

Melanoma biomarkers: current status and vision for the future

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SUMMARY

Melanoma is the leading cause of death from skin cancer in industrialized countries. Clinical and histological variables such as primary tumor invasion, ulceration, and lymph node status might fail to identify early-stage disease that will eventually progress. Tumor biomarkers might help to identify patients with early-stage melanoma who are likely to develop advanced disease and would benefit from additional therapies. These biomarkers offer the possibility of improved tumor staging through the molecular detection of microscopic lymph node metastases that are not visible on routine histological examination. We focus on biomarkers localized to the tumor tissue and those of prognostic value. We give an overview of the melanoma biomarkers that are most helpful for prediction of patients' outcomes, and discuss the primary melanoma biomarkers that have been shown to be of prognostic significance independent of primary tumor thickness and other common clinical prognostic indicators.

Although such tumor-associated biomarkers are thought to have the greatest potential, a lack of reliable data makes their true clinical utility difficult to determine. We conclude that several biomarkers show promise in early studies; however, additional large-scale studies are warranted. We suggest cautious optimism for the field of melanoma biomarkers, which we expect to be translated into clinical practice over the next few years.

KEYWORDS biomarker, Breslow thickness, histology, melanoma, prognosis

REVIEW CRITERIA

Data for this Review were obtained by searching the PubMed database without limitation by the date of publication. The following search terms were used: "melanoma", "biomarker" and "prognosis". Individual searches were also conducted on each biomarker uncovered by this search.

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INTRODUCTION

Promising developments in the field of tumor biomarkers have occurred as a result of advances in technologies. These improvements have led to increasingly sensitive and specific molecular detection capabilities, along with high-throughput assays that allow for rapid, large-scale molecular analyses. Although the clinical application of such technologies has improved tumor detection, diagnosis, and prognostic information for several malignancies, this has not been demonstrated for the melanoma field. The current staging classification system for melanoma accurately predicts outcomes in most patients with early-stage disease; however, prognostic algorithms could be significantly enhanced with accurate, prognostic, molecular biomarkers. The aggressive nature of melanoma has led many investigators to seek such molecular tools to aid in diagnosis and outcome prediction for patients who are at risk for the development of advanced disease.

The identification of such markers will not only help to establish personalized management strategies and risk assessments for patients with melanoma, but will also identify patients with sub-clinical metastatic disease, which could enable their immediate treatment and improved survival. Additionally, such prognostic markers might help to define molecular pathways involved in melanoma formation and progression that could be targeted therapeutically. The prognostic significance of such markers is particularly important since a number of patients with thin melanomas will develop recurrent disease and metastases; of 681 patients with melanomas less than 0.76 mm in thickness, 4.8% progressed to metastatic disease.¹ The early identification of such patients via specific and accurate prognostic biomarkers at the time of diagnosis would enable physicians to provide aggressive treatment from the start, with the expectation that such early interventions will lead to improved survival.

The main clinical and histopathological prognostic factors that are currently in use for melanoma include tumor depth (i.e. Breslow

thickness), diameter, ulceration, anatomic site (i.e. acral, mucosal, cutaneous) and sentinel-lymph-node status.² Many melanoma biomarkers identified previously have shown limited clinical utility and are of uncertain prognostic significance. These biomarkers include p53,² c-myc,² CD44,² Ki-67,² and Bcl-2.^{3,4} Other melanoma biomarkers are useful indicators of prognosis, independent of tumor thickness and other histological prognostic indicators; these biomarkers include MITF (microphthalmia-associated transcription factor), MMP2 (matrix metalloproteinase 2), p16, HIF2 α (hypoxia-inducible factor 2 α), CXCR4 (CXC chemokine receptor type 4), CEACAM1 (carcinoembryonic-antigen-related cell adhesion molecule 1), p-Akt, and β_1 and β_3 integrins.^{2,3,5–15} Other melanoma biomarkers, including tPA (tissue-type plasminogen activator), ICAM1 (intercellular adhesion molecule 1), ephrin A1, β -catenin, P-cadherin, pleiotropin, PLK-1 (polo-like kinase 1), and PUMA (p53 upregulated modulator of apoptosis), have been shown to be significantly associated with prognosis, although not independent of tumor thickness.^{2,3} Additionally, several markers of increased proliferation, such as the mitotic index and the Ki-67 marker expression index, have been shown to be associated with prognosis.¹⁶ Markers of melanocytic lineage that have been used for the highly sensitive detection of tumor burden in the blood or sentinel lymph nodes (SLNs) have also been evaluated in the context of improved staging of patients with melanoma. We focus on the primary melanoma biomarkers identified to date, and define their putative prognostic significance and clinical utility.

