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REVIEW Article

Immunotherapy as a Turning Point in the Treatment of Melanoma Brain Metastases

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ABSTRACT

The incidence of tumor metastases in the brain is many times more frequent than primary brain tumors, affecting a very large share of patients suffering from systemic cancer. Advanced malignant melanoma is well known for its ability to invade the brain space and current treatment options, such as surgery and radiation therapy, are not very efficient and cause notable complications and morbidity. The aim of this review is to explore the recent advances and future potential of using immunotherapy in the treatment of melanoma brain metastases. Several FDA approved immunotherapeutic drugs have shown to be able to at least double the overall survival rates in such patients. Clinical trials of varying phases are underway and available results are promising, significantly prolonging survival rates in patients with previously untreated melanoma brain metastases. Nevertheless, not all patients respond to these immunotherapies, facing a high percentage of resistant cases, without yet knowing the mechanisms and causes of resistance behind. Also, at the time of immunotherapy, a small percentage of patients is affected by pseudoprogression, which can be difficult to distinguish from true progression given the similarity of symptoms. Therefore, there is a pressing need for future research about treatment effectiveness in patients with brain metastases from melanoma, including outcomes from the perspective of patients.

SUMMARY

Keywords

Melanoma, brain metastasis, immunotherapy, clinical trial.

Abbreviations

Antigen Presenting Cells (APC); Blood-Brain Barrier (BBB); C-C Motif Chemokine Ligand 2 (CCL2); Guanosine–Adenosine Monophosphate (cGAMP); Central Nervous System (CNS); Cytotoxic T-Lymphocyte-Associated Protein 4 (CTLA-4); Connexin-43 (Cx43); Extracellular Matrix (ECM) ; Food and Drug Administration (FDA); High Dose Interleukin 2 (hdIL-2); Herpes Simplex Virus Type 1 (HSV-1); Immunoglobulin G1 (IgG1); Immunoglobulin G4 (IgG4); Interleukin 1 Beta (IL-1β); Interleukin 2 (IL-2); Interleukin 23 (IL-23); Interleukin 6 (IL-6); Interferon Alpha (INF-α); Lactate Dehydrogenase (LDH); Magnetic Resonance Imaging (MRI); Programmed Cell Death Protein 1 (PD-1); Programmed Death-Ligand 1 (PD-L1); Stereotactic Radiosurgery (SRS); Tumor Necrosis Factor Alpha (TNF-α); Tumor Node Metastasis (TNM); Regulatory T-Cells (Treg); Whole-Brain Radiation Therapy (WBRT).

1. Introduction

Brain metastasis, the spread of a tumor from a primary neoplasm to the brain, is about 10 times more frequent than a primary brain tumor[1]. Most common brain metastases have their primary tumor in the lung (~45%), breast (20%) and skin (e.g., melanoma, 10%)[2]. Brain metastases have a very poor prognosis and are characterized by a progressive central nervous system (CNS) damage and functional decline, significantly affecting quality of life and shortening survival rates. Advanced melanoma is well known for its potential to metastasize to the brain. However, current therapies are not very efficient and brain metastases are in most cases lethal.

Treatment of melanoma brain metastases with surgery and/or radiation therapy results in median overall survival of only about 4 to 6 months after diagnosis[3] and they cause notable complications and morbidity (stroke, radiation-induced necrosis and cognitive defects)[4]. New immunotherapies, such as the targeted or immunomodulatory drugs, many in clinical trials, have shown promise, with some immunomodulatory drugs being able to at least double the overall survival rates in melanoma brain metastases patients[5]. Immunotherapy uses components of the body's own immune system to fight against cancer. It works in several ways, for example by enhancing the capacity of the immune system to attack cancer cells or giving the immune system specific components artificially produced[6]. In particular, immunomodulators, antibodies stimulating T-cell function either by blocking or activating regulatory receptors, have been shown to cause regression of several types of tumors and an exponential number of clinical trials is underway. Remarkably, several immunomodulatory drugs/checkpoint inhibitors are already approved by the US Food and Drug Administration (FDA) for the treatment of melanoma, non-small cell lung cancer, breast cancer, bladder cancer, kidney cancer, and Hodgkin lymphoma[7,8].

2. Epidemiology of Malignant Melanoma

Malignant melanoma is the most life-threatening and deadly type of skin cancer, representing approximately 5-10% of all skin-cancers, but being responsible for more than 80% of deaths related to skin-cancer[9–11]. The other representants of skin cancer are basal cell carcinoma (BCC), squamous cell carcinoma (SCC) and Merkel cell carcinomas[9].

Recent data have shown that worldwide incidence of melanoma has been rising, making it the fifth most common type of cancer in adults, the first, second, third and fourth places being respectively occupied by breast cancer, lung and bronchus cancer, prostate cancer, and colon and rectum cancer[10,12,13].

Risk factors linked to melanoma development have been identified, including intense exposure (acute-intermittent rather than chronic) to sources of ultraviolet radiation (either natural – sunlight; or artificial – tanning bed), genetic predisposition, positive family history, compromised immune system, obesity, exposure to heavy metals and some pesticides, and alcohol consumption[9,13,14].

Outstandingly, amid all solid tumors, melanoma has the highest tendency for brain metastases[15,16].

3. Malignant Transformation of Melanocytes

Melanoma's cellular origin has been an important focus of research because of its doubtfulness. However, a recent study led by Kohler et al. has demonstrated that melanoma can arise from pigment-producing melanocytes residing in the interfollicular layer of epidermis[17].

