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INVITED REVIEW

Tissue prognostic biomarkers in primary cutaneous melanoma

Mario Mandalà · Daniela Massi

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Abstract Cutaneous melanoma (CM) causes the greatest number of skin cancer-related deaths worldwide. Predicting CM prognosis is important to determine the need for further investigation, counseling of patients, to guide appropriate management (particularly the need for postoperative adjuvant therapy), and for assignment of risk status in groups of patients entering clinical trials. Since recurrence rate is largely independent from stages defined by morphological and morphometric criteria, there is a strong need for identification of additional robust prognostic factors to support decision-making processes. Most data on prognostic biomarkers in melanoma have been evaluated in tumor tissue samples by conventional morphology and immunohistochemistry (IHC) as well as DNA and RNA analyses. In the present review, we critically summarize main high-quality studies investigating IHC-based protein biomarkers of melanoma outcome according to Reporting Recommendations for Tumor Marker Prognostic Studies (REMARK)-derived criteria. Pathways have been classified and conveyed in the "biologic road" previously described by Hanahan and Weinberg. Data derived from genomic and transcriptomic technologies have been critically reviewed to better understand if any of investigated proteins or gene signatures should be incorporated into clinical practice or still remain a field of melanoma research. Despite a wide body of research, no molecular prognostic biomarker has yet been translated into clinical practice. Conventional tissue biomarkers, such as Breslow thickness, ulceration, mitotic rate and lymph node positivity, remain the backbone prognostic indicators in melanoma.

Keywords Melanoma · Prognosis · Biomarkers · REMARK

Introduction

The global incidence of melanoma is increasing, with approximately 200,000 new cases and 65,000 melanoma-associated deaths each year [1, 2]. Overall, cutaneous melanoma (CM) accounts for only 4 % of all skin cancers; however, it causes the greatest number of skin cancer-related deaths worldwide [3].

As for many other cancers, mortality in melanoma is mainly related to metastatic spread to sites distant from the primary tumor and the prognostic parameters of the current melanoma American Joint Committee on Cancer (AJCC) staging system [4] represent the primary stratification criteria. Melanoma accurate prediction of prognosis is important to determine the need for further investigation, counseling of patients, to guide appropriate management (particularly the need for postoperative adjuvant therapy), and for assignment of risk status in groups of patients entering clinical trials.

However, according to AJCC staging system, the chances of relapse do not differ much between stage T2 and stage T3 patients; in addition also for melanoma detected at a very early stage (≤1 mm, T1a or T1b) the risk of recurrence ranges between 1 % and 12 % [5]. Such clinical observations suggest that melanoma represents a heterogeneous group of diseases with varied clinical behavior and response to therapy and there is a strong need for identification of additional robust prognostic factors to support decision making processes, since recurrence rate is largely independent from stages defined by morphological and morphometric criteria.

M. Mandalà
Unit of Clinical and Translational Research, Medical Oncology, Department of Oncology and Hematology, Papa Giovanni XXIII Hospital, Bergamo, Italy

D. Massi (✉)
Division of Pathological Anatomy, Department of Surgery and Translational Medicine, University of Florence, Largo Brambilla, 3, 50134 Florence, Italy
e-mail: Daniela.massi@unifi.it

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Novel technologies for molecular profiling, such as next generation DNA sequencing, have recently identified unique molecular profiles and mutation patterns in melanoma cells. At the RNA level, this has consisted of gene expression profiling using various robust and high throughput methodologies. In certain instances, markers identified using these platforms have been further examined and validated. However, at present, the contribution of such sophisticated and expensive techniques in predicting the prognosis of patients with melanoma, independently from conventional morphologically based tissue biomarkers, is not entirely clarified.

In 2000, Hanahan and Weinberg [6] reported six biological capabilities (hallmarks) acquired during the multistep development of human tumors: (i) sustaining proliferative signaling, (ii) evading growth suppressors, (iii) resisting cell death, (iv) enabling replicative immortality, (v) inducing angiogenesis, and (vi) activating invasion and metastasis [7]. In the last two decades, based on the emergence of new experimental data, two further hallmarks have been added to this list: (vii) reprogramming of energy metabolism and (viii) evading immune destruction. Such eight hallmarks, as distinctive and complementary capabilities that enable tumor growth and metastatic dissemination, may represent the logical framework in which organize and discuss the large amount of information related to prognosis beyond conventional prognostic biomarkers in CM patients.

To this aim, we performed an extensive "Medline" and Cancerlit literature review (1995–2013). Various combinations of search terms were used depending on the requirements of the database being searched. These terms included "melanoma," "prognosis," "prognostic," "prospective," "biomarkers," in combination with "genomic" or "postgenomic" or "immunohistochemical" or fluorescent in situ hybridization ("FISH") or comparative genomic hybridization ("CGH") or "molecular" or "incidence" or "management" or "recurrence" or "distant recurrence" or "survival", or "disease-free survival" or "trials" or "hallmarks" or "proliferative signaling" or "Ki67" or "phosphohistone H3" or "cyclin" or "growth suppressor" or "invasion" or "metastasis" or "lymphocytes" or "microenvironment" or "cell death" or "apoptosis" or "melanoma-specific survival," DNA microarray*, or RNA microarray*, or complementary DNA (cDNA) microarray,* or gene-expression profil*, or gene expression profil*, or gene expression signature*, or gene expression signature*, or gene microarray*. Figure 1 shows the key advances in prognosis melanoma determinants from conventional and molecular features.

Because acral lentiginous, mucosal, and uveal melanomas display different pathologic, molecular, and clinical features from more common cutaneous superficial spreading and nodular histological subtypes, studies describing results on specific melanoma subgroup, such as acral lentiginous, mucosal, choroidal, or uveal melanomas, were excluded.

The quality of each eligible study was assessed for adherence to Reporting Recommendations for Tumor Marker Prognostic Studies (REMARK) criteria [8–10]. According to Gould Rothberg et al. [11], high-quality studies should satisfy each of the following six criteria: (1) prospective or retrospective cohort design with a clearly defined source population and justifications for all excluded eligible cases; (2) assay of primary cutaneous tumor specimens; (3) clear descriptions of methods for tissue handling and IHC, including antigen retrieval, selection, and preparation of both primary and secondary antibodies, as well as visualization techniques; (4) a clear statement on the choice of positive and negative controls; (5) statistical analysis using multivariable proportional hazards modeling that adjusted for conventional clinical prognostic factors; and (6) reporting of the resultant adjusted hazard ratios (HRs) and their 95 % confidence intervals (CIs).

Herein, we followed the literature review strategy devised by Gould Rothberg [11] and Schramm and Mann [12], and we recorded additional eligible data for the subsequent 23 months.

Conventional tissue prognostic biomarkers

The clinical and histopathological characteristics that predict prognosis in primary CM have been studied for more than four decades. Presently, the following clinicopathologic prognostic markers have been consistently identified in several studies: age at diagnosis (with worse prognosis in older patients), male gender, growth phase (radial vs. vertical), Breslow thickness (BT), level of invasion, presence of ulceration and its extension, presence and density of tumor-infiltrating lymphocytes (TILs), presence of microsatellites, presence of vascular and/or lymphatic invasion, and the mitotic rate (MR).

However, when included in the more recent prognostic models [13–17], the absence or presence of regional nodal metastasis has been shown to be a strong prognostic factor that commonly negates all but a few of the variables associated with the primary tumor such as BT, MR, and ulceration.

