Abstract. In less than 10 years, melanoma treatment has been revolutionized with the approval of tyrosine kinase inhibitors and immune checkpoint inhibitors, which have been shown to have a significant impact on the prognosis of patients with melanoma. The early steps of this transformation have taken place in research laboratories. The mitogen-activated protein kinase (MAPK) pathway, phosphoinositol-3-kinase (PI3K) pathway promote the development of melanoma through numerous genomic alterations on different components of these pathways. Moreover, melanoma cells deeply interact with the tumor microenvironment and the immune system. This knowledge has led to the identification of novel therapeutic targets and treatment strategies. In this review, the epidemiological features of cutaneous melanoma along with the biological mechanisms involved in its development and progression are summarized. The current state-of-the-art of advanced stage melanoma treatment strategies and the currently available evidence of the use of predictive and prognostic biomarkers are also discussed.

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I. Epidemiology and risk factors

The worldwide incidence of cutaneous melanoma has been increasing annually at a more rapid rate compared to any other type of cancer (1). In 2012, 232,000 new cases of melanoma and 55,000 deaths were registered worldwide, ranking 15th among most common cancers worldwide (2). The incidence of cutaneous melanoma varies greatly between countries and these different incidence patterns are ascribed to variations in racial skin phenotype, as well as differences in sun exposure. Moreover, unlike other solid tumors, melanoma mostly affects young and middle-aged individuals (median age at diagnosis, 57 years). The incidence increases linearly after the age of 25 years until the age of 50 years, and then decreases, particularly in the female sex. When analyzing incidence data in relation to sex, women are more frequent in younger aged groups, while the male sex prevails from the age of 55 onwards (3).

Ultraviolet (UV) light radiation from sunlight is the main environmental risk factor for melanoma skin cancer development (4–6). The increased risk of melanoma due to sun exposure is directly associated with the UV level and in particular to the UV-B spectrum (5). In addition, sun exposure patterns and timing have been associated in a number of studies with an increased risk of melanoma. In particular, intense and intermittent sun exposure (typical of sunburn history) is associated with a higher risk compared to a chronic continuous pattern of sun exposure that is more frequently associated with actinic keratosis and non-melanoma skin cancers (7–10). Furthermore, a history of sunburn in childhood or adolescence is associated with the highest risk of developing melanoma and individuals experiencing >5 episodes of severe sunburn have a 2-fold increased risk (8,11). UV-A exposure from artificial sources has been also linked to an increased risk of developing melanoma. The follow-up of patients with psoriasis receiving UV-A radiation phototherapy, as well as in individuals using sunbeds
has revealed an increased risk of melanoma in this population (12,13). Specifically, several studies and a meta-analysis have demonstrated a positive association between the risk of developing melanoma and the amount of sunbed usage, particularly from a young age, thus raising a major public health issue (12,14,15). UV light from sunbeds has been formally classified as a human carcinogen (14). No other environmental factors, including tobacco/smoke addiction, have been associated with melanoma (1).

In addition, host risk factors, such as the number of congenital and acquired melanocytic nevi, genetic susceptibility and a family history play a central role in the development of melanoma and acquired melanocytic nevi, genetic susceptibility and a family history play a central role in the development of melanoma (16-18). Approximately 25% of melanoma cases arise on a pre-existing nevus (19). In this context, not only the total number of nevi, but also the size and type of nevi, are individually associated with an increased risk of melanoma (20-23).

As regards genetic susceptibility, the polymorphisms of the melanocortin 1 receptor (MC1R) gene, are responsible for the different human skin-color phenotypes. Individuals with characteristics, such as red hair, a light complexion and light eyes exhibit a low pigmentation, with a consequent heightened sensitivity to UV exposure (24). Approximately 7-15% of melanoma cases occur in patients with a family history of melanoma (25). However, true hereditary melanoma (i.e., multi-generational, unilateral lineage, multiple primary lesions and early onset of the disease) are infrequent; the familial clustering of the disease is considered to be responsible for the presence of a transmitted genetic mutation (25,26). Over the past years, melanomas have also been found to arise in families that are generally prone to specific patterns of malignancies, such as the familial atypical multiple mole-melanoma syndrome (FAMMM syndrome) and its variant, the melanoma-astrocytoma syndrome (MAS) (26). Germline mutations in cyclin-dependent kinase inhibitor 2A (CDKN2A or p16) and, less common, mutations in cyclin-dependent kinase 4 (CDK4) are the most frequent genetic abnormalities identified in these families (26-28). Other inherited conditions associated with an increased risk of developing melanoma are xeroderma pigmentosum, familial retinoblastoma, Lynch syndrome type II and Li-Fraumeni cancer syndrome (25).