One of the valuable roles of melanin is the creation of a sunshield protecting basal melanocytes from DNA damage induced by ultraviolet radiation[18]. Nonetheless, if DNA impairment occurs

and remains unrepaired, it can trigger mutations in the pigment-producing melanocytes, leading them to quickly multiply and undergo malignant transformation through a chain of reactions known as melanomagenesis[19]. The first stage in this process is the development of nevomelanocytes (an accretion of pigment cells) of benign/common nevi, which are cells characterized by atypical proliferation and arrested progression due to cellular senescence (a steady cessation of cell division occurring in response to several intrinsic and extrinsic factors despite the presence of mitogenic signals and optimal growth conditions)[14,18,20]. The second stage is the overriding of cellular senescence by enhancing both the cell cycle and the length of telomere[9]. This is one of the critical shifts leading to dysplastic nevi, which are cells characterized by atypical qualities and carrying the risk for melanoma development[10,14]. The third stage can be divided into two progressive phases: radial and vertical. The radial phase is characterized by an outward proliferation of melanoma cells, allowing them to spread across the epidermis or invade the papillary dermis. The vertical stage is characterized by the invasion of the dermis and the ability to disseminate or metastasize throughout the body[10,14,19]. The metastatic cells will first invade and proliferate at local or regional sites (e.g., regional lymph nodes) and then at distant sites, the most common being lung, liver, distant areas of the skin, brain, gastrointestinal tract, bone and adrenal gland[19]. The progression between successive stages of melanomagenesis is thought to be driven by the simultaneous accumulation of genetic, epigenetic and allogenetic variations[9–11]. Even though this model has been commonly accepted as a reference for the development of malignant melanoma, recent findings based on epidemiological, clinical and experimental data reveal that it only applies to a third of melanoma cases, thus evidencing that melanoma development might be more complicated and less stepwise as originally thought[10,14].

Malignant melanoma is the tumor with the highest number of mutations[10,21]. Wide-ranging cytogenetic and high-resolution genomic analysis have shown that genetic variations exponentially increase as it progresses from nevus to primary and later to metastatic melanoma[14]. Thus, a number of key genes and pathways have been revealed to play a role in melanoma development, progression and proliferation, ranging from signal transduction to developmental and transcriptional pathways and cell cycle deregulation. Several mutations, known as driver mutations (BRAF, NRAS, KIT, GNAQ, GNA11, NF1, and TERT), define most of the molecular subtypes of melanoma. However, studies have shown that these mutations alone are not enough to develop a straightforward tumorigenic phenotype. They require the presence of the so-called "supporting mutations". It is therefore important to keep searching for mutations (both driver and supporting) in melanoma in order to identify new molecular subtypes and, ultimately, guide targeted therapy choices to achieve long-lasting responses[11,22-26].

Although some key genetic stimuli are needed for melanomagenesis to occur, alone, they are not enough. Years of research have demonstrated that a synergetic interaction between environmental, genetic, and host factors is of vital importance for the malignant transformation of melanocytes. Tumor microenvironment is a complex and dynamic setting in close interaction with several structures, notably extracellular matrix, fibroblasts and microvasculature. It modulates the transformation process by influencing the concentration of key factors necessary for tumor cells to grow, these including growth factors, cytokines, nutrients (e.g., glucose), and metabolic gases (e.g., oxygen). Therefore, tumor microenvironment can either increase or decrease the likelihood for melanomagenesis to occur[11].

4. Melanoma Brain Metastases Origin and Development

4.1 Transmigration of Melanoma Cells to the Brain

Studies have shown that metastatic melanoma cells have evolved from their primary site and have acquired a selective brain tropism, thus enabling them to establish secondary neoplasms within the brain[27]. The mechanisms through which melanoma cells disseminate to the brain have remained unclear over the years, however the development of *in vivo*, live-cell imaging techniques provided new understandings about the underlying processes involved[16,28] (Figure 1).

In the initial phase of the metastatic cascade to the brain, melanoma cells enter the circulation and then undergo hematogenous spread towards the brain vasculature, where they arrest upon reaching the narrow blood vessel branch points and capillaries

