Paradigm of biomarkers in metastatic melanoma (Review)

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Abstract. Metastatic melanoma is an aggressive and deadly form of skin cancer, known for its rapid ability to spread to other organs. Melanoma metastasis involves several steps: Local invasion, lymphovascular invasion and proliferation to new sites. This process is facilitated by genetic alterations, interactions with the tumor microenvironment and evasion of the immune system. Despite advances in therapies, the 5-year survival rate remains low at ~22.5%. Notably, current research is focused on identifying patients who may benefit from specific treatments, considering factors such as mutational load and programmed death ligand 1 expression. BRAF inhibitors and immune checkpoint inhibitors have improved survival, although numerous patients do not respond or develop resistance, underscoring the need for novel biomarkers to optimize treatment and monitoring of the disease. In summary, the purpose of the present article is to review the different serological, histological, microRNA and circulating tumor cell biomarkers that have proven useful in the diagnosis, follow-up and prognosis of metastatic melanoma. These biomarkers represent a promising area for research and clinical application, with the aim of offering more precise and personalized treatments.

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1. Introduction

Metastatic melanoma represents one of the most aggressive and deadly forms of skin cancer, characterized by its ability to metastasize rapidly to other organs and tissues. Despite significant advances in the understanding and treatment of melanoma, metastatic disease remains a major clinical challenge with a rather limited prognosis. This article aims to explore advances in the identification and use of serological, histological, microRNA, and circulating tumor cell (CTC) biomarkers in the treatment of metastatic melanoma, providing a comprehensive view of the current situation and future prospects.

In terms of the epidemiology of stage IV melanoma, it stands out as being responsible for the majority of skin cancer-related deaths, despite constituting only a small percentage of all skin cancers. It should be noted that its incidence has increased significantly in recent decades, especially in populations of Caucasian origin. It is estimated that approximately 100,000 new cases of invasive melanoma will be diagnosed in the United States in 2024, with a mortality rate of approximately 8,000 cases per year. The incidence varies geographically, being higher in regions with greater exposure to ultraviolet radiation, such as Australia and New Zealand (1,2).

It should be noted that different risk factors include exposure to ultraviolet (UV) radiation, a family history of melanoma, the presence of dysplastic nevi, and the phenotype of fair skin. Intermittent and intense exposure to sunlight, which causes sunburn, is strongly associated with an increased risk of melanoma. In addition, the use of tanning beds has also been identified as a significant risk factor (2). On the other hand, genetic factors also play a crucial role in melanoma susceptibility. Mutations in genes such as CDKN2A and BRAF are associated with an increased risk of melanoma. In this sense, the CDKN2A gene, which encodes the proteins p16INK4A and p14ARF, is particularly relevant in familial melanoma, while BRAF mutations are common in sporadic melanomas (3,4).

The process of melanoma metastasis involves multiple steps, including local invasion, lymphovascular invasion, and proliferation in the new microenvironment. The ability of melanoma cells to perform these steps is mediated by a variety of molecular and cellular factors, including genetic and epigenetic alterations, interactions with the tumor microenvironment, and the ability to evade the immune system. All of this leads to histological aggressiveness that is quite prominent among all tumors. Despite this, survival from metastatic melanoma has improved in recent years thanks to advances in targeted therapies and immunotherapies. However, long-term survival remains limited. According to recent data, the five-year survival rate for metastatic melanoma is approximately 22.5%, although this figure varies depending on the location of the metastases and the tumor burden. Brain metastases, for example, are associated with a particularly poor prognosis (5,6).

In this sense, the identification of subgroups of patients who may benefit from specific treatments is an active area of research. Factors such as mutational load, PD-L1 expression, and the presence of mutations in genes such as BRAF and NRAS are being studied to better understand the heterogeneity of metastatic melanoma and develop more personalized therapies. Mutations in the BRAF gene, present in approximately 50% of melanomas, have allowed the development of targeted inhibitors such as vemurafenib and dabrafenib, which have shown improvements in survival. In addition, immune checkpoint inhibitors such as ipilimumab, nivolumab, and pembrolizumab have revolutionized the treatment of advanced melanoma by stimulating the body's own immune response against tumor cells (7). Despite these advances, many patients with metastatic melanoma do not respond to current therapies or develop resistance, underscoring the need to identify new biomarkers that can predict response to treatment, monitor disease progression, and develop personalized therapies.

This is why biomarkers play a crucial role in precision medicine, as they allow for better stratification of patients and more effective monitoring of the disease. In the context of metastatic melanoma, serological, histological, microRNA, and circulating tumor cell biomarkers offer a promising area of research and clinical application with the goal of improving diagnostic accuracy, treatment follow-up, and prognosis of patients with metastatic melanoma.

2. Serological markers

To date, LDH has been the only serological biomarker used to monitor tumor response to treatment, relating increasing values to disease progression. However, it has an important bias since an increase in these levels can also occur due to other causes (8-10).

According to the study carried out by Mancuso *et al* (8), it was observed that several serological markers have been associated with a worse prognosis in melanoma, and it can be determined that, in terms of tumor activity, high levels of Th2 cytokines [interleukin-4 (IL-4), IL-5 and IL-13)] and decreased levels of Th1 cytokines [IL-2 and interferon γ (IFN-γ)-interferon-γ] will condition the suppression of antitumor immunity.

