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Review

# Innovative Therapeutic Strategies for Effective Treatment of Brain Metastases

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**Abstract:** Brain metastases are the most prevalent of intracranial malignancies. They are associated with a very poor prognosis and near 100% mortality. This has been the case for decades, largely because we lack effective therapeutics to augment surgery and radiotherapy. Notwithstanding improvements in the precision and efficacy of these life-prolonging treatments, with no reliable options for adjunct systemic therapy, brain recurrences are virtually inevitable. The factors limiting intracranial efficacy of existing agents are both physiological and molecular in nature. For example, heterogeneous permeability, abnormal perfusion and high interstitial pressure oppose the conventional convective delivery of circulating drugs, thus new delivery strategies are needed to achieve uniform drug uptake at therapeutic concentrations. Brain metastases are also highly adapted to their microenvironment, with complex cross-talk between the tumor, the stroma and the neural compartments driving speciation and drug resistance. New strategies must account for resistance mechanisms that are frequently engaged in this milieu, such as HER3 and other receptor tyrosine kinases that become induced and activated in the brain microenvironment. Here, we discuss molecular and physiological factors that contribute to the recalcitrance of these tumors, and review emerging therapeutic strategies, including agents targeting the PI3K axis, immunotherapies, nanomedicines and MRI-guided focused ultrasound for externally controlling drug delivery.

**Keywords:** brain metastases; nanomedicine; immunotherapy; drug delivery

## 1. Introduction

The spread of cancer to the brain is a devastating complication associated with high morbidity and virtually 100% mortality. Brain metastases occur in 9–17% of cancer patients [1,2], most frequently in melanoma, lung and breast cancer [1,2]. They are broadly refractory to existing systemic therapies because of inefficient drug delivery, unique brain growth factors that promote resistance, and because they are usually detected at a late stage, when patients are already symptomatic. Solving these long-standing challenges around diagnosis and treatment requires innovation [3,4]. The risk of brain relapse for cancer patients today is higher than it was several decades ago [5–7]. In addition to population ageing, and improvements in the precision and accessibility of diagnostic imaging, this trend is also thought to be a consequence of contemporary systemic drug therapies that are more effective against extracranial than intracranial cancer deposits. Consistent with this, adjuvant treatment of human epidermal growth factor receptor-2 positive (HER2+) breast cancer with the anti-HER2 monoclonal antibody (mAb) trastuzumab (Herceptin®) increases the likelihood that the brain will be the first site of symptomatic recurrence [8]. The aims of this review are to outline conventional management strategies (Section 2), pathophysiological and molecular factors that underpin the recalcitrance of brain metastases (Section 3) and review new approaches to systemic therapy where compelling preclinical and/or clinical evidence indicates they could play a role in clinical management in the future.

## 2. Current Management

Brain metastases are usually diagnosed by magnetic resonance imaging (MRI) in patients who present with neurological symptoms, such as headache, cognitive deficits or seizures. Depending on the number, sizes and location of lesions, age and general health, management may involve surgical resection, stereotactic or whole brain irradiation, with long-term steroid treatment to limit intracranial edema. Based on the widely-utilized Graded Prognostic Assessment (GPA) prognostic index, the median survival of patients with brain metastases is 7.16 months [9]. However, clinical outcomes vary considerably depending on primary cancer histology, comorbidities and the extent of metastatic disease elsewhere in the body. For example, HER2+ breast cancer patients with isolated brain metastases or synchronous but stable extracranial disease may live 5 years or more with multi-modal treatment, while those with triple-negative breast cancer (TNBC), non-small cell lung carcinoma (NSCLC) and melanoma typically succumb within 12 months, even with aggressive treatment.

### 2.1. Surgery and Radiotherapy

Surgery is most valuable for symptomatic lesions that are accessible, in patients with controlled extracranial disease and good performance status [10]. It has the advantage of relieving symptoms in 60–90% of patients [11–13], achieving local control in 60–100% [12,14], and enabling diagnostic testing for biomolecular markers, which may differ from the primary tumor; for example, expression of therapeutically targetable receptors for estrogen, progesterone and/or epidermal growth factor (ER/PR/HER2) is discordant in 20–50% of breast cancer cases [15,16]. Local recurrence after surgery is a persistent problem, occurring in 5–40% cases [10]. It can be reduced by resection of infiltrating cells beyond the gliotic capsule [12,14] and en bloc resection to avoid local “spillage” of malignant cells [10], but this is not always safe or feasible, particularly in cystic or haemorrhagic metastases [17].

The addition of radiotherapy to surgery increases local control [10,18,19]. Historically, whole brain radiotherapy (WBRT) was used to try and also eliminate distant micrometastases, but randomized control trials showed no difference in survival [10] and there is evidence of delayed decline in cognitive function [20]. Consequently, many institutions currently offer post-operative focused stereotactic irradiation to the surgical resection cavity instead, although this has a higher risk of leptomeningeal or distant intracranial recurrence [18]. Radiotherapy, particularly stereotactic radiosurgery (SRS), may also be used as the primary treatment for local control, and is especially useful for deep, inaccessible lesions in patients with comorbidities. It is more effective for small tumors (<10 mm), and in certain

tumor types, such as breast cancer, compared to melanoma and colorectal metastases [21–23]. When treating multiple tumors concurrently, the total tumor volume (<250 mm<sup>3</sup>) is more important than the number of metastases (up to 12) in achieving disease control [22,23]. Stereotactic irradiation can often be repeated if new lesions arise [22,24]. In spite of this, there are still too many patients whose brain metastases cannot be controlled by surgery or radiotherapy.

## 2.2. Systemic Therapy

In the setting of metastatic cancer with brain involvement, any survival benefit conferred by existing therapeutics is primarily through stabilization of extracranial disease, as opposed to measurable effects on brain metastases. However, exceptions to this generality are continually emerging, particularly molecular-targeted agents where there is diagnostic evidence of drug sensitivity. For example, erlotinib, gefitinib and the third-generation tyrosine kinase inhibitor (TKI) osimertinib are active in patients with EGFR-mutant NSCLC brain metastases [25] (particularly in combination with radiotherapy [26]), and there is also evidence that B-Raf (BRAF) and mitogen-activated protein kinase kinase (MEK) inhibition with dabrafenib plus trametinib treatment is active in BRAF-mutant melanoma brain metastases [27]. The challenge now is to use these agents in structured regimens to further improve patients' longevity and maximize the arsenal of second- and subsequent-line therapies.