2. The genesis of malignant melanoma

Melanocytes are neural crest-derived cells that can be found principally in the basal epidermis and in hair follicles, along mucosal surfaces, meninges and in the choroidal layer of the eye (29). In response to UV-induced DNA damage, skin keratinocytes produce the melanocyte stimulating hormone (MSH) that binds the melanocortin receptor 1 (MC1R) on the melanocytes that than produce and release melanin. The melanin pigment ultimately operates as a shield for UV radiation, thus preventing further DNA alteration (30).

Cutaneous melanoma can be generally classified in the Caucasian population by its origin from chronically or intermittently sun-exposed skin that translate into different sites of origin, a degree of cumulative UV exposure, age at diagnosis, types of oncogenic drivers and mutational load (9). Indeed, melanomas in chronically sun-exposed skin usually appear in older-aged individuals (>55 years), on chronically sun-exposed areas, such as the head and neck, as well as the dorsal region of the upper extremities. The main genetic drivers are B-Raf proto-oncogene (BRAF), neurofibromin 1 (NF1) and NRAS mutations, and usually melanomas associated with chronically sun-exposed skin have a high mutational load related to UV exposure (9,31,32). On the other hand, melanoma associated with intermittent sun-exposed skin cases arise in younger-aged individuals (<55 years), on less sun-exposed areas, such as the trunk and proximal extremities, and are usually associated with BRAFV600E and a lower mutational load (31,32).

Over the past years, a deeper understanding of melanoma development and biology has been reached. It has become clear that the development of fully-evolved melanoma from pre-neoplastic lesions is not represented by a single evolutionary pattern. Each melanoma subtype can evolve from different precursor lesions, and can involve different gene mutations and stage of transformation (33). An interesting finding is that BRAF is mutated in up to 80% of benign nevi, resulting in limited melanocyte proliferation through the oncogene-mediated activation of cell senescence (34,35). These nevi remain indolent for decades also due to immune surveillance (36). Therefore, oncogenic BRAF alone is not sufficient for melanoma development and rarely benign nevi further progress to melanoma (33,37). When this usually occurs, it is associated with the acquisition of subsequent mutations in key genes, such as TERT or CDKN2A. On the other hand, melanomas associated with chronic sun-exposed skin usually do not arise form pre-existing nevi, but from melanomas in situ or dysplastic lesions and carry a different set of mutations (33). Histological characterization is the current mainstay of melanocytic neoplasia diagnosis and the definition of their malignant potential. However, histopathology is sometimes associated with the equivocal characterization of these lesions, leading to their improper risk stratification (38). The increasing understanding of the biological determinants of melanoma evolution and their potential integration in the management of melanoma patients may lead to an improve diagnosis and the earlier recognition of lesions at an increased risk of progression, thus improving patient risk stratification (Fig. 1).

3. Melanoma biology

Cutaneous melanoma is one of the most aggressive forms of skin cancer and one of the leading causes of cancer-related mortality due its metastatic power. Several studies have demonstrated that melanoma spreading is the result of genetic mutations and tumor microenvironmental alterations, characterized by the overexpression of proteins able to favor tumor invasion and surrounding infiltration (39-44). In particular, a key role is played by the overexpression of matrix metalloproteinases (MMPs), particularly MMP-9 and MMP-2, that induces the degradation of the components of the extracellular matrix, thus favoring tumor cell infiltration and spreading through the bloodstream (40-42). The overexpression of these proteins and tumor microenvironmental alterations are mediated by genetic alterations and the dysregulation of the nuclear factor (NF)-κB pathways. It has been demonstrated that MMP-9 overexpression observed in melanoma is caused by intragenic methylation phenomena that lead to protein overexpression (42). Furthermore, it has also been demonstrated that
NF-κB induces the overexpression of MMP-9 by the activation of osteopontin (OPN), another protein of the tumor microenvironment, thus playing a fundamental role in the development and progression of melanoma (43,44).