MELANOMA BIOMARKERS OF PROGNOSTIC SIGNIFICANCE

The biomarkers discussed below have all been shown to predict prognosis independent of Breslow thickness, and of many other common prognostic indicators such as ulceration and Clark level. Markers that fall into this category are particularly important as they help to identify patients with thin primary melanomas who should undergo aggressive treatment. Table 1 provides a summary of these studies and the prognostic significance of the markers assessed.

Microtubule-associated protein 2

Microtubule-associated protein 2 (MAP2) is a neuron-specific protein involved in mitotic spindle assembly during cell division. MAP2

stabilizes microtubules in the dendrites of post-mitotic neurons and is induced in primary cutaneous melanomas, but is absent in metastatic melanomas.¹⁷ Soltani and colleagues evaluated MAP2 as a melanoma biomarker in a prospective study of 37 patients.¹⁸ After 5 years of follow-up, patients who had primary tumors that were positive for MAP2 expression had significantly improved survival. After adjustments for age, sex, Breslow thickness, and Clark level, loss of MAP2 was an independent predictor of metastatic recurrence.¹⁸

Melanoma cell adhesion molecule

Adhesion molecules are important in cell–cell and cell–matrix interactions, which led to the hypothesis that the melanoma cell adhesion molecule (MCAM) might influence the metastatic potential of melanomas. MCAM is more strongly expressed in melanomas than in benign nevi.¹⁹ Pacifico *et al.* prospectively evaluated 76 melanoma specimens for expression of MCAM in order to determine its prognostic significance as a melanoma biomarker.²⁰ After 7–10 years of follow-up, the presence and intensity (mild, moderate or strong) of MCAM expression were identified as independent predictors of prognosis after adjustment for age, Breslow thickness and Clark level. MCAM expression was a stronger prognostic indicator than Breslow thickness.²⁰ The subgroup of melanomas of intermediate thickness (1.01–4.00 mm) were analyzed separately and also had a significant association with worse survival. However, a previous study that assessed MCAM in 386 specimens indicated that MCAM was not an independent prognostic predictor.²¹ The difference in the results of these studies was hypothesized to be a result of the antibody used.¹⁹ More data on the prognostic value of MCAM is needed to determine its importance as a melanoma biomarker.

Dysadherin

Dysadherin is a membrane glycoprotein that downregulates expression of the cellular adhesion molecule, E-cadherin. Surprisingly, E-cadherin expression has been found to be greatest in melanoma metastases and is rarely expressed in primary melanomas or benign nevi.²² Nishizawa *et al.* studied dysadherin expression in 115 melanoma samples.²³ A very high percentage of these tumors were acral lentiginous melanomas (55%), which are common in Asian and African-American populations. The follow-up

Table 1 Historical biomarkers of independent prognostic significance.