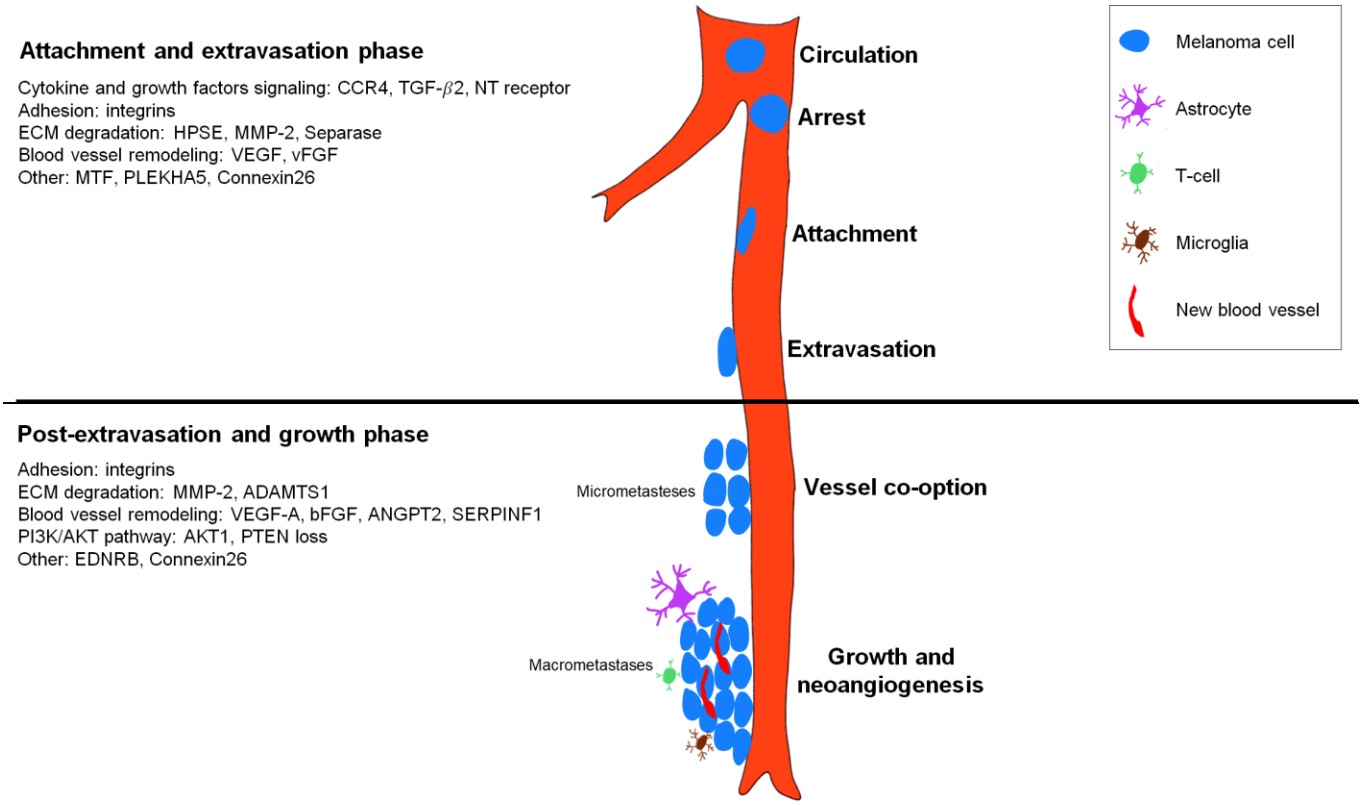

Figure 1. Process of Melanoma Transmigration to the Brain
The metastatic cascade to the brain involves a series of steps allowing melanoma cells to disseminate from their primary site to the brain parenchyma: 1) Cell inflowing to the blood circulation at primary site, 2) Cell arrest in the microvasculature, 3) Adhesion to the endothelial cells. 4) Extravasation to the brain parenchyma, 5) Vessel co-option allowing the formation of micrometastases. 6) Growth and neoangiogenesis empowering the magnification from micrometastases to macrometastases.

of the microvasculature[15,16]. There, they linger in an inert state for about 1–9 days (significantly slower cell migration when compared to that of other organs, which might explain the latency of melanoma brain metastasis development), allowing their adhesion to the endothelial cells[16]. In order to extravasate from the blood-brain barrier into the brain parenchyma, metastatic cells need to 1) push the endothelial cells apart through the action of mechanical forces (they become round cell and develop cytoplasmic protrusions), 2) disrupt tight junctions through the action of pro-invasive integrins (31, b3, 41) and proteases (cathepsin-S), and 3) degrade the basement membrane through the action of proteases (matrix metalloproteinases 2 and 9, heparanases)[15,16]. Studies have shown that some conditions might facilitate the transmigration process of melanoma cells, notably their affinity for soluble solutes produced within the brain (e.g., growth factors, cytokines) and shared transcriptomic lineage with brain cells. In fact, melanoma cells exhibit neurotrophin receptors with a high affinity for neurotrophins produced from the brain, indicating that these substances may play a role in their recruitment[15,27]. Once inside the brain parenchyma, melanoma cells initiate a vessel co-option and remain closely associated with the endothelial cells at the abluminal surface, where they start forming micrometastases and further invade the brain[15,16]. It has been observed that melanoma cells that did not have any contact with the blood vessels quickly die[16,27]. The brain microenvironment (notably astrocytes, microglia and T-cells) then influences the growth from micrometastases to macrometastases[15]. Further growth of metastases might involve the formation of new blood vessels at the tumor margin (neoangiogenesis)[15,16]. Mysteriously, some individual melanoma cells stay in a dormant state while co-opting the brain microvasculature, but still possessing the ability to migrate along it[16].

4.2 Brain Tumor Microenvironment

Tumor microenvironment is an important factor influencing all steps of metastasis development, from metastasis formation to its progression and response to different therapies, by providing pro-tumorigenic signals. These signals could be intrinsic or produced and secreted as a response to the metastatic process itself. Either way, they support viability, growth and proliferation of metastatic cells at secondary sites. In addition to the tumor cells, other types of cells can be found in the brain tumor microenvironment, including fibroblasts, immune cells, pericytes, and endothelial cells[27]. The main features distinguishing the brain tissue from any other tissues are the presence of blood-brain barrier (BBB) and unique resident cells (microglia, astrocytes and neurons), a distinctive immune advantage, and very high nutritional demands and energy consumption[27,29].

4.3 Blood-Brain Barrier

The blood-brain barrier, unique to the CNS, is located at the level of cerebral capillaries, and is a highly selective multicellular layer, that protects neural cells by restricting free movement of substances and cellular elements between the systemic circulation and brain tissue. Its exceptional structure is composed of tight junctions, which are dynamic arrangements located between endothelial cells and formed by transmembrane (occludins, cadherins, claudins and junctional adhesion molecules) and cytosolic (catenins and zonula occludens) proteins[15,30–32].

