Melanoma brain metastases: review of histopathological features and immune-molecular aspects

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Practice points

- Melanoma brain metastases (MBM) most commonly develop in the gray–white matter junction and major vascular border zones in the cerebral hemispheres, particularly the frontal lobe.
- MBM harbor additional oncogenic drivers not expressed in primary lesions and are associated with intertumoral and intratumoral genomic heterogeneity.
- Hyperactivation of the PI3K/AKT/mTOR signaling pathway represents a facilitator of melanoma progression and brain metastasis formation.
- Compared with other tumor types, the infiltrate of MBM shows the highest density of CD8⁺ T cells. Melanoma cells can evade adaptive immune response due to the overexpression of coinhibitory molecules, or recruitment of regulatory T cells, which suppress cytotoxic T lymphocytes response.

Patients with melanoma brain metastases (MBM) have a dismal prognosis, but the unprecedented advances in systemic therapy alone or in combination with local therapy have now extended the 1-year overall survival rate from 20–25% to nearing 80–85%, mainly in asymptomatic patients. The histopathological and molecular characterization of MBM and the understanding of the microenvironment are critical to more effectively manage patients with advanced melanoma and to design biologically driven clinical trials. This review aims to give an overview of the main histopathological features and the immune-molecular aspects of MBM.

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"There could well be survival advantage in being able to recognize the presence of cells carrying wrong molecular configurations and to eliminate them from further proliferation. It would profit the organism to maintain a surveillance over the orthodoxy of its chemical structure and to stamp out heresy before it could spread. To be able to do this would require just such a mechanism as is called for by the facts of immune tolerance."

Sir Frank Macfarlane Burnet
Nobel Lecture, 12 December 1960

Histopathology of melanoma brain metastases

Melanoma brain metastases (MBM) usually present as single or multiple well-circumscribed solid or partially cystic lesions in the brain parenchyma. MBM are often hemorrhagic, clinically appearing as intracerebral hemorrhage, and they are surrounded by substantial vasogenic edema, which causes mass effect with neurologic symptoms including headache, focal deficit and seizures [1–3]. The most common localization is the gray–white matter junction and the major vascular border zones in the cerebral hemispheres, particularly the frontal lobe [4,5].
Figure 1. Immunological players in melanoma brain metastases. The infiltrate of MBM is mainly composed of CTLs, which are situated around vascular structures and at the border zone between tumor tissue and peritumoral brain parenchyma. In addition, scattered Treg, memory T cells and B cells can be found. However, the lymphocytic infiltrate is absent in areas of necrosis or in regions of brain parenchyma other than the interface with MBM [6]. Brain-resident cells interacts with the tumor and the immune cells. CTL: Cytotoxic T lymphocyte; MBM: Melanoma brain metastases; MMP2: Matrix metalloproteinase-2; Treg: Regulatory T cells.

On histologic examination, MBM are characterized by variably pigmented spindled and epithelioid cells with abundant cytoplasm, large nuclei and prominent nucleoli, organized in nests frequently interlaced by extravasated red blood cells. MBM can show a significant lymphocytic infiltrate, primarily composed of cytotoxic T cells (CD8+) associated with interspersed regulatory T cells (FoxP3+), memory T cells (CD45RO+) and B cells (Figure 1) [6].

Immunohistochemically, the assessment of HMB-45 (human melanoma black-45), melan-A/MART-1 (melanoma-associated antigen recognized by T cells), tyrosinase and SOX10 (SRY-related HMG-box) expression can help orientate the diagnosis toward a MBM, especially when an undifferentiated brain tumor is found and metastatic amelanotic melanoma is suspected [7-9]. Protein S100 is highly sensitive, but not specific, as it is also expressed by neurons, astrocytes and glia [10]. Although immunohistochemistry remains the cornerstone of tumor diagnostics, it has been recently suggested that BrainMETH classifiers based on DNA methylation profiling could serve as an effective ancillary tool in accurately diagnosing challenging cases; specifically, patients with occult primary tumors or poorly differentiated brain metastatic lesions [11].

