doi:10.1093/brain/awab012 BRAIN 2021: 144; 1046–1066 | 1046

BRAIN
A JOURNAL OF NEUROLOGY

REVIEW ARTICLE

Emerging principles of brain immunology and immune checkpoint blockade in brain metastases

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Brain metastases are the most common type of brain tumours, harbouring an immune microenvironment that can in principle be targeted via immunotherapy. Elucidating some of the immunological intricacies of brain metastases has opened a therapeutic window to explore the potential of immune checkpoint inhibitors in this globally lethal disease. Multiple lines of evidence suggest that tumour cells hijack the immune regulatory mechanisms in the brain for the benefit of their own survival and progression. Nonetheless, the role of the immune checkpoint in the complex interplays between cancers cells and T cells and in conferring resistance to therapy remains under investigation. Meanwhile, early phase trials with immune checkpoint inhibitors have reported clinical benefit in patients with brain metastases from melanoma and non-small cell lung cancer. In this review, we explore the workings of the immune system in the brain, the immunology of brain metastases, and the current status of immune checkpoint inhibitors in the treatment of brain metastases.

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Keywords: Brain metastasis; immunotherapy; neuro-oncology; PD-1; PD-L1

Abbreviations: MHC = major histocompatibility complex; NSCLC = non-small cell lung cancer; PD-1 = programmed cell death protein 1; PD-L1 = programmed cell death 1-ligand 1; SCLC = small cell lung cancer; TAMs = tumour-associated myeloid cells and microglia

Introduction

Brain metastases are known to possess extraordinary intratumoural heterogeneity and special features that allow them to impede immunotherapy. Brain immunology, specifically, is governed by a set of doctrines that are different from the immune system elsewhere.[1] The blood–brain barrier plays an important role in dictating the entry of immune agents into the brain, leading to an immunosuppressive environment within the brain parenchyma.[2] As such, metastatic tumours are offered a safe haven in the brain, which makes them a major threat and an important target for therapeutic advancement.

In general, the brain boasts an immunosuppressive environment that does not permit the trafficking of immune cells.

Received May 25, 2020. Revised November 02, 2020. Accepted November 04, 2020. Advance access publication April 24, 2021

Discoveries in the past decade have shown that a special lymphatic system exists to drain from the dura mater of the brain to the peripheral lymph nodes.[3-7] It was further emphasized that endothelial, epithelial and glial brain barriers possess varied accessibility to different immune-cell subtypes.[8] New evidence continues to reveal a role for immune cells in the tumour microenvironment.[1] These results have debunked the widely held belief that the brain is an immune-privileged organ, which is free from immune activity. In fact, antigens derived from the brain are capable of inducing an immune reaction in cervical lymph nodes.[6,7,9,10] In addition, this heterogeneous blood–tumour barrier permeability in the setting of brain metastasis can facilitate the infiltration of multiple immune cells from the peripheral circulation.[11,12]

As our acumen on the immunology of brain metastases continues to grow, we learn that the CNS is home to a variety of antigen-presenting cells (APCs) such as microglia, dendritic cells and macrophages, as well as astrocytes.[8,13] T cells are now known to roam freely in the brain. Yet, this does not take away from the uniqueness of the brain immune system, as it continues to be more limited than the immune system in peripheral organs.

Brain metastatic cells are famous for their ability to manipulate immune responses.[14] While this ability might be helpful in limiting destructive inflammation, it can limit the access of T cells among others. This helps brain metastases escape antitumour immune responses,[15,16] promoting metastatic survival and chemoresistance. The large populations of microglia and monocytes in the brain further contribute to decreasing cell-mediated immunity,[17-19] leaving metastatic progression unchecked.

Reports in the literature continue to emphasize that the factors that contribute to immunosuppression at the primary tumour site are similar to those that contribute to it in the brain, highlighting the role of regulatory T cells and the expression of immune checkpoint programmed cell death 1–ligand 1 (PD-L1),[20,21] which is associated with T cell futility.[16,22] Nevertheless, recent trials with immune checkpoint inhibitors have shown survival and antitumour benefit in patients with melanoma brain metastases.[23]

With the brain as a common site of residence for metastatic cancerous and noncancerous cells, interactions between these cellular components seem inevitable. In this review, we explore the intricacies of the immune microenvironment of brain metastases and the efficacy of immune checkpoint inhibitors in the treatment of secondary tumours in the brain.

The immunology of brain metastases

Brain metastases exhibit one of the most diverse immune cell landscapes with substantial infiltration of T cells and neutrophils. In addition, a variety of immune agents, namely microglia and macrophages, lymphocytes and astrocytes complete the immune microenvironment of metastatic tumour cells.

Tumour-associated myeloid cells and microglia

Cells of myeloid origin are characterized by high plasticity and the ability to assimilate signals from cytokines, chemokines and growth factors. Their activation can promote cellular invasion, angiogenesis, metastasis and immune suppression.[24,25] It has been estimated that cells of myeloid origin comprise up to 32.7% of intratumoural cells in brain metastases.[26] Nevertheless, recent studies show that while tumour-associated myeloid cells and microglia (TAMs) can be significantly increased in gliomas compared with non-tumour tissue, lymphocytes are more prevalent in brain metastases.[27]

Myeloid cells arise from two distinct sources, including the periphery (bone marrow-derived macrophages; CD49D+) or the yolk sac (microglia; CD49D–).[28-30] They tend to accumulate in settings of higher brain tumour grade and engage in significant bidirectional crosstalk with the tumour cells.[31-33]

Microglia are a central component of the brain immune microenvironment.[34] Preclinical observations proposed a role for microglia in the extravasation stage of the metastatic process.[35] In the case of inflammation, myeloid cells are recruited from circulating monocytes.[36] The immune role of microglia involves the presentation of antigens, cytotoxic activity via nitric oxide and superoxide, and phagocytic capacity.[37] In brain metastatic settings, microglia show restricted upregulation of interleukin (IL)-6 that exerts immunosuppressive effects on T cells and mediates resistance to immune checkpoint blockade.[38] In addition, microglia limit the expression of TREM1 receptors, which modulate pro-inflammatory responses during neuroinflammation.[39,40] Microglia-released chemokines, such as C-X-C motif chemokine 5 (CXCL5) and CXCL8, are increased in the setting of brain metastases; these chemokines recruit immunosuppressed neutrophils into the metastatic niche.[27]

Microglia express a variety of proteins that play conflicting roles in immune induction and suppression. The expression of high mobility group box 1 protein (HMGB1) facilitates antigen presentation and activation of the adaptive immune system. However, TAMs can also express immunosuppressive proteins like PD-L1,[41,42] usually triggered by inflammation and/or necrosis.[43] These biological properties allow TAMs to regulate and preserve the immune balance, preventing any swelling and, ultimately, damage within the confined area of the skull. Nevertheless, metastatic tumour cells hijack the immunosuppressive ability of TAMs to evade the immune system and promote their own survival.[44] TAMs also secrete nitric oxide and drain the tumour microenvironment in the brain of amino acids that are essential for the activation of cytotoxic T cells, which leads to the blockade of immune signalling pathways, such as IL-2

signalling, an important stimulatory pathway for the immune system.[44,45] Moreover, the production of reactive oxygen species leads to the degradation of proteins, lipids and nucleic acids, pushing T cells to commit to apoptosis.[44] The coexistence of reactive oxygen species and nitric oxide within the same microenvironment further leads to the formation of peroxynitrites, leading to the nitrosylation of T-cell receptors. This disturbs the mechanism of interaction of T cells with tumour cells and may contribute to metastatic immune resistance.[45]

Microglia have also been shown to express neurotrophin (NT)-3 to regulate immune cellular activation. NT-3, similar to other neurotrophins, plays a major role in stimulating and controlling neurogenesis.[46] In metastatic settings, NT-3-expressing microglia are hijacked to assist in the formation of brain metastases.[47,48] In addition, the secretion of IL-10 and transforming growth factor beta (TGFβ) by myeloid cells and microglia induces the activity of M2 TAMs and regulatory T cells, which are known to suppress immune responses.[45] The role of microglia extends, in some cases, to guiding the invasion of metastatic cells into the brain.[44,49] Imaging studies have shown that a wall of microglial cells is situated at the border between the metastatic cells and the brain parenchyma.[50]

Macrophages are generally known to modulate between polarizing states: M1 and M2. While the M1 state is pro-inflammatory, inducing a Th1 response against foreign pathogens and cancer cells, the M2 state contributes to immunosuppression and cellular repair. Although understanding of macrophage polarization remains limited in the setting of brain metastasis, TAMs generally presume an M2 composition, causing immunosuppression and assisting tumour cells in evading immunity.[44] Nevertheless, polarization of macrophages differs between different regions of the brain. In parenchymal metastases, the release of cytokines such as lymphotoxin β and the increase in NF-κB1 activity demonstrate a direct involvement in the M2 polarization of macrophages.[51] Subsequently, this results in the secretion of growth factors, remodelling of the extracellular matrix (ECM), and angiogenesis.[52] The release of TGFβ, matrix metalloproteinases (MMP) 2, MMP9, cathelicidin, cathepsins and scavenger receptor class A (SRA) have been reported to play a major role in pro-metastatic ECM remodelling, whereas the secretion of vascular endothelial growth factor A (VEGFA) and platelet-derived growth factor (PDGF) contribute to angiogenic formations.[25,52]