Under physiological conditions, this semipermeable membrane only allows the passage of certain substances, either by passive diffusion (e.g., water, lipid-soluble molecules and gases) or active transport (e.g., nutrients, other molecules)[30]. A group of specific cells, namely endothelial cells, pericytes, astrocytes, microglia and neurons, forms the neurovascular unit, which regulates and supports tight junctions in a synchronized and coordinated manner[30,32]. Some studies suggest that the blood-brain barrier is compromised throughout the course of the metastatic proliferation to the brain, thus allowing the passage of certain substances, otherwise not possible under physiological conditions[15,30-42]. Some additional elements, particularly active transporters, adsorbent endocytosis and vesicular pathways, also contribute to the physiological function of the blood-brain barrier, however their role in the metastatic process is poorly recognized, thus evidencing the need for further studies[30].

In the setting of brain metastases formation, the blood-brain barrier holds a binary function: it protects the central nervous system from incoming cancer cells, but it also protects metastatic cells by supporting their transmigration, proliferation and survival inside the brain. In fact, after crossing the blood-brain barrier, metastatic cells escape the immune surveillance, and their growth is further potentiated by elements secreted by the barrier itself[32].

4.4 Interaction with Brain Parenchyma Cells

Once inside the brain, melanoma cells come into contact with multiple cell types and their interaction can have either tumor-suppressive or tumor-supportive effects[16].

Astrocytes represent roughly 50% of all cells in the brain and have an indispensable role in homeostasis. They support repair of brain tissue following injuries, and support endothelial cells in obstructing melanoma cells from entering the brain. Nevertheless, astrocytes are the most frequent cells implicated in brain metastasis development. They become activated upon interacting with tumor cells and start to secrete many soluble factors that support metastasis proliferation and survival in the brain microenvironment (Figure 2). The most well-known soluble factors secreted by the so-called reactive-astrocytes include neurotrophins (growth factors), chemokines and cytokines (IL-6, TNF-α, IL-1β, and IL-23). Remarkably, it has been shown that reactive-astrocytes also have the aptitude to induce the expression of several pro-survival genes (e.g., TWIST, BCL2L1) and extracellular matrix (ECM) degrading enzymes (e.g., metalloproteinases 2 and 9, heparanase) in tumor cells. Protocadherins and Connexin-43 (Cx43)-mediated gap junctions, where the transfer of the second messenger cytosolic guanosine–adenosine monophosphate (cGAMP) activates the STING pathway in astrocytes and instructs them to produce and secrete tumor-stimulating cytokines (e.g., INF-α, TNF-α) are thought to be the means by which tumor cells from brain metastasis communicate with local astrocytes.

These cytokines will then promote STAT1 and NF-κB-mediated survival and/or proliferation of cancer cells[16,17,27,30]. Gap junctions can be successfully targeted[33]. Astrocytes also regulate

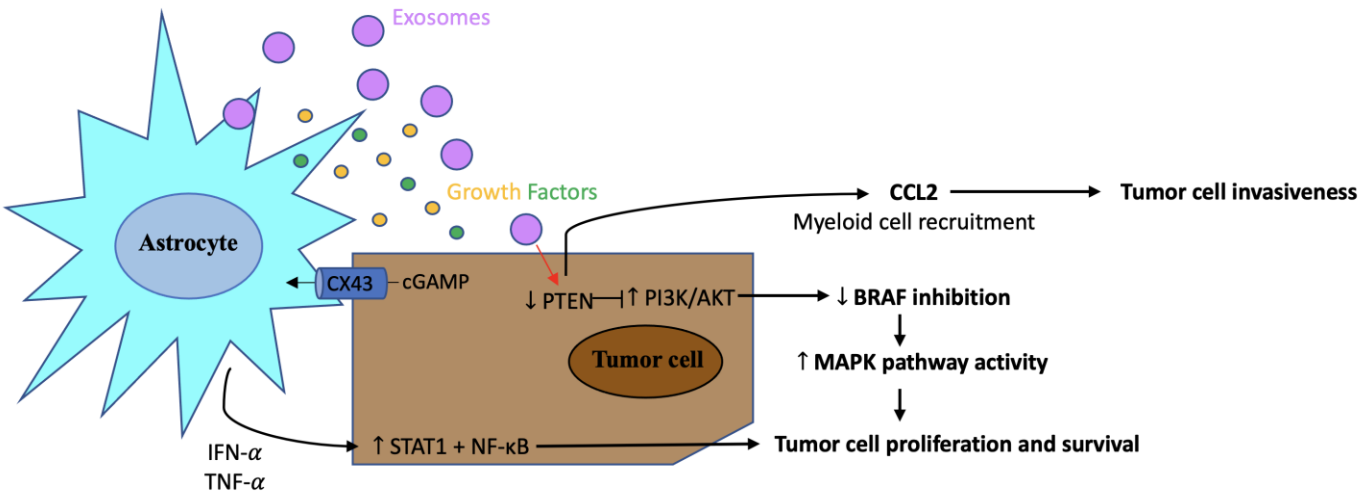

Figure 2. Pathways activated upon interaction between Astrocyte and Tumor cell
Second messenger cGAMP activates the STING pathway in astrocytes, thus allowing the release of specific cytokines triggering STAT1 and NF-κB-mediated survival and/or proliferation of tumor cells. The secretion of exosomes and growth factors silences PTEN, leading to the occurrence of two important phenomena: 1) the release of CCL2 from tumor cells, allowing myeloid cell recruitment via CCR2, which in turn promotes the chemotaxis and chemokinesis of tumor cells, and therefore tumor cell invasiveness; 2) activation of the PI3K/AKT pathway in melanoma cells, successively limiting their inhibition by BRAF kinase, which in turn upregulates the MAPK pathway, therefore their proliferation and survival.

tumor cell survival by means of epigenetic changes. They silence PTEN, a major tumor suppressor, by secreting exosomes containing micro-RNA-19a. This silencing will then activate the release of C-C motif chemokine ligand 2 (CCL2) from tumor cells, allowing myeloid cell recruitment via their C-C chemokine receptor type 2 (CCR2), which in turn will promote the chemotaxis and chemokinesis of tumor cells, and therefore tumor cell invasiveness[34].