According to the aim of this review, we will focus on conventional tissue prognostic biomarkers.

Breslow thickness

In 1970, Breslow [18] described a reproducible method of classification, based on the measurement in millimeters of the vertical thickness of the tumor. The BT is measured from the granular layer or, if the lesion is ulcerated, from the bottom of ulceration, up to the point of maximum infiltration.

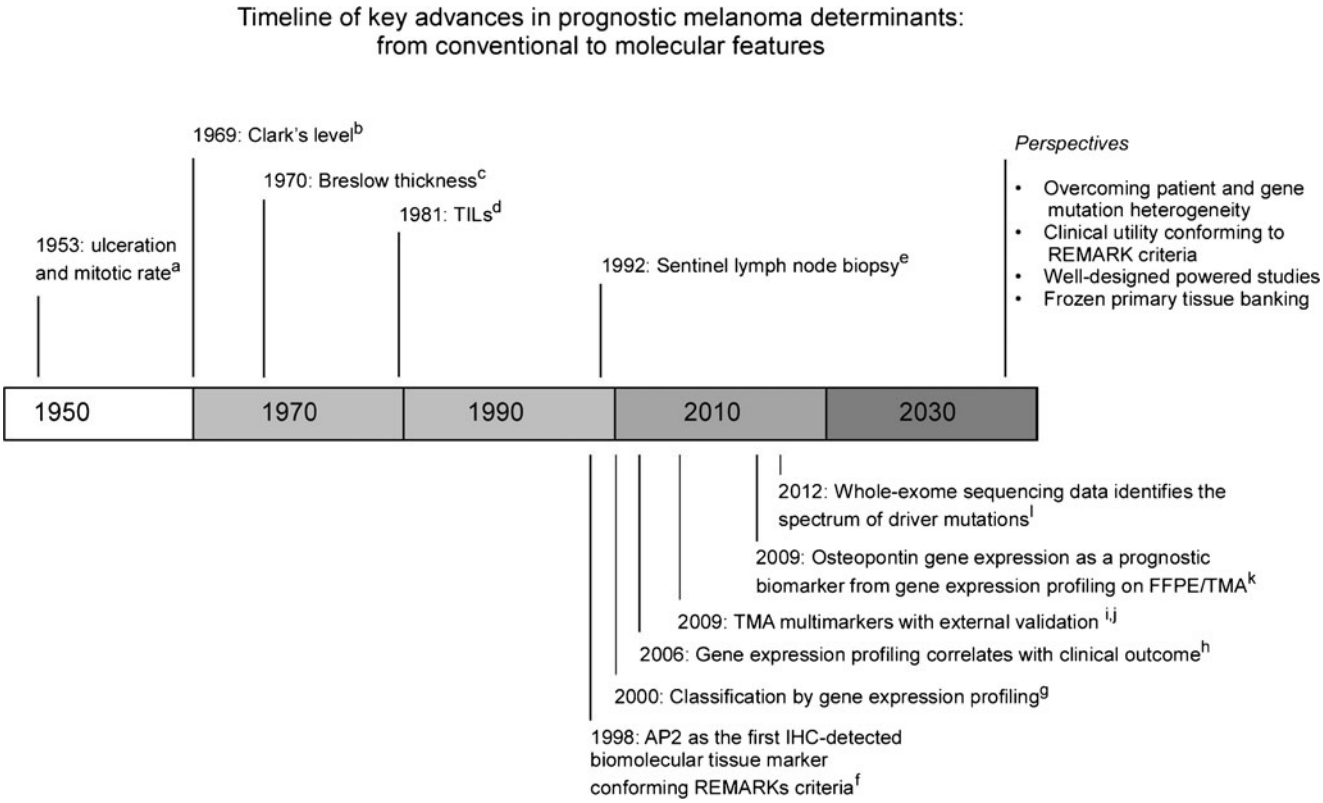

Fig. 1 Timeline of key advances in tissue prognostic biomarkers. **a** Allen AC, Spitz S [22]. **b** Clark et al. [35]. **c** Breslow [18]. **d** Day et al. [29]. **e** Morton et al. [19]. **f** Karjalainen et al. [72]. **g** Bittner et al. [87]. **h** Winnepenninckx et al. [90]. **i** Kashani-Sabet et al. [124]. **j** Gould Rothberg et al. [125]. **k** Conway et al. [96]. **l** Hodis et al. [20]

Adnexal involvement by melanoma is currently regarded as in situ disease, while in the case of periadnexal extension of melanoma, it is unclear from current evidence where the measurement of tumor thickness should be made to most accurately predict patient prognosis. It is generally agreed that thickness measurements should not be based on periadnexal extension, except when it is the only focus of invasion. In that case, BT may be measured from the inner layer of the outer root sheath epithelium or inner luminal surface of sweat glands, to the furthest extent of infiltration into the periadnexal dermis. The depth of extension of such foci beneath the granular layer of the epidermis may also be measured and reported (but not be recorded as the BT). Other problems in interpretation may arise when the nature of dermal cells is unclear (i.e., whether they represent melanoma or a pre-existing naevus) and in cases of tumors with exophytic/verruciform architecture. The thickness (measured from the top of the granular layer) of any zone of regression may also be recorded in the pathology report (but does not represent the true BT).

This classification system is more reproducible and reliable among pathologists and shows excellent correlation with mortality. The prognosis tends to worsen progressively in logarithmic function with increasing thickness, up to a thickness of 8 mm where it reaches a plateau, beyond which never reaches 100 % mortality [4]. In a population-based study of 548 patients, tumor thickness was the most significant prognostic factor for survival of patients with localized CM [21]. More recently, detailed evaluation of BT data recorded in the AJCC melanoma staging database, has confirmed that BT correlates with a highly significant decline in 5- and 10-year survival rates survival. Among patients with T1 melanomas (≤1 mm thickness), the 10-year survival was 92 %, but only 80 %, 63 %, and 50 %, in T2 (1.01 to 2.00 mm, T3 (2.01 to 4.00 mm) and T4 (>4 mm) melanoma patients, respectively [4].

Mitotic rate

In 1953, Allen and Spitz [22] reported the poor survival of patients who had primary melanomas with a high MR, but it was not until nearly 50 years later that MR began to be identified as an independent prognostic factor [23, 24]. In 2003, Azzola et al. [23] demonstrated that cellular proliferation within the primary tumor, as reflected by its MR, was a more powerful prognostic indicator than ulceration in a 3,661-patient, single-institution series.

In 2010, mitotic count was included in the pathological substaging of pT1 melanoma [4]. In the multivariate analysis, which formed the basis of these revised guidelines, mitotic

count was the second strongest prognostic factor after tumor thickness in localized melanoma [25]. The strongest prognostic impact of mitotic count was seen in the group of pT1 tumors, although it was also a significant factor for thicker lesions. In the current staging system [4], it is recommended that mitoses should be assessed in all primary melanomas. The MR should be expressed as the number of mitoses/mm^2 and rated in the invasive component of the melanoma from the areas with increased mitotic activity ("hot spots") and extending to adjacent fields for a total area of 1 mm^2. If there are no hot spots and mitoses are scattered randomly in the vertical growth phase component, in this case an area of 1 mm^2 around a representative mitosis should be assessed and the result expressed as the number of mitoses/mm^2. When the invasive component is <1 mm^2 the simple presence or absence of a mitosis can be designated as at least 1/mm^2 (mitogenic) or 0/mm^2 (non-mitogenic).