Apart from tumor microenvironmental alterations, melanomas are associated with one of the greatest burdens of somatic genetic alterations of all human tumors (45,46). The most frequent somatic mutations in chronically or intermittently sun-exposed skin melanomas affect genes that control central cellular process, such as proliferation (BRAF, NRAS and NF1), growth and metabolism [phosphatase and tensin homolog (PTEN) and KIT proto-oncogene receptor tyrosine kinase (KIT)], resistance to apoptosis [tumor protein p53 (TP53)], cell cycle control [cyclin-dependent kinase inhibitor 2A (CDKN2A) and replicative lifespan [telomerase reverse transcriptase (TERT)] (47,48). These genomic alterations typically lead to the aberrant activation of two main signaling pathways in melanoma: The RAS/RAF/MEK/ERK signaling cascade [also known as the mitogen-activated protein kinase (MAPK) pathway] and the phosphoinositol-3-kinase (PI3K)/AKT pathway (49).

The MAPK pathway is physiologically involved in the transduction of extracellular signals, such as growth factors and hormones, to the nucleus, leading to the expression of genes that are central drivers of cell proliferation, differentiation and survival (50,51). In addition, it has been shown that MAPK activation is a critical player in the biology of different types of cancer and is the most frequent pathway aberrantly activated in melanoma (52). The PI3K pathway is normally involved in cellular homeostasis and its activation has been demonstrated to be central in different cancer types, including melanoma where it is the second most frequently activated pathway (53,54).

Up to 90% of melanomas exhibit an aberrant MAPK pathway activation and this is a central step in melanoma development, being responsible for cell cycle deregulation and apoptosis inhibition (50,55,56). Among the different mechanisms responsible for abnormal MAPK pathway signaling in melanoma, the most frequent genetic abnormalities are, by far, BRAF mutations (37,47). Indeed, 37 to 50% of melanomas carry a somatic mutation in the BRAF gene with the highest frequency in cutaneous melanomas derived from intermittent sun exposure damage (approximately 60% carry a BRAF mutation) (31). Usually, BRAF mutations detected in cutaneous melanoma are missense mutations that determine amino acid substitution at valine 600. Approximately 80-90% of BRAF mutations are V600E (valine to glutamic acid), while 5-12% are valine to lysine substitution (V600K) and ≤5% are V600D (valine to aspartic acid) or V600R (valine to arginine) (57,58).

BRAF protein is a serine/threonine protein kinase of 766 amino acids organized in three domains: Two with regulatory function and one catalytic domain responsible for MEK phosphorylation (59). The catalytic domain is also responsible for maintaining the protein in its inactive conformation, through a hydrophobic interaction between the ‘so-called’ glycine-rich loop and the activation segment, making it inaccessible for ATP binding (59). In the BRAFV600E mutation, hydrophobic valine is replaced by polar, hydrophilic glutamic acid, resulting in an abnormal flip of the catalytic domain that generates a constitutive active conformation with a kinase activity 500-fold higher than wild-type BRAF kinase (60,61). Most of the non-V600E BRAF mutations act similarly through the alteration of glycine-rich loop and activation segment interaction, thus increasing BRAF kinase activity (61).

The second most common cause of aberrant signaling through the MAPK pathway in cutaneous melanoma is represented by NRAS activating mutations. NRAS is mutated in 15-30% of melanomas and in the majority of cases, these mutations are missense mutations of codon 12, 13 or 61 (the latter account for 80% of all NRAS mutations in melanoma) (31,62). Mutations of these codons lead to the prolongation of the NRAS-active GTP-bound state, thus abnormally maintaining NRAS signaling through both the MAPK and the PI3K pathways (47,63,64). Importantly, NRAS and BRAF mutations are considered mutually exclusive; however, co-mutations can rarely occur (approximately 0.5% in treatment-naïve patients) (64).