Upon settling in the brain parenchyma, metastatic tumour cells release factors to recruit TAMs to the tumour microenvironment (Fig. 1). Factors like VEGFA, chemokine ligand (CCL) 2, CCL5, CCL9, CCL18 and colony stimulating factor 1 (CSF1) have been reported to be released in such settings.[25,52,53] One of the therapeutic strategies used to target TAMs is the colony stimulating factor 1 receptor (CSF1R) inhibitors that inhibit the release of pro-survival factors by

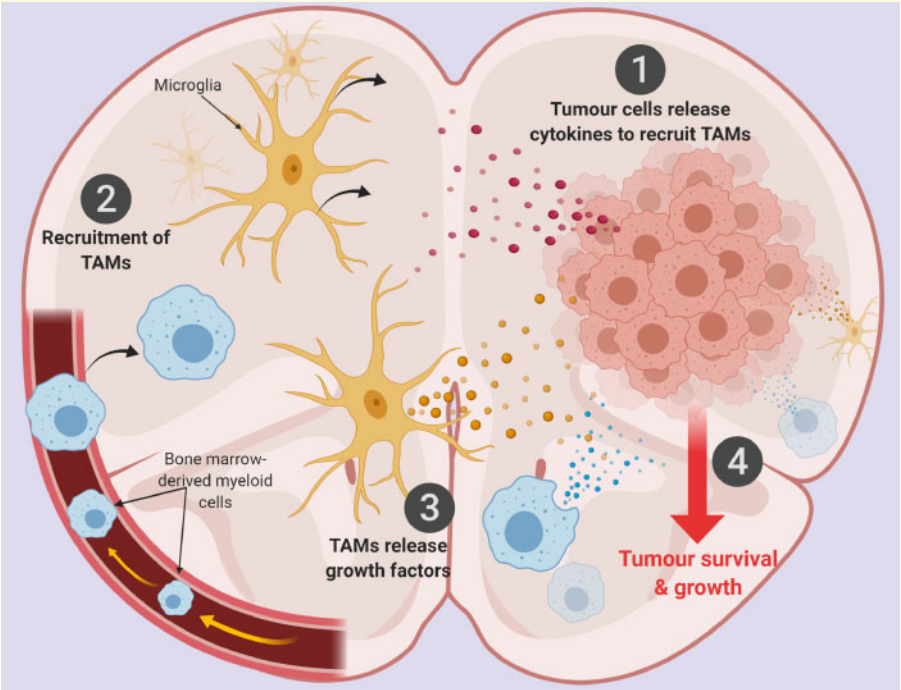

Figure 1 The interplay between metastatic tumour cells and TAMs in the brain microenvironment. (1) Upon entry into the brain parenchyma, tumour cells release cytokines and chemokines, such as CSF1, GM-CSF, MCP-1, HGF, SDF-1 and CX3CL1, to recruit myeloid cells from the periphery and brain-resident microglia into the tumour niche. (2) Myeloid cells derived from the bone marrow and the yolk sac enter the brain and microglia migrate towards the tumour cells. (3) TAMs release growth factors, such as EFG, IL-6, TGF-β, IL-1β and other proteases. (4) This promotes tumour survival and growth. Created with BioRender.com.

the TAMs. Inhibition of CSF1R either depletes or depolarizes TAMs.[31,32]

Unlike microglia, myeloid cells are poor APCs. They express isoforms of human leukocyte antigen (HLA)-G and HLA-E, in addition to the major histocompatibility complex (MHC) type I molecules that prevent the lysis of natural killer (NK) cells and T cells.[25] Nonetheless, their immunosuppressive ability is illustrated through the co-expression of inhibitory molecules, such as PD-L1 and PD-L2, and the release of inhibitory chemokines, such as IL-10, TGFβ, CCL5, CCL20 and CCL22, which prevent the activation of the adaptive immune response in various cancers.[25,44,54]

T cells and NK cells

Analysis of brain metastases confirmed a significantly higher proportion of lymphocytes, with melanoma brain metastases exhibiting the most lymphocytic infiltrates and CD8+ T cells predominating other lymphocytic fractions.[27] Yet, the level of inflammation around metastatic tumours is different from one patient to another and between cancer types, as the concentration of T cells around brain metastatic cells can vary from nil to very high.[55,56] The discrepancy in CD8+ T-cell density can be associated with the time at which brain metastases occurred.[57] Another explanation for this variation can be seen in the protein expression of metastatic cells. For example, high PD-L1 expression eases immune evasion and allows the metastatic cell to escape checkpoint by T cells (Fig. 2).[56] Moreover, expression of forkhead box p3 (FOXP3) as well as programmed cell death protein 1 (PD-1) by regulatory T cells favours immune suppression in the metastatic tumour microenvironment.[56,58]

In terms of clinical outcomes and survival, it has been demonstrated that patients with increased immune responses and cytotoxic T-cell infiltration of metastatic tumours show better prognoses.[58-60] Melanoma brain metastases exhibited

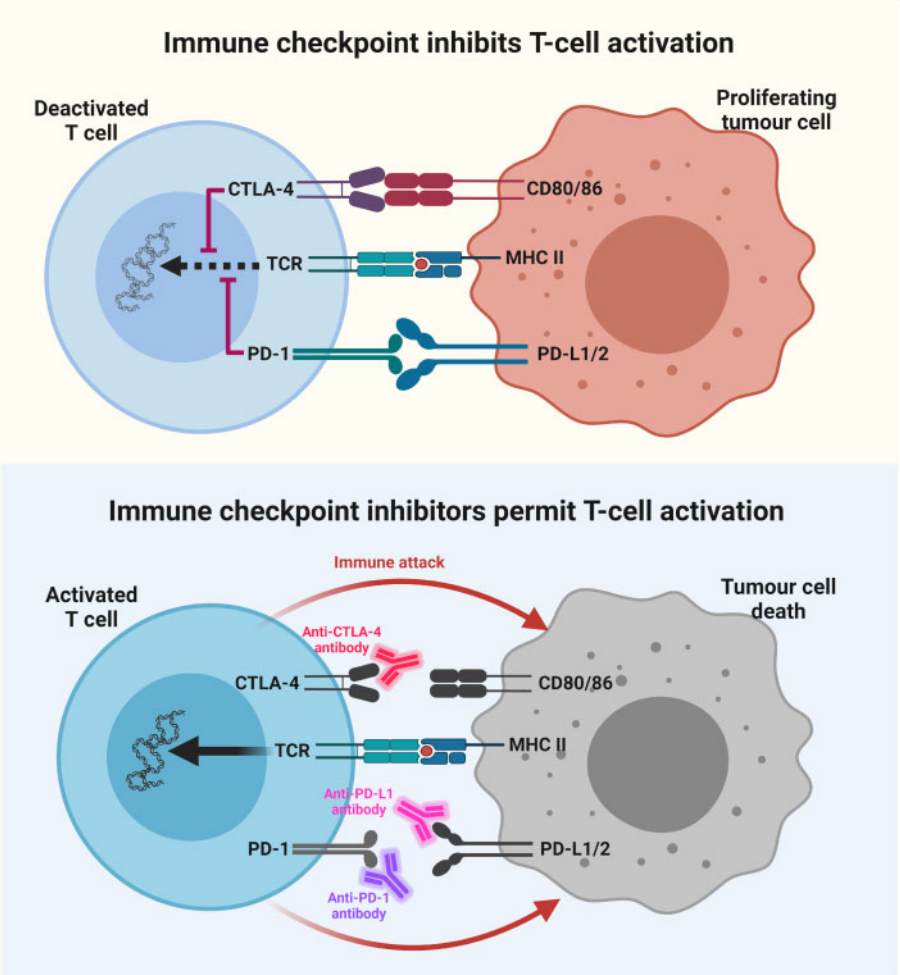

Figure 2 T-cell regulation through an immune checkpoint. *Top*: The expression of CTLA4 and PD-1 on T cells serves as an immune checkpoint through which tumour cells can bind and deactivate T cells. Suppression of effector T-cell responses provides metastatic cancer cells with the opportunity to proliferate. *Bottom*: Immune checkpoint inhibitors, such as anti-CTLA4, anti-PD-L1 and anti-PD-1 antibodies, prevent tumour cells from applying the brakes to T-cell activation, which subsequently leads to T-cell activation, immune attack and tumour cell death. Created with BioRender.com.

higher T-cell infiltration than breast cancer brain metastases.[61] This may be the reason behind the success of some immunotherapeutic regimens in improving survival in patients with metastatic melanoma to the brain.[62,63] Furthermore, the infiltration of brain metastases with high levels of effector CD3+, cytotoxic CD8+ or memory CD45RO+ T cells has been shown to improve survival.[61] In addition, increased T-cell trafficking into the brain metastatic areas decreases the integrity of white matter tracts, providing a method to identify immunologically active microenvironments in the brain using diffusion tensor MRI.[64]

The blood–brain barrier also plays a role in limiting the infiltration of cytotoxic T cells. Angiogenesis and neovascularization are key hallmarks of metastasis.[65] Nonetheless, the magnitude of angiogenic potential differs between cancer types.