Reference	Number of tumors and tumor type	Markers studied	Clinical outcome
Salti <i>et al.</i> (2000) ¹⁵	63 intermediate-thickness (1–4 mm) tumors	MITF	~150-month survival MITF positive: 86%; MITF negative: 58%
Väisänen <i>et al.</i> (1998) ⁷	50	MMP2	5-year survival ≥34% positive cells: 55%; <34%: 85% ≥34% and male: 39%
Hieken <i>et al.</i> (1999) ¹⁴	111 intermediate-thickness tumors	β ₁ Integrin β ₃ Integrin	~200-month overall survival β ₁ integrin-negative: 73%; β ₁ integrin-positive: 42% β ₃ integrin-negative: 90%; β ₃ integrin-positive: 40%
Straume <i>et al.</i> (2000) ¹²	202 vertical growth phase	p16	~140-month survival Moderate/strong p16: 65%; absent/minimal p16: 37%
Straume and Akslen (1997) ¹³	102 nodular	p16	~160-month overall survival Moderate or strong p16 expression: 65%; weak or absent p16 expression: 0%
Alonso <i>et al.</i> (2004) ⁶	60 vertical growth phase	p16	6-year overall survival p16 negative: 17%; p16 positive: 74%
Giatromanolaki <i>et al.</i> (2003) ¹¹	46	HIF2α	~175-month disease-specific survival HIF2α low: 87%; HIF2α high: 30%
Scala <i>et al.</i> (2005) ¹⁰	71 melanomas >1 mm thick	CXCR4	~60-month survival CXCR4-negative: 70% CXCR4-positive (low): 68%; CXCR4-positive (moderate): 25%; CXCR4-positive (high): 22%
Thies <i>et al.</i> (2001) ⁷⁷	100	CEACAM1	~120-month survival CEACAM1-negative: 92%; CEACAM1-positive: 30%
Dai <i>et al.</i> (2005) ⁸	107 melanomas <1.5 mm thick	p-Akt	~60-month overall survival Negative-to-moderate staining: 95%; strong staining: 83%

Abbreviations: CEACAM1, carcinoembryonic-antigen-related cell adhesion molecule 1; CXCR4, CXC chemokine receptor type 4; HIF2α, hypoxia-inducible factor 2α; MITF, microphthalmia-associated transcription factor; MMP2, matrix metalloproteinase 2

time ranged from 4 months to 285 months with a median of 69 months. Dysadherin expression was found to be an independent prognostic factor that correlated with reduced survival of patients after adjustment for patients' age and sex, and tumor subtype, site, cell type, growth pattern, Clark level, thickness, ulceration and lymph node metastases.²³ Importantly, acral lentiginous, mucosal, and cutaneous melanomas of sun-exposed and sun-unexposed sites might be distinct entities with different genetic pathways to melanoma.²⁴ Large-scale studies are expected to evaluate the prognostic significance of dysadherin expression in superficial and spreading, sun-exposed, cutaneous melanomas and mucosal melanomas, to determine whether this biomarker has clinical significance for only a subset of melanoma subtypes or for all genetic subclasses of melanoma.

β-Catenin

β-Catenin is involved in cellular adhesion through interactions with E-cadherin, and is also

involved in the Wnt signaling pathway. Nuclear β-catenin functions as a transcription factor in complexes with TCF (T-cell factor) or LEF-1 (lymphocyte enhancer factor 1) proteins to activate genes involved in melanoma tumorigenesis such as *c-myc*.²⁵ Bachmann and coauthors studied β-catenin expression in 133 primary melanomas (mostly nodular, some superficial and spreading), 58 paired metastases, and 32 benign nevi.²⁵ These investigators found higher expression of nuclear β-catenin in melanoma samples than in benign nevi. After adjustment for thickness, vascular invasion, Clark level, and Ki-67, loss of nuclear β-catenin was found to be independently associated with poor prognosis. In a previous study, however, loss of nuclear β-catenin was not found to be associated with poor prognosis in a series of mainly acral melanomas, which might represent a distinct entity.²⁶ Such disparate results suggest a possible prognostic significance for β-catenin gene expression in particular melanoma subtypes, which warrants further investigation.