NF1 is a tumor suppressor gene mutated in 10-15% of melanoma cases and is the third most frequently mutated gene in melanoma (65,66). The NF1 protein regulates the RAS family by converting the active RAS-guanosine triphosphate (RAS-GTP) to the inactive RAS-guanosine diphosphate (RAS-GDP), thereby inhibiting downstream RAS signaling (67). Therefore, NF1 loss-of-function determines the hyperactivation of NRAS protein and thus, increased MAPK and PI3K pathways signaling (65,67,68). NF1 genomic alterations are more frequent in melanomas associated with
chronically sun-exposed skin and are usually associated with a high number of various genomic mutations, including co-occurrence with BRAF or NRAS mutations (68,69).

The receptor tyrosine kinase KIT is physiologically involved in melanoma proliferation and survival through the PI3K/AKT and the RAS/RAF/MEK/ERK pathways. Somatic activating mutations in this gene have been found in 2-8% of all malignant melanomas and are more frequent in acral melanomas and with melanoma arising on intermittently sun-exposed skin (70,71).

BRAF, NRAS, NF1 and KIT genomic deregulations are considered driver alterations in melanoma development; however, a number of other genes are involved in the characterization of invasive and metastatic melanoma genotype. TERT promoter mutations confer proliferative advantage to melanoma cells and along with heterozygous CDKN2A alterations, have been frequently detected in in situ melanoma (72). The CDKN2A gene encodes for p16INK4A, a cyclin-dependent kinase inhibitor. The further bi-allelic inactivation of CDKN2A is a subsequent step to the melanoma invasive phenotype, rarely observed in precursor lesions (72-74). PTEN is a tumor suppressor gene involved in cell cycle progression control. PTEN dysregulation is usually detected in vertical growth phase melanoma and metastases with a frequency of 10-30% of cutaneous melanoma (47,75). Missense and frameshift mutations or chromosomal deletions are the most frequent alteration detected in PTEN but also epigenetic mechanisms and microRNAs post transcriptional regulation of PTEN expression have been found (76). The genomic alteration involving PTEN are usually mutually exclusive with NRAS mutations, but frequently co-occur with BRAF gain-of-function. This finds its biological rational in the loss of PTEN being associated with increased PI3K/AKT pathway activation (77,78). Indeed, BRAF mutations and PTEN loss-of-function together activate both the MAPK pathway and the PI3K pathway, thus being potentially equivalent to NRAS-only activation (78,79). In the clinical setting, PTEN

| Trial name   | Treatment                          | Overall response rate (%) | Median progression-free survival (months) | Median overall survival (months) |
|--------------|------------------------------------|---------------------------|------------------------------------------|---------------------------------|
| BRIM3 (87,121) | Dacarbazine versus vemurafenib | 5                         | 1.6                                      | 9.7                             |
| coBRIM (93)    | Vemurafenib versus vemurafenib + cobimetinib | 48                        | 5.3                                      | 13.6                            |
| BREAK-3 (86)   | Dacarbazine versus dabrafenib     | 7                         | 2.7                                      | Not reported                    |
| COMBI-d (90)   | Dabrafenib versus dabrafenib + trametinib | 50                        | 5.1                                      | 20                              |
| COMBI-v (89)   | Vemurafenib versus dabrafenib + trametinib | 51                        | 8.8                                      | 18.7                            |
| CA184-024 (122) | Dacarbazine versus dacarbazine + ipilimumab | 10.3                    | 3                                        | 9.1                             |
| CheckMate 066 (88) | Dacarbazine versus nivolumab  | 13.9                      | 2.2                                      | 10.8                            |
| CheckMate 067 (123) | Ipilimumab versus nivolumab  | 19                        | 2.9                                      | 19.9                            |
| CheckMate 067 (123) | Ipilimumab versus ipilimumab + nivolumab | 43.7                     | 6.9                                      | 37.6                            |
| KEYNOTE-006 (124) | Ipilimumab versus pembrolizumab q2w | 57.6                     | 11.5                                     | Not reached                    |
|               | pembrolizumab q3w                 | 33.7                      | 5.5                                      | Not reached                    |

q2w, every 2 weeks; q3w, every 3 weeks.
loss-of-function represents one of the mechanisms responsible for the acquired resistance of BRAF mutant melanoma treated with BRAF inhibitors (80).