Aside from expressing inhibitory proteins and receptors, such as cytotoxic T-lymphocyte associated protein 4 (CTLA4), PD-1, lymphocyte-activation gene 3 (LAG3), T-cell immunoglobulin and mucin-domain containing-3 (TIM-3), inducible T-cell costimulator (ICOS), and T cell immunoreceptor with Ig and ITIM domains (TGIT), regulatory T cells can also secrete large volumes of immunosuppressive cytokines. IL-10, IL-35 and TGFβ are reported to be released by regulatory T cells in the tumour microenvironment.[66] Moreover, regulatory T cells have been documented to drain the tumour microenvironment of immune-stimulatory cytokines, like IL-2, deactivating the antitumour Th1 immune response. More recently, it was shown that T-cell receptors that exhibit pan-cancer cell recognition via MHC class I-related proteins lacked detectable reactivity in melanoma. Providing the T cells with the MC.7.G5 T-cell receptors rendered them capable of killing autologous melanoma.[67]

NK cells also partake in the intratumoural immune response in the brain tumour microenvironment.[68] They have been shown to be present in metastatic brain tumours, gliomas and craniopharyngiomas.[69,70] Like cytotoxic T cells, NK cell functioning is often diminished in patients with brain tumours due to the release of anti-inflammatory molecules, such as TGFβ, by tumour cells. In addition, tumours that secrete inflammatory mediators like cyclooxygenases (COX) and prostaglandin E2 suppress NK cell antitumour activity.[71]

Neutrophils

Despite being the most abundant circulating immune cells, the function of neutrophils in the immune microenvironment of brain metastases remains unclear. Yet, recent advances have elucidated some of the mechanisms that govern neutrophil recruitment and activation in the brain metastatic microenvironment. Neutrophils can be recruited to the brain microenvironment through the secretion of CXCL8 chemoattractant by TAMs. The upregulation of ITGA3 and CXCL17 by brain metastatic cells further increases neutrophil abundance at the scene.[27] The overexpression of the adenosine receptor *ADORA2A* suppresses the pro-inflammatory phenotype of neutrophils.[27,72] Moreover, overexpression of *CD177* in brain metastatic cells affects neutrophil migration and activation and suppresses T-cell proliferation.[27] In addition, the upregulation of MET is associated with the recruitment of immunosuppressive neutrophils to the immune microenvironment in brain metastases.[27,73]

Recently, it has been shown that brain metastatic cells overexpress an epigenetic modifying protein, enhancer of zeste homolog 2 (EZH2), to stimulate signalling pathways that suppress recruited neutrophils.[74] In the setting of brain metastases, EZH2 phosphorylation changes its function from being a methyltransferase to a transcription factor that increases *c-JUN* expression.[74] This leads to the secretion of pro-tumorigenic inflammatory cytokines, including G-CSF, which recruits Arg1+ and PD-L1+ immunosuppressive neutrophils into the brain to drive metastasis outgrowth.[74] Blocking the influx of this subset of neutrophils by G-CSF-blocking antibodies or immune checkpoint blockade therapies combined with Src inhibitors impeded brain metastasis in multiple mouse models.[74] Furthermore, neutrophils can play a role in priming the brain metastatic niche. It has been shown that S100 calcium-binding protein A (S100A)-8 and S100A9 are upregulated in the pre-metastatic niche in the brain, leading to recruitment of neutrophils, which support subsequent metastatic seeding and colonization (Fig. 3).[75]

Neutrophils have a prognostic value in the setting of brain metastases. A high ratio of neutrophils to lymphocytes in the peripheral blood is associated with reduced survival time, even after surgical resection of brain metastases.[76] Furthermore, in patients treated with stereotactic radiosurgery, the post-treatment neutrophil-to-lymphocyte ratio was associated with poor overall survival.[77] Nevertheless, in patients with non-small cell lung cancer (NSCLC) who harbour EGFR mutations, a neutrophil-to-lymphocyte ratio $\leqslant 2.99$ was associated with prolonged survival.[78] Therefore, the neutrophil-to-lymphocyte ratio could serve as a useful prognostic biomarker in specific patients with brain metastases.

Astrocytes

Astrocytes are some of the most abundant cell types that are unique to the CNS and play important roles in mediating tissue-specific communication in the brain. Therefore, it is likely that metastases that preferentially grow within the brain must find ways to adapt and favourably interact with these unfamiliar cellular players.[8] Brain metastatic cells are known to express high levels of IL-1β upon interaction with astrocytes. This leads to the activation of the Notch signalling pathway, which increases stemness of cancer stem cells and drives their growth in the tumour microenvironment.[79] Moreover, it was shown that cancer cells within established brain metastases form functional gap junctions with astrocytes in the adjacent microenvironment, thereby creating a conduit for bidirectional communication to support

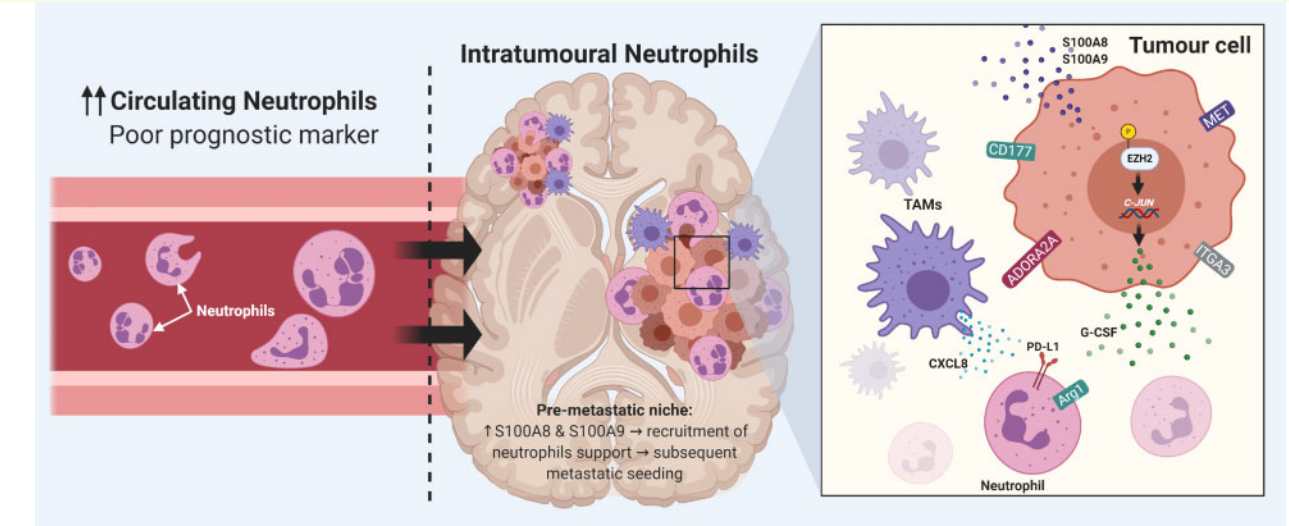

Figure 3 Role of neutrophils in brain metastases. *Left*: In the setting of brain metastases, a high neutrophil-to-lymphocyte ratio is associated with poor prognostic outcomes. *Middle*: In the tumour microenvironment, neutrophils are recruited through increased release of S100A8 and S100A9 proteins by tumour cells. *Right*: The increased expression of CD117, ADORA2A, ITGA3 and MET by brain metastatic cells leads to neutrophilic suppression in the immune microenvironment. The phosphorylation of EZH2 protein increases the expression of *c-JUN*, which leads to the release of G-CSF. G-CSF, in turn, leads to the recruitment of neutrophils that overexpress PD-L1 and Arg1. Furthermore, tumour-associated myeloid cells and microglia release chemokines, such as CXCL8, to suppress neutrophils in the tumour microenvironment. Created with BioRender.com.