Mediator PTEN is also a major regulator of the PI3K/AKT signaling pathway, and it has been shown that reduced PTEN expression is accompanied by elevated PI3K/AKT pathway activity in melanoma cells, which additionally limits their inhibition by BRAF kinase, thus upregulating the MAPK pathway (also known as the RAS/RAF/MEK/ERK signaling cascade) and subsequently promoting their proliferation and survival[16,17,27,30,35]. A recent retrospective analysis has also shown that concomitant occurrence of PTEN silencing with BRAF V600E mutation (the most common mutation in metastatic melanoma, which activates MAPK-ERK signaling pathway) revealed an earlier development of brain metastasis and consequently a shorter overall survival[16].

Microglia are the innate immune cells in the brain and resemble peripheral macrophages. They possess both tumor-suppressive and tumor-supportive effects. The main tumor-suppressive effects involve cell cytotoxicity mediated by nitric oxide, tumor cell phagocytosis, and activation of tumor-specific B- and T-lymphocytes while the main tumor-supportive effects involve expression of programmed death-ligand 1 (PD-L1) leading to inhibition of cytotoxic T-cells, and secretion of factors inducing cancer growth and invasion (e.g., growth factors, chemokines)[15,16,30]. There is ongoing research to determine if these cells are also involved in the Wnt-signaling pathway leading to metastasis invasion and colonization of the brain[15].

T-cells, also called T-lymphocytes, are major components of the adaptive immune system. Some subgroups exhibit tumor-suppressive effects (notably effector CD3+, cytotoxic CD8+ and memory CD445RO+), while some other subgroups exhibit tumor-supportive effects (regulatory FoxP3+ and immune tolerance PD-1+). Although their presence in brain parenchyma is quite rare under physiological conditions, it has been previouslyestablished[15] that melanoma brain metastases expressing PD-L1 have a higher infiltration of T-cells[15]. Furthermore, higher density of CD3 and CD8 tumor-associated lymphocytes has been correlated with increased survival[43]. Taking

into consideration these results, it makes sense to consider immunotherapy as a potentially promising tumor-targeting strategy in melanoma brain metastases. Recent clinical trials have confirmed this hypothesis to be correct.

4.5 Risk Factors and Metastasis Distribution

A set of risk factors has been explicitly linked to the development of melanoma brain metastases, including: male gender, age over 60 years, primary disease from mucosal surfaces or skin of the head, neck, scalp or trunk; acral, lentiginous, or nodular tumor histology; high Clark's level/Breslow thickness of the primary disease; occurrence of visceral or nodal metastases; unknown primary melanomas; increased serum lactate dehydrogenase (LDH) levels; presence of oncogenic BRAF and NRAS mutations; expression of CCR4 on melanoma cells; and activation of PI3K/AKT signaling pathway[16,31,44].

Melanoma brain metastases are the most frequent intracranial tumors in adults[27] and their location within the brain is well correlated with those areas receiving the highest blood flow: cerebral hemispheres (80%, from which 43.5% are located within the frontal lobe), cerebellum (15%) and brain stem (5%)[16,31,45].

5. Therapeutic Strategies for Melanoma Brain Metastases

Magnetic Resonance Imaging (MRI) is the gold standard for both diagnosis and monitoring of brain metastases[46]. According to the TNM (Tumor, Node, Metastasis) staging system, patients with melanoma can be clinically divided into three groups: patients with 1) local disease (stage I–II), 2) node-positive disease (stage III), and 3) advanced or metastatic disease (stage IV)[47].

When devising a therapeutic strategy in certain patients with melanoma metastases, important issues about therapeutic repercussions must be considered following prolonged survival and long-term remissions. As a result, for the correct treatment of a patient with brain metastases, a multidisciplinary strategy that examines all possible treatment modalities is required. For the correct designing of a comprehensive therapeutic approach, important aspects need to be considered, notably the clinical features of brain metastases (e.g., number, size, location, and extent of CNS symptoms), extracranial systemic disease, presence of BRAF mutation, patient performance status (patient's ability to perform daily activities without any help), associated comorbidities, and prior exposure to intracranially effective therapy (e.g., immunotherapy, BRAF/MEK inhibitors, stereotactic radiosurgery)[44,48].

In earlier times, the only options to control brain metastases were locoregional therapies such as surgical excision and/or radiation therapy (whole-brain radiation therapy - WBRT, and stereotactic radiosurgery - SRS)[44,49]. In addition to being generally inefficient, with a median overall survival of only 4-6 months following diagnosis, they also cause notable complications and morbidity[4]. Method WBRT is the standard treatment for metastatic brain tumors, with WBRT and surgical removal being used for multiple and/or large tumors and MRI-assisted SRS for smaller tumors. Tumor Treating Fields method is an additional option used in treating brain metastasis[50,51]. Focal therapies such as SRS and surgery are limited to the treatment of the area of interest, which may result in tumor relapse from other non-treated sites which were under the limit of detection of available imaging methods[52]. In general, SRS is preferred to WBRT in the treatment of melanoma brain metastasis[53]. Melanoma cells usually have a powerful DNA damage repair machinery, thus resulting in the need of delivery of larger fractions/doses of radiotherapy[54].