Metallothioneins

Metallothioneins are heavy-metal binding proteins that function in heavy metal detoxification, ultraviolet protection, modulation of free radicals, and in the inhibition of apoptosis.²⁷ Weinlich *et al.* evaluated metallothionein expression in the melanomas from 1,270 patients.²⁷ The median follow-up was 42.5 months (range 3–143 months). Metallothionein expression was independently associated with both progression to metastasis, as well as poor survival rate. Furthermore, metallothionein-positive melanomas of less than 1 mm in thickness were associated with a higher risk of progression to advanced disease compared with their metallothionein-negative counterparts (5.30% versus 0.28%). In addition, patients with thin, metallothionein-positive melanomas were at similar risk of progression to patients with tumors 2.1–4.0 mm thick whose melanomas were negative for metallothionein expression. The authors suggest that in patients with metallothionein-positive melanomas careful follow-up, as well as an SLN biopsy, would be a reasonable management approach.²⁷

Minichromosome maintenance complex proteins

Minichromosome maintenance complex proteins (MCMs) unwind DNA at the initiation of replication during cell division, and are critical mediators of cellular proliferation. In one study, gene-expression profiling was used to assess primary human melanomas; 254 genes were associated with distant-metastasis-free survival in patients with primary melanoma.²⁸ The MCM genes *MCM4* and *MCM6* were among the 254 identified. Expression of *MCM4* and *MCM6* protein levels were evaluated by immunohistochemistry in 176 primary melanomas and was significantly associated with overall survival. Furthermore, *MCM4* and *MCM6* protein expression was associated with decreased survival after adjustment for thickness, ulceration, age, and sex, which suggests that they could be used as an independent prognostic marker for melanoma.²⁸ Further prospective studies will need to be performed to determine the true prognostic value of *MCM4* and *MCM6* expression in primary melanomas.

Nuclear receptor coactivator 3

Rangel *et al.* studied the expression of nuclear receptor coactivator 3 (NCOA3) in primary melanomas from 343 patients.²⁹ NCOA3 is a member of the steroid receptor coactivator 1 family that

directly binds nuclear receptors and stimulates transcriptional activity in a hormone-dependent fashion.³⁰ The gene mapped to a region on chromosome 20q,³¹ which has been shown to be amplified in melanomas.³² In this study, NCOA3 protein expression was associated with an increased incidence of SLN metastases and reduced relapse-free and disease-specific survival. High expression levels of NCOA3 protein significantly increased the risk of melanoma relapse (52.2% versus 35.9%, $P=0.010$) and reduced the relapse-free survival of patients with melanoma in this cohort, in a Kaplan-Meier analysis ($P=0.021$). High NCOA3 protein expression was also associated with increased risk of death due to melanoma (31.9% versus 18.5%, $P=0.021$) and reduced disease-specific survival by Kaplan-Meier analysis ($P=0.030$). Notably, desmoplastic melanomas, which infrequently lead to lymph node involvement, rarely showed high levels of NCOA3 expression. During follow-up (median 45 months), NCOA3 was found to be independently associated with a poor prognosis after adjustment for Clark level, thickness, ulceration, site, age, sex, mitotic rate, degree of vascularity, vascular involvement, microsatellites, and regression. Importantly, NCOA3 was a stronger predictor of disease-specific survival than all other variables, including tumor thickness.²⁹

Human natural killer antigen

The human natural killer antigen (HNK-1) is a glycotope that glycosylates cell adhesion molecules and enables cell migration.³³ HNK-1 is known to be crucial for cell migration in the neural crest; however, HNK-1 expression is lost as neural crest cells differentiate.³⁴ Thies *et al.* assessed HNK-1 expression in 100 primary melanomas, 19 distant cutaneous metastases, 6 lymph node metastases, and 12 benign nevi.³³ While nevi and lymph node metastases failed to express HNK-1, 8 of 19 distant metastases were positive for HNK-1 expression. The lack of expression in the SLN was postulated to be caused by nodal cytokines that downregulate HNK-1 expression. In patients with primary melanomas who were followed up for up to 10 years, HNK-1 expression was an independent indicator of a significantly worse prognosis after adjustment for thickness and ulceration. Stage I melanomas were also associated with a significantly higher risk of metastasis when they were positive for HNK-1 expression than when they were negative.³³ This finding suggests that patients with HNK-1-positive melanomas might merit close follow-up and possibly require SLN biopsy for stage I disease.