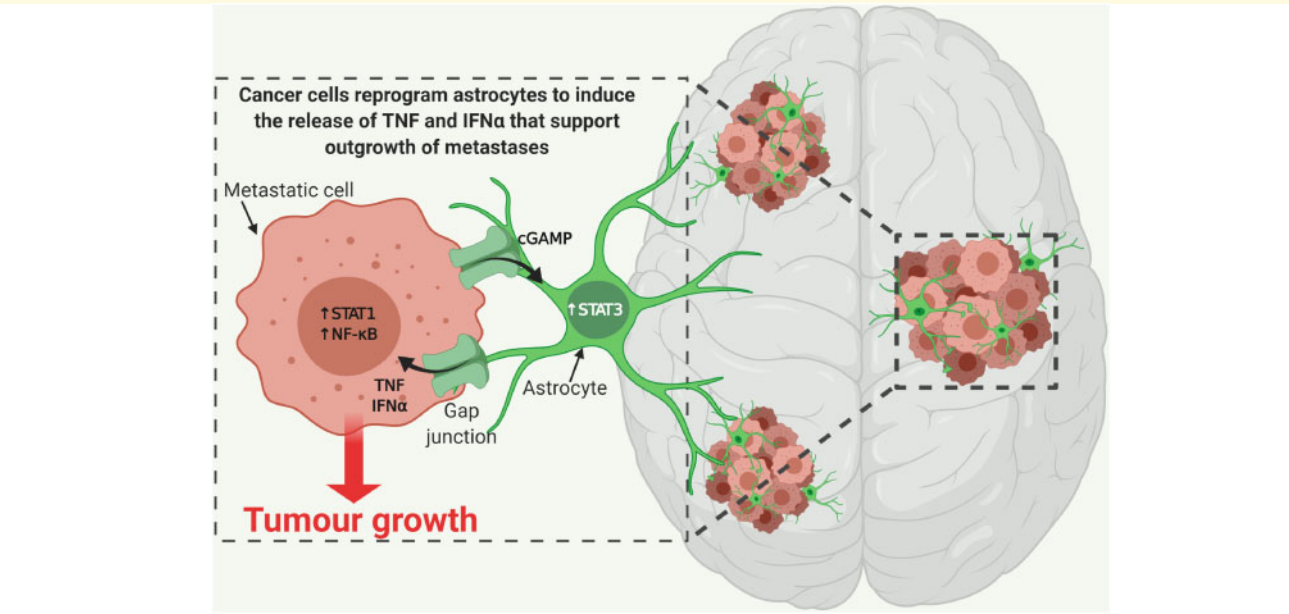

Figure 4 Interaction of astrocytes with brain metastatic cancer cells. Metastatic cancer cells release cGAMP to reprogram astrocyte behaviour and increase STAT3 signalling. In turn, astrocytes form gap junctions with metastatic cancer cells in the brain to exchange stimulatory signals, such as TNF and IFNα, which activate STAT1 and NF-κB pro-tumorigenic signalling. Created with BioRender.com.

outgrowth.[80,81] Through these gap junctions, cancer cells reprogram astrocytes by providing cGAMP to induce a pro-inflammatory program, characterized by the production of interferon alpha (IFNα) and tumour necrosis factor alpha (TNFα).[80,81] In turn, these cytokines support outgrowth of metastases by activating signal transducer and activator of transcription (STAT) 1 and nuclear factor-κB (NF-κB) signalling within cancer cells (Fig. 4). By interfering with the formation of gap junctions through pharmacological regimens, this heterotypic signalling loop can be blocked, thus

mitigating brain metastasis outgrowth.[81] In addition, astrocytes possess the capability to release factors that can remodel the ECM, such as MMPs and heparanase, which contribute to the invasion of metastatic cells across the blood–brain barrier and facilitate subsequent metastatic colonization.[82,83]

Brain metastatic cells induce and maintain the co-option of a pro-metastatic program driven by STAT3 in a subpopulation of reactive astrocytes surrounding metastatic lesions.[84] These reactive astrocytes benefit metastatic cells by their modulatory effect on the innate and acquired immune system. Blocking STAT3 signalling in reactive astrocytes reduces experimental brain metastasis from different primary tumour sources, even at advanced stages of colonization.[84,85] A safe and orally bioavailable treatment that inhibits STAT3 exhibits significant antitumour effects in patients with advanced systemic disease that included brain metastasis.[84]

Immune checkpoint in brain metastases

From an evolutionary perspective, cancer cells have developed various features to escape immune surveillance. PD-L1 is an immune-checkpoint molecule that regulates immune homeostasis and prevents autoimmunity.[86] In pathological conditions, such as pancreatic cancer, the expression of PD-L1 shields the tumour cells from the activation of cytotoxic T cells.[87] Unlike other immune-surveillance molecules like CTLA4, the expression of PD-1 is not limited to T cells, but occurs in B cells as well.[88] This highlights the wider range of functions exhibited by PD-L1.[89]

It is unclear how metastatic brain cells develop mechanisms to overcome the effect of T cells. Heavy reliance on breaking the interaction between T cells and metastatic brain cancer cells and the ablation of specific metabolic pathways result in activation of pro-survival mechanisms in the cancer cells, presenting a potential therapeutic avenue against lethal advancement. The PD-1 receptor was first identified on thymocytes in response to a pro-apoptotic stimulus. Further studies linked the function of this receptor to immunological tolerance. Like CTLA4, PD-1 belongs to the CD28 family and is known for its inhibitory effect. Upon antigenic stimulation, the tail region of PD-1 is phosphorylated, leading to the recruitment and activation of SHP-2.[90] This is followed by complex formation with SRC-based family receptors, resulting in the activation of ERK and RAS signalling pathways in primary brain tumours.[91] The exact mechanism by which this occurs is still unclear but it seems to be related to the ability of PD-1 to inhibit the production of IFN-induced nitric oxide,[92] suppress glucose metabolism, and inhibit arginine and tryptophan amino acids.[93]

Most recently it was shown that therapeutic reduction of PD-1 expression leads to cytoprotective autophagy induction in tumour cells, thereby increasing their survival. Two PD-1 receptor ligands are known: PD-L1 and PD-L2 belonging to the B7 family.[94,95] PD-1, PD-L1 and PD-L2 were observed to be differentially expressed between primary and metastatic tumours.[95] Further histopathological examination of these immune checkpoints in metastatic lesions revealed that their expression is correlated with shorter overall survival.[95]

The inhibition of PD-1 and CTLA4 on T cells in brain metastases seems to also depend on the status of the systemic disease. In melanoma brain metastases, the efficacy of intracranial immune checkpoint inhibition was observed only when an extracranial tumour was present.[96,97] Extracranial presence further increased the trafficking of CD8+ T cells and TAMs to brain tumours, in response to immune checkpoint therapy. Moreover, tumour-induced peripheral immunosuppression promotes brain metastases in patients with NSCLC. Increased expression of PD-L1 on peripheral TAMs decreases T-cell activation and trafficking, leading to poor outcomes.[98]

Potential mechanisms that limit immune responses to checkpoint inhibitors also include brain access and vascular permeability. Despite being leaky to a variable extent, blood vessels in brain tumour settings are less permeable than in extracranial tumours.[2,11] Anti-PD-1 and anti-CTLA4 blocking antibodies have limited access to intracranial tumours and thus their ability to reduce PD-1 and CTLA4 inhibition of T cells is diminished. Nevertheless, effective immune responses against brain metastatic cells can be dependent on the production of antigen-specific T cells as a result of PD-1 blockade or CTLA4 inhibition in the extracranial tumour site.[97,99] Moreover, tumour antigens of brain metastases may reach draining lymph nodes, leading to the induction of T-cell priming and the release of antigen-specific T cells from checkpoint inhibition in the extracranial tumour-draining lymph nodes.[97]

Brain metastatic cells can be genetically and phenotypically different from their primary tumour origin. Genomic characterization of brain metastases revealed that in 53% of cases, clinically informative alterations in brain metastases were not detected in the matched primary tumour sample.[100] Distal extracranial and regional lymph node metastases were highly divergent from brain metastases, whereby detected alterations associated with sensitivity to PI3K/AKT/mTOR, CDK and HER2/EGFR inhibitors in the brain metastases.[100] These differences between primary and metastatic tumour cells further include alterations in immune responses, inflammation-related pathways, NF-kB1 activity and cytokine profiles.[51] For example, lymphotoxin β expression was directly correlated with M2 polarization of parenchymal macrophages in the setting of brain metastases.[51]

The expression of immune checkpoint molecules in the immune microenvironment of brain metastases can affect the immune modulatory response against tumour cells. Studies exploring PD-1 expression on T cells in the setting of brain metastases showed wide discrepancies in results, with PD-1 expression noted in 3.1–68% of brain metastatic samples.[21,101,102] PD-L1 expression was detected in 25–28% of immune cells in the setting of brain metastatic cells.[101,103,104] On tumour cells, PD-L1 expression ranged from 22% to 75% in brain metastatic samples.[21,101-107]

Therapeutic advancements against metastasis are particularly successful when targeting multiple pathways of the metastatic process. In the clinical setting, therapeutic efficacy and significant prolongation of survival were observed with interventions that simulate anticancer responses mediated by T cells. Ongoing preclinical studies demonstrate survival benefit in models of melanoma, lung and breast cancers upon blockade of interactions between T cells and cancer cells.[108] Nonetheless, the mechanism of interplay remains wide open as clinical translation of such therapies has only been able to demonstrate benefit in melanoma brain metastasis.[23]

Clinical outcomes with immune checkpoint inhibitors

Anti-CTLA4 therapy

Ipilimumab is a fully human monoclonal antibody against CTLA4, which plays a pivotal role in downregulating the production of cytotoxic T cells.[109] The effect of ipilimumab was first noted intracranially in a phase 3 trial in patients with metastatic melanoma, in which an improvement in survival was seen.[110] This was subsequently followed by a phase 2 trial that focused on patients with melanoma brain metastases; however, ipilimumab only showed activity in small and non-symptomatic brain lesions.[111,112] Patients who were initially asymptomatic from a neurological standpoint had a 24% intracranial response rate, whereas symptomatic patients on corticosteroids had a 10% response rate.[111] Overall survival rate amongst patients with melanoma brain metastases at 1 year was ~20% across studies.[111-113] Moreover, baseline metabolic tumour volume measured on ^{18}F-fluorodeoxyglucose PET/CT appears to be a strong independent prognostic factor in melanoma patients treated with ipilimumab.[114] This technique could be used to personalize immunotherapy in future clinical studies.

