

The inflammatory microenvironment in brain metastases: potential treatment target?

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Abstract: Brain metastases (BM) are frequent in cancer patients and are associated with high morbidity and mortality. The incidence of BM is increasing and only limited treatment strategies are available. Immunomodulatory agents are emerging as effective cancer therapeutics and a deeper understanding of the inflammatory microenvironment in BM might reveal new treatment possibilities. Although the brain has long been considered an ‘immune-privileged’ organ with limited capacity for inflammatory response, BM do contain tumor infiltrating lymphocytes (TILs). Moreover, dense infiltration with TIL showed an association with favorable patients’ overall survival times. Microglia/macrophages were shown to be involved in the pivotal steps of BM formation by providing a “pre-metastatic niche” attracting BM initiating cells and to promote growth and survival of tumor cells in the CNS microenvironment. The anti-cytotoxic T lymphocyte antigen 4 (CTLA4) immune checkpoint inhibitor ipilimumab showed activity against melanoma BM in a clinical trial, thus providing proof of concept for effective therapeutic immunomodulation in patients with CNS metastases. Collectively, the available data show that BM harbors an active inflammatory microenvironment that may be exploited as treatment target.

Keywords: Brain metastases (BM); tumor infiltrating lymphocyte (TIL); inflammatory microenvironment; checkpoint inhibitor

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Introduction

Brain metastases (BM) are devastating complications occurring in up to 40% of cancer patients. Only limited treatment options exist upon the occurrence of BM, resulting in the limited survival prognosis of BM patients ranging from only about few weeks to months (1). There is a strong need for the identification of novel treatment modalities to improve patient outcomes.

Recently the inflammatory microenvironment was identified to have prognostic and therapeutic implications in various malignancies. Infiltration with T-cell as well as tumor associated macrophages (TAMs) correlate with patients’ survival times in several extracranial malignancies (2-5). Of particular importance is the recent introduction

of effective immunomodulatory anti-cancer agents. The monoclonal antibodies ipilimumab, nivolumab and pembrolizumab activate the anti-tumor T-cell response by interfering with tumor-associated immunosuppression induced via the immune checkpoint molecules. Ipilimumab inhibits the T-cell co-receptor cytotoxic T lymphocyte antigen 4 (CTLA4), while nivolumab and pembrolizumab inhibit the T-cell co-receptor programmed cell death 1 (PD1). These drugs have shown remarkable and durable responses in several tumor types including melanoma, lung cancer, Hodgkin’s lymphoma and others (6-8). A large number of clinical trials evaluating immune checkpoint inhibitors in various malignancies are underway and immunotherapy is very likely to quickly become a standard treatment modality in oncology. However, so far little

is known about the inflammatory microenvironment of BM and whether it could serve as feasible treatment target. The CNS has long been considered an “immune-privileged” organ with limited capacity of inflammatory response. However, this concept has been challenged and it is becoming clear that neoplastic processes in the brain may induce prominent anti-tumor immune responses. In the following we will discuss the characteristics of the inflammatory microenvironment in BM and its potential as clinical biomarker and therapeutic target.

Interaction of immune system and BM initiating cell in the brain metastatic cascade

Immune escape is an emerging hallmark of cancer (9). Presumably, BM initiating cancer cells have to evade immune attack several times during the brain metastatic cascade. However, besides immune escape, immune cells were postulated to have supporting functions in the process of metastatic spread and are adapted by the cancer cell to support their growth. In the following paragraph we discuss the steps of the brain metastatic cascade and the involvement of the immune system in the single steps of BM development.

First BM initiating cells have to disconnect from the primary tumor (or an extra-cranial metastasis) and enter the blood stream. Here, TAMs were shown to have a metastasis promoting function as they contribute to the degradation of the extracellular matrix and thereby facilitate intravasation (2). Macrophages were identified to enhance cancer cell intravasation upon physical contact as observed by using high resolution imaging techniques including repetitive *in vivo* multi-photon laser microscopy. Cancer cell—macrophage contact promotes formation of actin-rich degradative protrusion enabling the cancer cell to degrade matrix barriers in the process of trans-endothelial migration (10). Further, tumor cells were shown to synthesize colony-stimulating factor 1 (CSF1) to stimulate macrophages. This causes the clustering of TAMs and cancer cells around blood vessels and promotes intravasation and the formation of metastases (11). In line, experiments with macrophage-deficient mice showed normal progression of the primary tumor but prevention of metastases outgrowth (12).

