyen et al. established the Memorial Sloan Kettering—Metastatic Events and Tropisms (MSK-MET) pan-cancer database resource containing clinical and genomic alteration data across 50 different tumor types [55]. Metastatic burden for some of the cancer types was strongly correlated with chromosomal instability, but not for others, highlighting both the intrinsic cancer and target organ differences. For BrM specifically, it was found that BrMs derived from lung adenocarcinoma had a higher incidence of TP53, EGFR mutations, and TERT amplification, while *PTEN* mutations and *PI3K* pathway alterations were common in melanoma derived BrMs [55]. Another recent study conducted whole-exome sequencing on patient primary tumors and matched brain metastases deriving from lung (n = 38), breast (n = 21) and RCCs (n = 10). They detected genetic vulnerabilities in pathways such as PI3K/AKT/mTOR, CDK, and HER2/EGFR that could be exploited for targeted treatments. Importantly, many of the BrM alterations were not present in matched primary tumor samples, indicating that the primary tumors and metastases had very divergent developmental trajectories [56]. Similarly, another recent study found *MYC*, *YAP1*, and *MMP13* to be important genomic alterations in BrM in a cohort of 105 patients with lung adenocarcinoma [57].

scRNA-sequencing

Aimed to discover universal BrM characteristics, independent of the primary tumor of origin, Gonzalez et al. performed single cell RNA sequencing (scRNA-seq) in a large-scale study on more than 100,000 malignant and non-malignant cells harvested from patients' BrMs. This comprehensive study on 15 human parenchymal BrMs described two distinct and functional BrM archetypes: one proliferative and one inflammatory, as well as characterized the immune infiltrate [58]. Another study utilized scRNA-seq to examine patient breast (n = 3) and lung (n = 2) cancer derived BrMs. They uncovered tumor-associated fibroblasts that produce high levels of collagen and facilitate TME remodeling. Furthermore, they identified tumor-specific collagen receptors that have the potential to become biomarkers of BrM patient survival [59]. These relatively large scale BrM profiling serve as valuable resources to guide further research. However, such studies are still largely under sampled. Additionally, it is currently not possible to examine the impact of different clinical treatments or predict therapy response.

Similarly, there is a great need for further pre-clinical studies exploring the role of systemic factors on BrM development.

Multi-omics profiling

The molecular profiles of melanoma-derived BrM have been examined in 88 patient-derived samples and 42 matched extracranial lesions through a combination of RNA, whole exome, T cell receptor (TCR) sequencings, and IHC [60]. It was found that melanoma-derived BrMs had high oxidative phosphorylation (OXPHOS) levels and utilizing an OXPHOS inhibitor could abrogate BrM in mouse xenografts [60]. For lung (n=21) and breast (n=9) cancer derived BrM patients specifically, a different study performed whole-exome sequencing, RNA sequencing, flow cytometry, immunofluorescence, and tissue imaging on surgically collected tissue. This study revealed that the immune infiltrate was unique even among BrMs derived from within a single primary tumor of origin group and correlated *TP53* mutations with overall infiltration level across all tumors [61]. Another multiome profiling study conducted RNA, exome, low-pass whole-genome sequencing, and global DNA microarrays on metastases from 55 breast cancer patients, of whom 11 were diagnosed with BrM and 28 had liver metastases. In these patient cohorts, patterns of DNA hypermethylation around the genes of major histocompatibility complex (MHC), Class I A, *HLA-A*, were associated with lower expression of MHC molecules and reduced immune cell infiltration [62]. Yet another valuable profiling study focusing on the immune compartment utilized RNA-seq (n=48), flow cytometry (n=100), IF, and ELISA assays to directly compare the immune infiltrate between patient gliomas and BrMs originating from melanoma, breast, and lung cancer. They found that globally, compared to gliomas BrMs exhibited greater immune cell diversity as well as distinct transcriptional programs in the myeloid compartment. Specifically, they observed upregulation of neuroinflammation mediators CSF2 and IL23A as well as the receptor MARCO in BrM microglia and infiltrated macrophages [63].

Cerebrospinal fluid liquid biopsy

Although the characteristics of the immune infiltrate are often used to predict immune checkpoint treatment response, it is often difficult to obtain biopsies from BrM lesions. Thus, a recent study explored the immune composition of cerebrospinal fluid (CSF) of six BrM patients with matched BrM lesions and found that the degree of CD8 T cell infiltration into the tumor can be predicted from T cell abundance in the CSF. Interestingly, TCR clonotypes expanded in BrM lesions can also be detected in the CSF, pointing to active cellular exchange between these two tissue compartments [64]. Another study examined the immune composition of

melanoma BrM and leptomeningeal melanoma metastases (LMM). In total, they examined 8 skin samples, 14 melanoma BrMs, and 19 LMM specimens. It was shown that the LMM immune landscape was more immuno-suppressive compared to BrM or primary melanoma and that response to anti-PD-1 therapy was correlated with increased levels of T cells and dendritic cells in the CSF of patients [65].