As underlined by Scolyer and Thompson [26] MR should be recorded as the number of mitoses per mm^2 and not per high power field (HPF), for at least three reasons: 1) comparison of MRs reported as n/HPF may not be reliable, because the field diameter of an HPF can vary greatly between microscopes; 2) there is an excellent inter-observer reproducibility when the hot spot method has been reported in a number of studies, whereas there is a poor inter-observer reproducibility for MR when it was recorded per HPF; 3) MR has been reported as an independent prognostic factor only when the hot spot method, as recommended by the current version of the AJCC staging manual, has been used.

Ulceration

The presence of ulceration must be microscopically evaluated in each primary melanoma specimen and the interpretation of melanoma ulceration is one of the most reproducible histopathological features. Ulceration is characterized by: i) full-thickness epidermal defect (including absence of stratum corneum and basement membrane); ii) evidence of reactive changes (fibrin deposition and neutrophils); iii) thinning, effacement, or reactive hyperplasia of the surrounding epidermis in the absence of trauma or a recent surgical procedure. It has been recently reported that extent of ulceration (measured either as diameter or percentage of tumor width) provides more accurate prognostic information than the mere presence of ulceration [27] but the evaluation of such parameters is not currently included in the pathological staging.

Thicker melanomas are more commonly ulcerated. In a population-based series, the incidence of melanoma ulceration in thin melanomas was only 6 %, while thick melanomas were ulcerated in 63 % of cases [21]. Ulceration is a factor that predicts disease outcome independently of tumor thickness. The presence or absence of primary melanoma ulceration was the third most powerful survival predictor in the analysis of the current AJCC staging system [4]. Patients with ulcerated melanomas had a twofold higher estimated risk of dying due to melanoma compared to those with non-ulcerated tumors [4].

Interestingly, the presence of ulceration diminishes survival rates to the same level as for the patients with non-ulcerated melanomas of the next, larger thickness group. In the last AJCC melanoma staging database, 5-year survival was 79 % for T3a non-ulcerated melanomas, and was 82 % for T2b ulcerated melanoma. T4a non-ulcerated melanoma showed a 5-year survival of 71 %, similar to that of T3b ulcerated melanoma with a 68 % rate. T4b ulcerated melanoma were associated with a 5-year survival of 53 % [4].

Tumor-infiltrating lymphocytes

The metastatic process involves a complex series of interactions between the tumor and the host. Several data, first from animal models and then from studies in cancer patients, support the hypothesis that the immune system can recognize and reject tumors, suggesting that the ability of the tumor to evade immune recognition (immune surveillance) often determines the clinical course of the disease [28]. In melanoma tissue specimens, it has long been recognized that lymphocytes may be observed in intimate association with melanoma cells.

In 1981, Day et al. [29] reported a significantly better prognosis in patients with a marked lymphocytic infiltrate within primary CM than those with absent TILs. Clark et al. [30] later classified TILs according to their distribution and intensity as follows: (1) "brisk," if the lymphocytes were present throughout the substance of the vertical growth phase or were present and infiltrating across the entire base of the vertical growth phase; (2) "non-brisk," if the lymphocytes were in one focus or more foci of the vertical growth phase, either dispersed throughout or situated focally in the periphery; and (3) "absent," if there were no lymphocytes or if the lymphocytes were present but did not infiltrate the melanoma. Clark et al. [30] demonstrated that TILs had prognostic significance only if observed in the vertical growth phase but not in the radial growth phase.

The role of TILs as prognostic factors has been suggested by several reports, although conflicting data have been reported so far [21, 31–34]. Several reasons can be taken into account to justify such discrepancies: (1) inclusion of predominantly thin and radial growth phase melanomas vs. thick melanomas; (2) difficulty in some cases to grade a TILs infiltrate using the brisk and non-brisk categories; (3) studies may be underpowered to demonstrate an independent prognostic factor; and (4) scarce information regarding the immunology and pathobiology of TILs.

Recently, Azimi et al. [34] reported a four-tier system for grading TILs infiltrates based on the distribution and the density of TILs observed in the dermal component of the

tumor. By using this classification, TIL grade has been reported as an independent predictor of survival and sentinel lymph node (SLN) status in patients with melanoma [34]. Although this study suggests that absence of TILs predicts SLN positivity and a poorer prognosis, melanoma progression still occurs, in most cases, despite lymphocytic infiltration. Such observation, in turn, implies the inability of TILs to mount an effective immune response. Whether TILs in such cases are functionally defective, incompletely activated, or anergic is still open to further investigation. Moreover, a better molecular characterization of TILs in PCM patients mandates further studies.

Clark's level

In 1969, Clark et al. [35], for the first time, formulated a histopathological classification of melanoma based on the level of invasion of the anatomical layers of the skin, demonstrating that the level of invasion was closely related to survival. In level I, melanoma is limited to the epidermis (in situ) and without risk of distant metastases. In level II, superficial extension to the papillary dermis, still guarantees an excellent prognosis. Invasion of level III (infiltration of the papillary dermis up to the reticular dermis) of IV (invasion of the reticular dermis) and V (invasion of subcutaneous fat) instead provides a gradual increase in the risk of metastasis and mortality. When considered as a single variable, Clark level of invasion is strongly associated with melanoma outcome. In a population-based series, patients with level II melanoma had 98.8 % 5-year survival, which dropped to 92.5 % in patients with level III melanoma, 76.7 % in patients with level IV melanoma, and 75 % in patients with level V melanoma [21].

In the 2010 AJCC classification, Clark level of invasion is no longer incorporated as a staging criterion, since when MR is considered in the multivariate analysis, level is not an independent prognostic factor. Level has been replaced by MR in the 2010 AJCC classification for subclassifying pT1 lesions as T1a or T1b, but in the text and in a table comment of the AJCC chapter [4], Clark level IV or V is referred to as a tertiary criterion for T1b in cases with no ulceration and "if MR cannot be determined." Clark level should therefore be reported whenever it would form the basis for upstaging T1 lesions.

Tumor growth phase

CM originates from the proliferation of melanocytes in the basal layer of the epidermis and then expand radially at a later time and invades deep into the dermis. The radial phase is the not tumorigenic phase, characterized by the proliferation of melanocytes in the epidermis and/or in the papillary dermis, without formation of tumor nodule. The vertical growth phase is that where the tumorigenic melanoma acquires the ability to metastasize and is morphologically characterized by the presence of an expansive nodule larger than the intraepidermal aggregates and/or by the presence of mitotic figures in the invasive component.

Tumor regression

Regression is the segmental replacement of the melanoma by fibrosis, with increased vascularity and melanophages, and a lymphocytic infiltrate of variable density, with or without residual epidermal component. Regression can be defined as partial (involving less than 75 % of the lesion) or extensive (involving 75 % or more of the lesion). This phenomenon may be viewed into three temporal stages: early, intermediate, and late. In most reports assessing the prognostic significance of regression, intermediate, and late regression have not been differentiated and the prognostic significance of regression remains unclear. Some studies report that it indicates a worse prognosis (particularly in thin melanomas) [36, 37], whereas others did not report poorer outcome [38]. Difficulties in interpreting such studies include lack of a standardized diagnostic criteria and poor interobserver reproducibility.