Even though a conclusive model of recurrent alterations leading to metastatic progression has yet to be elucidated, β-catenin-mediated WNT signaling activation has been shown to be associated with metastatic dissemination, as well as melanoma formation (37,81). CTNNB1 (β-catenin) gene mutations are detected in 2-4% of malignant melanomas and act through the stabilization of β-catenin and increased transcription of TCF/LEF-responsive target genes (82).

4. Principles of medical treatment

The majority of patients with newly-diagnosed melanoma have early-stage disease. For these patients, surgical excision represents the treatment of choice and is curative in the majority of cases (83). However, some patients will later relapse with disseminated disease, while approximately 10% of melanoma cases are diagnosed at an advanced stage, and are unresectable or already metastatic. Among stage IV tumors, approximately one-third have visceral and brain involvement at diagnosis, with a severe prognosis and lower probability to have a sustained response to treatment (84). For patients facing advanced-stage disease, melanoma treatment has been revolutionized since 2010, with the approval of several therapeutic agents. These agents include RAF and MEK kinase inhibitors, as well as immune checkpoint inhibitors [anti-cytotoxic T-lymphocyte-associated antigen 4 antibodies (anti-CTLA4) and anti-programmed cell death protein 1 antibody (anti-PD1)]. Indeed, in the advanced-stage setting, anti-PD1 and anti-CTLA4 antibodies (such as nivolumab, pembrolizumab and ipilimumab), as well as selective BRAF inhibitors ( vemurafenib and dabrafenib) alone and/or in combination with MEK inhibitors ( cobimetinib and trametinib) have shown promising results in clinical trials (Table I) (85-93). Currently, only the presence of BRAFV600E mutation is evaluated in the clinical setting, as it is essential to drive the appropriate treatment strategy. Other driver mutations, such as NRAS, NF1, CKIT, CDKN2A and PTEN, have not yet been included in standard clinical practice. However, the identification of these genomic alterations can identify patients who may benefit of experimental approach in clinical trials.

Immunotherapy and kinase inhibitors are nowadays the backbone of systemic therapy, while chemotherapy is considered a second-line, or even further, treatment option (94-96) (Fig. 2). Anti-PD1 antibodies and, with lower magnitude anti-CTLA4 therapeutic agents, offer lower response rates, but potentially long durable responses (85,91,92). In BRAFV600E melanoma, there has been a reasonable approach to the use of BRAF inhibitors with MEK inhibitors. The combination has led to high response rates (70%) and a rapid response induction and symptom control, with a progression-free survival of approximately 12 months (89,90,93). To date, however, there are no available data from prospective trials on the optimal choice for frontline treatment and treatment sequence, at least to the best of our knowledge. Nivolumab and pembrolizumab have shown to be effective on BRAF mutant melanoma after BRAF inhibitor resistance has risen, but there are no similar data for ipilimumab or for BRAF-inhibitor therapy in those with primary or secondary resistance to anti-PD-1 therapy (97-99). Currently, the combination of two different immune checkpoint inhibitors or the combination anti-PD1/anti-CTLA4 with targeted therapy must be considered an experimental approach in clinical trials. Each strategy has a clear benefit and basic research has demonstrated significant synergistic effects that need to be weighted with the potential increase in toxicity (100,101).