Chemotherapy was previously the only approved medication for metastatic melanoma, but the results in melanoma patients with brain metastasis were disappointing, similar to those obtained in melanoma treatment in general, with only 5-20% of patients having their tumor shrink, but no improvement in overall survival, despite it[55].

In recent years, the development of new systemic treatment modalities such as immune check point inhibitors, and BRAF plus MEK inhibitors provides an alternative for patients suffering from melanoma brain metastases by virtue of their intracranial efficacy[44]. FDA-approved targeted therapies such as vemurafenib, trametinib, dabrafenib, and some of their combinations, act by blocking BRAF with activatory mutations such as V600E or V600K[56,57]. However, in spite their intracranial efficacy, resistance develops in the majority of treated cases. The occurrence of resistance in melanoma brain metastases is poorly understood, and the specific CNS microenvironment may contribute to different resistance mechanisms

than those previously described in extracranial melanoma[58,59]. Remarkably, immunotherapy has demonstrated tremendous promise, being able to at least double the overall survival rates for patients with melanoma brain metastases[5]. Outstandingly, radiation has the ability to enhance these treatments[60], while also reducing their side effects (e.g., neurotoxicity)[43].

6. Immunotherapy as a Turning Point in the Treatment of Melanoma Brain Metastases

6.1 Definition

The regulation of the immune system is a highly complex process. It involves a multitude of components, one of these being immune checkpoints, which are responsible for self-tolerance, the immune system's ability to recognize what is 'self' and not react against or attack it[61-62]. Immune checkpoint inhibitors, like anti-PD-1/PD-L1/CTLA-4, are a form of immunotherapy regulating this process by boosting immune reactions against tumor cells, while also endorsing autoimmunity. Through the action of interferon gamma, these molecules are upregulated by the inflammatory response[63–65].

6.2 Mechanism of action

Research studies have demonstrated that CD4 and CD8 lymphocytes are required for limitation or prevention of brain metastasis, with an important role assigned to the regulatory T-cells (Treg)[66].

The most important molecules as immune checkpoints are the programmed cell death protein 1 (PD-1) and its ligand programmed death-ligand 1 (PD-L1) and the cytotoxic T-lymphocyte-associated protein 4 (CTLA-4). Protein PD-1, also known as CD279, is mostly found on the activated CD8+ T-cells, but also on the surface of dendritic cells, macrophages, and B-cells. Despite of its similarity to CD28, it interacts with its specific ligands: 1) PD-L1, which is expressed on the surface of various cells, including hepatocytes, myocytes, cancer cells, immune cells, pancreatic islet cells, endothelial cells, thyroid cells, and many other cells; and 2) PD-L2, which is only expressed on the surface of macrophages and dendritic cells. The binding of PD-1 to its ligands will induce an inhibitory effect on cytotoxic T-cells activity by regulating their glucose metabolism (decreased glucose uptake and gluco-neogenesis) and triggering their apoptosis, while also forestalling the co-stimulatory pathway of CD28-CD80/86[67–70]. Treg cells are the only cells escaping apoptosis as they are able to suppress cytotoxic CD8+T-cell proliferation, therefore supporting the immune escape of tumor cells[67,68,70,71]. It has been shown that PD-L1 are mostly present in inflammatory settings due to the fact that they are strongly regulated by interferon gamma[70,72]. Hence, chronic inflammation surrounding tumors could explain the limited destruction of cancer cells in such scenarios[70,73]. Furthermore, tumor aggressiveness has been shown to be directly proportional to the expression of PD-L1: the higher the PD-L1 expression, the greater the tumor aggressiveness[74]. CTLA-4, also known as CD152, is a co-stimulatory glycoprotein expressed on the surface of CD4+ and CD8+ T-cells[64], which downregulates effector T-cells activation[68]. Despite its similarities to CD28, CTLA-4 has a 20-fold higher binding affinity to B7 glycoproteins: 1) B7.1 or CD80, and 2) B7.2 or CD86[68,69]. This limits activation of effector T-cells proliferation[5], henceforth backing up the immune escape of tumor cells[70]. Both pathways are significant modulators of immune-tumor interaction and targeting them focused significant energy in the past several years, with notable successes[43]. However, because they regulate different phases of the immune response (CTLA-4 regulates the early stages of T-cell activation, whereas PD-1 is expressed after T-cell activation) and act at different sites (tumor microenvironment for PD-1/PD-L1 and draining lymph nodes for CTLA-4), it is fathomable that their effects and adverse events differ[70,75,76]. Noteworthy, it has been shown that anti-PD-1 have a more specific effect, with less severe adverse events[70,75,77].

Stimulation of T-cells in the periphery with immunomodulators have also beneficial effects against CNS tumors. A recent study has shown that pembrolizumab-induced PD-1 inhibition results in 20-30% responses in CNS, in patients with melanoma or non-small lung cancer CNS metastasis. Moreover, combined regimen of nivolumab and ipilimumab, which acts by both inhibiting PD-1 and CTLA-4 has notable 55% CNS response in melanoma brain metastasis patients[43]. Additionally, radiation therapy (e.g., SRS) is known to sensitize melanoma brain metastases to the action of checkpoint inhibitors, such as ipilimumab[78].