In other studies such as that of Paganelli *et al* (11), the behavior of IFN-γ can be studied in depth, demonstrating that patients with an early stage in diagnosis (I-II) had elevated levels of IFN-γ in the blood, which progressively decreased with the metastatic development of the disease. Thus demonstrating the importance of IFN-γ in the antitumor immune response.

It has also been observed (8,11) that the presence of both the proinflammatory cytokine IL-17A and high levels of IL-10 (mainly immunosuppressive activity) and transforming growth factor beta (TGF-β) are associated with a poor prognosis [findings that can also be observed in studies (12-14)].

In this section, it is important to highlight the role of S100 proteins, particularly S100B, which are of special interest for use as diagnostic markers in melanoma due to their higher expression in tumor tissues compared to healthy tissues. Recently, elevated levels of S100B in the plasma of melanoma patients have been associated with a poorer prognosis, underscoring their relevance in assessing disease progression (15-18).

On the other hand, it has been observed that increasing levels of granulocyte-macrophage colony-stimulating factor (GM-CSF) can promote tumor cell migration *in vitro* and *in vivo* in multiple cancer types. Conversely, some studies have suggested that GM-CSF has inhibitory effects on tumor progression (19). Despite the lack of consensus on the exact role this factor plays, it has a component in tumor progression, which supports its inclusion in biomarker studies. The role of serum dermcidin (an antimicrobial peptide that can stimulate keratinocytes for the production of cytokines through the G protein and the subsequent activation of protein kinase) as a marker of metastatic progression should be highlighted (8).

Other studies reviewed, such as that of Paganelli *et al* (11), have corroborated the information described above, identifying the direct relationship between the levels of certain cytokines in the blood with the clinical course of the disease, and there may be a direct association with tumor progression.

Therefore, with the information provided by the different studies reviewed, we can conclude that the joint evaluation of elevated serum levels of IL-4, as well as the decrease in levels of GM-CSF, IFN-γ and DCD would be associated with a worse prognosis of the disease. Despite this, to date there are still no validated serological markers for determining the prognosis of the disease, since the marker that shows the greatest sensitivity in tumor staging is still the Breslow index.

3. Histological markers

Histologic markers are crucial for diagnosing and classifying melanoma, as well as for assessing its prognosis and

determining appropriate treatment. It is important to recognize that immunohistochemistry (IHC) should be interpreted in the context of clinical and histological findings and not in isolation. The following are some key histologic markers used in the study of melanoma, which are summarized in Table I.

Melanocytic lesions encompass a variety of skin tumors, from various benign nevi to malignant melanoma. IHC plays an important role in the treatment of cutaneous melanocytic tumors because the presence of melanin pigment indicates melanocytic differentiation, and in most cases, IHC is unnecessary. However, in difficult cases, IHC helps confirm the diagnosis. In some cases, pigment production may be minimal or absent, which can lead to a misdiagnosis such as carcinoma or poorly differentiated lymphoma. Immunohistochemical markers for melanocytic differentiation, such as Melan A, MART-1 (melanoma antigen recognized by T lymphocyte 1), HMB-45 (black human melanoma 45) (all cytoplasmic), and S-100 (nuclear), are frequently positive in melanocytic tumors but negative in epithelial or mesenchymal tumors (20,21). Among these markers, S-100 is the most sensitive, showing almost universal positivity in melanocytic tumors (22). Melan A and HMB-45 are more specific for melanocytic differentiation but less sensitive. HMB-45 can distinguish between benign and malignant melanocytic lesions; deeper parts of a nevus show reduced expression of HMB-45, while melanoma exhibits uniform positivity (23). It is important to note that some malignant melanoma variants, such as desmoplastic melanomas, are almost always negative for Melan A and HMB-45, but show S-100 expression. Therefore, the use of a combination of melanocytic markers is advisable in complicated diagnostic cases. SOX10, a member of the Sry HMG box (Sox) family of transcription factors, is a crucial transcription factor in the differentiation of neural crest cells. It is a newly described marker for melanocytic tumors, showing strong and diffuse nuclear positivity in these tumors. In reference to MITF, SOX10 directly regulates the expression of MITF, playing a crucial role in melanocyte development and melanoma progression. It is also useful for diagnosing desmoplastic melanoma and differentiating it from other spindle cell and scar cell tumors (24,25). Like other melanocytic markers, SOX10 should be used in conjunction with other markers and not in isolation.

IHC is usually not required to differentiate between benign melanocytic nevi and melanoma. The presence of pigmented nevi cells at various stages of maturation is usually sufficient for diagnosis. However, in certain cases, such as nevoid melanomas, differentiation of nevi such as Spitz's nevus is necessary, which exhibits transepidermal migration and pagetoid dissemination. Proliferative markers may be particularly important in these situations, as melanomas exhibit a much higher proliferation rate compared to nevi. Although there is no universally accepted cut-off point, the mean Ki-67 proliferation rate in Spitz's nevus is usually less than 2%, while in melanomas, the proliferation rate is 10% or more (26).