One reason for the slow development of effective treatments is an historic paucity of research investigating the molecular vulnerabilities and treatment barriers unique to brain metastases, using appropriate preclinical models and clinical samples [4]. Patients with symptomatic brain disease have been actively excluded from clinical trials because their prognosis is broadly incompatible with trial endpoints. Where they have been included, there was usually minimal or no impact on intracranial progression according to standard Response Evaluation Criteria in Solid Tumors (RECIST), which was widely interpreted to be a consequence of drugs not crossing an intact blood-brain-barrier (see below), and/or the disease being categorically chemoresistant. Both are assumptions we now know to be inaccurate, owing to a substantial increase in research activity over the last decade, and development of specific criteria to standardize the assessment of intracranial responses across trials [28]. This growing body of evidence suggests that to consistently achieve durable responses and good quality-of-life for our patients, we need compounds that target vulnerabilities specific to each cancer, with pharmacodynamic properties that overcome physiological drug delivery barriers at this site.

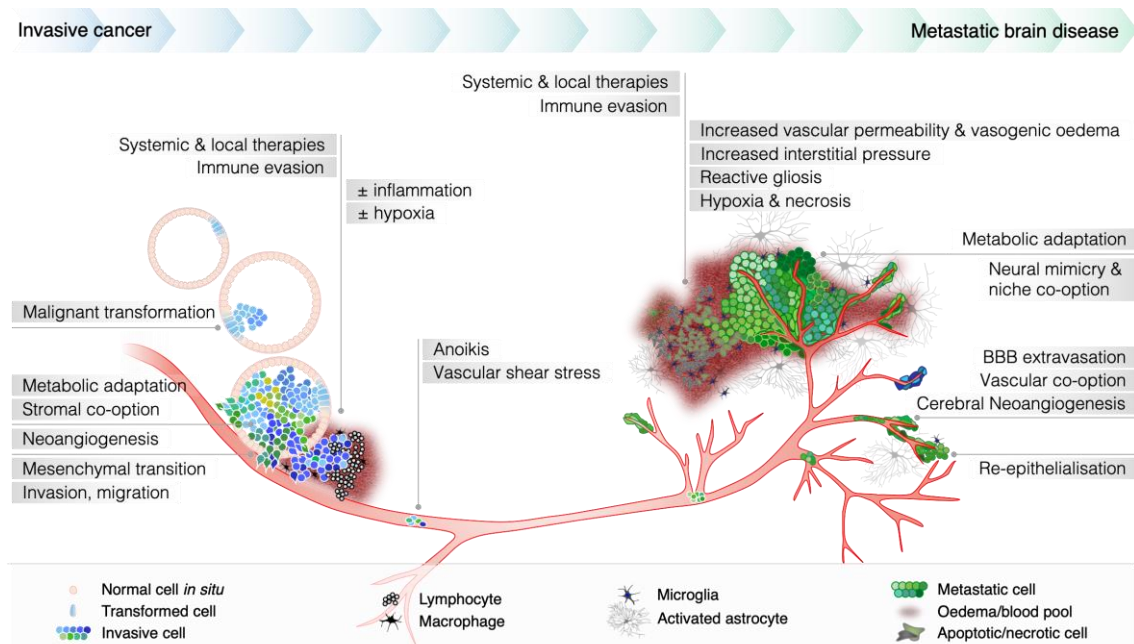
## 3. Why Are Brain Metastases Refractory to Conventional Treatment?

### 3.1. Protection of Micrometastases from Systemic Therapy

The pathophysiology of metastatic brain relapse is complex, impacted by a multitude of extrinsic selection pressures occurring over variable periods of time (Figure 1). The passage of molecules from the blood into the brain is tightly regulated by specialized blood vessels that perfuse the central nervous system (CNS). Tight junctions between vascular endothelia in this organ create a biological seal that effectively limits the diffusion of large molecules across paracellular clefts. Unlike peripheral blood vessels, this endothelium lacks fenestrations and only permits free diffusion of gases and small lipophilic molecules (<500 Da). Astrocyte foot processes further reinforce the endothelial layer via cellular communication and physical support [29,30]. Sandwiched between endothelia and foot-processes, pericytes and a basal lamina envelop the cerebral vasculature, where they regulate barrier function [31–34]. This specialized regulator complex is colloquially referred to as the blood-brain-barrier (BBB).

To colonise the brain, tumor cells must actively cross the BBB and evade local defences; or at least reach equilibrium with the ensuing inflammatory milieu. Evidence suggests they preferentially attach to endothelium that is inflamed and focally discontinuous, followed by enzymatic degradation of tight junctions and trans-endothelial migration [35,36]. Micrometastatic deposits remain dormant in the perivascular space for variable periods of time, protected from therapeutics and peripheral immune