Further, T cells were shown to impact the metastatic potential of a primary tumor. Dense infiltration with tumor infiltrating lymphocytes (TILs) was shown to correlate with survival prognosis in various extracranial malignancies,

arguing that TIL density might reduce metastatic spread (3). In line, preclinical models revealed that depletion of CD4⁺ and CD8⁺ T cells results in the increased development of BM (13).

Upon arrival in the brain vasculature, cytokines were shown to promote the site-specific metastatic behavior of BM initiating cancer cell. Of particular interest in the brain metastatic cascade are the chemokine receptor CXCR4 and its ligand CXCL12 (stromal cell derived factor 1 alpha). The chemokine pair CXCR4/CXCL12 were shown to contribute to brain-specific metastatic behavior by promoting adhesion of the BM initiating cells to the brain vasculature and further facilitate the migration through the brain microvascular endothelial cells (14,15).

The process of extravasation from the vasculature into the parenchyma was shown to be promoted by TAMs in the case of extracranial malignancies. Co extravasation might have tumor promoting properties, as TAMs can express growth and survival factors, promote angiogenesis and remodeling of the extracellular matrix like during the process of intravasation (2,12,16). The specific recruitment of TAMs might even precede the BM development as they were shown to be involved in the preparation of the “pre-metastatic niche”, i.e., the preparation of a certain site for the homing of circulating tumor cells (17). Further, preclinical models suggest that myeloid derived suppressor cells are also involved in the formation of the “pre-metastatic niche” in breast cancer BM (18). Importantly, inhibition of TAMs as well as of myeloid derived suppressor cells was postulated to have preventive potential, as the interaction of these two cell types with BM initiating cells represents a pivotal step in the brain metastatic cascade (18,19). However, so far only few preclinical models have investigated this theory and further studies on the exact interaction of host T cells and BM initiating cells are warranted.

Due to increased expression of vascular endothelial growth factor (VEGF) by TAMs, vascular permeability is increased and thereby extravasation is facilitated. After the trans-passing of the endothelium, TAMs promote cell survival, growth and invasion via the maintenance of CSF1 signaling within the perivascular niche (20,21). However, the exact involvement of TAMs and especially the importance of microglia cells with phagocytic activity in comparison to blood derived macrophages within the brain metastatic cascade remain to be investigated.

TAMs were associated with the “angiogenic switch”, i.e., induction of angiogenesis during the outgrowth from micro- to macrometastases. Neoangiogenesis is

promoted by the production of VEGF by TAMs (22). This is of major interest in the brain metastatic cascade, as the induction of neoangiogenesis is considered one of the pivotal steps during BM outgrowth. The formation of NSCLC macrometastases was shown to depend on the induction of neoangiogenesis, as the prophylactic application of the VEGF antibody bevacizumab resulted in demise of micrometastases before the outgrowth of macrometastases (23).

Further, TAMs were shown to express interleukin 10 and transforming growth factor β (TGF- β) and thereby stimulate regulatory T cells, an immunosuppressive subpopulation of T cells characterized by the expression of FOXP3, which contribute to an immunosuppressive tumor microenvironment (24). In line, the combination of dense infiltration with TAMs and a high ratio of regulatory versus cytotoxic T cells are associated with poor survival in breast cancer (2).

Overall, the immune system seems to be important throughout multiple steps of BM formation and deeper insight into the interaction of cancer cells and the immune system may offer opportunities for rational drug-mediated interference with the goal to prevent of CNS metastases in cancer patients.

Composition and potential clinical value of the inflammatory microenvironment of established brain metastases (BM)

The main cell types involved in the inflammatory microenvironment of BM microglia/macrophages, B lymphocytes and T lymphocytes.