There are prognostic and predictive markers in melanoma that are crucial in selecting the most appropriate treatment for patients. Melanoma is often characterized by activation of the RAS-RAF-MAP kinase signaling pathway. The BRAF mutation is considered a key initial event in 50-60% of melanoma cases. Detection of the BRAF mutation is of great clinical importance because BRAF inhibitors are used to treat advanced melanoma. The most commonly seen BRAF mutation in melanoma is BRAF V600E. Genetic sequencing is the gold standard for detecting BRAF mutations. However, a commercially available antibody (VE1 clone) can now detect this specific mutation with high sensitivity and specificity (27). Tumor-infiltrating lymphocytes (TILs) are important for tumor immunosurveillance, as they can inhibit tumor growth. The TIL response in melanoma can be classified into three types: absent, non-intense, and intense. Programmed death ligand 1 (PD-L1) expressed by tumor cells can interact with PD receptors on T cells, leading to their inactivation. PD-1 inhibitors, such as pembrolizumab, have been introduced as a treatment for metastatic melanoma as part of immunotherapy. The use of this drug requires prior detection of PD-L1 expression by tumor cells and TILs, as it predicts response to treatment (28,29). IHC is essential for detecting PD-L1 expression, although staining patterns and diagnostic criteria vary across clones (30).

Therefore, proper application and interpretation of histologic and immunohistochemical markers, combined with clinical and histologic findings, are vital for accurate diagnosis, prognosis, and treatment selection in the treatment of melanoma. The continued evolution and integration of these markers into clinical practice promises significant improvements in the outcomes of melanoma patients (30).

Table I presents a summary of the main histologic biomarkers used in the diagnosis, surveillance, and prognosis of metastatic melanoma. It includes details on the type, usefulness and references of each biomarker, providing a comprehensive overview of clinical and research applications (30).

4. Genetic markers

It should be noted that there is a relationship between genetic, epigenetic, and environmental factors that justify the increase in the incidence of cutaneous melanoma since the 1960s (31,32). Currently, phenotypic characteristics, such as fair skin, freckles and red hair (phototypes I and II) are identified as risk factors for the development of this neoplasm. Likewise, ultraviolet radiation (UVR) represents a direct risk factor for direct DNA damage as it induces several mutations in the different molecular pathways, thus conditioning the development of one of the types of melanoma. These processes are factors that facilitate the appearance of malignant melanoma and, therefore, currently represent a public health problem (31,33).

Exposure of the skin to ultraviolet radiation has positive and negative effects. Among the effects of exposure to ultraviolet radiation are the regulation between the local neuroimmunoendocrine system and central homeostasis. Among the mechanisms proposed to reinforce this conception is what is stated in Slominski *et al* (34), where it is proposed that ultraviolet radiation, both UVA and UVB, produce physicochemical changes (at the level of pH, ions, reactive oxygen species and reactive nitrogen species) and that these changes could induce the activation or alter the functioning of sensory nerve endings, resulting in the transmission of signals to the brain or stimulation of the sympathetic nervous system (SNS) or autonomic nervous system (ANS), resulting in reflex-based responses.

Table I. Histologic biomarkers in metastatic melanoma.

First author/s, year	Biomarker	Marker type	Diagnostic or prognostic utility	(Refs.)
Davey *et al*, 2000	S-100	Histological	Neural crest origin tumor marker (including melanoma)	(15)
Davey *et al*, 2000; Petersson *et al*, 2009	Melan A, MART-1	Histological	Melanosomal proteins and differentiation markers in melanocytic lesions	(15,16)
Oberholzer *et al*, 2008	HMB-45	Histological	Melanosomal proteins and differentiation markers in melanocytic lesions	(17)
Hong, 2016; Viray *et al*, 2013	SOX10	Histological	SOX10 directly regulates the expression of MITF, serving a crucial role in melanocyte development and melanoma progression	(19,20)
Weinstein *et al*, 2014	Ki-67	Histological	It indicates the rate of proliferation; higher in melanomas than in nevi	(21)
Dorizzi *et al*, 2005	BRAF V600E	Histological	Mutation detection for targeted therapy with BRAF inhibitors	(22)
Rothberg *et al*, 2008; Ramos-Herberth *et al*, 2010	PD-L1	Histological	Predicts response to PD-1 inhibitors in immunotherapy	(23,24)

The table summarizes the key histologic biomarkers used in the diagnosis, follow-up and prognosis of metastatic melanoma. The type, utility and reference for each biomarker are included to provide a comprehensive overview of clinical and research applications. MART-1, melanoma antigen recognized by T lymphocyte 1; MITF, microphthalmia-associated transcription factor; PD-1, programmed cell death protein 1; PD-L1, programmed death ligand 1.

If ultraviolet exposure is not controlled, as Cole *et al* (35) commented, these physicochemical changes will accumulate in skin cells and trigger cascades of signaling pathways that cause alterations in collagen homeostasis, being the link with skin aging, thus leading to immunosuppression (35-38).

A result of ultraviolet radiation is that, when absorbed by DNA, it will carry out the production of pyrimidine cis-syn cyclobutane (CPD) dimers (39) as well as the photoproduct 6-4PPs (40), leading to oxidative stress (37). The accumulation of DNA damage can lead to mutations in key genes to maintain homeostasis, such as the p53 gene, which acts as a tumor suppressor (41).