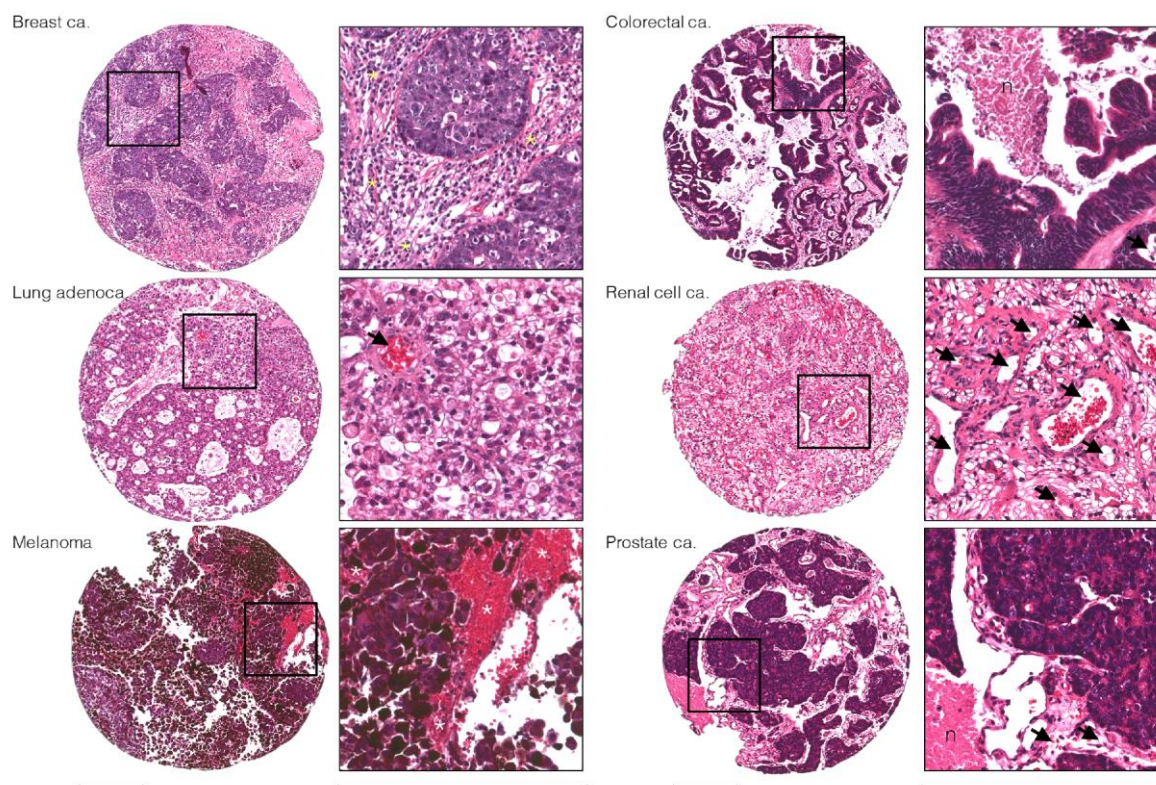
surveillance by the BBB for days, weeks or even years, until new adaptations (genomic, epigenetic and/or transcriptomic changes) tip the balance in favour of outgrowth. Astrocytes and microglia are involved, with tumor cell communication and growth factor co-option facilitating proliferation and immune avoidance [37–40]. Analysis of microenvironmental gene expression differences between brain metastases with low and high vascular permeability revealed that secretion of interleukin (IL)-6 and C-C motif chemokine ligand 2 (CCL2) by reactive (inflammatory) astrocytes contributes to BBB permeability [41]. Brain metastatic lung cancer cells can release IL-8, macrophage inhibitory factor, and plasminogen activator inhibitor-1, stimulating astrocytes to secrete tumor growth factors [42,43]. Tumor cells may also subvert astrocytic attack by producing serpins [44,45].



**Figure 1.** Schematic illustrating pathophysiological aspects of metastasis to the brain. Brain metastasis is an inefficient process marked by high rates of cellular attrition, and impacted by a myriad of selection pressures that drive clonal evolution. This includes requirements intrinsic to the metastatic cascade (capabilities for “metastatic fitness” are indicated in horizontal tracks) as well as extrinsic factors that drive the selection of particular tumor cell clones able to successfully establish brain metastases (vertical tracks; e.g., chemotherapy, radiotherapy or surgery) (adapted from [46]).

### 3.2. Microenvironmental Adaptation and Clonal Selection

Cells that successfully invade the perivascular space and survive seem to migrate along paths of least resistance. Some proliferate in the perivascular pathway, forming sheaths that co-opt the vessels; while others take interstitial tracks and form well-demarcated parenchymal lesions that presumably depend more on neo-angiogenesis for a constant supply of metabolic substrates. The degree to which these mechanisms are used varies according to tumor histology. For example, melanoma brain metastases are highly vascular and tend to grow as haemorrhagic, perivascular proliferations, while carcinomas are more likely to develop in the parenchyma with pushing margins [47–49] (Figure 2).



**Figure 2.** Typical histological appearance of brain metastases from different primary tumor types. Hematoxylin and eosin (H&E)-stained tissue sections show that brain metastases usually exhibit morphology typical of the primary tumor of origin. Features often seen in brain metastasis surgical specimens are highlighted: intact and dilated blood vessels (arrows; high microvascular density in the renal cell carcinoma-brain metastasis shown), hemorrhagic regions typical of melanoma brain metastases (white asterisks), necrotic tissue (“n”) and reactive gliosis around tumor cell nests with pushing margins (yellow asterisks). Scale bars = 250  $\mu\text{m}$ . ca., carcinoma; adenoca. adenocarcinoma.

Tumor cells use remarkable mechanisms to co-opt the neural niche. There is evidence that they can repurpose neurotransmitters as metabolic substrates, recruit and promote the differentiation of neural progenitors into tumor-supporting reactive astrocytes, and mimic neural traits to effectively “plug-and-play” with the new soil. Brain growth factors play critical roles in driving speciation of cancer cells and development of drug resistance, particularly mechanisms that promote phosphoinositide 3-kinase (PI3K) activity [3,50–54]. Molecular profiling of surgical samples has identified frequent activating alterations in *PIK3CA*, amplification and overexpression of upstream growth factor receptors (e.g., HER2, HER3, c-MET [55–58]) and repression of its suppressor, phosphatase and tensin homolog (PTEN), through genomic loss, inactivating mutation or promoter methylation [57,59–61]. Post-transcriptional silencing has also been identified as a mechanism suppressing PTEN in brain metastases from breast cancer, resulting from the delivery of *PTEN*-specific miRNAs to tumor cells by re-educated astrocytes in the tumor microenvironment [62]. These studies and others collectively indicated that inhibiting the PI3K/Akt/mTOR pathway is a promising strategy for treatment.