Anti-PD-1/PD-L1 therapy

The PD-1/PD-L1 pathway may be a promising target for immune checkpoint inhibition. Anti-PD-1/PD-L1 antibodies such as nivolumab and pembrolizumab have been approved for advanced melanoma, kidney cancer and NSCLC. A phase 2 study with pembrolizumab on patients with brain metastatic melanoma and NSCLC observed partial responses in 25% of the patients with melanoma and 44% of the patients in the NSCLC arm, with a median overall survival of 7.7 months.[115-117] Patients with melanoma brain metastases treated with nivolumab or pembrolizumab showed a median overall survival of 9.9 months.[118] Moreover, a phase 2 trial with pembrolizumab in melanoma brain metastases induced a median progression-free survival and an overall survival of 2 and 17 months, respectively; 48% of patients were alive after 2 years.[119] In patients with NSCLC brain metastases, nivolumab achieved an overall response rate of 17%.[120] In addition, the use of the anti-PD-L1 antibody atezolizumab in patients with brain metastases has shown an overall survival benefit of 16 months.[121] Atezolizumab also increased progression-free survival when compared with docetaxel.[122] Nevertheless, overall survival was similar when comparing patients with NSCLC who were treated with nivolumab alone versus docetaxel (Table 1).[123]

Often, patients with brain metastases are given corticosteroids to decrease symptoms of tumour-related inflammation. While patients with melanoma brain metastases who received ipilimumab and corticosteroid treatment showed worse outcomes,[111] patients with NSCLC brain metastases do not seem to show the same with nivolumab. In a case report, the effect of nivolumab was obtained despite concomitant high-dose corticosteroid therapy.[124] Partial shrinkage of the brain tumour was detected with no unexpected adverse events.[124] More studies are needed to ascertain the reason why patients taking corticosteroids had worse outcomes with ipilimumab. It is possible that these patients' tumours had unfavourable prognostic features at baseline, or that corticosteroid use counteracted the effectiveness of the checkpoint inhibitor.

Combination of immunotherapies

Combination immunotherapy can have better therapeutic effects than monotherapy. Using high-dimensional single-cell profiling, combination therapy with anti-CTLA4 plus anti-PD-1 elicited cellular responses that are partially distinct from those induced by either monotherapy.[127] The combination mediated a switch from expansion of phenotypically exhausted CD8 T cells to expansion of activated effector CD8 T cells.[127] Recently, the combination of ipilimumab and nivolumab showed the best benefit with an estimated 12-month survival reaching 81.5% in patients with melanoma brain metastases.[23] A recent update of the study reported an intracranial objective response rate of 54%; the 18-month survival rate was 75%.[126] In a subgroup of patients with symptomatic brain metastases, the 6-month overall survival rate reached 66% at a median follow-up of 5.2 months.[126] In a similar trial with a median follow up of 17 months, ipilimumab and nivolumab demonstrated intracranial responses in 16 of 35 patients (46%), whereas only 5 of 25 patients (20%) who received nivolumab, and one of 16 patients (6%) who received ipilimumab showed similar responses.[125] The combination therapy further saw six patients (17%) achieving a complete intracranial response, whereas only three patients (12%) who received nivolumab had a similar response.[125]

Immunotherapy and radiation

Stereotactic radiosurgery or whole-brain radiation therapy with or without surgery has been the mainstay for the treatment of patients with symptomatic brain metastases.[128] As various studies point out, the effects of radiotherapy on metastatic tissue combine DNA damage,[129,130] possible

Table 1 Clinical findings for immune checkpoint inhibitors as monotherapy or in combination in brain metastases

Strategy	Study type	Selected patients	Primary tumour	Results	Reference
Ipilimumab	Phase 3	82	Melanoma	Ipilimumab improved overall survival by 3–4 months. Adverse events were reversible with appropriate treatment.	Hodi *et al.*[110]
Ipilimumab	Retrospective	165	Melanoma	Overall survival rate at 1 year was 20%. Treatment was relatively safe.	Heller *et al.*[113]
Ipilimumab	Phase 2	72	Melanoma	Ipilimumab had 24% intracranial response rate when metastases were small and asymptomatic. The drug had no unexpected toxicity.	Margolin *et al.*[111]
Pembrolizumab	Phase 2	10	NSCLC	Partial responses were recorded in four patients. No grade $\geqslant$ 3 adverse events.	Goldberg *et al.*[116]
Pembrolizumab	Phase 2	17	Melanoma, lung cancer	Partial responses were observed in three patients. Grade 3 liver function abnormalities were noted in one patient.	Kluger *et al.*[115]
Pembrolizumab	Phase 2	36	Melanoma, NSCLC	Response was achieved in 4 (22%) of 18 patients with melanoma and 6 (33%) of 18 patients with NSCLC.	Goldberg *et al.*[117]
Nivolumab	Retrospective	46	NSCLC	At time of last assessment, 33% of patients had no evidence of CNS progression (stable/decreased brain lesions).	Goldman *et al.*[123]
Nivolumab or pembrolizumab	Retrospective	66	Melanoma	Response rate was 21% and disease control rate was 56%. Median overall survival was 9.9 months. Patients requiring corticosteroids had shorter overall survival (4.8 versus 13.1 months).	Parakh *et al.*[118]
Atezolizumab	Phase 3	85	NSCLC	Median overall survival for patients treated with atezolizumab was 20.1 months versus 11.9 months for patients treated with docetaxel.	Rittmeyer *et al.*[121]
Nivolumab and corticosteroid	Case report	1	NSCLC	Nivolumab was effective in shrinking symptomatic brain metastases in a patient with first-line chemotherapy failure.	Pluchart *et al.*[124]
Nivolumab and ipilimumab	Phase 2	94	Melanoma	At 14 months, the rate of intracranial clinical benefit was 57%. The rate of complete response was 26%, the rate of partial response was 30%, and the rate of stable disease for at least 6 months was 2%.	Tawbi *et al.*[23]
Nivolumab and ipilimumab	Phase 2	35	Melanoma	At 17 months, intracranial responses were achieved by 16 patients (46%). Intracranial complete responses occurred in six patients (17%).	Long *et al.*[125]
Pembrolizumab	Phase 2	23	Melanoma	Six patients (26%) had a response. Median progression-free and overall survival were 2 and 17 months, respectively. Most responders had higher pretreatment tumour CD8 cell density and PD-L1 expression.	Kluger *et al.*[119]
Nivolumab	Retrospective	409	NSCLC	Disease control rate was 39%: four patients had a complete response, 64 a partial response and 96 showed stable disease. The median overall survival was 8.6 months.	Crino *et al.*[120]
Atezolizumab	Phase 3	123	NSCLC	In patients with asymptomatic metastases, median overall survival was longer with atezolizumab than with docetaxel (16.0 versus 11.9 months).	Gadgeel *et al.*[122]
Nivolumab and ipilimumab	Phase 2	119	Melanoma	In asymptomatic patients, the clinical benefit rate was 58.4%. In symptomatic patients, the clinical benefit rate was 22.2%.	Tawbi *et al.*[126]

More information can be found in the Supplementary material.

release of damage-associated molecular patterns or stress molecules,[131] and the expression of MHC-I molecules for effective antigen presentation.[132,133] However, overall, radiation fails to produce a durable anticancer effect. Simultaneously, developing immune checkpoints by cancer cells and the suppressive microenvironment contribute to tumourigenicity.[134-136]

Several studies have shown that the combination of stereotactic radiation and ipilimumab is safe in the management of melanoma brain metastases.[137-140] In addition, the combination of nivolumab and stereotactic radiation improved overall survival and progression-free survival as compared with historical controls treated with radiation alone.[140] A meta-analysis of patients with brain metastases from melanoma, NSCLC and renal cell carcinoma showed that the use of immune checkpoint inhibitors alongside stereotactic radiation (median 20 Gy in one fraction) improved 1-year overall survival to 65%, as compared to 52% in cases treated with monotherapy.[141] Another study also showed an improved median overall survival of 24.7 months compared to 12.9 months with radiation therapy alone (Table 2).[154] Radiation leads to the upregulation of PD-L1 on cancer cells, which sensitizes them to anti-PD-L1 therapy.[155,156] In addition, it increases blood–brain barrier permeability allowing better penetration of immunotherapy into the brain. Nevertheless, the high doses of steroids administered with whole-brain radiation therapy to decrease intracranial oedema pose a problem due to their immunosuppressive effects.[111] This has caused many centres to move away from using steroids routinely for all patients with brain metastases.[142]

Immunotherapy and other therapies

Chemotherapy may indeed potentiate the effect of immune checkpoint inhibitors by increasing T-cell responses and disrupting the activity of TAMs.[157] Nevertheless, its exact mechanism of action in the setting of brain metastasis remains unknown. In NSCLC, pembrolizumab in addition

Table 2 Clinical findings for combinatory approaches with immune checkpoint inhibitors and radiotherapy in brain metastases