Lymphovascular invasion

Vascular invasion is identified by the histopathological demonstration of melanoma cells within the lumina of blood vessels or lymphatics, or both. It is an uncommon finding in the excision specimens of primary CM (up to 8 %) [39], and is generally regarded as a marker of poor prognosis [40, 41]. IHC-detected LVI ranges from 16 % to 47 % [40, 42, 43] whereas BVI is uncommon in primary melanoma (3–4 % incidence) [42, 43]. The presence lymphatic vessels invasion and blood vessels invasion detected by IHC has been associated to melanoma prognosis in many studies [40, 41, 43–47].

Microsatellites

Microsatellites are defined as microscopic and discontinuous cutaneous and/or subcutaneous metastases>0.05 mm in diameter found on pathologic examination, adjacent to a primary melanoma (separated from the main invasive component by a distance of at least 0.3 mm). Microsatellites are cutaneous or subcutaneous deposits of melanoma trapped within the lymphatics between the primary tumor and the regional lymph node basin. Microsatellitosis defines a subgroup of patients at higher-risk for regional and systemic recurrence.

Melanoma histotype

The common melanoma histotypes (superficial spreading melanoma, nodular melanoma, acral lentiginous melanoma and lentigo maligna melanoma) are of little if any prognostic significance, independent of tumor thickness. Their interpretation is subjective and prone to interobserver variation.

Lymph node positivity

Melanoma commonly metastasizes by the lymphatic route, and the disease status of regional lymph nodes is considered as the most important prognostic indicator for patients with melanoma. In order to assess the regional lymph node status, SLN biopsy was developed as a standard procedure technique in patients deemed to have significant risk of clinically occult nodal metastases. The aim of this procedure is to detect micrometastatic lymphatic disease for selective lymphadenectomy. Depending on the extent of the pathology protocol used and the BT of the population studied, SN positivity ranges from 15 % to 33 % [48–50]. The more extensive sectioning protocols, like the standardized EORTC MG protocol, increases the detection rates of metastatic melanoma in SLN and also reveals larger deposits.

Among patients with localized melanoma undergoing SLN biopsy, the status of the sentinel node is the most important prognostic factor. According to the 2010 AJCC classification, clinically occult nodal metastases of melanoma found by SLNB or elective lymphadenectomy are defined as micrometastases, whereas nodal metastases found by clinical or radiologic evaluation and confirmed pathologically are classified as macrometastases.

Among patients with nodal metastases (Stage III), the number of metastatic nodes, clinical nodal status (macrometastases vs. micrometastases), the sentinel node tumor burden (represented by maximum diameter of the largest tumor lesion), and microanatomic tumor location within a sentinel node are the most important predictors of survival [51, 52]. In patients with nodal metastases, MR, primary ulceration, and primary thickness are all independent prognostic factors at multivariate analysis. In contrast, for patients with nodal macrometastases, the number of tumor-containing nodes and primary ulceration independently predict survival at multivariate analysis.

IHC-based novel tissue biomarkers: independent or redundant prognostic classifiers?

Validation issue strategy and related currently open issues

In the last two decades, several studies evaluated single biomarkers for outcome prediction in melanoma patients. The overall quality of the studies was generally poor if benchmarked against the REMARK guidelines [8]. In particular, a minority of the studies stated explicitly that they conformed to the REMARK checklist [11].

According to a recent meta-analysis, among 1,797 manuscripts reporting new tissue biomarkers for predicting outcome in melanoma, only 37 cohort studies, collectively describing 87 assays on 62 distinct proteins, conformed to the REMARK criteria by Gould Rothberg et al. [11].

In the present review, we updated data reported from Gould Rothberg et al. [11] and summarized the results of the most significant studies in Tables 1, 2, 3, 4, and 5. High-quality clinical and translational studies evaluating new potential IHC-based tissue biomarkers in melanoma, according to the REMARK criteria, are reported. Described biomarkers have been clustered according to the eight hallmarks described by Hanahan and Weinberg [6, 7] (Fig. 2).

Even taking into account that these studies met the REMARK criteria, a number of considerations should be raised to understand whether these biomarkers are fully ready to be transferred into clinical practice:

(i) *small series*: many of the studies included a limited number of patients and this has reduced the statistical power and widened the CIs, making extrapolation of results in the general patient population very difficult to implement;
(ii) *heterogeneous/lack of staging information*: most of the studies did not report the TNM classification and included patient cohort with heterogeneous clinical and pathological characteristics, such as patients with node positive and negative, that cannot be considered as a single entity neither from a clinical nor from a biological point of view;
(iii) *retrospective studies*: the design of the studies was generally based on retrospective series and limitations due to patient selection criteria cannot be ruled out;
(iv) *external/internal validation*: only 2 out of 63 studies reported an independent predictive molecular prognostic assay for primary melanoma, replicating the independent prognostic effect of molecular markers: (a) in a data set drawn from a completely different patient population, (b) across different tissue platforms (tissue microarray in the initial study cohort vs. tissue sections in the replication cohort), and (c) using different measurement techniques (pathologist scoring vs. digital imaging analysis);
(v) *pre- and post-SNLB era*: most prognostic models with long-term follow-up have been developed with patients who did not receive a SLN biopsy and these models have been based on clinical factors and histologic findings in the primary tumor. Thus, these results should not be extrapolated to patients treated in the era of SLNB;

Table 1 Prognostic biomarkers: cell cycle and proliferation

Author year	Biomarker	Method/tissues	Derivation cohort (*n*)	Statistical analysis	Covariates MVA	Outcome	HR (95 % CI)	*P* values
Alonso et al. [53]	BCL6	IHC/FFPE (TMA)	60° RTV+validation cohort 72° RTV	MVA	BT	OS	BCL6 6.1 (1.92–20.5)	0.002
	KI67						Ki67 Not significant	–
	p16						p16 0.14 (0.04–0.45)	0.001
	p21						p21 not significant	–
Alonso et al. [54]	PKCα	IHC/FFPE* (TMA)	34°* RTV+validation cohort 127° RTV	MVA	BT	DFS	2.3 (1.3–4.0)	0.004
Berger et al. [55]	ATF2	IHC/FFPE (TMA)	269° RTV	MVA	BT, U, TILs Ms, CL	MSS	1.8 (1.34–2.4)	0.0002
Florenes et al. [56]	Cyclin A, Ki67	IHC/FFPE (whole sections)	172° RTV	MVA	BT, Age AS, U, G, Ki67	DFS OS	Cyclin A, 3.7 (3.4–4.1)	0.0003
							Ki67, Not significant	–
							Cyclin A, Not significant	–
							Ki67, Not significant	–
Florenes et al. [57]	Cyclin D2	IHC/FFPE (whole sections)	172° RTV	MVA	BT, Age AS, U, G	DFS	Not significant	–
	Cyclin D3					OS	Not significant	
Li et al. [58]	SKP2, $p27^{Kip1}$	IHC/FFPE (whole sections)	104 Stages I–III RTV	UVA	–	OS	Not significant	–
Niezabitowski et al. [59]	PCNA	IHC/FFPE (whole sections)	91 Stages I–III RTV	MVA	BT	OS DFS	PCNA, 2.27	0.03
							Ki67, 5.17	0.002
	Ki67						Ki67, Not significant	0.03
							PCNA 4.44	–
Rangel et al. [60]	NCOA3	IHC/FFPE (TMA)	343° RTV	MVA	BT, Age AS, U, G	MSS	1.91	0.021
						DFS	1.69	0.0095
Straume and Akslen [61]	ID1	IHC/FFPE (TMA)	119 Stages I–III RTV	MVA	BT, AS, U G	MSS	Not significant	–
Straume et al. [62]	Ki67	IHC/FFPE (whole sections)	187 Stages I–III RTV	MVA	BT, U, VI, AS	OS	3.7 (1.6–8.9)	0.003
Straume et al. [62]	p53	IHC/FFPE (whole sections)	187 Stages I–III RTV	MVA	BT, U, VI, AS	OS	8.9 (2.7–29.0)	0.0003
Piras et al. [63]	p53	IHC/FFPE (whole sections)	68 Stages I–II RTV	MVA	BT, CL, Stage	OS	Not significant	–
Rangel et al. [64]	RGS1	IHC/FFPE (TMA)	301° RTV	MVA	BT, Age, AS, U, G SLN	MSS	1.74	0.004
						DFS	1.63	0.007
Reschke et al. [65]	HER3	IHC/FFPE (TMA)	130° RTV	MVA	BT, Age U, G	OS	2.6 (1.04–6.6)	0.041
Ladstein et al. [66]	PHH3	IHC/FFPE (whole sections)	338° RTV	MVA	BT, U, MR	OS	1.9 (1.04–3.36)	0.027
Ladstein et al. [67]	PHH3	IHC/FFPE (TMA)	202° RTV	MVA	BT, U, MR	OS	Not significant	
Chen et al. [68]	Skp2	IHC/FFPE (TMA)	249° RTV	MVA	Age, G, BT, U, AS	OS	Not significant	–
						MSS	Not significant	–
Gimotty et al. [69]	Ki–67	IHC/FFPE (whole sections)	396 T≤1 mm RTV	MVA	Age, G, BT	OS	3.4 (1.6–7.0)	0.001