Expression of p53 upregulated modulator of apoptosis

The mitochondrial protein, p53 upregulated modulator of apoptosis (PUMA), results in induction of melanoma-cell apoptosis when upregulated by E2F1 (E2 family of transcription factors 1).³⁵ Karst and coauthors evaluated 107 primary melanomas, 51 melanoma metastases, and 64 dysplastic nevi for PUMA expression.³⁶ In this study, dysplastic nevi showed considerable PUMA expression compared with primary melanomas. PUMA expression was significantly higher in primary melanomas than metastases. The authors also tested four melanoma cell lines and observed that levels of apoptosis correlated with PUMA overexpression in a dose-dependent fashion. After adjustment for patients' sex and age, and tumor thickness, ulceration, subtype and site, the loss of PUMA expression was independently associated with both disease-specific and overall 5-year survival.³⁶

Melastatin

Melastatin is a member of the transient receptor potential superfamily of ion channel proteins, and is important in cell-cycle control and cell survival.^{37,38} Melastatin is expressed by nevi and *in situ* melanomas, and its expression is down-regulated in invasive and metastatic melanomas.³⁹ Duncan *et al.* evaluated melastatin expression in 150 patients with stage I and II melanomas.³⁸ They found that after adjustment for Breslow thickness, mitotic rate, ulceration, and subtype, loss of melastatin was independently associated with worse 8-year disease-free survival, as well as a sixfold increase in metastasis risk.³⁸

The presence of melastatin mRNA was studied in 30 primary melanomas in patients with negative SLN biopsies who developed recurrent melanomas.³⁷ The investigators found that increased loss of melastatin mRNA significantly correlated with increased tumor depth and increased microsatellites; multivariate analysis revealed a significant effect on disease-free survival. However, overall survival was not significantly correlated with melastatin expression. These data suggest the potential prognostic utility of melastatin expression in melanoma, but large-scale analyses will be required to determine the clinical value of this marker.

Analysis of GADD153

Growth arrest and DNA-damage-inducible (GADD) proteins facilitate DNA repair and can block the cell cycle at the G1 or G2 phases after

DNA damage. Korabiowska and colleagues evaluated the expression of GADD genes in primary melanomas to determine their prognostic significance.⁴⁰ This study followed on from previous work that demonstrated marked downregulation of GADD gene expression in melanomas compared with benign nevi.⁴¹ Of the GADD genes studied, GADD153 was the only one to demonstrate independent prognostic significance for the 106 melanomas (24 superficial spreading, 82 nodular), after adjustment for the effects of thickness and other markers.⁴¹

Basic fibroblast growth factor

Straume *et al.* evaluated 202 vertical-growth-phase melanomas for expression of several markers associated with angiogenesis including basic fibroblast growth factor (bFGF), vascular endothelial growth factor C (VEGF-C), fms-related tyrosine kinase 4 (FLT-4), fibroblast growth factor receptor 1 (FGFR-1), interleukin 8 (IL-8), Eph-related receptor tyrosine kinase ligand 1 (ephrin-A1), and ephrin type A receptor 2 (EphA2).⁴² The median follow-up was 76 months (range 13–210 months). In this study, a minority (30%) of the tumor cells expressed bFGF; however, 79% of the endothelial cells within tumors showed expression of bFGF. Expression of bFGF in tumor-associated endothelial cells was an independent prognostic factor after adjustment for thickness, Clark level, ulceration, vascular invasion, proliferation, and vascular density.⁴² Interestingly, bFGF expression was also required for the continued proliferation of melanomas *in vivo* through support of tumor-associated angiogenesis.⁴³