Considering genetic mutations, 50–55% of MBM are BRAF mutated, 15–22% NRAS mutated and 11% KIT mutated, while multiple mutations are found in only 2% of cases [7,12-14]. BRAF and NRAS genetic testing can contribute to the diagnosis of poorly differentiated metastatic melanoma versus sarcoma when immunostaining results are negative [15]. Ninety percent of all BRAF mutations involve the substitution of amino acid valine by glutamic acid in the activating segment of the kinase domain of BRAF (Val600Glu or V600E), compared with a smaller percentage of cases in which lysine substitutes valine (Val600Lys or V600K), resulting in a constitutive activation of the mitogen-activated protein kinase (MAPK) signal transduction pathway, which leads to proliferation, invasion and metastatic tumor potential [16,17]. Remarkably, MBM not only diverge from primary melanoma, as they harbor additional variants not expressed in primary lesion, but also show interlesional and even intralesional genomic heterogeneity [18]. In relation to the progression of melanoma from its precursors, the polyclonality typically occurs at the later stages of evolution, when melanoma cells become invasive [19].

The crucial initial steps necessary for melanoma metastases are local invasion, epithelial-to-mesenchymal transition and intravasation; these processes require melanoma cell motility, remodeling of extracellular matrix (ECM) and stromal interaction [20]. The dissemination to the brain is hematogenous. Upon reaching the brain vessels,
metastatic melanoma cells arrest by size restriction in capillary bed at branch points, attach to endothelial cells, actively extravasate, transmigrate across the blood–brain barrier (BBB) via disruption of tight junctions and degradation of ECM proteins, and finally, seed into the brain parenchyma [21,22]. Extravasated cells must perpetuate a close perivascular position in order to survive, and they initially proliferate by co-opting existing microvessels [23]. The angiotropic dissemination of melanoma cells is predominantly due to extravascular migration along the external walls of brain microvasculature, and correlates with the expression of Serpin B2 that is the plasminogen activator inhibitor type 2 [24]. Angiogenesis is essential to proliferation and survival of melanoma cells and is promoted by different factors: VEGF attracts angioblasts, induces endothelial cell proliferation, vasculogenesis and vascular remodeling; bFGF contributes to vascular formation; IL-8 increases the permeability of the blood–tumor barrier; matrix metalloproteinases fragment ECM; integrins enhance the expression of matrix metalloproteinases [25,26]. Bevacizumab, a recombinant humanized monoclonal antibody against VEGF-A, has been used in advanced melanoma and is currently in clinical trials in patients with MBM (Table 1). In addition, VEGF has pleiotropic effects: it causes inhibition of dendritic cell maturation and antigen presentation as well as influences lymphocyte vascular trafficking [27]. Indeed, combination of antiangiogenic therapy targeting the VEGF-A pathway and ipilimumab resulted in endothelial activation-favoring lymphocytes migration into tissues and an increase in circulating memory T cells [27].

The brain is not an innocent bystander in MBM formation. Interaction between melanoma cells and astrocytes facilitates invasion resulting in the release of IL-23 by astrocytes and the upregulation of matrix metalloproteinase-2 in melanoma cells (Figure 1) [28]. Furthermore, metastasis-associated astrocytes produce CXCL10, a proinflammatory chemokine that attracts T cells but also melanoma cells via CXCR3 and it has been found to be elevated in the cerebrospinal fluid of patients with MBM [29,30]. Given that melanocytes and neuronal cells share common embryologic origin from the neural crest, melanoma expresses neurotrophin receptors (p75NTR and TrkC), regulated by NGF and neurotrophin-3, which can be secreted by astrocytes at the stromal-tumor border, thus promoting invasion and supporting melanoma metastasis formation [31]. Incipient MBM determine both astrogliosis, which is the primary response of astrocytes to brain insult, characterized by proliferation, migration to the site of invasion and upregulation of glial fibrillary acidic protein and neuroinflammation, associated with increased BBB permeability [32]. Microglia, the brain-resident macrophages and effector cells of the innate immune system, have a direct tumoricidal activity, mediated by the production of nitric oxide [33], and contribute to the adaptive immunity via activation of tumor-specific T cells and B cells. Conversely, microglia may promote neoplastic invasion by expression of PD-L1 (programmed death-ligand 1) and inhibition of tumor-specific cytotoxic T cells [22,34].