Perspectives in translational brain metastasis research

A recent viewpoint article by the experts in the field has provided informative insights on the pressing needs in translational BrM research [66]. Moreover, a recent virtual National Cancer Institute (NCI) workshop consisting of BrM patents, advocates, scientists, and clinicians convened in 2022. Together, they outlined unmet needs in translational BrM research and offered guidelines for how to better align translational research with patient-defined meaningful advancements [6]. Here, we will highlight some key points from the workshop and provide additional viewpoints for future BrM research.

Immunotherapy beyond immune checkpoint blockade therapy

Although ICT has been extremely successful in treating certain types of cancer, for patients who do not respond to ICT there are other emerging modalities for enhancing the immune response against metastasized cancer cells [12]. For example, engineered chimeric antigen receptor (CAR) T cells targeting CD19 has been revolutionary in treating hematologic malignancies, such as acute lymphoblastic leukemia (ALL) [15]. More recently, CAR-T cells have been applied to CNS cancer such as glioblastoma. In a 2020 study, Wang et al. exploited a previously described scorpion venom protein chlorotoxin (CLTX) that binds specifically to glioblastoma cells to generate their CAR construct. It was found that CLTX CAR-T therapy results in GBM regression with no observable off target effects in mouse GBM models [67]. Following this study, a Phase I clinical trial was initiated to assess the clinical efficacy of CLTX CAR-T in patients with recurrent glioblastoma, malignant glioma, and WHO grade II and III gliomas (NCT04214392). For CAR T therapy to be effective in BrM, the specific tumor antigen to be targeted needs to be considered carefully, as off target effects in the brain could lead to devastating side effects. A 2018 study in an orthotopic xenograft model of breast cancer-derived BrM demonstrated that local, intracranial delivery of HER2-CAR T cells had potent in vivo effects on reducing BrM burden [68]. Thus, the ability to identify the specific molecular subtype of the primary tumor of origin is

of paramount importance for further CAR T developments in BrM treatment.

The lack of clinical efficacy for ICT in certain solid tumors is at least partially attributed to an immunosuppressive microenvironment fueled by tumor-associated macrophages [69]. Indeed, CNS-native microglia and infiltrated macrophages are known to influence BrM outgrowth, consequently, blockade of the macrophage colony-stimulating factor 1 receptor (CSF1R) in a murine breast cancer BrM model led to reduced tumor burden. This response is more durable with dual targeting of CSF1R and microglial STAT5 signaling [70]. Therefore, in addition to CAR-T cell therapy, another immunotherapy approach is to focus efforts on reprogramming the myeloid cell compartment to elicit more efficient cancer cell phagocytosis (directly) or boost the cytotoxic T and natural killer (NK) cell response (indirectly), and potentially serve as drug delivery conduits [69]. The therapeutic utility of myeloid cell engineering has been demonstrated recently. Ex vivo engineered IL-2 secreting myeloid cells can readily cross the BBB after i.v. administration and prolong survival in a mouse model of glioma [71]. As bone marrow derived myeloid cells (BMDMs) are known to infiltrate the brain during BrM [72, 73], studies exploring myeloid cell-based therapies could prove to be very valuable.

Translation-oriented pre-clinical models and experimental designs

Historically, the most widely used models to study BrM rely on the utilization of brain-seeking cell lines generated through multiple rounds of in vivo selection of the original parental cell line with lesser metastatic propensity. Cancer cells are typically delivered via intracardiac (left ventricle) or intracarotid artery (ICA) injections [74]. Although the models provided novel insights into basic BrM biology, the limited genetic repertoire of cancer cell lines, common utilization of immunocompromised hosts, and treatment-naïve conditions have reduced the clinical relevance and translational impact of these findings. In this section, we will highlight some emerging pre-clinical models and future study design priorities for addressing the historical BrM pre-clinical research limitations.