Comparing patient-matched pairs of primary and metastatic tumor tissue can reveal differences that arise from selective pressure imposed by treatment and/or the microenvironment [57,60,63,64]. Brastianos and colleagues sequenced the exomes of 86 trios for deep analysis of clonal selection: primary breast tumors, metastases (mostly brain, including a handful of cases with sequential brain tumors) and blood as a source of germline DNA [60]. The majority of cases exhibited branching evolution, with a “trunk” of shared changes and a series of “private” mutations reflecting continual

independent evolution. Alterations predicting sensitivity to PI3K/AKT/mTOR, cyclin-dependent kinases (CDKs) and HER2/EGFR inhibitors were identified; and importantly, in 53% of patients, clinically informative mutations were not detected in the primary tumor tissue sample. While extracranial metastases may be more accessible for biopsy than brain metastases, they were found to be highly divergent in this study. These and related studies indicate that ideally, management decisions should be based on direct diagnostic profiling wherever possible, rather than information inferred from analysis of the primary tumor or other metastases. Although it is recommended that metastatic breast cancer treatment be based on the biomarker profiles of available metastases [65], brain tumor biopsy is often challenging or not possible. There is a clear unmet need for less invasive modalities to measure biomarker expression in metastatic brain lesions.

Treatment can also be a key driver of extrinsically driven clonal evolution in brain metastases. For example, radiotherapy is a critical component of clinical management that on balance, is effective in slowing relapse and improving quality-of-life via debulking. However, there is also evidence that it promotes progression in hypoxic tumor niches [66], where an accumulation of hypoxia-inducible factor-1 alpha (HIF1- $\alpha$ ) has likely already primed cells for survival and tissue regeneration. A local deficiency of molecular oxygen can limit the extent of DNA damage caused by ionizing radiation, expediting clonal evolution rather than the intended effect of catastrophic genome instability and apoptosis. Paradoxically, hypoxia also characterizes regions of post-treatment radiation necrosis [67], and thus overall, is a potent barrier to effective radiotherapy in brain and other solid tumors [68–70]. Preclinical studies have implicated c-Met signaling in hypoxia- and radiotherapy-associated protection from apoptosis [71,72]. Supporting this, two case studies recently reported slower disease progression in brain metastasis patients treated with c-Met inhibitors, crizotinib or cabozantinib [73,74].

### 3.3. Abnormal Perfusion Dynamics and High Tumor Interstitial Pressure

In established, symptomatic lesions, an abnormal capillary network that evolves in parallel with the tumor takes over from the BBB as the chief opponent of drug delivery. Brain metastases are characterized by perpetual, spatially overlapping cycles of proliferation, localized hypoxia and angiogenesis. The tips of sprouting vessels are intrinsically “leaky” because the immature BBB is devoid of tight junctions [3,75], and continual vascular remodelling produces a tortuous, dysfunctional vascular bed with heterogeneous, but overall elevated permeability. The crucial, detrimental consequence is that this abolishes the pressure gradient between the vascular compartment and surrounding brain tissue, resulting in edema and locally elevated interstitial fluid pressure that physically oppose penetration of circulating compounds into the tumor. Moreover, blood flow becomes largely static, exacerbating hypoxia and poor drug accumulation in an environment that is, paradoxically, highly vascularized [76–79]. Particular to brain metastases is that this occurs in the closed cranial cavity, which has little biophysical capacity to diffuse additional fluid pressure. Therefore, established, symptomatic brain metastases present unique barriers to simple convective uptake and retention of circulating drugs [80].

### 3.4. Late Clinical Detection

A critical outstanding question is whether earlier diagnosis and treatment of brain metastases would materially improve clinical outcomes. Currently, brain imaging is not routinely performed after treatment for primary cancer unless there is a specific indication or new symptoms warranting investigation. The decision to not perform ongoing surveillance for metastatic disease is largely economic, but also clinical, as conventional MRI can detect abnormalities of unknown significance, prompting repeat scanning or biopsy for a definitive diagnosis. This presents the potential to cause physical harm, and also psychological harm due to an uncertain diagnosis, or a diagnosis with no actionable treatment plan. Even if surveillance was routinely conducted in high-risk patients, by the time brain metastases are detected or symptomatic they are already resistant to multiple lines

of therapy and are equipped for efficient adaptation to new selection pressures. So long as they are identified at this late stage, treatment benefits will continue to be incremental [3].

If detection and treatment of small, asymptomatic lesions is to be considered, we also need diagnostic tools with precision and specificity that exceed the standard achievable with diagnostic MRI. With significant research efforts being directed towards the application of machine learning methods in image analysis [81,82], it is becoming possible to correlate more subtle image parameters with the development of brain metastases [83,84]. However, simultaneous acquisition of positron-emission tomography (PET)-based tumor biomarker information will likely be required to achieve the specificity and sensitivity required for clinical implementation as a surveillance modality.

#### 4. Innovative Strategies to Overcome Treatment Barriers Unique to Brain Metastases

Considering the combination of pathophysiologic and clinical factors limiting the delivery and efficacy of systemic therapy in brain metastases, it is clear that development of more effective strategies will require significant investment in innovative research that crosses disciplinary boundaries. This section will review emerging approaches that may be able to overcome some of the current limitations, and/or have strong clinical momentum in this setting (Figure 3).

Class	Targets	Examples
Molecular-targeted agents	Phosphoinositide 3-kinase (PI3K)	alpelisib, buparlisib, dactolisib
	Human epidermal growth factor receptor 3 (HER3)	patritumab (AMG-888), lumretuzumab (RG7116), seribantumab (MM121), elgentumab (LJM716)
	Vascular endothelial growth factor receptor (VEGFR)	bevacizumab, cabozantinib*, axitinib*
	Poly(ADP-Ribose) Polymerase (PARP)	veliparib
Drug transporters & pumps	low-density lipoprotein-related protein 1 receptor (LRP1)	ANG1005
	P-glycoprotein (P-gp)	elacridar, tariquidar, zosuquidar
Immune checkpoint inhibitors	programmed cell death-1 (PD-1)	pembrolizumab, nivolumab
	cytotoxic T-lymphocyte-associated protein 4 (CTLA-4)	ipilimumab
Myeloid-derived suppressor cell (MDSC)-targeted therapy	Vascular endothelial growth factor receptor (VEGFR)	bevacizumab, cabozantinib*, axitinib*
Vaccines	Tumour-specific antigens	irradiated self-tumour cells; autologous, antigen-pulsed dendritic cells; oncolytic viruses
Nanomedicines	Encapsulated cytotoxics	Doxil®, Abraxane®, Marqibo®
	Tumour-specific biomarkers	Not yet available
MRI-guided, focused ultrasound	Endothelial tight junctions	Not yet available

\* Additional receptor tyrosine targets apart from VEGFR

**Figure 3.** Summary of the approaches discussed in Section 4, including therapeutic classes, their molecular targets and examples of experimental or clinically approved agents.