Dysregulated melanogenesis, together with the depletion of essential cellular antioxidants and the production of reactive oxygen species (ROS), as well as the direct influence of quinone and semiquinone intermediates on RNA, DNA, and regulatory proteins, will result in an environment conducive to mutations that favors melanomagenesis (42,43).

To understand melanogenesis, it is essential to consider melanin as a pigment whose production is regulated by exposure to ultraviolet radiation, hormones, and cytokines (42). As a result, it should be noted that in addition to its protective role against UVR, melanin may play a role in the malignant transformation of melanocytes (44,45). There are two main types of melanin: eumelanin and pheomelanin (46,47). While eumelanin offers radiation protection and photoprotection by acting as an antioxidant and an efficient sunscreen, pheomelanin, due to its lower stability in the face of light, can create an environment prone to mutations after exposure to shortwave ultraviolet radiation (44,48,49).

According to the 2018 WHO classification, we can find three types of melanoma: those associated with cumulative sun damage (CSD), those not associated with cumulative sun damage, and nodular melanoma (50). Melanoma pathways associated with CSD include superficially disseminated, lentigo maligna, and desmoplastic melanomas, with a subdivision between high sun damage and low sun damage based on histopathological findings (38PO). Melanomas not associated with CSD are subclassified into spitzzoid, acral, mucus, and uveal melanomas and melanomas arising in congenital and blue nevi. This new classification describes in more detail the mutagenic changes found in the formation of melanoma.

Each subtype of melanoma is the result of the evolution of a precursor pathway (e.g., mutations in BRAF and NRAS are not accepted as features of the ultraviolet light-induced mutation (32,50). Each precursor has a variable risk of progression. It should be noted that nodular melanoma is classified in isolation in the new WHO classification because it can originate from any precursor pathway (51).

The role of fibroblasts in the evolution of melanoma has been reviewed, noting that the removal of reactive oxygen species interferes with the accumulation of HIF-1 in fibroblasts associated with hypoxia and reduces the expression of IL-6, VEGF-A and SDF-1. This phenomenon suggests that hypoxia-driven oxidative stress plays a crucial role in regulating the angiogenic and inflammatory response during melanoma progression (52).

Therefore, molecular and genetic markers have been postulated as a great tool for the diagnosis and targeted treatment of melanoma, facilitating the development of new lines of treatment (53). All these biomarkers are summarized in Table II.

Genetics of metastatic spread. In the melanoma progression model, we can find somatic alterations, such as activation of the mitogen-activated protein kinase (MAPK) pathway, activation

Table II. Genetic biomarkers in metastatic melanoma.

First author/s, year	Biomarker	Marker type	Diagnostic or prognostic utility	(Refs.)
Chatterjee and Bhattacharjee, 2018	BRAF	Genetic	Most common mutation in cutaneous melanoma; target for BRAF inhibitors	(30)
Chatterjee and Bhattacharjee, 2018	NRAS	Genetic	It is associated with nodular melanoma and chronic sun-damaged skin; limited targeted therapy options	(30)
Schneider *et al*, 2017	CDKN2A	Genetic	Common in hereditary melanoma; associated with multiple melanomas and dysplastic nevi	(36)
Bobos, 2021; Chatterjee and Bhattacharjee, 2018	TERT	Genetic	Activation is associated with a poor prognosis, particularly in sun-damaged melanoma	(27,30)
Vollmer, 2004	GNAQ/ GNA11	Genetic	Common in uveal melanoma; it is not usually associated with metastatic progression of cutaneous melanoma	(26)
Zorina *et al*, 2022	BAP1	Genetic	Tumor suppressor gene; associated with uveal melanoma and other cancer types	(37)
Vollmer, 2004	SF3B1, EIF1AX	Genetic	Mutations in these genes lead to an improved prognosis in uveal melanoma	(26)
Vollmer, 2004; Chatterjee and Bhattacharjee, 2018	VDR	Genetic	Inverse association between VDR and tumor progression, as well as overall survival and disease-free survival	(26,30)
Naylor *et al*, 2011	MC1R	Genetic	Increases risk of melanoma in individuals with CDKN2A mutations	(38)
Kripke *et al*, 1992	MITF	Genetic	It is associated with nodular and fast-growing melanoma; diagnostic marker of metastatic melanoma	(39)
Vollmer, 2004	HAPLN1	Genetic	Expression levels associated with aging and melanoma progression	(26)

The table summarizes the main genetic biomarkers used in the diagnosis, follow-up and prognosis of metastatic melanoma. The type, utility and reference for each biomarker are included to provide a comprehensive overview of clinical and research applications.

of the PI3K pathway, upregulation of telomerase activity, loss of control of the G1/S checkpoint, modulation of chromotine, and alteration of the p53 pathway (51,53).

The importance of the mitogen-activated protein kinase (MAPK) pathway is that it encodes the regulation of cell growth, proliferation, differentiation, and apoptosis (54). Mutations along this pathway result in an overamplification of signaling, leading to cell cycle dysregulation and uninhibited cell growth.

According to the pattern of the most prevalent mutations that activate the MAPK pathway, a genomic classification has been established into four major subtypes: mutated BRAF, mutated NRAS, mutated NF1 and the triple wild type. It should be noted that in all these subtypes, with the exception of the triple wild type, markers of sun damage due to ultraviolet radiation are found (51).