AS Anatomic site, *ATF2* activating transcription factor 2, *BT* Breslow thickness, *CL* Clark's level, *CI* confidence interval, *DC* derivation cohort, *DFS* disease-free survival, *FFPE* formalin-fixed paraffin-embedded, *G* gender, *HR* hazard ratio, *ID1* DNA-binding protein inhibitor1, *MCAM* melanoma cell adhesion molecule, *MSS* melanoma-specific survival, *MVA* multivariate analysis, *MR* mitotic rate, *Ms* microsatellitosis, *MSS* melanoma-specific survival, *NCOA3* nuclear receptor coactivator-3, *OS* overall survival, *PCNA* proliferating cell nuclear antigen, *PHH3* phosphohistone H3, *PKCα* protein kinase Cα, *RGS1* regulator of G protein signaling 1, *RTV* retrospective, *SKP2* S-phase kinase-associated protein-2, *SNL* sentinel lymph node, *TMA* tissue microarray, *tPA* tissue type activator of plasminogen, *U* ulceration, *UVA* univariate analysis, *VC* validation cohort, *VI* vascular invasion, *60°* no TNM information [additional metastases (8 of 88 cases) from patients from whom primary tumors could not be obtained were also included in the study], *34°, 269°, 127°, 172°, 214°, 301°, 343°, 338°, 202°, 130°, 259°* incomplete TNM information, *34°** training set for gene expression profiling, *IHC/FFPE* (TMA)* validation cohort

Table 2 Prognostic biomarkers: growth suppressors

Author year	Biomarker	Method/tissues	Derivation cohort (n)	Statistical analysis	Covariates MVA	Outcome	HR (95 % CI)	*P* values
Berger et al. [70]	AP-2α	IHC/FFPE (TMA)	214° RTV	MVA	BT, U, CL, TILs, Ms	MSS	2.14 (1.22–3.76)	0.0082
Berger et al. [71]	HDM2	IHC/FFPE (TMA)	200° RTV	UVA		OS	–	0.03
						MSS		0.03
Karjalainen et al. [72]	AP-2	IHC/FFPE (whole sections)	273 Stages I–II RTV	MVA	BT	DFS	3.12 (1.42–6.82)	0.0026
						OS	Not significant	
Korabiowska et al. [73]	Ku70	IHC/FFPE (whole sections)	76° RTV	MVA	BT	OS	Ku 70 0.87 (0.82–0.92)	<0.001
	Ku80						Ku 80 0.85 (0.79–0.92)	<0.001
McDermott et al. [74]	nm23	IHC/FFPE (whole sections)	145° RTV	UVA MVA	Stage	OS	Not significant	–
Pacifico et al. [75]	nm23	IHC/FFPE (TMA)	120 Stages I–III RTV	MVA	Age, BT, U, CL	OS	Not significant	–
Soltani et al. [76]	MAP2	IHC/FFPE (whole sections)	37° RTV	MVA	Age, G, BT	DFS	0.18	0.003
Straume et al. [62]	p16	IHC/FFPE (whole sections)	167 Stages I–IIII RTV	MVA	BT, U, vascular invasion, AS	OS	2.5 (1.5–4.2)	0.0008
						DFS	2	0.007
Li et al. [77]	ING4	IHC/FFPE (TMA)	101° RTV	MVA	Age, G, BT, U, AS	OS	2.50 (1.09–5.74)	0.031
						MSS	2.97 (1.02–8.63)	0.045
Lin et al. [78]	SNF5	IHC/FFPE (TMA)	88° RTV	MVA	Age, G, BT, U, AS	OS	5.145 (1.48–17.89)	0.010
						MSS	4.637 (1.15–18.63)	0.031
Jonsson et al. [79]	RBM3	IHC/FFPE (TMA)	215 Stages I–III RTV	MVA	Age, G, MR, CL, BT	DFS	Not significant	–
						OS	0.33 (0.18–0.61)	0.001
Jafarnejad et al. [80]	Sox4	IHC/FFPE (TMA)	89°* RTV	MVA	Age, G	OS	1.9 (1.078–3.362)	0.026
						MSS	1.99 (1.087–3.658)	0.025
Chen et al. [81]	SATB1	IHC/FFPE (whole sections)	47 Stages I–II RTV	MVA	BT	OS	9.92 (1.18–83.78)	0.03

AP-2 activator protein-2, *AS* Anatomic site, *BT* Breslow thickness, *CL* Clark's level, *CI* confidence interval, *DFS* disease-free survival, *FFPE* formalin-fixed paraffin-embedded, *G* gender, *HDM2* human homolog of murine double minute 2, *HR* hazard ratio, *ING4* inhibitor of growth 4, *MAP-2* microtubule-associated protein-2, *MCAM* melanoma cell adhesion molecule, *MSS* melanoma-specific survival, *MVA* multivariate analysis, *MR* mitotic rate, *Ms* microsatellitosis, *MSS* melanoma-specific survival, *nm23* nonmetastatic 23, *NR* not reported, *OS* overall survival, *PKCα* protein kinase C α, *RBM3* RNA-binding motif protein 3, *RTV* retrospective, *SATB1* special AT-rich sequence-binding protein-1, *SNF5* core subunit of SWI/SNF chromatin remodeling complexes, *SNL* sentinel lymph node, *SOX4* sry-related high-mobility group box transcription factor, *TMA* tissue microarray, *U* ulceration, *UVA* univariate analysis, *214°, 200°, 76°, 145°, 37°, 101°, 88°, 89°* incomplete TNM information, *89°** disease-free and overall survival in high-risk patients: BT>1.5 mm