##### 4.1. New Molecular-Targeted Agents

The PI3K/Akt/mTOR signaling pathway is a potent mediator of cell survival and drug resistance, and a key driver of brain metastasis [62,85–87], with more than 50% of breast and lung cancer brain metastases harboring *PIK3CA* mutations or genomic amplification [57,60]. PI3K inhibitors including alpelisib, buparlisib and dactolisib are currently being evaluated in the setting of advanced cancer where there is diagnostic evidence of mutation. In the SOLAR-1 registrational trial, alpelisib reduced the risk of progression or death by 35% in patients with ER+, HER2-negative, *PIK3CA*-mutant advanced breast cancer [88], but assessment of CNS progression is currently not planned. On the

other hand, buparlisib is brain-penetrant, inhibits intracranial outgrowth in preclinical models, was well-tolerated in phase-I studies and reduced metastatic brain tumor volume by 28% in one patient [89–91]. It has also been evaluated in ER+, HER2-negative advanced breast cancer, and in both trials increased progression-free survival, but produced significant toxicity [92,93]. This side-effect profile could be problematic in patients with brain involvement, and thus a carefully designed phase-II trial is required to assess overall clinical benefit and identify additional predictive markers to help further refine the treatment population.

Kodack and colleagues recently demonstrated that HER3 induction was an effective escape mechanism for experimental brain metastases treated with buparlisib, but that sensitivity could be restored by simultaneously treating mice with a HER3-targeted mAb [87]. Since HER3 induction and activation are common in brain metastases from a multitude of cancers [55,57,58], combination strategies with HER3 inhibition may be more effective than PI3K monotherapy. The central role of HER3 in chemotherapeutic resistance has led to development of HER3-targeted monoclonal antibodies (mAbs), which are being clinically evaluated in various solid cancers [94,95]; for example, patritumab (AMG-888; Daiichi-Sankyo), lumretuzumab (RG7116; Roche), seribantumab (MM121; Merrimack), elgemtumab (LJM716; Novartis). HER3 is induced in brain metastases compared to matching breast and lung primary tumors, is frequently phosphorylated in brain metastases with various histologies, and in primary breast cancers, its overexpression increases the likelihood that the brain will be the first site of relapse [55,58,96,97]. Activation of PI3K/MAPK signaling through HER3 could also explain resistance to other targeted agents in intracranial versus extracranial metastases [98,99]. Therefore, there is accumulating evidence in favor of trialing anti-HER3 therapy in a variety of contexts for cancer patients with brain involvement.

Targeting vascular endothelial growth factor (VEGF) activity has emerged as another promising strategy that acts largely by suppressing neo-vascularization and normalizing perfusion in hyperpermeable tumors. The VEGF-receptor inhibitor (VEGFRi), bevacizumab (Avastin<sup>®</sup>), has limited activity as a single agent, but improves delivery and potentiates the effects of others [77,100]. At least 22 phase-II clinical trials are ongoing to test the clinical benefit of combining bevacizumab with other agents for patients with brain metastases [101]. The VEGFR2i, cabozantinib, is also being evaluated in combination with trastuzumab in HER2+ breast cancer patients with brain metastases (NCT02260531) on the basis of its broader range of tyrosine kinase receptor targets, including c-MET, RET and AXL. In addition to directly improving oxygenation and drug delivery in solid tumors, VEGFR inhibition may also enhance anti-tumor immunity, as VEGF has a multitude of immunosuppressive effects, including direct action on immune cells ([102], see Section 4.3).

Poly(ADP-Ribose) Polymerase inhibitors (PARPi) represent a new class of agents that exploit dsDNA break repair defects and addiction to alternative, PARP-mediated DNA repair in cancer cells. This genetic theory of “synthetic lethality” has been validated in breast cancer patients with germline loss-of-function mutations in BRCA1 or BRCA2. Research identifying ways to maximize clinical effectiveness is still underway, with potential approaches being to give PARPi as chemo- or radio-sensitizing agents. Genome instability and BRCA1 dysfunction characterize some breast cancer brain metastases, and may predict benefit from PARPi [103,104]. The PARPi veliparib was investigated in combination with WBRT in brain metastases from solid tumors. Results from the phase-1 dose-escalation study were encouraging, but the second-phase randomized trial comparing WBRT with and without veliparib in NSCLC brain metastases revealed no significant differences in disease progression between treatment groups [105,106]. It is currently being investigated in combination with cisplatin in TNBC or BRCA-associated breast cancer with brain metastases (NCT02595905). A phase 2 trial of iniparib with irinotecan to treat patient brain metastatic TNBC reported modest survival benefit [107]. It is not clear whether this outcome is attributable to PARP inhibition directly, and/or an increase in reactive oxygen species [108].

#### 4.2. Drug-BBB Transporter Conjugates

The selective passage of nutrients and metabolites essential for neuronal function from the bloodstream to the brain parenchyma is regulated by transporter proteins that are differentially expressed on the luminal and abluminal membranes of brain endothelia. In an attempt to increase drug delivery to the CNS, innovative therapeutic strategies are being developed that exploit three major BBB transcytosis pathways:

1. Adsorptive transcytosis occurs when cationic molecules bind to negatively-charged clathrin-coated or caveolar vesicles on brain endothelial cells. Drug delivery strategies harnessing this pathway involve direct chemical modifications; such as cationization of therapeutic molecules, which promotes adhesion to the anionic cell membrane [109]. However, since anionic sites are found in all living cells, the risk of off-target drug toxicity is significant [110].
2. Carrier-mediated transporters specifically translocate small molecule nutrients such as glucose, amino acids, amines, nucleosides and small peptides. Drug targeting using carrier-mediated transport involves conjugation to its endogenous substrate or mimic ligand. Glucose transporters are highly expressed by the BBB, are well-characterized and have evolved to meet the metabolic demands of the brain, making them good drug delivery vehicle candidates. One study reported that the density of ligands decorating the conjugate correlated with blood-brain transport efficiency [111]. Liposomes coated with a high density of the glucose derivative glycosyl achieved close to 3-fold higher brain uptake than unconjugated liposomes or liposomes with low glycosyl density [111]. Supporting this, glucose-coated paclitaxel nanoparticles were effective in a mouse model of glioma [112].
3. Receptor-mediated drug transport strategies rely on molecular ligand mimicry to induce endocytosis and transport to the abluminal membrane opposing the brain parenchyma. Referred as the “trojan horse”, this approach has garnered more attention in the field than the former, because of the potential for ferrying larger cargoes conjugated to molecules like transferrin and insulin. These conjugates have been developed and successfully applied in neurological conditions; for example, the large neutral amino acid transporter (LAT1) has been exploited to transport gabapentin in epilepsy [113], L-DOPA in Parkinson’s disease [114] and baclofen for patients with cerebral palsy [115]. Another example is a bispecific antibody against the transferrin receptor (TfR) and  $\beta$ -secretase, an Alzheimer’s disease drug target developed by Genentech in 2011 [116]. Interestingly, high-affinity monoclonal antibody (mAb) resulted either in the conjugate being sorted to lysosomal degradation or remaining trapped to the receptor after abluminal trafficking, resulting in TfR deficiency in the brain. On the other hand, a lower affinity mAb transcytosed and successfully dissociated from TfR, resulting in more drug uptake in the brain parenchyma and no associated neurotoxicity [116,117].

BBB transporter-taxol conjugates are also beginning to show potential for treating brain tumors. ANG1005 comprises paclitaxel linked to Angiopep-2, a peptide ligand for low-density lipoprotein-related protein 1 receptor, which is highly expressed on BBB endothelia. Preclinical studies showed that ANG1005 significantly prolonged the survival of mice after intracerebral injection with NSCLC cells [118]. Further, a mouse brain perfusion study indicated that ANG1005 also bypassed p-glycoprotein-mediated efflux, leading to greater uptake compared to unconjugated paclitaxel [118]. Clinically, it produced survival benefit in patients with both primary and secondary brain cancers [119–122], was granted orphan drug status by the U.S. Food and Drug Administration (FDA) for treating the aggressive primary brain tumor glioblastoma (GBM) in 2014.

Preclinical and clinical studies continue to be invaluable for developing a better understanding of the caveats of receptor-mediated drug transport, highlighting several key pharmacodynamic considerations, including competition with endogenous substrates, ligand-binding affinity, effect on normal receptor trafficking and interaction with xenobiotic efflux pumps like P-glycoprotein (P-gp; encoded by ATP-binding cassette sub-family B member 1 (ABCB1)) and Breast Cancer Resistance

Protein (BCRP; ABCG2). Preclinical studies with the P-gp inhibitors elacridar, tariquidar and zosuquidar hinted at the possibility that inhibiting efflux could improve the efficacy of other treatments in the brain [123–125]. However, the pharmacokinetic profile of P-gp inhibitors presents significant challenges to clinical translation, related to non-specific interaction with cytochrome P450 enzymes, competition for target binding with other therapeutics and the ubiquitous expression of efflux pumps throughout the body. Several clinical studies reported increased toxicity in patients treated with P-gp inhibitors [126–129], and so far, improvement in clinical outcomes of patients with solid tumors have been mediocre [128,130,131].

### 4.3. Immunotherapy

The brain is an immune-specialized site with unique defenses. It is protected by an independent innate immune system comprising glia, ependymal cells and neurons, all capable of antigen presentation [132–134]. Brain-resident macrophages (microglia) monitor the microenvironment and react to non-self-antigens by adopting an inflammatory phenotype, involving increased motility, phagocytosis and proliferation. Reactive astrocytes also participate by expressing inflammatory mediators and exhibiting phagocytic activity [135–137]. Though devoid of typical lymphoid tissue, the brain has specialized lymphatic vessels that drain antigens and antigen-presenting cells (APCs) from the dural sinuses to cervical lymph node chains [138], where they are capable of inducing responses that involve infiltration of the brain parenchyma by peripheral effector cells via the BBB, choroid plexus epithelium and glia limitans [139–142].

#### 4.3.1. Immune Checkpoint Inhibitors

T-cells become reactive when the T-cell receptor binds an antigen presented by APCs via the major histocompatibility complex (MHC), with appropriate “danger”-induced co-stimulatory molecules. If a T-cell simultaneously binds a co-inhibitory ligand (“negative checkpoint”), this impairs the T-cell response. Inhibitory checkpoint activation is a physiological negative feedback mechanism to avoid excessive reactivity and maintain self-tolerance. It is strongly promoted by chronic exposure of activated effector cells to tumor antigens and the immunosuppressive microenvironments of advanced cancers, and is considered to be a key driver of immune evasion during progression. Targeting checkpoints that constrain anti-tumor immune responses has emerged as a revolutionary approach allowing long-term survival of a consistent proportion of patients with disseminated cancer. Great efforts are being made to develop co-stimulatory receptor agonists (e.g., OX40, 4-1BB) and inhibitory receptor antagonists (e.g., CTLA-4, PD-1/PD-L1, VISTA, LAG-3, TIM-3) to restore and/or reinvigorate anti-tumor T-cell responses. In the clinical setting, immune checkpoint inhibitors (ICIs) can trigger clinically relevant anti-tumor responses not only in classically immunogenic tumor types like melanoma, but also other solid tumors.

New findings have revealed that ICIs can also produce durable effects against brain metastases—the first truly optimistic results for this patient population in decades [143–147]. For example, intracranial response rates of 20–30% were observed in melanoma and NSCLC patients treated with pembrolizumab (anti-programmed cell death-1 (PD-1)), and nivolumab plus ipilimumab (anti-PD1 and -cytotoxic T-lymphocyte-associated protein 4 (CTLA-4)) produced objective CNS responses in 55% of melanoma patients [148,149]. Interestingly, preclinical studies suggest that ICIs are most effective against brain metastases when there is concurrent extracranial disease. Intracranial responses were attributed to a substantial increase in CD8+ T-cell trafficking into the brain after expansion of homing-competent T-cells in the periphery, potentiated by induction of T-cell entry receptors on brain microvasculature [150]. The field eagerly awaits results from trials investigating the benefit of combining ICIs with chemo- and radiotherapy, which enhance the production of neoantigens that can subsequently stimulate anti-tumor immunity. The initial results available seem promising, as shown by a recent report in metastatic melanoma, where concurrent SRS and ICI therapy improved intracranial control and survival compared with historical controls patients [151].