The inclusion of patient characteristics [biochemical parameters of melanoma kinetics, such us lactate dehydrogenase (LDH)] and expected toxicity profile, as well as comorbidities and patient personal preferences are central elements to be taken into account for frontline treatment strategy definition. In this rapidly evolving landscape, it is of great importance the participation of patients in randomized clinical trials.
5. Predictive biomarkers in melanoma

The identification of biomarkers that can predict patient benefits towards specific treatment strategies is a central goal of cancer research. BRAF mutations, particularly BRAF\(^{V600E}\), is a typical predictive marker of response to RAF inhibitors. However, these patients almost invariably develop disease progression after a variable period of time and some patients can display primary resistance to BRAF (+/- MEK) inhibitors. Several studies have described the central role of acquired genetic mutations affecting the Ras/Raf/MEK/ERK and PI3K/PTEN/Akt/mTOR signaling pathways in inducing resistance to both chemotherapy and targeted therapy in melanoma and other tumor types (102-104). In particular, the mechanisms responsible for BRAF (+/- MEK) inhibitor resistance can be divided into genomic (NRAS/KRAS mutation 20%, BRAF splice variants 16%, BRAF amplification 13%, MEKI/2 mutation 7%, bypass track mutations 11%), immunologic (epigenetic and transcriptomic changes of molecules involved in antigen presenting mechanisms) and a combination of both (105,106).

Currently, the detection of the mechanisms responsible for BRAF and MEK inhibitor resistance is not part of standard clinical practice; however, the development of non-invasive techniques for tumor mutational status assessment may lead to more rapid changes in this setting (107). The technique termed ‘liquid biopsy’ enables the detection of tumor-derived circulating cell-free DNA (ctDNA) in the plasma and is emerging as a promising blood-based biomarker for monitoring the melanoma disease status. Several studies have indicated that BRAF\(^{V600E}\) detection through ctDNA prior to the commencement of treatment is predictive of the response to RAF kinase inhibitors, and that high basal ctDNA levels are associated with a lower response rate and progression-free survival (107-109). Moreover, ctDNA is an indicator of tumor burden and tumor dynamics, and it has been demonstrated that an increase in ctDNA levels during treatment is indicative of disease progression and acquired resistance (107,109). Notably, ctDNA can be used also for the detection of mutations responsible for resistance to BRAF targeted therapies and in the future, this can be used to guide subsequent treatment strategies (107,109).

Immune checkpoint inhibitors are associated with a low overall response rate (ORR). This has driven considerable research efforts in the identification of biomarkers able to predict which patients will more likely benefit from these treatments. At this point, PD-L1 immunohistochemistry on tumor specimens is not a candidate marker for PD-1 inhibitor treatment response, due to the extremely heterogeneous results obtained from clinical trials (88,99). Several other predictive biomarkers are currently under investigation. The specific components of melanoma microenvironment and in particular the CD8\(^+\) T cell activation, through IFN-\(\gamma\) gene expression signature, has been associated with immunotherapy response (110,111). Moreover, several studies have demonstrated the mechanisms through which specific genomic alterations can drive immune checkpoint resistance through the alteration of antigen-presenting mechanisms and IFN-\(\gamma\) production (112-114). Recently, in humans, it has been demonstrated that specific gut microbiota compositions can drive differential responses to immune checkpoint inhibitors (115-117). This is not only a new intriguing field of research for immunotherapy biomarkers, but it also paves the way for the potential modulation of human gut microbiota composition to improve the immunotherapy response. As regards the identification of complex biological interactions among different pathways and their interplay with the immune system, bioinformatics has yielded promising results. In this context, computational models can simulate biochemical, metabolic and immune mediated interactions and characterize how they are potentially involved in melanoma development (118,119). Overall, computational approaches may potentially lead to the identification of novel therapeutic targets and may accelerate the drug discovery process (120).

6. Conclusions

Marked improvements in melanoma treatment have been achieved over the past decade. The tireless efforts of researchers have shed light on essential mechanisms involved in melanoma biology, paving the way for targeted treatment and immunotherapy. However, melanoma remains a lethal type of cancer, particularly when diagnosed at an advanced stage. Further elucidation of melanoma biology and evolution also in presence of treatment-selective pressure represent a central goal of cancer research in this field and may ultimately improve patient care and prognosis.

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Authors’ contributions

GCL and LF wrote the manuscript and were involved in data collection. JAM, RS and AZ contributed to enriching the knowledge concerning the genesis of melanoma, the current medical treatment and the predictive biomarkers tested for the diagnosis of melanoma. SC was involved in the creation of the figures. ML, SC and DAS conceived the review and revised the manuscript. All authors edited the manuscript and have approved its final version.

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