Microglia cell/macrophages

Microglia cells are the main effector cells of the innate immune system in the brain parenchyma (25). Under healthy condition, microglia cells are motile and constantly move through the brain parenchyma. Therefore a heterogeneous distribution of microglia cells can be observed within the healthy brain (26). Upon activation, microglia cells may exert several functions. First, microglia cell can induce cytotoxicity via the release of nitric oxide (NO). However, only minor expression of factors associated with NO release was observed within and around human BM, indicating that this cytotoxic pathway has only minor importance in BM (27). A second function of microglia encompasses phagocytosis (25). Microglia cells

with phagocytic properties belong to the population of TAMs within BM. The population of TAMs however also encompasses blood-derived macrophages and no clear cut difference between macrophages of microglia origin and of blood origin can be made by the current available methods (21). Two functional phenotypes of TAMs were postulated. The M1 TAMs are defined by tumor suppressive functions like inhibition of cell proliferation. The M2 TAMs are postulated to have rather tumor promoting functions including suppression of adaptive immune response and promotion of tumor cell migration, invasion, as well as intravasation into the vascular system, angiogenesis and persistent growth (28,29).

A further important function of microglia cells in the inflammatory microenvironment of BM is T-cell activation via expression of the HLA ABC/major histocompatibility antigen class I. Therefore, microglia activation is mandatory for the induction of a specific immune response including T and B cells (25). However, microglia cells are also capable of immune suppressive functions. PD-L1 expression, the ligand of the inhibitory T-cell co-receptor PD1, was observed on microglia cells (30). Via activation of CTLA4 or PD1, microglia cells can suppress the anti-tumor T-cell response and participate in the generation of an immune suppressive tumor microenvironment. Microglia has also been described to enhance invasion and colonization of brain tissue by cancer cells by serving both as active transporters and guiding rails (31).

Myeloid-derived suppressor cells

Myeloid-derived suppressor cells are a heterogeneous population of immature myeloid cells and are involved in several immunosuppressive functions. Within cancer tissue they were shown to be highly involved of the formation of the “pre-metastatic niche” as they prepare the tissue for the infiltration of metastatic tumor cells (32). Further, myeloid-derived suppressor cells are known to inhibit T-cell activation via cytokine production and promote angiogenesis and facilitate tumor metastasis and invasion (3). Increased circulating of myeloid-derived suppressor cells is associated with poor prognosis in lung cancer patients (33).

T cells

T cells are the main effector cells of the adaptive immune system. Lymphocytes are mostly absent in the healthy brain parenchyma and migrate into the CNS under pathological condition (34). Various subtypes of TILs can be observed

in the inflammatory microenvironment of BM and they differ in function and prognostic relevance (35). CD3⁺ TILs reflect the overall population of effector T cells. CD8⁺ TILs represent cytotoxic T-cell and CD45RO⁺ TILs, the so-called memory T cells, which are observed after an initial immune response as a sign of immunological memory. FOXP3⁺ TILs are so called regulatory T cells and have immunosuppressive function. They suppress the function of CD8⁺ cytotoxic T-cell and participate in the generation of an immune suppressive microenvironment. Importantly, dense infiltration with CD3⁺, CD8⁺ or CD45RO⁺ TILs is associated with improved survival prognosis in several extracranial malignancies like colorectal cancer, breast cancer and ovarian cancer (4,36,37). Dense infiltration with the immunosuppressive subtype of FOXP3⁺ TILs however is associated with impaired survival prognosis e.g. in lung cancer and breast cancer (38,39). Similar impact has been observed in liver and lung metastases of colorectal cancer as well as in BM, as patients with dense infiltration of TILs presented with an improved survival prognosis as compared to patients with only sparse or absent TIL infiltration in the resected metastases (35,40,41).

B cells

The role of B cells in the inflammatory microenvironment has only been poorly investigated so far. Fewer B cells were observed in the matched BM as compared to the primary tumor in triple negative breast cancer (42). The involvement of B cell is supported by the observation that in preclinical models the metastatic burden is increased as a result of B cell depletion (43). The B cell depletion causes reduced T-cell response, thus arguing for a strong interaction of B cells and tumor repression via T-cell activation (16).

Potential role of TILs infiltration of brain metastases (BM) as biomarker

TIL infiltrates of variable density can be observed in BM and are typically composed of various cell types with a higher fraction of T cells than B cells (16,35,44). An accumulation of TILs was reported within the tumor stroma and in the border region to the surrounding brain parenchyma, while the intratumoral density was less pronounced. The lymphocytic infiltrates presented with a mixed composition of both immuno-activating TIL subsets such as CD3⁺ effector and CD8⁺ cytotoxic TILs, memory TIL subsets CD45RO⁺ and immunosuppressive TIL subsets such as FOXP3⁺, and PD1⁺ TILs. A strong

correlation with survival prognosis was observed as patients with dense infiltration with of CD3⁺, CD8⁺ and CD45RO⁺ TILs presented with a significantly prolonged survival as compared to patients with only sparse or scattered TIL infiltration. Furthermore, the Immunoscore, which is based on automated calculation of the CD3⁺/CD8⁺ ratio among TILs and has been elaborated in colorectal cancer specimens, showed an independent and strong impact also in BM patients (5,35). These findings argue for an impact of TIL infiltration irrespective of tumor stage and strongly support the application of immune modulatory agents also in patients with metastatic disease. Future studies are needed to determine whether TIL infiltration correlates with response to immunomodulators and may serve as clinically relevant predictive biomarker.