(vi) *heterogeneous follow-up strategy*: in retrospective trials the follow-up strategy has not been homogeneous for all enrolled patients, therefore a lead time bias due to different follow-up timing cannot be excluded;

(vii) *intratumoral heterogeneity/serial tissue sections bias*: all the reported studies measured the new biomarker in a tissue section that might have been distant from the original sections used to assess conventional prognostic histopathological factors. Future studies should include a protocol wherein these biomarkers are read on the same or adjacent serial sections for all lesions, in order to exclude biased results determined by tumor heterogeneity;

(viii) *tissue microarray (TMA) vs. whole tissue sections*: discrepancies are reported in results obtained in TMA vs. whole sections [66, 67]. TMA sections are less suitable for recording unevenly distributed markers in tumor tissues, therefore, the results obtained by TMA studies cannot be automatically translated into clinical practice where standard whole sections are used; and

(ix) *undemonstrated clinical utility*: although several studies demonstrated a strong correlation between a tissue biomarker and DFS or OS, this does not imply per se that the marker should be used to direct patient care. A new tissue biomarker should demonstrate clinical utility, namely, that the use of the marker test to drive patient management results in a favorable balance of benefits to harm, leading to superior outcomes compared with nonuse of the marker test. Improvement in outcome may relate to OS, DFS, quality of life, or cost of care.

Table 3 Prognostic biomarkers: resisting cell death

Author year	Biomarker	Method/tissues	Derivation cohort (*n*)	Statistical analysis	Covariates MVA BT, MR, U, NP	Outcome	HR (95 % CI)	*P* Values
Divito et al. [82]	Bcl-2	IHC/FFPE (TMA)	159° RTV	MVA	Age, gender, BT, U, CL	MSS	Bcl-2 0.64 (0.48–0.86)	0.026
Ekmekcioglu et al. [83]	iNOS*	IHC/FFPE (whole sections)	132 RTV Stage III	MVA	Age, gender, SNL Status, In transit disease	MSS	iNOS PC 4.63 (2.60 8.25)	<0.0001
							iNOS SI 7.69 (3.76 15.74)	<0.0001
Bachmann et al. [84]	TNFα	IHC/FFPE (TMA)	133 RTV Stages I–III	MVA	BT, U, Age, G, SNL Status	MSS	2.7 (1.5–4.8)	0.001
Piras et al. [85]	Survivin	IHC/FFPE (whole sections)	50 RTV Stages I–II	MVA	BT	DFS	Survivin 7.320 (1.43, 37.38)	0.017
Piras et al. [63]	Survivin	IHC/FFPE (whole sections)	68 RTV Stages I–II	MVA	BT, CL, Stage	OS	Not significant	–
Zhuang et al. [86]	GRP78	IHC/FFPE (whole sections)	92° RTV	MVA	BT, U, Age, G, MR	OS DFS	Not significant	–

Bcl-2 B-cell lymphoma 2, *BT* Breslow thickness, *CI* confidence interval, *CL* Clark's level, *DFS* disease-free survival, *FFPE* formalin-fixed paraffin-embedded, *G* gender, *HR* hazard ratio, *GRP78* glucose-regulated protein 78, *iNOS** inducible nitric oxide synthase: number of positive cells (PC) and staining intensity (SI), *MCAM* melanoma cell adhesion molecule, *MSS* melanoma-specific survival, *MVA* multivariate analysis, *MR* mitotic rate, *MSS* melanoma-specific survival, *OS* overall survival, *RTV* retrospective, *SNL* sentinel lymph node, *TMA* tissue microarray, *TNF* tumor necrosis factor, *U* ulceration, *UVA* univariate analysis, *159°, 92°* incomplete TNM information

For the above considerations, notwithstanding that hundreds of such studies sought to assess the potential prognostic value of IHC-detected protein biomarkers in predicting the clinical course of melanoma patients, no biomolecular profile correlated with outcome can be considered ready to enter routine clinical practice.

Genetic microarray signatures: impact on prognosis and technical shortcomings

Genomic and transcriptomic technologies make the analysis of gene expression signatures and mutation status possible so that tumors may be classified more accurately with respect to diagnosis and prognosis.

In their pioneering study, Bittner et al. [87] reported, through gene expression profiling analysis, two molecular subtypes of metastatic melanoma with possible prognostic significance. Since the publication of this first signature in the late 1990s, high-throughput gene expression analysis has revolutionized genetics over the last 13 years. Nevertheless, scientific excitement about the attractiveness of molecular technologies has been temperate by results that did not reach scientific evidence of clinical benefit. Furthermore, it is not still clear whether these expensive and complex techniques can be applied extensively for routine use.

Recently, molecular studies reporting gene expression profiles in melanoma have been reviewed [88]. From more than 100 articles available in the literature, only 14 gene expression studies that identified biomarkers associated with prognosis fulfilled the REMARK criteria [54, 89–100, 124]. These studies included patients with different BT, patients with superficial spreading and nodular melanoma, with or without SLN involvement, with different follow-up strategy and treatment in the adjuvant and metastatic setting and heterogeneous rationale of dataset construction. Samples were obtained from different sources: primary tumor, lymph nodes, and distant metastases. Only 4 out of 14 studies provided estimated effects with CIs from an analysis in which the marker and standard prognostic variables were included. Furthermore, most of these studies did not describe the flow of patients through the study, including the reasons for dropout. Most of the above reported reasons may partially justify the poor signature overlap across these 14 studies. However, other technical reasons should be considered.

MHC II molecules were found to correlate with prognosis in two studies [89, 93]. However, biopsies with infiltrated metastatic lymph nodes were evaluated in both studies and thus a small amount of residual lymphoid tissue, expressing HLA class II molecules, could have been present.

Tumor heterogeneity, namely, non-malignant and malignant melanocytes for DNA and/or RNA-based studies is a critical issue in polymerase chain reaction and cDNA arrays in which cells are disrupted to release genomic material from different cell types. The scarcity of tumor samples amplifies this confounding heterogeneity.

Furthermore, as compared to other solid tumors technical limitations in the availability of frozen tissue from primary CM-limited large gene expression profiling study from patients with long clinical follow-up, since it is custom to fix and embed in paraffin for histologic diagnosis the whole primary tumor.

Finally, there is evidence that mutation frequency varies by several orders of magnitude across patients with a given