For the correct study of the different genetic markers present in melanoma and their clinical usefulness, they will be grouped according to the latter.

Prognostic or diagnostic markers

GNAQ/GNA11. They produce an overamplification of signaling through the MAPK and PI3K pathways by blocking GTPase activity (31). These mutations are mutually exclusive and will be found at a rate of 80-90% in uveal melanoma (55). In the case of cutaneous melanomas, these are very rare mutations (56).

According to the review by Yang *et al* (31), it was determined that the presence of mutations in GNAQ or GNA11 are not a priori related to metastatic progression.

CDKN2A. This marker is the most common in cases of hereditary melanoma, being present in up to 20% of subjects with a family history (51,57). Thus, individuals with the CDKN2A mutation develop multiple melanomas and significantly more dysplastic nevi, including dysplastic nevus syndrome (58). In the study by Yang *et al* (31), a correlation was observed between the mutation of this gene and the damage caused by UVA radiation. On the other hand, in the study by Helgadottir *et al* (59), it was observed that patients with melanoma and the *CDKN2A* mutation had an earlier age of onset and worse survival than those without the mutation.

BAP1. BAP1 is a tumor suppressor gene with a poorly understood mechanism in the development of melanoma. Mutations in BAP1 are associated with monosomy 3, which is associated with metastatic uveal melanoma (31). Notably, germline mutations of BAP1 have been identified, suggesting an inherited form of uveal melanoma. In fact, BAP1 is the most common mutation found in familial uveal melanoma, with an estimated frequency of 8 to 50% (60). In the study by Newton-Bishop *et al* (32), the relationship between this mutation and lung cancer or meningioma is also identified.

SF3B1 and EIF1AX. In the review by Yang *et al* (31), it is shown that the mutation of both genes (independently) results in a better prognosis in the case of uveal melanoma.

VDR. This is the vitamin D receptor, which has been shown to be a protective factor against melanoma by inhibiting proliferation, regulating growth factor activity, and promoting apoptosis. That is why a low expression of this receptor leads to a worse prognosis in melanoma (31,51,61), with a lower survival rate (62). This opens up promising horizons in the treatment of melanoma, with several clinical trials in countries such as Belgium or Australia, included in the study by Yang *et al* (31) that try to evaluate treatment with high doses of vitamin D in patients after surgical resection of melanoma.

MC1R. Melanocortin receptor 1 (MC1R) has been identified as an important gene in the development of sporadic melanoma, as MC1R variants increase the penetrance of CDKN2A mutations, significantly increasing the risk of melanoma development (63).

MITF. MITF is a regulatory gene for the development and differentiation of melanocytes, therefore, it is also associated with the development and progression of melanoma. This variant has been associated with a fast-growing, nodular melanoma variant, implying a worse prognosis (51). In turn, MITF is useful as a sensitive (88-100%) and specific marker for the diagnosis of metastatic melanoma of histologically similar non-melanocytic tumors (64).

It is interesting to note that this marker plays a key role in determining the behavior of melanoma cells based on their activity levels. When MITF is expressed at high levels, the cells respond by proliferating or differentiating, while at low levels, they acquire stem cell-like characteristics, leading to increased invasive capacity (65,66). On the other hand, prolonged suppression of MITF induces cellular senescence (melanoma regression) (66,67).

These fluctuations in MITF activity are driven by microenvironmental signals and epigenetic modifications, with direct genetic alterations having less influence on its activity (66,68). MITF is not an easy target for direct drug treatment, so therapeutic approaches focus on modulating the signaling pathways that regulate it (66). The inhibition of the MAPK pathway combined with histone deacetylase inhibitors (HDACi) has been shown to prevent MITF-mediated drug resistance in melanoma cells (69). For all these reasons, we can conclude that MITF is a genetic marker of great interest, and we will likely see new applications for it in the future (66).

HAPLN1. This molecule is directly related to aging, as fibroblasts secrete fewer components of the ECM with hyaluronan and proteoglycan binding protein 1 (HAPLN1) (31,70). Age-related changes degrade the extracellular matrix (ECM) in the skin and thus promote melanoma growth and migration (71), so determining HAPLN1 expression levels may be particularly useful in elderly patients (31).

Additionally, this marker is particularly interesting given that aged fibroblasts activate a signaling cascade in melanoma cells that decreases the levels of β-catenin and (MITF). This process contributes to the increased resistance of melanoma cells to targeted therapy with vemurafenib (71).

TERT. Activation of the telomerase pathway is a common event in the melanoma progression model, regardless of subtype (with or without sun damage). TERT-promoting mutations have been associated with a worse prognosis in melanoma, primarily in sun damage-related melanomas, while TERT amplification has been associated with a worse prognosis in acral melanoma (32,51,72).

Therapeutic targets

BRAF. BRAF is a kinase that is involved in signaling the MAPK pathway. The expression of BRAF-V600E acts synergistically with the loss of tumor suppressors (PTEN or p16INK4A), thereby leading to melanoma with metastatic potential (73). In fact, the different mutations that we will find in this pathway constitute the most common genetic alteration in cutaneous melanoma, representing between 40 and 60% of the total mutations present in this subtype of melanoma (31). Some of the BRAF mutations are V600E, V600K and V600R, with V600E being the most common of them, reaching 80% (74). It should be noted that mutations in BRAF are directly related to exposure to UV rays, thus conditioning different mutations depending, in part, on the presence or absence of chronic sun damage. For example, V600E expression is not associated with chronic sun damage, so it occurs in younger patients. On the contrary, the V600K mutations do have a direct relationship with chronic sun damage, taking place in areas of the body with sustained sun exposure (for example, the head) and, therefore, in elderly patients (75).