#### 4.3.2. Myeloid-Derived Suppressor Cell (MDSC)-Targeted Therapy

In cancer and other chronic inflammatory conditions, the differentiation of immature myeloid cells becomes biased toward expansion of myeloid-derived suppressor cells (MDSCs) at the expense of the monocyte and neutrophil populations [152]. MDSCs promote cancer progression in multiple ways, including active suppression of T lymphocytes and Natural Killer cells, and as such they are a key target of interest in oncology. A reduction in absolute numbers and function of MDSCs correlate with the efficacy of VEGF-targeted therapy, and recent findings have implicated this mechanism in intracranial activity of VEGFRi. For example, bevacizumab significantly delayed intracranial progression amongst stage-IV EGFR-mutant lung cancer patients, associated with a higher ratio of effector T-cells to peripheral MDSCs, and pre-treatment MDSC levels correlated with progression-free survival in this trial [153]. Likewise, axitinib prolonged survival of experimental mice bearing melanoma-brain xenografts, and this was associated with MDSC differentiation toward an antigen-presenting phenotype with reduced suppressor activity [154]. More clinical studies are warranted to investigate the optimal contexts for MDSC-targeted approaches in brain metastatic cancer. Notably, there are currently no clinically approved agents that directly target this cell population.

#### 4.3.3. Cancer Vaccines

A vaccine is a therapy targeted at acquiring long-term immunity. Expressed, somatically mutated genes sometimes encode neoantigens that are potentially immunogenic and therapeutically targetable, but clinical implementation of an optimized process for identifying, validating and developing such precision vaccines remains complex. Contrary to early expectations, overexpressed self-antigens, particularly those with direct roles in tumorigenesis, can be highly immunogenic, and in fact comprise the largest class of candidate targets for vaccine development [155]. In contrast to neoantigen vaccination strategies, this class is applicable to larger patient groups and is amenable to development of an “off-the-shelf” suite of cancer vaccines that could be combined in personalized, polyvalent cancer vaccines. PERCELLVAC3 (NCT02808416) is an ongoing phase I/II trial evaluating the safety and efficacy of personalized cellular vaccines in patients with brain metastases. Patients are immunized with autologous, irradiated tumor cells, or APCs that have been pulsed with tumor antigen-encoding RNA identified by profiling tumor material after surgery.

Oncolytic viruses that selectively infect tumor cells are emerging as a novel class of cancer immuno-therapeutics that may be considered a particular form of vaccination against cancer. In fact, oncolytic viruses induce immune responses not only against viral antigens but also against tumor antigens and can thereby also target non-infected metastases. To overcome the hurdles of systemic delivery, it has been proposed to arm mesenchymal stem cells with oncolytic viruses. In a preclinical model, injection of these cells in combination with PD-L1 blockade increased interferon gamma (IFN $\gamma$ )-producing CD8<sup>+</sup> tumor-infiltrating T lymphocytes and significantly prolonged survival [156].

#### 4.3.4. Clinical Considerations for Treating Brain Metastases with Immune-Modulating Therapies

Used to limit peritumoral edema and intracranial pressure, dexamethasone is a core component of management for brain metastasis patients. But corticosteroids like dexamethasone are also potent immunosuppressants that counteract the effects of immunotherapy. In contrast to asymptomatic melanoma patients with brain metastases, ipilimumab conferred no survival benefit to patients being managed with corticosteroids [145]. Furthermore, other melanoma and lung cancer trials also reported ICIs were more effective when prednisone or dexamethasone were not required for symptom control [157,158]. Ideally, ICIs should be given before corticosteroids, which would require diagnosis before the onset of neurological symptoms, and so the promise of immunotherapy poses a new rationale for earlier detection of smaller lesions through surveillance of high-risk patients. In general, the main limitation of ICIs is the possibility of T-cell activation causing an adverse immune reaction, but there are additional risks associated with therapeutically stimulating immune responses within

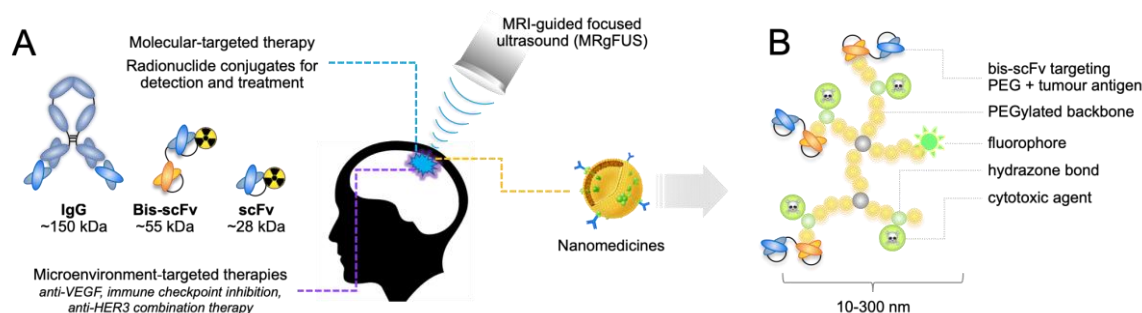
the cranial cavity, where sharp increases in pressure from cytokine storming and edema could be life-threatening. However, the incidence of grade-3/4 adverse neurological events in early GBM and brain metastatic melanoma trials was not particularly frequent (~7% of cases); considered to be acceptable in these groups that already have a very poor prognosis.