Table 4 Prognostic biomarkers: invasion and metastasis

Author year	Biomarker	Method/tissues	Derivation cohort (n)	Statistical analysis	Covariates MVA	Outcome	HR (95 % CI)	*P* Values
Ferrier et al. [101]	tPA	IHC/FFPE (whole sections)	214 Stage II (T_3–T_4 N0M0) RTV	MVA	BT, U, Age	OS	1.9 (1.00–3.76)	0.043
						DFS	Not significant	–
Ilmonen et al. [102]	Tenascin-C	IHC/FFPE (whole sections)	98° RTV	MVA	BT U	OS DFS	Not significant	–
Karjalainen et al. [103]	CD44	IHC/FFPE (whole sections)	292 RTV Stages I–II	MVA	BT	OS DFS	Not significant 1.74 (1.05–2.87)	0.03
Pacifico et al. [104]	CD44vr	IHC/FFPE (TMA)	120 RTV Stages I–III	MVA	BT, U, CL, Site, Age, Gender	OS	Not significant	–
Pacifico et al. [105]	P-Cadherin	IHC/FFPE (TMA)	120 RTV Stages I–III	MVA	BT, U, CL, Site, Age, Gender	OS	Not significant	–
Pacifico et al. [106]	MCAM	IHC/FFPE (TMA)	76 RTV Stages I–III	MVA	BT, U, CL, Site, Age, Gender	OS	Not significant	–
Pearl et al. [107]	MCAM	IHC/FFPE (TMA)	76 RTV Stages I–II–III	MVA	BT, U, Age, Gender	DFS	14.8	0.01
Rangel et al. [108]	Osteopontin	IHC/FFPE (TMA)	345° RTV	MVA	BT, U, Age, Gender	MSS	1.55	0.04
Scala et al. [109]	CXCR4	IHC/FFPE (whole sections)	71 RTV Stages II–III	MVA	BT, U, Age, Gender, SLN status	DFS	1.65 (0.10–0.90)	0.0148
						OS	2.07 (0.14–1.31)	0.0150
Thies et al. [110]	L1CAM	IHC/FFPE (whole sections)	100 Stages I–II RTV	MVA	BT, MR, U	DFS	4.384 (2.082–9.229)	<0.0005
Thies et al. [111]	CEACAM1	IHC/FFPE (whole sections)	100 Stages I–II RTV	MVA	BT, MR, U	DFS	7.170 (3.222–15.945)	<0.0005
Weinlich et al. [112]	Metallothionein	IHC/FFPE (whole sections)	1,270° RTV	MVA	BT, U, Age, Gender	DFS	3.94 (2.77–5.6)	<0.001
						OS	3.49 (2.25–5.43)	<0.001
Weinlich et al. [113]	Metallothionein	IHC/FFPE (whole sections)	158° RTV	MVA	BT, U, Age, Gender, SNL Status	DFS OS	2.86 (1.31–6.77) Not significant	0.009
Bachmann et al. [114]	αv Integrin	IHC/FFPE (TMA)	133° RTV	MVA	BT, U, Age, Gender, Ki67	MSS	2.3 (1.0–5.1)	0.049
Boone et al. [115]	Rhoc	IHC/FFPE (whole sections)	123° RTV Stages I–II–III	MVA	BT, U	OS	Not significant	–
Kreizenbech et al. [116]	E-Cadherin P-Cadherin	IHC/FFPE (whole sections)	201° RTV	MVA	BT, U	OS	Not significant	–
Buljan et al. [117]	Galectin-3	IHC/FFPE (whole sections)	104 Stages I–III	MVA	BT	DFS MSS	Not significant Not significant	–
Lade-Keller et al. [118]	Low E- and high N-cadherin	IHC/FFPE (TMA)	393 Stages I–III	MVA	BT, U, tumor stage	MSS	1.96 (1.0–3.7)	0.04
						DMFS	2.1 (1.1–3.9)	0.02

BT Breslow thickness, *CEACAM* carcinoembryonic antigen-related cell adhesion molecules, *CL* Clark's level, *CI* confidence interval, *CXCR4* chemokine receptor 4, *DMFS* distant metastasis-free survival, *DFS* disease-free survival, *FFPE* formalin-fixed paraffin-embedded, *HR* hazard ratio, *L1CAM* cell adhesion molecule L1, *MCAM* melanoma cell adhesion molecule, *MSS* melanoma-specific survival, *MVA* multivariate analysis, *MR* mitotic rate, *OS* overall survival, *Rhoc* Ras homolog gene family, member C, *RTV* retrospective, *SNL* sentinel lymph node, *TMA* tissue microarray, *tPA* tissue-type activator of plasminogen, *U* ulceration, *UVA* univariate analysis, *98°, 345°, 1270°, 158°, 133°, 201°* no complete TNM information

Table 5 Prognostic biomarkers: replicative immortalization, (lymph)-angiogenesis, cell metabolism, immune evasion

Author year	Biomarker	Method/ tissues	Derivation cohort (*n*)	Statistical analysis	Covariates MVA	Outcome	HR (95 % CI)	*P* values
Replicative immortalization								
Bachman et al. [119]	BMI-1	IHC/FFPE (TMA)	127 RTV Stages I–III	MVA	BT, U, CL, Ki-67	MSS	2.9 (1.1–3.7)	0.024
Lymphangiogenesis								
Straume et al. [120]	LYVE-1 Podoplanin	IHC/FFPE (whole sections)	166° RTV	MVA	BT, U, Vascular invasion, CL, Ki67	OS	MVD High 1.8	0.04
							LVDpt Low 2.1	0.009
Xu et al. [41]	D2-40/S100	IHC/FFPE (whole sections)	251 RTV Stages I–II	MVA	BT, U, AS, MR	DFS	2.2	0.026
Cell metabolism								
Zhuang et al. [121]	LDH-5	IHC/FFPE (whole sections)	86° RTV	MVA	BT, U, CL, MR,	DFS OS	Not significant	–
Immune evasion								
Hofbauer et al. [122]	MAAs HLA class I	IHC/FFPE (whole sections)	91° RTV	UVA No	–	OS	–	–
Svobodova et al. [123]	CTAg	IHC/FFPE (whole sections)	321 Stages I–II RTV	MVA	BT, U, MR	DFS OS	1.715 (1.140–2.580) Not significant	0.010
Multimarkers								
Kashani-Sabet et al. [124]	Multimarkers# NCOA3, RGS1, SPP1	IHC/FFPE (TMA)	395° RTV+External validation cohort 141	MVA	BT, U, SLN Status	MSS	1.34	0.001
Gould Rothberg et al. [125]	Multimarkers* ATF2, p21, p16, fibronectin, β-catenin	IHC/FFPE (TMA)	192 RTV Stage II+external validation cohort 246	MVA	BT, Age, Gender, SLN Status	MSS	2.72 (1.12–6.58) High risk vs. low risk	0.027

ATF2 activating transcription factor 2, *BMI-1* B-cell-specific Moloney murine leukaemia virus integration site *1*, *BT* Breslow thickness, *CI* confidence interval, *CL* Clark's level, *CTA* cancer testis antigen, *DC* derivation cohort, *DFS* disease-free survival, *FFPE* formalin-fixed paraffin-embedded, *HR* hazard ratio, *LDH* lactate dehydrogenase, *LVDpt* lymphatic vessel density intra- and peri-tumoral, *MAAs* melanoma-associated antigens, *MCAM* melanoma cell adhesion molecule, *MR* mitotic rate, *MSS* melanoma-specific survival, *MVA* multivariate analysis, *MVD* microvessel density, *NCOA3* nuclear receptor coactivator-3, *NP* lymph node positivity, *RGS1* regulator of G protein signaling 1, *OS* overall survival, *RTV* retrospective, *SNL* sentinel lymph node, *SPP1* secreted phosphoprotein 1, *TMA* tissue microarray, *U* ulceration, *UVA* univariate analysis, *VC* validation cohort, *91°, 86°, 187°, 395°* incomplete TNM information

[#] Each marker was given a score of +1 (overexpressed) or −1 (underexpressed) A prognostic index was calculated that reflected the net score (the sum of the scores of the three individual markers), resulting in a seven-point scale ranging from −3 (all markers below their cut-points for the given outcome measure) to +3 (all markers above their cut-points for the outcome measure). A high-risk group was defined as all patients with positive net multimarker index scores and a low-risk group as all patients with negative net multimarker scores