Cyclin A

Cyclin A is a mitotic cyclin that is necessary for DNA replication in the S-phase of the cell cycle. A study of 66 melanomas and 60 benign nevi showed that melanomas stained preferentially for cyclin A compared with benign nevi.⁴⁴ Florenes *et al.* further evaluated the expression of cyclin A from 172 primary melanomas (110 superficial spreading, 62 nodular). The median follow-up time was 151 months (range 26–172 months).⁴⁵ Nevi rarely exhibited positive staining for cyclin A. Superficial spreading and nodular melanomas were evaluated separately, and after adjustment for depth, subtype, location, sex, age, and Ki-67 expression, superficial spreading melanomas in which 0–5% of the cells expressed cyclin A, were independently associated with poor relapse-free survival but not overall survival.⁴⁵

Assessment of HLA-DQ

Ostmeier *et al.* stained for 7 markers in 688 primary melanomas from patients without metastatic disease at the time of excision.⁴⁶ Human leukocyte antigen DQ (HLA-DQ) was the only marker to attain independent prognostic significance with a 1.5-fold increased relative risk after a median follow-up of 85 months (range 60–173 months). HLA-DQ expression was evaluated in 452 patients and multivariate analysis showed that thickness, sex, mitotic rate, age, localization, and ulceration were not prognostic factors.⁴⁶

B-cell lymphoma 6 protein

B-cell lymphoma 6 protein (Bcl-6) is a transcriptional repressor that is involved in immune system development.⁴⁷ Bcl-6 regulates germinal center B-cell differentiation and inflammation, and is commonly mutated in lymphomas; however, its function in melanoma is unknown. Alonso *et al.* evaluated 165 melanomas from 88 patients for 39 antigens previously shown to be associated with melanoma progression.⁶ Follow-up data were available for 60 patients. After a 6-year follow-up period, the two antigens that had significant independent prognostic value were Bcl-6 and p16. This analysis included biomarkers of univariate prognostic significance as well as Breslow thickness. Median follow up was 61 months (range 2–186 months). Interestingly, several of the markers did not have independent prognostic significance in this cohort, such as cyclin A, HLA-DQ, Ki-67, and p53. This study did not correct for multiple comparisons in its significance thresholds. The authors independently validated their results in 72 vertical-growth-phase melanomas, which were immunostained for the biomarkers associated with prognostic significance.⁶ The data they acquired were very similar to their initial set; however, multivariate analysis of the validation set did not include tumor thickness, which limited its utility.

Lymphangiogenesis

Massi and coauthors studied the expression of the vascular endothelial marker D2-40 in 15 melanoma samples that were associated with SLN metastases and 30 melanomas without metastases.⁴⁸ Lymphangiogenesis has been associated with both progression and increased survival in other cancers, but its role in melanoma had not been studied previously.⁴⁸ The follow-up period was 42 months (range 1.4–63.3 months). This study found that both peritumoral and intratumoral

lymphatic density was associated with poor survival and lymph node metastases. Peritumoral lymphatic density was the only independent prognostic factor. In a multivariate analysis, the area of the intratumoral lymphatic system was of borderline significance ($P=0.07$).⁴⁸