6.3 Advances in Immunotherapy

The first immunotherapeutic to show effect against melanoma brain metastases was high dose interleukin 2 (hdIL-2). Melanoma patients with CNS involvement require higher doses of IL-2, which is challenging due to adverse events such as neurotoxicities and the need for hydration to counteract the induced vasodilation[30,79]. Recently, several immunomodulatory drugs were approved for melanoma treatment, with a recent study showing that the immune checkpoint blocking immunotherapy can double survival rates for patients with melanoma brain metastases[5]. Patients receiving these immunomodulatory drugs showed a mean survival of ~12.5 months compared to ~5.2 months for those not receiving immunotherapy, with a 4-year survival of ~28% versus only ~11%[5,80].

6.4 Clinical Trials

It is important to point out that, currently, there are several clinical trials underway for melanoma brain metastases. Clinical trials are research studies conducted in volunteers and designed to evaluate the efficacy of new interventions. According to the general rules, any clinical study, including clinical trials in patients with brain metastases, needs to follow a strict protocol established prior to the beginning of the study. These protocols will specify the eligibility criteria, the number of participants, the length of the study, whether there will be a control group or any other way to limit research bias, the posology and route of administration, and the method of data analysis. Due to high mortality rates in patients with melanoma brain metastases, there is a pressing need for the discovery of new agents to effectively treat patients who have failed standard therapies. In the past, patients with brain metastases have been excluded from clinical trials, however their inclusion has been rising nowadays[81]. And the discovery of immunomodulatory drugs led to the development of many clinical studies targeting such patients.

The results of already finished clinical trials have shown that immunotherapy significantly prolongs survival in patients with previously untreated melanoma brain metastases. The combination of CTLA-4 inhibitor ipilimumab with the PD-1 inhibitor nivolumab is the preferred treatment modality for patients with asymptomatic, untreated brain metastases from melanoma. Data supporting its use in this population comes from an open-label single-arm phase II trial (CheckMate-204), in which 101 patients were treated. This combination demonstrated an intracranial clinical benefit of 57%, which was superior to previously reported with ipilimumab (24%) or nivolumab (22%) alone, and with ipilimumab plus fotemustine (50%). The rate of adverse events associated with these agents was similar between the group tested and patients without brain metastases, with a low percentage of severe neurotoxicity[44,82]. The ABC phase II trial compared combination immunotherapy with single-agent nivolumab in 60 asymptomatic patients with no prior treatment, again showing a higher rate of intracranial response with the combination (46%) than with nivolumab alone (20%)[44,83]. To further consolidate such findings, another randomized phase III trial (NIBIT-M2) including 80 patients with untreated asymptomatic brain metastases, demonstrated a higher overall-survival rate in patients treated with combination nivolumab plus ipilimumab (29.2 months), than those treated with fotemustine alone (8.5 months) or in combination with ipilimumab (8.2 months)[44,84].

For symptomatic brain metastases from melanoma, the available data regarding the efficacy of immunotherapy as a single prime therapy is very limited. Such patients often require glucocorticoids, surgical resection and/or SRS to treat neurological symptoms prior to beginning immunotherapy[44].

6.5 Limitations

Immunomodulatory drugs, such as PD-1/PD-L1 or CTLA-4 inhibitors, have a great therapeutic potential in metastatic melanoma, including melanoma brain metastases. Yet, only a small percentage of the patients are actually responding to these immunotherapies, with a high percentage of resistant cases. An extensive understanding of these mechanisms and causes of resistance for brain metastases is required in order to overcome this resistance.

One limitation to these investigations is the current methods used to investigate the tumor and in situ tumor microenvironment of the brain, which provide limited information of a heterogeneous tissue, spatially and dynamically, in time[51]. Another limitation is the lack of preclinical models which can mimic with high accuracy human brain metastases and that can recapitulate all the steps of brain metastases development[46]. As some research groups