Patient-derived xenografts (PDXs) from BrM are valuable models typically generated by transplanting patient tumors subcutaneously or orthotopically into immune-deficient mice to expand the tumors, followed by systemic or local injection for BrM establishment or generation of patient-derived cultures that can be cultured at low passages. PDX models have the advantage of more faithfully recapitulating the diverse and heterogenous genomic features of patient tumors [74]. However, in vivo PDX models in immune-deficient hosts lack immune elements which

are critical for BrM success and immunotherapy testing. The recent progress in creating humanized mouse models holds potential for overcoming the limitation of current PDX models [75].

The prevailing BrM models based on tumor cell injection only recapitulate the later steps of the metastatic cascade. The ideal mouse model of BrM would rely on spontaneous metastasis, originating from either a syngeneic cell line implanted orthotopically or naturally developed in genetically engineered mouse models (GEMs). These model systems would recapitulate the entire metastatic cascade as well as allow for the examination of the pre-metastatic niche. Unfortunately, very few cell lines can efficiently metastasize to the brain in an immunocompetent host, limiting their practicality in a preclinical setting [74]. Development of primary tumor models that have more reliable and reproducible spontaneous BrM capability would significantly enhance both mechanistic and translational BrM studies.

Very few pre-clinical studies at present include clinically relevant neurosurgical resection, radiation therapy and/or concurrent systemic treatments. Most pre-clinical BrM research is conducted in treatment-naïve conditions, yet patients diagnosed with BrM have often undergone several rounds of systemic therapy for the primary tumor, followed by neurosurgery, radiotherapy, chemotherapy, targeted therapy, or even ICT, upon BrM diagnosis [74]. As radiotherapy (SRS or WBRT) is the standard of care in the clinical management of brain metastasis patients regardless of primary tumor of origin [3, 8, 9, 76–81] (Table 1), future preclinical studies under the context of radiation or systemic treatment would reveal valuable mechanisms that could be more relevant to clinical trial settings. A recent 2019 review article outlined the utility and pitfalls of various mouse models and currently available small animal radiotherapy platforms. This review can serve as a resource for pre-clinical researchers interested in incorporating radiotherapy modalities most applicable to the current clinical practice of tumor radiotherapy treatment [82].

Finally, as outlined in the 2020 NCI workshop patient-guided priorities, there is a critical need to improve health span metric data collection in BrM clinical trials [6]. The BrM intracranial mass leads to the development of a unique and devastating set of neurological symptoms and cognitive decline which vary depending on tumor size and anatomical location within the brain. The aggressive treatments used in BrM management such as radiotherapy and surgical resection can further exacerbate neurocognitive symptoms [83]. As this aspect of the disease can be a significant concern for the patient and their family, a high priority for developing novel pre-clinical animal models is incorporating assessments of overall health span metrics and neurological function, as compared to only assessing gross tumor burden.

Novel technologies

High throughput transcriptomic techniques such as scRNA-seq have revolutionized our understanding of cellular heterogeneity of tissues in steady state and various diseases. However, as these techniques are not performed *in situ*, they do not provide information about the spatial relationship among different cells within the critical niche of the metastasis. The advent of next-generation sequencing (NGS)-based and imaging-based spatial transcriptomics technologies provides a novel avenue for exploring the tumor and metastatic microenvironment [84]. Although NGS-based spatial transcriptomics platforms such as Visium have advanced our understanding of tumor microenvironment architecture [85, 86], they are not yet able to capture transcriptomes at a single cell level. To circumvent inherent limitations of both single cell and spatial methods, recent efforts have explored ways to integrate scRNA-seq, single-nucleus RNA-seq, and spatial information. Given the unique tissue architecture of the BrM microenvironment, jointed analysis of single cell transcriptome and spatial transcriptomic analyses could provide unique mechanistic insights into the spatial relationship between BrM cancer cells and the niche, which could potentially infer novel therapeutic targets. For example, a 2022 study led by Biermann et al. conducted single cell/nucleus RNA-seq, spatial single-cell transcriptomics and TCR sequencing in treatment naïve melanoma BrM patients [87]. They made key discoveries in both metastasized cancer cells and immune infiltrates. In cancer cells they found high levels of chromosomal instability, neuronal-like programs, and spatially variable metabolic alterations. In the BrM TME, lymphoid aggregates harboring B cells actively differentiating into plasma cells, infiltration of monocyte-derived macrophages, and dysfunctional T cells [87]. In addition to these transcriptomic analysis advancements, on the protein level, advances in tissue sequential immunofluorescence (IF) approaches such as the COMET™ (Lunaphore) platform will allow researchers to multiplex many more proteins on the same slide.