The inflammatory microenvironment as a potential therapeutic target in brain metastases (BM)

Recently a new class of anti-cancer agent, namely immune checkpoint inhibitors, has shown remarkable clinical activity in several extracranial malignancies, including some of the most frequent primary tumors causing BM, such as melanoma and non-small cell lung cancer (8,45,46). Immune checkpoint inhibitors exert their anti-cancer activity via the activation of the T-cell response, as they target inhibitory co-receptors of the T-cell receptor and thereby boost the anti-tumor T-cell response (47). The so far approved agents targeted the CTLA4 (ipilimumab) and the PD1 (pembrolizumab, nivolumab) axis.

Ideally BM patients would be selected based on predictive biomarkers for new targeted therapies, however little is known so far on predictive biomarkers for the response to immune checkpoint inhibitors (48). Dense infiltration with TILs was postulated as a predictive marker for response to ipilimumab, as in theory the increased T-cell response is more potent in an immune active microenvironment (49). An immune active microenvironment, as resembled by a dense infiltration of TILs, was observed in neuro-surgically resected BM, especially in melanoma, renal cell carcinoma and NSCLC BM (35). As re-biopsy is not always feasible, radiological surrogate parameters for the identification of an immune active microenvironment are of special interest in BM patients. Interestingly, a dense infiltration with CD8⁺ TILs showed an association with large peritumoral edema, which could serve as a surrogate parameter (50). Prospective clinical trials are warranted to further investigate the correlation of TIL infiltration and radiological parameters

as well as the response to immune checkpoint Inhibitors.

Another proposed predictive biomarker for response to PD1 axis targeting immune checkpoint inhibitors is expression of the ligand of PD1, namely PD-L1, on the cancer cells (51). PD-L1 expression was frequently observed in melanoma and NSCLC BM specimens, indicating that the PD1 axis is also active BM and might serve as a potential treatment target (44,52).

Clinical data on immune checkpoint inhibitors in BM patients are scarce. So far only treatment with ipilimumab in patients with newly diagnosed melanoma BM has been investigated in a prospective clinical trial. ipilimumab monotherapy presented with intracranial response rates up to 24% in patients with newly diagnosed, asymptomatic melanoma BM, while symptomatic patients in need for steroid treatment presented only with response rates around 10% (53). The extracranial response rate for ipilimumab in newly diagnosed melanoma patients also ranges around 25%, indicating that the intracranial response rate in patients not in need for steroid treatment is comparable to the extracranial one (54). The reasons for the different response rates between symptomatic and asymptomatic patients might be caused by the steroid treatment. Steroid treatment is routinely used in BM patients to reduce the peritumoral edema and might resemble a challenge in the treatment of BM patients with immune checkpoint inhibitors as steroids suppress the immune response and therefore the immune checkpoint inhibitor treatment might not reveal its full potential in symptomatic patients under steroid treatment (53). So far little is known on the interaction of steroid treatment and the possibility of continued response to Immune Checkpoint Inhibitors. Retrospective investigations suggest that patients with immune related adverse events caused by immune checkpoint inhibitors might still respond after the need of steroid treatment (55). However, the up-front steroid treatment in BM patients might have a negative impact on antineoplastic efficacy of immune checkpoint inhibitions (56). Another challenge in the treatment of BM patients is the possibility of pseudo-progression and subsequent symptom burden due to increased edema. Patients might present with stabilization or even an increase in tumor size due to infiltrating lymphocytes before actual tumor shrinkage (57). In the special situation of BM, this phenomenon may be of particular concern and may require symptomatic treatment e.g., with steroids, which might influence the treatment potency. A possibility to overcome the clinical challenges associated with intracranial