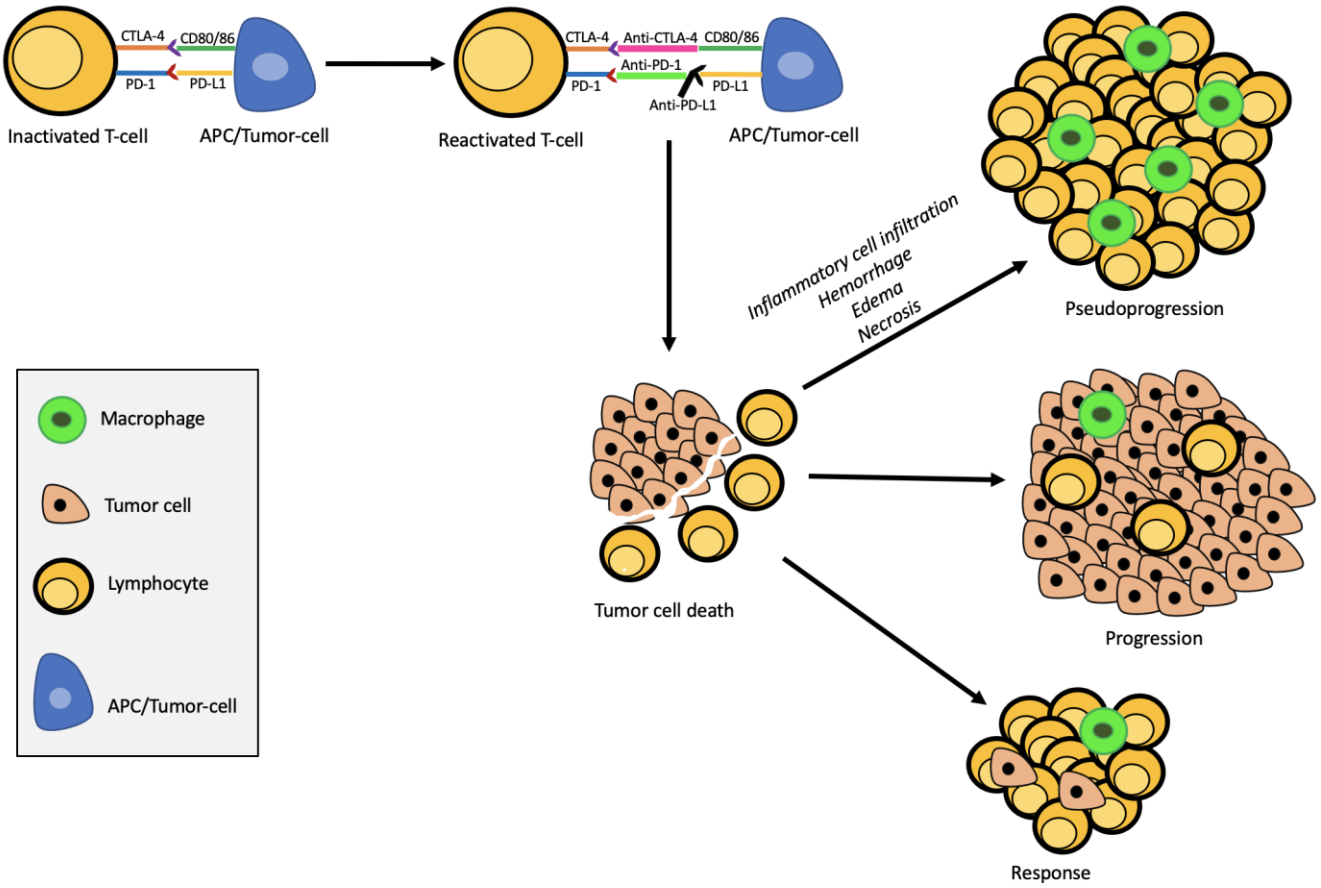

Figure 3. Pseudoprogression following Immunotherapy
Immune checkpoint inhibitors reactivate T-cells heretofore inactivated by PD-L1 and CTLA-4 presentation by tumor cells or antigen-presenting cells (APCs), thus allowing them to successively invade and kill tumor lesions. The concomitant occurrence of inflammatory cell infiltration, hemorrhage, edema and necrosis promotes gradual lesion expansion as seen in imaging methods, thus indicating pseudoprogression.

suggest, the development of intravital microscopy technologies for high resolution imaging of brain metastases can be an important step forward[51]. The lack of predictive biomarkers of response and toxicity is another limitation of immunotherapy[10]. Additionally, some treated patients with brain metastases may need control of their symptoms with steroids, which can make immunotherapy ineffective[43].

Taking into consideration these facts, significant research has to be further performed in order to clearly define which patients respond to immune checkpoint inhibitors and how to sensitize the non-responders to these therapies.

6.6 Pseudoprogression

A small number of patients with melanoma brain metastases experience pseudoprogression at the time of immunotherapy. Even though there is still no agreement on its precise molecular mechanism, it is believed to result from an invasion of lymphocytes leading to the formation of new tumor lesions, or the growth of existing ones, before their subsequent regression during continued therapy (or rarely, after discontinued treatment).

At first, T-cells are inactivated subsequent to PD-L1 and CTLA-4 presentation by tumor cells or antigen-presenting cells (APCs). They are then reactivated following the administration of immune checkpoint inhibitors, namely anti-PD-1/PD-L1/CTLA-4. Activated T-cells will successively invade and kill tumor lesions, resulting in the release of antigens as they die, which attracts more inflammatory cells. Tumor shrinkage can lead to rupture of blood vessels and the formation of hemorrhages in locoregional lesions, which can lead to edema of the lesions along with an inflammatory response. Moreover, as the necrotic byproducts of dead tumor cells cannot be immediately absorbed, they accumulate in locoregional lesions. Therefore,

the concomitant occurrence of inflammatory cell infiltration, hemorrhage, edema and necrosis causes gradual lesion expansion as seen in imageology studies, thus indicating pseudoprogression (Figure 3)[85].

Pseudoprogression can be difficult to distinguish from true progression given the similarity of symptoms. As a result, the clinical treatment becomes more difficult, and patients and their families may get confused. Because immunotherapy is a relatively new treatment, there is limited data to guide clinical decision-making in such patients[30,86].

7. Conclusion

Brain metastases are about 10 times more frequent than a primary brain tumor, being present in 20-40% of adults with systemic cancer. Malignant melanoma is the deadliest form of skin cancer, and its worldwide incidence has been increasing over the years. Advanced melanoma is well known for its propensity to metastasize to the brain and patients diagnosed with melanoma brain metastases have an overall survival of only 4 to 6 months with standard available treatments, such as surgery and radiation therapy. This is definitely not the desired outcome and sustained efforts are currently underway to develop better therapies.

Immunotherapy brings great promise as new tools for melanoma treatment, in particular, and for the treatment of other types of malignancies in general. This new modality is able to at least double the overall survival rates for patients with melanoma brain metastases. However promising, they require additional investigation. It is now imperative to detect better biomarkers within the CNS which can guide the therapeutic strategy and can predict the response to therapy. Although there has been great progress in recent years, there are still many challenges and limitations to overcome, and thus, a need to investigate, understand, and develop effective therapies to treat patients with melanoma brain metastases in a cost-effective manner with greater value to patients.

Conflict of Interest

The author declares no conflicts of interest.

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