This route is of great importance as it also conditions therapeutic options. For example, drugs such as vemurafenib, dabrafenib, and encorafenib are inhibitors of BRAF V600 mutations (currently approved by the FDA). However, the combination of BRAF and MEK inhibitors (trametinib and cobimetinib) has now been shown to be more effective due to the joint inhibition of both pathways, thus reducing the resistance that develops to the use of BRAF inhibitors in monotherapy (31). Therefore, recent studies have shown that patients with mutated BRAF have a better prognosis than patients with native BRAF due to the difference in therapeutic options (51). Therefore, due to the success of targeted therapies, it is critical to identify melanoma patients with BRAF mutations.

NRAS. NRAS is critical in the transduction of extracellular growth signals through the MAPK and PI3K/AKT pathways. Melanomas with NRAS mutations correlate with the nodular subtype and are found in patients who have undergone CSD (76). As with BRAF mutations, we found direct sun damage (51). The prognostic value of NRAS mutation identification is unclear; this is because therapeutic options targeting this mutation remain scarce (31). However, in some studies, such as that of Jakob *et al* (77), the role of the NRAS mutation is described as an independent marker of poor prognosis, with decreased survival in patients diagnosed with metastatic melanoma. Speaking of mutations in BRAF and NRAS, it is worth highlighting the development of circulating DNA tests for use as possible prognostic factors. Thus, according to the study by Tímár and Ladányi (53), the detection of BRAF and NRAS mutations in peripheral blood would constitute markers of poor prognosis, which would imply the presence of molecular residual disease.

C-KIT. Unlike the BRAF mutation, this mutation implies a worse prognosis compared to wild-type melanomas. C-KIT is

a receptor tyrosine kinase that constitutes the first signal of the MAPK and PI3K pathways. This mutation is found in mucosal and acral melanomas and in those derived from chronic sun damage (31,78). Drugs such as imatinib and nilotinib are currently available for the treatment of melanomas with this mutation (78).

Immune checkpoint (CTLA-4, PD-1, PD-L1). This immune checkpoint is of great importance, as its inhibition causes increased activity and proliferation of T cells in peripheral tissue (31). The interaction between PD-L1 on tumor cells and PD-1 on T lymphocytes is crucial for tumor immune evasion. This binding activates a signal that deactivates the T cell receptor (TCR) response, thus inhibiting the secretion of growth factors and promoting tumor survival by blocking the immune response (79,80). Therefore, both the use of drugs such as ipilimumab (CTLA-4 antibodies) or Pembrolizumab or Nivolumab (anti-PD-1) will be very useful to improve the response to the tumor. Because of that, identifying PD-L1 expression in melanoma is the first effective step in treatment planning, as it is a first-line treatment in positively expressed tumors. In the case of tumors with negative expression, it will constitute a supportive treatment (31). Some studies, such as that of Pistillo *et al* (81), have shown that serum CTLA-4 levels could predict a favorable clinical outcome in patients treated with antibodies against CTLA-4 such as ipilimumab. The early identification of these markers, for all of the above, will directly condition both the treatment and the prognosis of the patient.

Therefore, to conclude, we can determine that higher-risk genes, particularly CDKN2A, have been identified. Familial melanoma genes are associated with an increased number of melanocytic nevi. At the somatic level, the most common driver mutation is BRAF and the second most common is NRAS, thus identifying a growing number of additional mutations that are less common, such as in TP53, but that may be very useful in the future (32).

Knowledge of the molecular pathology of melanoma represents a significant advance towards early diagnosis (diagnostic aid if histology is not decisive) and the optimization of treatment. The identification of common mutations (BRAF or NRAS) is of great help in directing therapy, and these pathways are still under study with possible promising results in the coming years, especially for the development of new therapies. Expanding knowledge about vitamin D is still an area under study, but interesting results are expected.

As mentioned in one study (82), vitamin D3 exerts various impacts, such as anti-melanoma activities and protective or restorative functions against oxidative stress and DNA damage caused by ultraviolet radiation. In addition, there is increasing evidence that vitamin D deficiency, defined as ≤20 ng/ml (50 nmol/l) of 25(OH)D3, and alterations in vitamin D signaling, involving VDR and CYP27B1 in the canonical pathway, influence the risk of developing melanoma and the evolution of the disease, affecting OST, DFST, and response to treatment.

5. Circulating tumor cells

Circulating tumor cells (CTCs) are cancer cells that break off from a primary tumor and travel through the bloodstream or lymphatic stream. They are markers of cancer spread and can be detected in peripheral blood. Studies of CTCs in melanoma in recent years have made it possible to assess tumor progression, disease recurrence, and response to treatment without the need for tumor biopsies (83).