Dadras *et al.* visualized lymphatic vessels and studied levels of lymphatic vessel endothelial receptor 1 (LYVE-1) in 18 patients with primary melanomas and early nodal metastasis, and 19 patients with melanoma that had not metastasized.⁴⁹ The density of lymphatic vessels was increased in the melanomas compared with adjacent normal skin, and was higher in primary tumors that had metastasized than in those that had not. In addition, the lymphatic size and peritumoral area was larger in metastatic melanomas than nonmetastasized melanomas. The authors did not find a difference in the thickness of primary lesions between the two groups. Multivariate analysis that controlled for vascular invasion, ulceration, microsatellites, peritumoral inflammation, site, regression, and mitosis uncovered a significant independent association of lymphangiogenesis with time to lymph node metastasis. Multivariate analyses did not assess survival; however, univariate analysis showed that high levels of lymphangiogenesis were associated with reduced disease-free survival and overall survival (Table 1).⁴⁹ In 2005, the investigators evaluated 45 patients with primary melanomas who had undergone SLN biopsies.⁵⁰ Of those patients, 27 had negative SLNs while 19 had positive nodes. Levels of LYVE-1 were assessed in the lymphatic vessels. Although both the area and density of the lymphatic system were prognostic indicators in a univariate analysis, after adjustment for thickness and other variables, only the peritumoral lymphatic area was significantly associated with prognosis. Of note, the correlation between peritumoral lymphatic area and prognosis was more significant than correlations of all other variables, including thickness.⁵⁰ Unfortunately, the survival data was not reported, so the magnitude of this prognostic association is unclear. Large studies will need to be done to validate the use of lymphangiogenesis in the prognostic assessment of aggressive, thin melanomas that are likely to metastasize to lymph nodes.

Straume *et al.* evaluated the lymphatic vessel density of 202 nodular melanomas with LYVE-1 and podoplanin staining.⁵¹ Median follow-up was 76 months (range 13–210 months). A decrease in both peritumoral and intratumoral lymphatic

vessel density was independently associated with a significantly improved overall 5-year survival after adjustment for thickness, Clark level, vascular invasion, ulceration, Ki-67, and mean blood vessel density.⁵¹

PROGNOSTIC MELANOMA BIOMARKERS IN LYMPH NODES

SLN evaluation has been established as an important prognostic indicator in melanoma, and could lead to different management strategies for patients with nodal disease. The rationale behind a SLN biopsy is that the lymph node(s) that drain the skin region surrounding the melanoma are the most likely sites to which the melanoma will first metastasize. Negative SLNs are, therefore, thought to greatly diminish the chances of a patient having other affected nodes in the same lymphatic basin. The decision for or against performing a regional lymphadenectomy can be made after the sentinel nodes are evaluated for the presence of metastatic melanoma. Patients who might benefit from adjuvant and investigational immunotherapy can also be identified with the use of this technique.^{52,53} Unfortunately, SLN biopsy is not a perfect technique for the detection of micrometastatic lymph node disease, and 20–30% of patients with negative SLNs develop recurrent melanoma within 10 years.⁵⁴ Thus, biomarkers that can detect micrometastatic disease in lymph nodes could become very important for staging, and for decisions about whether to use lymphadenectomy and adjuvant therapy. Several groups have investigated panels of markers in SLNs for their associations with tumor prognosis, some of which are discussed below. These biomarkers are specifically associated with melanocytic lesions but lack prognostic significance in primary melanomas.

Takeuchi *et al.*⁵⁴ reviewed several markers for lymph node detection of melanoma that had been investigated before 2004. To date, tyrosinase has been the most widely studied marker. Several studies indicate that tyrosinase is a more sensitive marker for the detection of lymph node micrometastasis than hematoxylin and eosin histological examination.^{55–58} Interestingly, tyrosinase expression in lymph nodes has been found to correlate with the thickness of the primary melanoma.⁵⁸ Multivariate analyses, performed in two studies, found that tyrosinase positivity in SLNs was independently associated with poor disease-free survival.^{59,60} In one study, the probability of recurrence-free survival at 24 months was 90% for patients with negative SLN tyrosinase

by reverse transcriptase polymerase chain reaction (RT-PCR) versus 75% for patients with histologically negative but RT-PCR positive lymph nodes.⁵⁹ The other study found a statistically significant difference in disease-free survival of 97% versus 85% ($P=0.02$), and an overall survival difference of 100% versus 92% ($P=0.02$).⁶⁰ Another study used RT-PCR to assess the combination of the melanoma-associated markers MAGE-3 (melanoma-associated antigen 3), MART1 (melanoma antigen recognized by T-cells 1), and tyrosinase in SLNs, and found that in a multivariate analysis, expression of two to three of these markers independently predicted an increased risk of disease recurrence compared with none or one of these markers.⁶¹