#### 4.4. Nanomedicines

Studies from the 1970s–90s reported intracranial responses in up to 59% of metastatic breast cancer patients treated with cytotoxic chemotherapy, including cyclophosphamide, doxorubicin, vincristine, methotrexate and 5-fluorouracil [159,160]. Mechanistically, this makes sense as brain metastases exhibit rapid proliferation and genomic instability—hallmarks of chemosensitivity [161]. In current practice, chemotherapy is usually prescribed secondary to local treatment, and/or with the aim of stabilizing extracranial disease, with poor CNS penetration and drug resistance often cited as the rationale. However, in early trials, intracranial effects paralleled systemic responses [159,160], consistent with the notion that the sensitivity of the underlying tumor histology to a particular therapy is more important than its apparent CNS penetrance [162]. It has also been challenging to exploit such intrinsic vulnerabilities in this patient population, where the comorbidity burden is high and the therapeutic indices of most cytotoxics are already narrow.

The emergent nanomedicine field offers possibilities to circumvent toxicity through drug design. Nanomedicine is the application of nanotechnology in medicine to diagnose, prevent and treat disease. Integrating bioengineering with immuno/oncology and nuclear medicine is an exciting prospect for cancer management; particularly brain tumors, which present unique drug delivery challenges and where there is great opportunity to positively impact patient outcomes [4]. Key design features include (Figure 4):

1. Shell material to enhance solubility and delay clearance by minimizing immune recognition. For example, polyethylene glycol (PEG) minimizes recognition by mononuclear phagocytes. PEGylation also reportedly increased distribution of cytotoxic drugs in the brain and provided resistance to enzymatic decay [163–165].
2. Molecular targeting functionality, generally via conjugated mAbs or mAb fragments. This is achieved using a number of approaches, including engineered proteins with dual specificity to tether the shell material to tumor antigens [166].



**Figure 4.** Emerging strategies for detection and treatment of brain metastases. (A) Bioengineering and molecular oncology research are beginning to identify approaches that specifically target vulnerabilities of brain metastases, including microenvironmental adaptations. Through flexibility of design, nanomedicine approaches offer exciting possibilities for overcoming drug delivery and therapeutic index challenges in the metastatic brain cancer patient population. Externally-augmented drug release and/or activation may be a useful therapeutic tool in some circumstances. (B) Typical structure of a polymer-based nanocarrier with various functional groups. bis-scFv, bispecific single-chain variable fragment; IgG, immunoglobulin; kDa, kilodalton; nm, nanometer.

Using this technology, therapeutics with proven efficacy in particular settings can be redesigned to mitigate physicochemical, biological and physiological barriers, targeting payloads with anatomic and molecular precision [167–170]. This can improve pharmaco-kinetic and -dynamic profiles, increase drug accumulation and specificity, and reduce systemic toxicity. At least 50 nanocarriers have been clinically approved (e.g., Doxil<sup>®</sup>, Abraxane<sup>®</sup>, Marqibo<sup>®</sup>), with another 80 or so being tested in ongoing trials [171]. Most are relatively simple conjugates, but translational research efforts are now focusing on parallel diagnostic and therapeutic applications for bespoke carriers. With further development, patients at high risk of brain relapse could be administered with tumor-targeted carriers loaded with a PET isotope, then if disease is detected in the brain using diagnostic PET-MRI, administered with equivalent carriers carrying a therapeutic payload. This approach could also allow personalized, early treatment without the need for a tissue biopsy. The tunability, synthetic flexibility, and longer circulatory half-lives of nanomedicines offer significant potential in precision medicine, including the treatment or prevention of recurrent brain tumors.

#### 4.5. MRI-Guided, Focused Ultrasound

The BBB presents a significant challenge for delivering systemic agents to brain micrometastases and regions of established tumors where it is still intact. Remodelling the tumor vasculature using MRI-guided, focused ultrasound (MRgFUS) is showing promise for brain tumor management [172,173]. Gas-filled, lipid microspheres (microbubbles, MB) have been used in echocardiography for many years but more recently it has been shown that in combination with MRgFUS, they disrupt tumor vasculature and increase drug delivery [174]. Due to the mismatch in acoustic impedance between their gaseous cores and surrounding tissue, MBs resonate when excited by ultrasound. Under low acoustic pressure, MBs undergo repeated expansion and contraction, (cavitation) which generates flow around their surfaces (microstreaming), causes shear stress to cell membranes in the vicinity and disruption of endothelial tight junctions [175,176]. This transiently increases vascular permeability and the uptake of circulating compounds, including engineered nanomaterials [174].

Arvantis and colleagues found that MRgFUS increased the uptake of trastuzumab, the mAb-cytotoxic conjugate, ado-trastuzumab emtansine (T-DM1) and doxorubicin in experimental brain metastases by 2–7-fold [177]. Pharmacokinetic modelling indicated a virtually linear increase in transvascular flux through BBB pores up to 50 nm in diameter, theoretically permissive to small nanoparticles. Preclinical GBM modelling has shown 2-fold higher uptake of paclitaxel-loaded liposomes of 120 nm diameter after administering MBs with FUS [178], though generally, smaller particles are likely to be most effective due not only to BBB pore size limitations, but also susceptibility to interstitial hypertension during the post-extravasation phase, where movement through the extracellular matrix is largely via passive diffusion [179–181]. Although still an investigational procedure, a recent study in patients with malignant glioma suggests that MRgFUS is safe, feasible, and can increase the concentration of systemically delivered chemotherapy by temporarily disrupting the BBB [182].

## 5. Concluding Remarks

This review illustrates the importance of pharmacodynamic and pathophysiological context to the systemic treatment of metastatic brain disease. Historically, interpretation of clinical trials failed to adequately consider the pathophysiological context of each treatment, and there was a disproportionate focus on the BBB as an immutable obstacle to effective systemic therapy. However, it has become clear that the barriers to successful treatment are different in early and later stages of brain metastasis development [46], and it is crucial that future designs establish the disease entity being targeted to ensure success. From a biological point-of-view, the goals of immuno-/oncologic therapy could be broadly divided as follows:

1. Specifically target subclinical deposits with an intact BBB:

- to prevent outgrowth in patients at high risk of brain relapse who are undergoing first-line treatment. This has the greatest potential to impact clinical outcomes, but requires accurate risk prediction protocols;
  - as an adjunct to local treatment to limit post-treatment recurrence, or new recurrences that arise from “self-seeding”.
2. Specifically target established, symptomatic tumors that are not suitable for surgery or radiotherapy using innovative drug conjugates and combination approaches to overcome physiologic barriers to the simple convective delivery.

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