Strategy	Study type	Selected patients	Primary tumour	Results	Reference
Ipilimumab and WBRT	Retrospective	27	Melanoma	Median survival was 21.3 months. The Diagnosis-Specific Graded Prognostic Assessment score was the most significant predictor of overall survival.	Knisely *et al.*[138]
Ipilimumab and SRS	Retrospective	25	Melanoma	Ipilimumab neither increased toxicity nor improved intracerebral disease control in patients with limited brain metastases who received SRS.	Mathew *et al.*[139]
Ipilimumab and SRS or WBRT	Retrospective	33	Melanoma	Patients had an overall median survival of 18.3 months. Ipilimumab was associated with a significantly reduced risk of death in patients with melanoma brain metastases who underwent radiotherapy.	Silk *et al.*[137]
Ipilimumab with WBRT or SRS	Case series	16	Melanoma	Favourable systemic response was more likely when ipilimumab was administered within 3 months of radiation.	Schoenfeld *et al.*[142]
Ipilimumab and SRS	Retrospective	46	Melanoma	Patients treated with SRS during or before ipilimumab had better overall survival and less regional recurrence than those treated with SRS after ipilimumab (1-year survival: 65% versus 56% versus 40%)	Kiess *et al.*[143]
Nivolumab and SRS	Retrospective	26	Melanoma	Eight patients (11%) had a $\geqslant$ 20% increase in tumour volume. Median overall survival was 12 months.	Ahmed *et al.*[140]
SRS with ipilimumab or nivolumab/pembrolizumab	Retrospective	33	Melanoma	Concurrent use of immunotherapy and SRS resulted in a greater reduction in lesion volume. Reduction in lesion volume was greater for nivolumab/pembrolizumab than ipilimumab.	Qian *et al.*[144]
SRS with ipilimumab, nivolumab and/or pembrolizumab	Retrospective	48	Melanoma	One-year intracranial control rates for SRS + checkpoint inhibitors ranged from 60% (local) to 85% (distal). SRS + checkpoint inhibitors significantly reduced tumour recurrence compared with SRS alone.	Acharya *et al.*[145]
SRS with ipilimumab or pembrolizumab	Retrospective	18	Melanoma	Concurrent checkpoint inhibitors and SRS significantly improved progression-free survival and increased lesion regression compared with SRS alone.	Yusuf *et al.*[146]
Ipilimumab and WBRT or SRS	Phase I	16	Melanoma	Ipilimumab + WBRT led to progression-free survival of 2.5 months and overall survival of 8 months. Ipilimumab + SRS led to progression-free survival of 2.5 months and overall survival was not reached.	Williams *et al.*[147]
Ipilimumab and SRS	Retrospective	25	Melanoma	Median overall survival was 35.8 months and local control was 94.8%. Regional brain control and time to progression were significantly improved when SRS was delivered $\leqslant$30 days after ipilimumab.	Skrepnik *et al.*[148]
Ipilimumab and SRS	Retrospective	20	Melanoma	Use of ipilimumab within 4 months of SRS was safe. This treatment regimen was not associated with improved outcomes.	Patel *et al.*[149]
SRS with ipilimumab, BRAF inhibitor and/or pembrolizumab	Retrospective	179	Melanoma	SRS with checkpoint inhibitors and BRAF inhibitor was highly protective. Overall survival was best in BRAF-wild-type patients receiving SRS + pembrolizumab (12.26 months), and in BRAF-mutated patients receiving SRS + BRAF inhibitors + pembrolizumab (14.82 months).	Gaudy-Marqueste *et al.*[150]
SRS and ipilimumab	Retrospective	46	Melanoma	The effect of SRS and ipilimumab on local recurrence-free duration seemed greater when SRS was performed before or during ipilimumab treatments. The timing of immunotherapy and SRS may affect local recurrence-free duration and post-SRS oedema.	Cohen-Inbar *et al.*[151]
SRS with ipilimumab or pembrolizumab	Retrospective	39	Melanoma	Median survival with SRS + ipilimumab was 7.5 months, and 20.4 months with SRS + pembrolizumab. Median brain control was 7.5 months with SRS + ipilimumab, and 12.7 months with SRS + pembrolizumab.	Choong *et al.*[152]
Nivolumab and SRS	Retrospective	17	NSCLC	The 6-month rate of distant brain control for patients treated with SRS during or prior to nivolumab therapy was 57% compared to 0% among patients who received nivolumab before SRS.	Ahmed *et al.*[153]
SRS with ipilimumab, nivolumab and/or pembrolizumab	Retrospective	79	Melanoma, NSCLC, renal cell carcinoma	Median overall survival was 24.7 months. SRS with concurrent checkpoint inhibitors was associated with improved survival compared with SRS alone and non-concurrent SRS and checkpoint inhibitors.	Chen *et al.*[154]

SRS = stereotactic radiosurgery; WBRT = whole brain radiation therapy. More information can be found in the Supplementary material.

to chemotherapy has become the standard-of-care therapy.[158] Clinical trials focusing on patients with brain metastases who are receiving immunotherapy in addition to targeted therapy are lacking.[159] Although, in some trials, patients with untreated, asymptomatic and small (maximum diameter 15 mm) brain metastases are allowed, the percentage of these patients and their outcomes are not reported.[158] Secondary analyses focusing on small subgroups of patients with brain metastases report conflicting results. In small cell lung cancer (SCLC) brain metastases, adding atezolizumab to chemotherapy did not increase overall survival[160]; however, adding durvalumab to chemotherapy showed survival benefit in similar patients with brain metastases.[161] Combining ipilimumab with fotemustine (an alkylating agent used in the treatment of metastatic melanoma) yielded an overall survival of 27.8% at the 3-year interval (Table 3).[162-164]

Targeted therapy in addition to immune checkpoint blockade can further enhance therapeutic effects. Inhibition of the MAPK pathway increases the efficacy of dendritic cells, T cells and NK cells, and decreases the activity of tumour-associated fibroblasts and TAMs in brain tumours.[165-167] The addition of Toll-like receptor 1/2 (TLR1/2) ligand to anti-CTLA4 antibody in a mouse model of melanoma enhanced the antitumour efficacy of anti-CTLA4 by increasing Fcγ receptor IV expression in macrophages, which in turn increased the depletion of tumour-infiltrating regulatory T cells.[168] These findings are likely extensible to other checkpoint antibodies in cancer patients. In melanoma, trials are now ongoing with combinations of PD-L1 or CTLA4 inhibitors with BRAF and/or MEK inhibitors. Unfortunately, no trials specifically report or exclusively focus on patients with brain metastases.

Angiogenesis in the brain can affect immune function. Vascular abnormalities are a hallmark of brain metastases and facilitate immune evasion. Elevated levels of proangiogenic factors, such as VEGF and angiopoietin 2, play a major role in this process.[169] Nevertheless, angiogenesis can be a double-edged sword. While the formation of new vasculature may increase inflammation in the tumour immune microenvironment and the trafficking of effector T cells into the scene, the abnormal vasculature can limit the movement of immune cells. VEGF alters the expression of adhesion molecules on endothelial cells and immune cells, limiting proper adhesion and subsequent passage into the tumour microenvironment.[2] As such, the inhibitors of angiogenesis may normalize the vasculature and increase T-cell trafficking to the location of metastases, switching the immunosuppressive tumour microenvironment to an immunosupportive one and increasing the therapeutic potential of immune checkpoint inhibitors, as can be seen in malignant gliomas.[170] Furthermore, the combination of both regimens can decrease the collection of inflammatory molecules and oedema around the tumour site and subsequently reduce the need for steroidal therapy. Moreover, bevacizumab reduces TAMs that express S100A9, which reduces immunosuppression and restores anti-tumour immunity.[171]

Table 3 Clinical findings for combinatory approaches with immune checkpoint inhibitors and targeted therapy in brain metastases

Strategy	Study type	Selected patients	Primary tumour	Results	Reference
Ipilimumab + fotemustine	Phase 2	20	Melanoma	Ten patients (50%) achieved disease control	Di Giacomo *et al.*[162]
Ipilimumab + fotemustine	Phase 2–follow-up	20	Melanoma	Overall survival and 3-year survival rate were 12.7 months and 27.8%, respectively. Increase from baseline to week 12 in 'memory' but not in 'naïve' T cells conferred better survival. The neutrophil-to-lymphocyte ratio correlated with significantly better survival at early time points	Di Giacomo *et al.*[163]
Atezolizumab + carboplatin + etoposide	Phase 1/3	22	SCLC	Adding atezolizumab to carboplatin and etoposide produced a significant improvement in overall survival and progression-free survival in first line extensive-stage SCLC	Mok *et al.*[160]
Durvalumab + etoposide	Phase 3	28	SCLC	At median follow-up of 14.2 months, disease progression was noted in 17 of 28 patients (61%)	Paz-Ares *et al.*[161]

More information can be found in the Supplementary material.

Bevacizumab in addition to atezolizumab and chemotherapy is a first-line therapeutic option in patients with advanced NSCLC.[172] Interim analyses of a phase 2 trial with pembrolizumab and bevacizumab for melanoma and NSCLC brain metastases showed that 12 of 20 patients (60%) exhibited an objective tumour response; seven complete responses and five partial responses.[173] Unfortunately, patients with brain metastases continue to be excluded from such trials.