CRISPR/Cas9 based genetic screening technology is another emerging technology that could significantly reduce the time required to identify novel druggable targets in BrM and could work synergistically with profiling approaches described above. In the classical CRISPR “drop-out” genetic screen, depletion of specific guide RNAs (gRNAs) identifies genes which are essential for survival under the specified selection conditions [88]. Such *in vitro* screens conducted on various cell lines elucidated novel synthetic lethality and drug resistance pathways [89, 90]. Although valuable, this simplified 2D *in vitro* screening approach cannot completely model the heterogeneous *in vivo* metastatic microenvironment. Thus, more recent studies are exploring organoid [91] and *in vivo* CRISPR screening approaches alongside

scRNA-seq or using patient-derived xenografts (PDXs) [88, 92–95], further broadening the scope of information that can be obtained.

Pushing the boundary of clinical diagnosis

Clinical presentation of an intracranial BrM lesion includes symptoms such as headache (50% patients), papilledema (15–25%), focal neurological deficits (40%), and seizures (15–20%). Currently, the gold standard for diagnosing BrM is histopathological analysis of brain tissue involved. Neuroimaging techniques such as brain contrast-enhanced magnetic resonance imaging (MRI) can also be used to assess the global BrM burden [1]. Moreover, MRI imaging techniques are used throughout the treatment process to monitor therapeutic effects in a less invasive manner. Novel imaging modalities such as quantitative magnetic transfer (qMT) and magnetic resonance spectroscopy (MRS) are currently being evaluated for their sensitivity in distinguishing between different types of brain tumors and detection thresholds and is an emerging area with several clinical trials underway [96].

Pushing the sensitivity of imaging modalities is highly impactful for BrM early detection. One promising approach to increase the sensitivity of existing MRIs is increasing image contrast with BrM-targeted nanoparticles. A phase 1 clinical trial was conducted where 15 patients were injected with a single intravenous dose of gadolinium(Gd)-based AGuIX nanoparticles [97]. Notably, these BrM patients represented four different primary tumors of origin: melanoma, lung, colon, and breast cancer. Upon administration, the nanoparticles were able to home to the brain and increase MRI image contrast quality [97]. Similarly, a combination of neuroimaging and nanoparticles can be used to assess the status of the BBB for both integrity and heterogeneity. Currently, contrast-enhanced MRI is the gold standard for BBB assessment, however positron emission tomography (PET) and intravital microscopy can also be utilized. These approaches are an emerging clinical priority, not just for the detection of BrM lesions, but additionally for assessing BBB/BTB heterogeneity before and after implementing therapeutic interventions [28]. Perspectives in preclinical BrM imaging have been surveyed in a valuable 2021 review, which discussed recent MRI and PET strategies currently being applied in animal BrM models and provided recommendations for clinical translation [98].

Conclusion

In this article, we reviewed recent research efforts related to the translational perspectives of brain metastasis. The rise of single-cell omics technologies has opened a new era of unbiased studies for clinical BrMs and provided much-needed

Table 2 Summary of key knowledge gaps and recommendations

Main gaps in knowledge	Future pre-clinical study priorities
Establishing dosing regimes for combinatorial treatments, e.g. SRS/WBRT with ICT	Including radiotherapy and other regimens in pre-clinical studies
Limited permeability and heterogenous nature of BTB and BBB	Novel BTB/BBB disruption approaches
Treatment naive pre-clinical models do not reflect the majority of BrM patients	Including radiotherapy and other regimens in pre-clinical studies
Limitations in cancer cell lines used most often in BrM pre-clinical studies	Effective utilization of available patient data sets and novel technologies
Sparse inclusion of health span metrics and neurological function in pre-clinical studies	Expanding treatment efficacy endpoints to include behavioral and health metrics
Low sensitivity of imaging modalities to detect early BrM lesions	Improving imaging resolutions for enhanced sensitivity
Lack of specific molecular targets, mechanisms, and role of the heterogeneous brain TME	Mechanistic study designs informed by single cell analysis of BrM and BrM TME

resources and insights for dissecting the extremely heterogeneous BrM ecosystem. However, the gap between preclinical studies and clinical profiling studies has widened. Given that BrM is a clinical challenge, there is a pressing need to develop preclinical models and conduct preclinical research under more clinically relevant treatment contexts. Here, we have summarized the potential high priority areas for future pre-clinical studies (Table 2). Continuing to expand preclinical research efforts on basic cellular mechanisms with more considerations to the current clinical practices will be pivotal to reduce the gap between basic discoveries and novel therapeutics. We believe this can be achieved through leveraging novel technologies adopted from different fields with emerging pre-clinical models.

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Declarations

Competing interests The authors declare no competing interests.

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