pseudoprogression might be the combination with an anti-angiogenic agent like bevacizumab. The VEGF antibody bevacizumab has shown favorable results in the treatment of peritumoral edema and (radio)necrosis in BM patients and might further have synergistic treatment effects (50). Further, BM patients have an impaired survival prognosis and are in need for an immediate tumor control. Therefore, BM patients might not experience the delayed tumor response of immune checkpoint inhibitors. The combination of a local radiotherapy with an immune checkpoint inhibitor treatment might therefore be a treatment option, proving local control and time for the immune checkpoint inhibitor to reveal its therapeutic potential (58). Further the so-called “abscopal” effect is of special interest in BM patients. The abscopal effect refers to the observation that an increased response to Immune Checkpoint Inhibitor treatment may occur in non-irradiated lesions after radiation of distinct tumor manifestations. In theory, the radiation causes increased antigen reveal and therefore an increased activation stimulus for the T cells (59). Some retrospective observations underscore the abscopal effect theory as patients receiving ipilimumab before or after radiation had an improved survival as compared to patients receiving ipilimumab before radiation only (58). However, increased rates of radio necrosis were observed in patients with melanoma BM treated with the combination of ipilimumab and stereotactic radiosurgery (58).

A further challenge is the response assessment as already mentioned an initial increase in tumor size and well as number of metastases followed by delayed response can be observed (57). Therefore, the Response Assessment in Neuro-Oncology (RANO) group founded a dedicated subdivision to formulate intracranial response criteria for patients treated with an immune checkpoint inhibitor (iRANO). Currently some studies investigate the impact of Immune Checkpoint Inhibitors as well as the sequencing with radiotherapy in patients with BM (Table 1).

Conclusions

Several lines of evidence show a prominent involvement of the immune system in the pathobiology of BM. The interaction of cancer cells and the immune system seems to crucially involved in several steps of BM formation and established BM contain considerable inflammatory infiltrates composed of various immune cells. The density of TILs in BM has been described to correlate with patient

Table 1 Selected on-going, recruiting trials on immune checkpoint inhibitors in brain metastases (source: www.clinicaltrial.gov)

| Entity | Immune checkpoint inhibitor | Combination therapy | Biomarker | Primary endpoint | Design | ClinicalTrials.gov identifier |
|---|-----------------------------|--|--|---|----------|-------------------------------|
| Newly diagnosed melanoma BM | Ipilimumab | WBRT | None | 1 year survival rate | Phase II | NCT02115139 |
| Previously untreated or progressive diagnosed asymptomatic melanoma BM | Ipilimumab | SRS | MRI parameters | Local control rate | Phase II | NCT02097732 |
| Newly diagnosed asymptomatic melanoma BM | Ipilimumab; nivolumab | none | PD-L1 status; blood biomarkers: T-cell subsets, myeloid derived suppressor cells | Intracranial response rate | Phase II | NCT02374242 |
| Newly diagnosed or progressive after local treatment but asymptomatic melanoma BM | Ipilimumab; nivolumab | none | None | CNS clinical benefit rate | Phase II | NCT02320058 |
| Newly diagnosed melanoma BM | Ipilimumab | WBRT or SRS | None | Maximum tolerated dose of Ipilimumab | Phase I | NCT01703507 |
| Newly diagnosed melanoma BM | Ipilimumab | SRS | None | Number of adverse events and severity of adverse events | Phase I | NCT01950195 |
| Melanoma BM | Ipilimumab | SRS | Blood biomarkers: absolute lymphocyte count, T-cell activation markers, cytokines, T-regulatory markers, co-stimulators molecules, T-cell activation markers, T-cell suppression markers | Progression free survival | Phase II | NCT02107755 |
| Previously untreated asymptomatic NSCLC BM with no evidence of cerebral edema | Ipilimumab; nivolumab | Gemcitabine; cisplatin; pemetrexed; carboplatin; bevacizumab | None | Safety and tolerability | Phase I | NCT01454102 |
| Previously untreated asymptomatic NSCLC or melanoma BM | Pembrolizumab | none | None | Response rate | Phase II | NCT02085070 |
| BM, brain metastases. | | | | | | |

survival times and emerging data support a clinically relevant activity of immunomodulatory drug therapy with immune checkpoint inhibitors in patients with BM. More research is needed to better understand the role of the immune system in BM and clinical trials should specifically enroll BM patients to investigate the risks and benefits of immune therapies in this patient population.

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