Hyperactivation of the PI3K/AKT/mTOR signaling pathway represents a facilitator of melanoma progression and brain metastasis formation, as it has been evidenced in BRAF mutated, stage IIIB/IIIC melanomas [21,35]. It most commonly results from inactivation or deletion of phosphatase and tensin homolog (PTEN), a lipid phosphatase, which dephosphorylates phosphatidylinositol-3,4,5 triphosphate to phosphatidylinositol-4,5 biphosphate, thus antagonizing the pro-oncogenic effect of phosphatidylinositol 3-kinase (PI3K) and reducing AKT phosphorylation [21]. The brain microenvironment, in turn, favors PI3K/AKT/mTOR pathway aberrant activation, because astrocytes secrete exosomes containing miRNAs, which epigenetically, induce PTEN loss in melanoma cells through reversible PTEN mRNA and protein downregulation [36]. Because of these findings, targeting PI3K/AKT pathway may represent a treatment opportunity in patients with MBM [37]. Buparlisib, a pan-class I PI3K inhibitor, halts hyperactivated AKT and induces apoptosis in melanoma cells [38]. Unfortunately, buparlisib in combination with MAPK inhibitors determined increased toxicity [39], this was also observed with uprosertib, an ATP-competitive pan-AKT inhibitor, in association with trametinib [40]. In order to overcome drug-related toxicity limits, isoform-specific PI3K inhibitors could be used or PIK3CA mutant-selective inhibitors could be developed [41].

**Molecular immunological features of melanoma brain metastases**

The brain is an immunologically unique and specialized organ, given the existence of a highly selective barrier (BBB) and the presence of a functional meningeal lymphatic system that drains cerebrospinal fluid to the deep cervical lymph nodes, instead of classical lymphatic drainage [42–45]. The immune response against melanoma is innate and adaptive. Melanoma is an immunogenic tumor, characterized by the expression of multiple antigens targetable by the host immune system such as MAGE-A (melanoma-associated antigen), MART-1, tyrosinase (an enzyme involved in melanin synthesis) or glycoprotein 100 (gp100, a transmembrane glycoprotein found in melanosomes) [46,47]. Compared with other primary tumor types, the infiltrate of MBM shows the highest density of CD8+ T cells [48]. In order to be activated against melanoma, CD8+ T cells need three ‘positive signals’, which

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**Table 1**

| Therapy | Result |
|---------|--------|
| VEGF    | Increases permeability of BBB |
| Bevacizumab | Inhibits dendritic cell maturation |
| CXCL10  | Attracts T cells and melanoma cells |
| PTEN    | Downregulated in melanoma cells |
| PD-L1   | Inhibits tumor-specific cytotoxic T cells |
| BRAF    | Mutated in stage IIIB/IIIC melanomas |
| PI3K/AKT/mTOR | Alters melanoma progression |
Table 1. Ongoing and recent clinical trials of immunotherapies and targeted therapies in melanoma brain metastases according to clinicaltrials.gov (as of February 2020).

| Disease subtype | Intervention | Target | Phase | Study number | Open Status |
|-----------------|--------------|--------|-------|--------------|-------------|
| BRAF V600-mutated MBM | Vemurafenib + cobimetinib | post-SRS | Phase II | NCT0391859 | R (2019) |
| BRAF V600-mutated MBM | Encorafenib + binimetinib | pre-WBRT/SRS | Phase II | NCT03930360 | R (2019) |
| BRAF V600-mutated MBM | Vemurafenib + cobimetinib | post-SRS | Phase II | NCT02049316 | Y R (2018) |
| BRAF V600-mutated MBM | Encorafenib + binimetinib | pre-WBRT/SRS | Phase II | NCT03930360 | R (2019) |
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| BRAF V600-mutated MBM | Encorafenib + binimetinib | pre-WBRT/SRS | Phase II | NCT03930360 | R (2019) |
### Table 1. Ongoing and recent clinical trials of immunotherapies and targeted therapies in melanoma brain metastases according to clinicaltrials.gov (as of February 2020) (cont.).