Cell surface-specific melanoma-associated antigens (MAAs) are very limited, with limited availability of antibodies specific to these antigens. Analysis of MAA biomarkers by RT-PCR suggests that many CTCs are present in the peripheral blood of melanoma patients. The different levels of expression between MAA biomarkers during follow-up demonstrate the heterogeneity of CTCs. The utility of MAA multi-marker RT-PCR provides clinical information that correlates with disease outcomes in treated and untreated patients. From a clinical point of view, the enrichment of CTCs is crucial to increase the concentration of these cells in peripheral blood samples, facilitating their detection and analysis. Advances in enrichment methods have allowed for standardized and reproducible evaluations of multiple genetic alteration profiles of DNA, mRNA, and MAA. For example, the CELLSEARCH® system has been approved by the FDA to analyze CTCs in metastatic cancers such as breast, colon, and prostate, using ferrofluids with CD146 antibodies to enrich CTCs and a specific antibody against MAA for detection in melanoma (84).

One study (85) evaluated the detection of CTCs in patients with metastatic melanoma using techniques such as immunocytochemistry, RT-PCR, and digital droplet PCR to analyze both the presence of CTCs and circulating tumor DNA (ctDNA). High heterogeneity was identified among melanoma CTCs, with multiple distinct subpopulations. Detection of CTC in this study was associated with shorter, progression-free overall survival. In addition, correlations were observed between CTC scores and plasma ctDNA concentrations, suggesting that these biomarkers may have prognostic utility.

Assessment of tumor response in patients with advanced melanoma treated with checkpoint inhibitors (CIIs) or combination therapies is a challenge without real-time biomarkers, considering the possibility of objectifying pseudoprogressions in radiological imaging evaluations. A prospective study evaluated whether the molecular profiling of CTCs in blood can facilitate the follow-up of patients with metastatic melanoma during their treatment with CII (86). Panels of mRNA/DNA biomarkers were used in patients with melanoma during treatment, including LDH, CTC-mRNA, and tumor BRAF. These panels allow patients to be stratified into two groups: low-risk and high-risk. High-risk patients had lower disease-free survival and shorter overall survival. Patients who overexpressed beta catenin 1 on RNA sequencing had an increased risk of disease progression in contrast to patients who responded completely to treatment. On the other hand, CTC sequencing allowed the identification of subclinical disease in patients who developed progressive disease during treatment and follow-up.

In a study conducted in China (87), it was investigated whether there was a correlation between the number of CTCs before and after treatment with immunotherapy and BRAF-targeted therapy in patients with melanoma, including 49% in stages III-IV. It was found that an elevated baseline CTC count was associated with deep local invasion, nodal metastases, and distant metastases, as well as poorer overall survival, progression-free survival, and

disease-free survival. These findings suggest that baseline CTC count may be an important prognostic and predictive factor in patients with advanced melanoma.

Hoon *et al* (88) demonstrated the usefulness of CTCs in patients with stage I-IV cutaneous melanoma, positively correlating MAA markers detected by RT-PCR with stage, disease progression, and survival. They highlight the transcription factor associated with microphthalmia (MITF), crucial in the development of melanocytes and the growth of melanoma. The detection of MITF increases with tumor stage and its presence after treatment is associated with lower disease-free and overall survival (89).

Studies such as that of Koyanagi *et al* (90) have explored the usefulness of CTC screening as a predictor of response to treatment in metastatic melanoma. They evaluated the expression of MAAs mRNA biomarkers (B4GALNT1, MAGEA3, MITF, MLANA, and PAX3) by RT-PCR in a prospective multicenter phase II clinical trial. They observed a decrease in the number of MAA-positive CTCs during treatment, which was associated with improved response and increased survival. Surgical treatment has also been shown to significantly reduce the number of CTCs at all stages of melanoma (I-IV) compared to healthy patients (91).

The analysis of CTCs is constantly expanding thanks to the development of new technologies for their enrichment and detection. Recent advances in molecular profiling have the potential to enrich the information derived from CTCs, thereby increasing their clinical utility for patients with advanced melanoma.

6. MicroRNA

MicroRNAs (miRNAs) were first discovered in 1993 as a group of non-coding RNAs with a length of 21 to 23 nucleotides (92). Since their discovery, miRNAs have become an exciting area of research. In recent years, the value of miRNAs in melanoma according to their tumor inhibitory properties has been studied, classifying them as oncogenes or tumor suppressor genes (93). Importantly, miRNA expression levels have been clinically related to the response rate, efficacy, and side effects of treatments (94). Some key aspects of the involvement of miRNAs in melanoma are described below.

Fibroblasts play a suppressive role in the early development of melanoma. However, with tumor growth, fibroblasts are reprogrammed into cancer-associated fibroblasts (CAFs) to promote tumor progression. Before the invasion of melanoma cells, melanoma cells release some melanocytes into the dermis. These melanoma-derived exosomes contain a large amount of non-coding RNAs that are biologically active. The absorption of these miRNAs by fibroblasts can lead to the formation of CAFs, which are especially important in the formation of the metastatic niche (95).

Therefore, miRNAs can act as oncogenes or tumor suppressors. In this sense, miR-21 is frequently overexpressed in melanoma, which favors cell proliferation and resistance to apoptosis (96), while miR-34a acts as a tumor suppressor (97). On the other hand, some miRNAs, such as miR-10b and miR-182, are associated with the promotion of invasion and metastasis in melanoma cells, facilitating tumor progression to more aggressive stages (98).