[*] The following five markers and associated cut points have been reported: ATF2 ratio more than −0.052 (>cytoplasmic concentration), β-catenin more than 38.68, fibronectin≤57.93, $p16^{INK4A}$ ratio≤ −0.083 (>nuclear concentration), and $p21^{WAF1}$ more than 12.98. Low risk: 4–5 markers, high risk: 0–3 markers. Overall, assignment to the low-risk group requires elevated levels of overall β-catenin and nuclear $p21^{WAF1}$, decreased levels of fibronectin, and distributions that favor nuclear concentration for $p16^{INK4A}$ but cytoplasmic concentration for ATF2

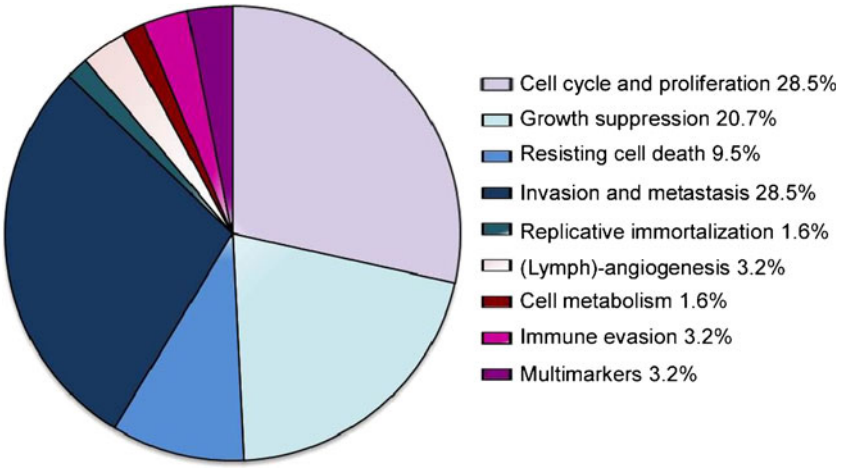

Fig. 2 Main prognostic biomarkers conformed to the REMARK criteria reported in primary cutaneous melanoma patients and clustered according to hallmarks of cancer (Hanahan and Weinberg [7])

cancer type and there is also variability among individual patients. These issues should be considered since current analytic methods could fail to account for mutational heterogeneity that affects the mutational background mutation rate [126].

BRAF/NRAS: prognostic or predictive biomarkers?

In patients with CM, the AJCC staging criteria and currently used conventional prognostic models define subgroups of patients with different survival. However, within each patient subgroup, there still remains significant variability in clinical outcome. With the advancement of cancer genomics, it is clear that the genetic features of melanoma are heterogeneous and relevant to melanoma progression. Driver mutations of genes associated with the development of tumor cells, such as genes controlling cell division and apoptosis, invasion and metastasis, growth suppressor, and immune evasion have been identified in melanoma.

There is increasing evidence that CM is a genetically heterogeneous disease, and genetic alterations can be used to classify primary tumors into distinct subtypes. Loss of PTEN (25–50 %) and amplification of microphthalmia-associated transcription factor (MITF; 10–15 %), CDK2/4 (20 %), and Cyclin D (30 %) are frequently observed [127]. Mutation of c-KIT occurs in 20–40 % of acral and mucosal tumors [127]. More than half of primary CM show activating mutations of NRAS (15 %) or BRAF proto-oncogenes (50 %), which are components of the RAS-RAF-MEK-ERK signal transduction pathway [128]. Gain-of-function BRAFV600E mutation accounts for more than 70 % of the BRAF alterations described in melanoma, with alternative point mutations at the same position (V600D, V600K, V600R) contributing as following: 10–30 % are V600K, while V600R mutations constitute approximately 3–7 % of all BRAF mutations.

Several studies evaluated the impact of NRAS and/or BRAF mutations on outcome in patients with primary CM [129]. In an attempt to correlate BRAF and NRAS mutational status with features known to influence tumor behavior including age, gender, Breslow depth, Clark level, MR, the presence of ulceration and AJCC staging Ellerhorst et al. [130] performed a microdissection of 223 primary melanomas. In this study, survival did not differ between Stage III patients whose primary tumors did or did not carry mutations, even though the mutated tumors tended to produce larger volume nodal disease.

Edlundh-Rose et al. [131] analyzed a total of 294 melanoma tumors from 219 patients. Mutations in BRAF exons 11 and 15 were identified in 156 (53 %) tumors and NRAS mutations in 86 (29 %) tumors. BRAF and NRAS mutations did not influence the overall survival from time of diagnosis.

BRAF mutation has been tested in 197 Australian patients with metastatic melanoma in order to identify clinicopathologic variables correlated with BRAF mutation status; furthermore, a survival analysis was conducted [132]. Features of the antecedent primary melanoma significantly associated with a BRAF mutation were the presence of mitoses, single or occult primary melanoma, truncal location, and age at diagnosis of primary tumor≤50 years. BRAF mutation was found to be prognostically relevant in metastatic melanoma but not in primary CM. Furthermore, the presence of mutant BRAF did not seem to have any impact on the disease-free interval from diagnosis of first-ever melanoma to first distant metastasis.

Recently, Si et al. [133] reported the prevalence and the prognostic relevance of BRAF V600E in 396 Chinese patients. This retrospective study suggested that BRAF/NRAS may be independent adverse prognostic factors in melanoma. However, mucosal and unknown primary melanoma have been included; furthermore, it is not clear whether these data obtained in Chinese patients can be extrapolated in Caucasian population.

Finally, Devitt et al. [134] reported a prospective cohort of 249 patients. When compared to WT, multivariate analysis of melanoma-specific survival (MSS) identified NRAS mutations as an adverse prognostic factor but not BRAFV600E mutations. However, only eight events occurred for NRAS analysis; therefore, further study is warranted with a larger prospective cohort to fully address this issue.

A recent meta-analysis found a prognostic effect for BRAF mutation [129]. However, in this study, patients with limited as well as metastatic disease were included in the analysis.

Overall, the take-home message from the above-reported studies is that BRAF and NRAS mutations are not independent prognostic biomarkers in patients with limited (Stages I–III) radically resected primary CM.

Perspective

The past decades witnessed important molecular advances in the field of melanoma research. Despite a wide body of research, no molecular prognostic biomarker has yet been translated into clinical practice. Several biomolecular prognostic factors have been reported but a limited number has been validated in independent datasets with sufficient follow-up, and few studies include complete clinical and pathological information. Accordingly, at present, no single molecular biomarker is routinely or broadly used to guide patient care decisions. Conventional tissue biomarkers, such as BT, ulceration, mitotic rate, and lymph node positivity, remain the backbone prognostic indicators in melanoma.

More recently, the powerful and independent prognostic efficacy of combined marker derived from gene expression profiling analyses has been demonstrated. The prognostic role of multimarker molecular assays, which partially capture melanoma heterogeneity and describe signalling pathways important in melanoma biology, has been validated in independent dataset [124, 125, 135]. Thus, in the forthcoming years, validated multimarker molecular assays will emerge to supplement available clinicopathological parameters for refining prognosis in melanoma patients.

Several issues remain open and represent a call to action for future research:

(1) To improve the quality of clinical research and specifically the adherence to higher standards for reporting prognostic translational biomarker studies;
(2) To identify molecular markers establishing clinical utility according to REMARK recommendation; and
(3) To develop methods that would correct for patient- and gene-specific mutational heterogeneity and be capable to identifying much shorter lists of plausible biological genes useful for clinical decision-making process.

Conflict of interest None

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