| Disease subtype | Intervention | Target | Phase | Study number | Open | Status |
|-----------------|--------------|--------|-------|--------------|------|--------|
| **BRAF** V600-mutated MBM | Vemurafenib + cobimetinib | BRAF + MEK1 | Phase II | NCT02230306 | N  | T (2016), HR |
| **BRAF** V600-mutated MBM | Dabrafenib + trametinib + SRS | BRAF + MEK1/MEK2 | Phase II | NCT01721603 | N  | T (2016), HR |
| **BRAF** V600-mutated MBM | Dabrafenib + trametinib | BRAF + MEK1/MEK2 | Phase Ib | NCT01978236 | N  | T (2017), HR |
| MBM | SRS + ipilimumab | CTLA-4 | Phase I | NCT01950195 | N  | T (2016), NRA |
| Symptomatic MBM | Ipilimumab + nivolumab | CTLA-4 + PD-1 | Phase II | NCT02621515 | N  | T (2018), NRA |
| Asymptomatic, untreated MBM | Fotemustine or fotemustine + ipilimumab or ipilimumab + nivolumab | CTLA-4 ± PD-1 | Phase III | NCT02460068 | N  | T (2018), NRA |
| MBM | γ-secretase inhibitor (RO4929097) + WBRT | γ-secretase | Phase I | NCT01217411 | N  | T (2011), HR |
| MBM not eligible for surgery or SRS, failed prior BRAF ± MEK-I therapy and immunotherapy | Buparlisib | PI3K | Phase II | NCT02452294 | N  | US (2015), NRA |
| MBM | Nab-paclitaxel + temozolomide + bevacizumab | Microtubules + NF-κB + VEGF | Phase I | NCT02065466 | N  | W (2014), NRA |
| Recurrent MBM | IL-2 + ipilimumab post-SRS/WBRT | CTLA-4 | Phase Ib–II | NCT03297463 | N  | W (2018), NRA |
| Asymptomatic **BRAF**V600-mutated MBM | Uprosertib + dabrafenib + trametinib | Akt + BRAF + MEK1/MEK2 | Phase I–II | NCT01902173 | N  | Suspended |

Akt: AKT serine/threonine kinase; BRAF: B-Raf proto-oncogene, serine/threonine kinase; BRAF-I: BRAF inhibitor; C: Completed (study completion date); CDK: Cyclin-dependent kinase; CTLA-4: Cytotoxic T-lymphocyte antigen 4; HER2: Human EGF receptor 2; HR: Has results; LITT: Laser interstitial thermotherapy; MBM: Melanoma brain metastases; MEK: Mitogen-activated protein kinase kinase; MEK-I: MEK inhibitor; N: No; NF-κB: Nuclear factor κB; NR: Not recruiting; NRA: No results available; NYR: Not yet recruiting; PD-1: Programmed cell death protein 1; PDGF: PDGF receptor; PI3K: Phosphatidylinositol 3-kinase; R: Recruiting (study start date); RTK: Receptor tyrosine kinase; SRS: Stereotactic radiosurgery; T: Terminated (study termination date); US: Unknown status (study start date); VEGF: VEGF receptor; W: Withdrawn (study start date); WBRT: Whole-brain radiotherapy; Y: Yes.
are provided by the T-cell receptor direct recognition of tumor-specific antigens presented by the antigen-presenting cell (APC) in association with MHC class I, costimulatory interaction between CD28 (on T cell) and CD80 (on APC) and the production of cytokines.