Surgery and immunotherapy

The role of neurosurgery and its effect on immunotherapeutic efficacy remains unclear. Nevertheless, early data show that the two modalities can potentiate therapeutic effects and improve patient outcomes. Advances in neurosurgery have significantly improved the safety of surgical resection of brain metastases.[174,175] Surgical resection can improve patient performance and decrease the use of steroids. In a small study of 12 patients with melanoma brain metastases whose treatment regimens involved craniotomies and ipilimumab, nine patients had better performance status after surgery.[176] Furthermore, the sequence and timing of neurosurgery and immunotherapy contributes to overall outcomes. A clinical study of 142 patients with melanoma brain metastases showed that immunotherapy-naïve patients who underwent surgical resection prior to immune checkpoint blockade had longer median overall survival (22.7 months) than patients treated with immunotherapy alone (10.8 months) or immunotherapy followed by surgery (9.4 months).[177] In glioma, early clinical trials show improvement in survival with surgery and PD-1 blockade.[178,179]

Discussion

Patients with brain metastases continue to be excluded from clinical trials due to their dismal outcomes and poor prognoses.[98] Many prospective and retrospective immunotherapeutic studies exclude patients with brain metastases despite increasing data that point to potential efficacy against intracranial metastases.[180] In a multivariate analysis, brain metastases were not associated with poorer survival in patients treated with immune checkpoint inhibitors in NSCLC.[181] Stable patients with brain metastases without baseline corticosteroids and a good diagnosis-specific graded prognostic assessment (DS-GPA) classification have the best prognosis.[181] Combining different immune checkpoint inhibitors together or with radiation, chemotherapy, targeted therapy, antiangiogenic therapy and/or neurosurgery seems to potentiate their effect in the setting of brain metastases. Therefore, randomized controlled trials for patients with brain metastases are needed to fully understand the exact clinical benefit of immunotherapy as monotherapy or in combination. In addition, the exact biological mechanism that governs the function of immune checkpoint inhibitors in the immunosuppressive environment of the brain needs to be determined.

Immunotherapy has not yet been brought to the brain metastasis space in patients with breast cancer. Despite clinical benefit being achieved in patients with brain metastases from melanoma and NSCLC,[23,117] breast cancer remains out of bounds. Preclinical studies in metastatic breast cancer have illustrated a lower immune content in brain metastases.[182] The development of immune strategies to understand and alter the complex brain immune microenvironment is needed. Few clinical trials are exploring this avenue (Table 4). One trial is studying the effect of atezolizumab in combination with HER2-targeting agents in HER2-positive breast cancer, and atezolizumab with doxorubicin and cyclophosphamide in HER2-negative breast cancer (NCT02605915). Another trial is exploring the combination of atezolizumab with stereotactic radiotherapy in patients with brain metastases from triple-negative breast cancer (NCT03483012). Radiotherapy can be an important tool to induce the immunogenicity of 'cold' tumours, such as breast cancer brain metastases. Doses and fractionation that trigger inflammatory responses in combination with immune checkpoint inhibitors can trigger the effector immune cells in the brain tumour microenvironment.[186] Radiation has the ability to upregulate MHC-I expression on tumour cell surfaces and to allow antigen presentation of tumour-specific peptides for recognition by cytotoxic T cells.[187] Furthermore, DNA damage induced by radiation can lead to the production of new antigens that can be recognized by the immune system.[188] In addition, radiation activates the stimulator of interferon genes (STING) pathway, which plays a central role in anti-tumour immunity.[189,190] Nevertheless, radiotherapy can also lead to pro-tumorigenic effects. DNA damage induced by radiotherapy may increase tumour resistance and aggressiveness. In addition, brain metastatic cancer cells can employ the STING pathway and produce factors that activate the STAT1 and NF-κB pathways to support tumour growth and resistance.[81] As such, more research is warranted to acquire a comprehensive biological insight into the immune mechanisms induced by radiotherapy. In clinical trials, the combination of radiotherapy and immunotherapy shows signs of promising intracranial response but marked improvement in overall survival is yet to be reported. Tumour-instigated immunosuppression remains dominant over attempts to instigate anti-tumour immunities. Thus, modalities that aim to deplete the immune microenvironment of TAMs and inhibit the activity of suppressive cytokines in the immune milieu can lead to better activation of effector T cells.[191,192]

There is a dire need for predictive biomarkers and prognostic scales that permit prospective evaluation of patients on immune checkpoint inhibitors.[193] Diagnostic clinical trials in patients with brain metastases are scarce and suffer from poor trial design and follow-up.[194] The characterization of circulating tumour cells and cell-free DNA can help reveal the molecular foundations of brain metastases and offers the chance to observe possible changes in response to immunotherapy.

Table 4 Ongoing clinical trials using immune checkpoint blockade for brain metastases

NCTID	Strategy	Phase	Start date	Number enrolled	Primary tumour	Primary outcome	Notes and preliminary results
NCT02460068	Fotemustine versus fotemustine + ipilimumab versus ipilimumab + nivolumab	3	2012	168	Melanoma	Overall survival	Recruitment status unknown
NCT02097732	Ipilimumab + SRS	2	2014	4	Melanoma	Intracranial control rate	Active, not recruiting patients
NCT02696993	Nivolumab + SRS ± ipilimumab	1/2	2016	88	NSCLC	Dose-limiting toxicity, progression-free survival	Concurrent SRS with nivolumab/ipilimumab is safe with durable tumour responses[183]
NCT02681549	Pembrolizumab + bevacizumab	2	2016	53	NSCLC, melanoma	Objective response rate	12 of 20 patients had a response: 7 complete and 5 partial[173]
NCT02858869	Pembrolizumab + SRS	1	2016	30	NSCLC, melanoma	Dose-limiting toxicity	The 6-month local control (94.0%), progression-free survival (50%), and overall survival (77.4%) were higher than for monotherapy alone, with no added toxicity[184]
NCT02716948	SRS + nivolumab	1	2016	90	Melanoma	Incidence of adverse events	Recruiting patients
NCT03325166	Pembrolizumab + ferumoxytol	2	2017	20	NSCLC	Sensitivity and specificity of relative cerebral blood volume	Recruiting patients
NCT02978404	SRS + nivolumab	2	2017	26	NSCLC, SCLC, melanoma, renal cell carcinoma	Progression-free survival	In 3 of 8 patients, there was 1 partial response and 2 cases of stable disease. SRS + nivolumab is safe with no acute toxicity[185]
NCT03175432	Bevacizumab + atezolizumab ± cobimetinib	2	2017	60	Melanoma	Objective response rate, safety profile	Recruiting patients
NCT03563729	Steroid + pembrolizumab or ipilimumab + nivolumab	2	2018	80	Melanoma	Progression-free and overall survival	Recruiting patients
NCT03728465	Ipilimumab + nivolumab	2	2018	68	Melanoma	Intracranial control rate	Recruiting patients
NCT03340129	Ipilimumab + nivolumab with SRS	2	2019	218	Melanoma	Neurological cause of death	Recruiting patients
NCT04047602	Checkpoint inhibitor + SRS	Prospective	2019	42	Any solid tumour	Symptomatic radiation necrosis rate	Recruiting patients
NCT04021420	Sonocloud + nivolumab	1	2019	21	Melanoma	Most successful dose	Recruiting patients
NCT04461418	Checkpoint inhibitors + steroids	2	2020	20	Any solid tumour	Objective response rate	Not yet recruiting
NCT03955198	SRS + durvalumab	2	2020	100	NSCLC	Progression-free survival	Recruiting patients
NCT04434560	Neoadjuvant nivolumab versus ipilimumab	2	2020	40	Solid tumour	Proliferation of circulating T cells	Recruiting patients
NCT04187872	LITT + pembrolizumab	1	2020	15	Solid tumour	Immune effect	Recruiting patients
NCT04348747	Anti-HER2/3 dendritic cell vaccine + pembrolizumab	2	2020	23	Breast cancer	Objective response rate	Not yet recruiting patients

SRS = stereotactic radiosurgery; WBRT = whole brain radiation therapy. More information can be found in the Supplementary material.

The integration of machine learning in the assessment and classification of patients with brain metastases seems inevitable to identify tumour-infiltrated diagnostic regions and assess the response of metastatic lesions faster and more accurately.[195] In addition, future studies should continue to explore the genetic, phenotypic and immune regulatory differences between primary tumours and brain metastases.

There are many opportunities to improve the efficacy of immune checkpoint inhibitors in light of reported observations. The timing and sequence of administration of immunotherapy alongside other regimens can be crucial to the overall clinical outcome. In addition, the receptor expression patterns on T cells differ at different anatomical locations.[96] Therefore, enhancing organ-specific T-cell trafficking to the brain is an important goal to achieve. Furthermore, NK cells have been shown to be increased in response to immune checkpoint inhibitors; however, the mechanism of action and ways to potentiate this response remain to be elucidated.

The role of B cells in enhancing the response to immune checkpoint blockade remains unclear. Cell depletion studies reveal that NK cells and CD8+ T cells are required for intracranial anti-PD-1/anti-CTLA4 efficacy.[96] Nevertheless, new studies show that the incidence of B cells in human tumours, such as melanoma and sarcomas, is associated with better response to immunotherapy.[196-198] In primary brain tumours, it was revealed that TAMs can transfer functional PD-L1 via microvesicles to confer on regulatory B cells the ability to suppress CD8+ T-cell activation.[199] Similar mechanisms may be occurring in the setting of brain metastases. In glioma, a B cell-based vaccine, with CD40 agonism and IFNγ stimulation, migrates to key secondary lymphoid organs and is proficient at antigen cross-presentation, which promotes both the survival and the functionality of CD8+ T cells. Combining this vaccine with radiation and PD-L1 blockade conferred tumour eradication in 80% of treated tumour-bearing animals.[200] As such, designing strategic immune therapies that can address B cell responses in brain metastases is essential for more effective treatments.