It should also be noted that miRNAs are also implicated in resistance to conventional and targeted therapies. For example, miR-200c (99) has been linked to resistance to BRAF inhibitors, limiting the first-line therapeutic arsenal in these patients.

In addition, miRNA expressions in blood or biopsies in melanoma patients have been shown to be closely related to response to immunotherapy treatments, suggesting that miRNAs could serve as practical biomarkers to predict the response rate or survival time of patients treated with immunotherapy (100,101).

miRNAs can modulate the activity of different immune cells. For example, miR-155 is crucial for the function of T cells and macrophages, as it influences the immune response against melanoma. In this context, it has been observed that a high level of miR-155 expression after receiving anti-PD-1 treatment correlates with prolonged overall survival in patients with melanoma (102,103). In addition to miR-155, an elevated level of other miRNAs has been shown to indicate a positive response to PD-1 inhibitors. The expression of miR-100-5p and miR-125-5p has been shown to be positively correlated with the overall survival of melanoma patients treated with PD-1 inhibitors (104). Therefore, the interaction of miRNAs with immune checkpoint inhibitors, such as PD-1 and CTLA-4 inhibitors, is an area of great interest. miRNAs can affect the efficacy of these therapies and also be involved in the appearance of adverse effects related to the immune system.

In summary, miRNAs are crucial components in melanoma biology and have the potential to revolutionize both the diagnosis and treatment of this aggressive disease.

7. Neuroendocrine markers

In other sense recent research, including studies by Scheau *et al* (105), has significantly expanded our understanding of the neuroendocrine factors involved in melanoma pathogenesis, highlighting their roles in directly influencing tumor cell proliferation and metastasis or indirectly through modulating immune and inflammatory processes that impact disease progression. This emerging evidence suggests that neuroendocrine factors, including neurotransmitters like catecholamines and neuropeptides such as alpha-MSH, may offer novel serological markers for melanoma (105,106).

The intricate interactions of melanoma cells with the neuroendocrine system, as described in Scheau's comprehensive review, underscore a complex network where melanoma cells not only respond to neuroendocrine signals but also produce these factors to modulate their microenvironment. For instance, the production of CRH and ACTH by melanoma cells can lead to systemic effects, potentially altering the HPA axis and influencing systemic immune responses. This phenomenon could explain the elevated levels of certain hormones and neurotransmitters in patients with advanced melanoma, which might serve as potential biomarkers for disease status and progression (105,106).

Moreover, the ability of melanoma to drive systemic changes through neuroendocrine manipulation suggests that these markers could be pivotal for understanding the tumor's influence on systemic disease mechanisms.

However, in line with previous references to serological biomarkers, the study of serological and neuroendocrine biomarkers opens up a wide horizon. The reviewed studies observed that cross-evaluating the levels of serological markers alongside the Breslow index offers greater prognostic value in staging metastatic melanoma. Integrating these insights not only aids in the detection and monitoring of melanoma but also enhances our understanding of its systemic impact. This holistic approach is crucial for developing more precise and effective management strategies for patients with this aggressive cancer.

8. Conclusions

Treatment of metastatic melanoma remains a significant challenge due to its aggressive nature and ability to spread rapidly to other organs. Although advances in targeted therapies and immunotherapies have improved the outlook, the long-term survival rate remains low. However, the identification and use of various biomarkers offer a promising avenue to improve the diagnosis, follow-up and personalized treatment of this disease.

Serological biomarkers, such as LDH and certain cytokines, have been shown to be useful in providing information about tumor progression and response to treatment. However, more research is needed to validate its clinical application routinely. On the other hand, histological biomarkers, such as Melan-A, HMB-45, and S-100, are critical in the diagnosis and classification of melanoma, as they help distinguish between benign and malignant lesions and identify specific subtypes.

Similarly, microRNAs and circulating tumor cells (CTCs) have shown great potential to improve the treatment of metastatic melanoma. MicroRNAs can act as oncogenes or tumor suppressors, influencing disease progression and response to treatment. While CTCs allow tumor progression and response to treatment to be evaluated without the need for invasive biopsies, representing a less invasive tool for continuous patient monitoring.

In summary, advances in the identification and utilization of serological, histological, microRNA, and CTC biomarkers represent a very promising area of research and clinical application. These biomarkers are essential for improving the diagnosis, follow-up, and prognosis of metastatic melanoma, and have the potential to offer more precise and personalized treatments, significantly improving the approach to patients with metastatic disease.

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Authors' contributions

LP, ASC, YDYJ, LRO, BIP, LB, EDRC, CSC, JC, LT, AQC, MAS, MAM and MAO were involved in the conceptualization of the study. MAO and MAM were involved in funding acquisition. MAO was involved in project administration. LP, ASC, YDYJ, LRO, BIP, LB, EDRC, CSC, JC, LT, AQC, MAS, MAM and MAO were involved in the investigative aspects of the study. LP, ASC, YDYJ, LRO, BIP, LB, EDRC, CSC, JC, LT, AQC, MAS, MAM and MAO were involved in data validation. Data authentication is not applicable. All authors have read and approved the final manuscript.

Ethics approval and consent to participate

Not applicable.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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