There is evidence that APCs in the CNS may be dendritic cells [45,49,50]. The immune response to melanoma is controlled both by intrinsic regulatory mechanisms, which are constituted by activation of inhibitory receptors expressed by tumor-specific T cells (such as cytotoxic T-lymphocyte-associated protein 4 [CTLA-4], programmed cell death protein 1 [PD-1], lymphocyte-activation gene-3 [LAG-3], T-cell immunoglobulin mucin-3 [TIM-3] and T-cell immunoreceptor with immunoglobulin and ITIM domains [TIGIT]), generation of Treg or anergy (i.e., when the T cell becomes unresponsive to the antigen), and by melanoma cells, which implement strategies for the purpose of evading the host defense, thereby continuing to effectively proliferate [47]. In this respect, one of the possible mechanisms of immune evasion displayed by melanoma cells is represented by the inhibition of the effector function of tumor-specific CD8+ T cells via overexpression of PD-L1 and indoleamine-pyrrole 2,3-dioxygenase [51,52]. The blockade of intrinsic ‘immune checkpoints’ that downregulate T-cell activity (CTLA-4 and PD-1) is currently among the treatments of advanced melanoma [53–58]. In fact, anti-CTLA-4 and anti-PD-1 monoclonal antibodies block the transduction of the inhibitory signal to the tumor-specific T cell, thus reinforcing the antitumor activity of the adaptive immune response [59,60]. Combination immunotherapy anti-CTLA-4/anti-PD-1 benefits from the complementary and nonredundant coinhibition of T cells [61] and increases long-term progression-free survival and overall survival of patients with MBM [62,63]. CTLA-4 binds to CD80 approximately 48 h after T-cell activation, thus at an earlier stage than PD-1, which, for its part, contributes to T-cell functional inactivation at the effector phase [64]. In a melanoma transplantation murine model with extracranial (subcutaneous) plus...
intracranial tumors, the contemporary blockade of these two immune checkpoint inhibitors enhanced trafficking of CD8+ T cells to the brain (almost 14-fold), and also increased that of macrophages and microglia, as well as upregulated genes involved in activation of T cells, natural killer (NK) cells and microglia/macrophages [65]. It is important to note that, while increase in CD4+ T cells, Treg and effector T cells were independent of extracranial tumor, CD8+ T cells enhancement in MBM following anti-PD-1/anti-CTLA-4 therapy relied upon the presence of subcutaneous melanoma [65]. This finding is consistent with the notion that the adaptive responses against antigens in the CNS are initiated in the periphery and propagated to the CNS by central memory T cells [66]. Despite this, intracranial response to pembrolizumab has been shown in patients with brain only involvement, but this could be due to undetectable microscopic extracerebral disease [53,67]. It has been recently evidenced that MBM differ from extracranial disease in terms of significant immunosuppression and enrichment of oxidative phosphorylation (OXPHOS). Giving the increased utilization of OXPHOS, melanoma cells metabolically compete with immune cells, thus antagonizing the immune response in the brain [68]. An OXPHOS inhibitor, currently in early-phase clinical trials in acute myeloid leukemia (NCT02882321), lymphoma and advanced solid tumors (NCT03291938), in murine models with MAPK inhibitor-resistant intracranial melanoma xenograft has resulted in improved survival [68]. Since resistance to BRAF inhibitor and MEK inhibitor-targeted therapies can be mediated by OXPHOS [69,70], the inhibition of this metabolic program has promising future applications in those patients who experience failure of targeted therapies [68].

Conclusion
MBM are a true challenge for successful treatment of patients with stage IV melanoma. The genetic, molecular and metabolic changes in the tumor cells at the primary site and later in the brain are crucial for melanoma to establish and grow in a new microenvironment. Nevertheless, the immune cells maintain a surveillance over the tumor and attack melanoma both at the periphery and in the brain. The immune response can be evaded by melanoma through immunosuppressive pathways, but checkpoint inhibitors restore the adaptive response against neoplastic cells and represent one of the treatment of MBM.

Future perspective
We are currently experiencing a fascinating time with the recent advances of combined immunotherapy in the management of metastatic melanoma, but there are still gaps in our knowledge and numerous uncertainties. The focus of basic research on the potential novel molecular targets in melanoma cells, the mechanisms of immune surveillance in MBM and the CNS microenvironment will offer opportunities for successful targeted and immune-based treatment. The inclusion of patients with active MBM and/or leptomeningeal disease in future clinical trials will be extremely important in paving the way for curing melanoma metastatic to the brain. It will be of outstanding interest to see future applications of a newly discovered T-cell population that specifically targets melanoma cells while sparing healthy cells via recognition of the monomorphic MHC class I-related protein MR1 [71].

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