Brain metastatic cancer cells arising from primary tumours have the propensity and the necessary metabolic properties to colonize the brain. The brain is the organ with the highest energy demand and the ability to rewire its metabolism in response to varying stressors.[201] It poses metabolic challenges to a colonizing cancer cell, ranging from fuel and oxygen availability to oxidative stress. As such, brain metastases display a remarkable metabolic flexibility by utilizing acetate, glutamine and branched-chain amino acids as alternative sources of fuel.[202] Brain metastases upregulate the synthesis of acetyl-CoA synthetase enzyme 2, which allows them to fuel the tricarboxylic acid cycle and convert acetate to acetyl-CoA for energy metabolism.[203] Furthermore, brain metastases oxidize branched-chain amino acids and glutamine to survive and proliferate in the absence of glucose.[204] In addition, brain metastases can produce glucose by upregulating fructose-1,6-bisphosphatase 2 for gluconeogenesis.[204] Comparing the molecular profiles and gene expression patterns of melanoma brain metastases with patient-matched extracranial metastases identified significant immunosuppression and enrichment of oxidative phosphorylation in brain metastases, which was confirmed by direct metabolic profiling and [U-^{13}C]-glucose tracing.[15] Increased oxidative phosphorylation leads to oxygen deprivation and hypoxia in the immune microenvironment, hampering immune cell function. Effector T cells suffer from metabolic insufficiency and dysfunction due to nutrient depletion.[205] Though oxygen metabolism is less vital for T-cell effector function, oxygen consumption is necessary for memory formation and T-cell proliferation.[206] As such, the inhibition of oxidative phosphorylation in the immune microenvironment of brain metastases can help lift the immunosuppression and lead to better immune activity against brain metastatic cells. Metabolic interventions in combination with immune checkpoint inhibitors can further enhance the therapeutic effect against brain metastases.

Delivery of immune therapies and the trafficking of cytotoxic T cells to the brain are hampered by the presence of the blood–brain barrier. Overcoming this barrier is essential to achieve therapeutic benefit akin to what is achieved in extracranial disease. A permeable blood–brain barrier may facilitate the presentation of tumour-associated antigens and enhance the effects of immune cells in the tumour microenvironment. Therefore, strategies that involve physical disruption of the blood–brain barrier can improve antigen presentation, enable immune checkpoint inhibitor transmigration, and increase immune cell trafficking into the immune microenvironment to further sensitize brain metastases to immunotherapies. In recent years, a number of strategies have been explored to hijack barriers posed by the blood–brain barrier. Recent preclinical developments with focused ultrasound allow the delivery of therapeutic agents to the brain that were once deemed undeliverable.[207] In a clinical trial on patients with recurrent glioblastoma, pulsed ultrasound in combination with systemically injected microbubbles showed that the approach is safe and well tolerated.[208] Through focused ultrasound sonication, engineered NK cells against the HER2 receptor were capable of crossing the blood–brain barrier in the setting of breast cancer brain metastases.[209] The use of whole-brain radiation therapy has shown conflicting results in regard to its capability of enhancing the delivery of therapeutic molecules across the blood–brain barrier. In a self-controlled pilot study, whole-brain radiation therapy failed to boost intracerebral gefitinib concentration in patients with brain metastatic NSCLC who were treated with the drug for 14 days.[210] Yet, the permeability of gefitinib after treatment for 30 days increased in accordance with the escalated dose of whole-brain radiation therapy in NSCLC brain metastases.[211] Intravital microscopy and computational modelling show that a single, low dose of radiation therapy can induce transient, dynamic and localized vascular bursting, as well as enlarge blood vessel volume. Increased permeability can facilitate extravasation of immune checkpoint inhibitors from blood vessels in tumours.[212] Nevertheless, the downfalls of whole-brain radiation therapy are its side effects. Cerebral oedema may be

induced or worsened after the initiation of whole-brain radiation therapy. As a result, whole-brain radiation therapy is usually preceded by corticosteroid therapy, which may affect the efficacy of immune checkpoint blockade.[213] Therefore, finding the right balance between the safety profile and the therapeutic benefits of interventions, such as focused ultrasound and whole-brain radiation therapy, is vital in assessing ways to enhance the penetration of immune checkpoint inhibitors into the tumour microenvironment of brain metastases. Moreover, the translation of some of these preclinical modalities to the brain metastatic clinical setting is necessary for proper evaluation of efficacy and outcomes.

Exploring new models for the induction of immune checkpoint blockade is necessary to potentiate the effects of immunotherapy. Preclinically, nanotherapy in conjunction with photothermal induction and immune checkpoint blockade has been shown to be effective in brain tumours.[214] Targeted nanoscale immunoconjugates presented on a natural biopolymer scaffold, poly(β-L-malic acid), with covalently attached anti-CTLA4 or anti-PD-1 antibodies for systemic delivery across the blood–brain barrier and activation of local brain anti-tumour immune response resulted in an increase in CD8+ T cells, NK cells and macrophages along with a decrease in regulatory T cells in the tumour microenvironment of gliomas.[215] It also significantly increased survival when compared to animals treated with single checkpoint inhibitor-bearing nanoscale immunoconjugates or free anti-CTLA4 or anti-PD-1 antibodies.[215] Moreover, therapeutic targeting of PD-L1 on TAMs using nano-immunotherapy synergizes with radiation in primary brain tumours.[216] Similar strategies can be used against brain metastases. Furthermore, preclinical data demonstrate that neural stem cells can deliver systemic agents that target brain metastases from melanoma and breast cancer.[217,218] In addition, delivering apoptosis-promoting genes to the immunosuppressive TAMs in brain metastases can be done through haematopoietic stem cells.[26] Thus, utilizing the inherent ability of stem cells to cross the blood–brain barrier and rocket towards tumours can help in increasing immune targeting of brain metastases. Moreover, oncolytic viruses have shown promise in targeting primary brain tumours and potentiating the immune response against tumour cells.[219] The clinical delivery of oncolytic viruses to brain metastases has yet to be done. Preclinical data show that intravenous delivery of an oncolytic *Orthoreovirus* can immunologically prime brain metastases against PD-1 blockade.[220] In addition, the utilization of CAR T cells to target HER2-positive breast cancer brain metastases offers a new immunotherapeutic medium for exploration.[221]

The rise of cancer neuroscience as a field opens a window to explore how the immune microenvironment interacts with its neural counterpart in the setting of brain metastases. Given how astrocytes affect the immune response towards tumour cells, it is rational to believe that the nervous tissue contributes in one way or another to the immunogenicity of brain metastases. Recent discoveries reveal that excitatory neurotransmission enables NMDAR signalling in brain metastases.[95] Breast cancer cells that have metastasized to the brain upregulate neurotransmitter receptor expression and extend perisynaptic processes to receive neuronal activity-dependent neurotransmitter signals that trigger a receptor-mediated signalling cascade, induce inward currents in the malignant cells, and drive growth of breast cancer brain metastases.[95] In gliomas, glutamatergic synaptic input and electrochemical communication drives brain tumour progression.[222,223] Dissecting the neural–immune–cancer interactions is necessary to obtain a comprehensive picture of how brain metastases progress through immune checkpoint evasion. In fact, these interactions may be contributing to the effect of immunotherapies in the brain.

Conclusion

The brain continues to be a special organ in terms of immune regulation, with the blood–brain barrier being a major contributor to this immune state. The immunosuppressive role of the tumour microenvironment in the setting of brain metastases emphasizes the need for new therapeutic strategies that can reverse immunosuppression. These strategies should aim to favour the M1 state of macrophages or help in the recruitment of cytotoxic T cells. They can also target immunosuppressive elements like regulatory T cells and TAMs. So far, immunotherapy has failed to show significant benefit in breast cancer brain metastases (unlike in metastatic melanoma and NSCLC). As such, approaches that aim to flip cold brain metastatic tumours to hot ones should be emphasized and the potential risk of inducing autoimmunity should be evaluated. It is also important to consider that immunosuppression is a protective mechanism that keeps the brain safe from excessive inflammation and subsequent oedema that may harm the sensitive structures of the brain, leading to damage. Understanding the biological underpinnings and detailed mechanisms that are implicated in immunosuppression of brain metastases is vital to design novel immune interventions that are safe, efficacious and can elicit an enduring response. Embracing combination therapy with immune checkpoint inhibitors seems to yield better clinical outcomes and survival benefit. As such, strategies that are based on the biological mechanisms of these inhibitors should be adopted. Developing appropriate preclinical models that recapitulate the immune microenvironment in the setting of brain metastases is a necessity for the advancement of novel therapies that can be translated to clinical practice.

Funding

This work was supported by NIH grants R35CA197725 (M.S.L.), R01NS87990 (M.S.L.) and R01NS093903 (M.S.L.), and by the Ministry of Science and Higher Education of the Russian Federation grant №075-15-2020-926 (I.U.).

Competing interests

The authors report no competing interests.

Supplementary material

Supplementary material is available at *Brain* online.

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