Takeuchi and colleagues evaluated the expression of four melanoma-associated genes and their ability to predict tumor recurrence and survival.⁵⁴ The four genes were *MART1*, *MAGEA3*, *CSGALNACT*, and *PAX3*. *MART-1* is a T-cell antigen important in melanoma structure and differentiation.⁶² *MAGE-A3* is also a T-cell antigen found in cancer cells. *CsGalNAc-T* is a glycosyltransferase involved in the synthesis of melanoma surface markers, and *Pax3* is a transcription factor important in melanin synthesis, migration, and apoptosis.⁵⁴ *MAGEA3*, *CSGALNACT* and *PAX3* were not expressed in benign nevi; all four genes were often expressed in melanomas.⁵⁴ In total, 308 lymph nodes from 215 patients were used in the evaluation. Of the lymph nodes known to be positive, 96% expressed one or more of these markers and 38% of tumors expressed all four. No false positives occurred among the 39 normal lymph nodes that were tested. After a follow-up of more than 8 years, 24% of the patients with histologically negative lymph nodes had experienced disease recurrence. Of the patients with histologically negative nodes who did not express any of the four markers, 12 out of 114 (11%) developed recurrences compared with 27 out of 48 (56%) of the patients whose nodes expressed one or more markers. Recurrence occurred in 12 of the 29 patients (41%) who expressed one marker, 9 of the 13 (69%) who expressed two markers, and 6 (100%) of those patients who expressed three markers in the lymph nodes. The results were adjusted for tumor thickness, Clark level, tumor site, sex and age, and revealed an independent association with significantly worse prognosis for those patients with histologically negative lymph nodes that expressed one or more markers, compared with those that lacked marker

Table 2 Summary of cutaneous biomarkers with independent prognostic significance.

Reference	Number of tumors and tumor type	Markers studied	Clinical outcome
Soltani <i>et al.</i> (2005) ¹⁸	37	MAP2	~60-month disease-free survival MAP2-negative: 80%; MAP2-positive: 35% Estimated independent survival % MAP2-negative: 77%; MAP2-positive: 30%
Pacifico <i>et al.</i> (2005) ²⁰	76	MCAM	5-year survival MCAM-negative: 94%; MCAM-positive: 46%
Nishizawa <i>et al.</i> (2005) ²³	115	Dysadherin	~120-month survival Dysadherin-negative: 95%; 1–20% dysadherin positive: 34%; 20–100% dysadherin positive: 45%
Bachmann <i>et al.</i> (2005) ²⁵	133	β-Catenin	5-year and 10-year survival β-catenin positive: 76%, 72%; β-catenin negative: 55%, 40%
Weinlich <i>et al.</i> (2006) ²⁷	1,270	MT	~120-month survival MT-positive: 92%; MT-negative: 55%
Winnepenninckx <i>et al.</i> (2006) ²⁸	176	MCM4 MCM6	4-year overall survival MCM4-negative: 83%; MCM4-positive: 66% MCM6-negative: 81%; MCM6-positive: 67%
Rangel <i>et al.</i> (2006) ²⁹	343	NCOA3	~12-year disease-specific survival NCOA3-negative: 67%; NCOA3-positive: 18%
Thies <i>et al.</i> (2004) ³³	100	HNK1	~120-month survival HNK1-negative: 70%; HNK1-positive: 37%
Karst <i>et al.</i> (2005) ³⁶	107	PUMA	~60-month survival PUMA-positive: 85%; PUMA-negative: 64%
Hammock <i>et al.</i> (2006) ³⁷	30	Melastatin	~20, 40, and 60-month disease-free survival ≤75% mRNA loss: 68%, 25%, 6% >75% mRNA loss: 17%, 0%, 0%
Duncan <i>et al.</i> (2001) ³⁸	150	Melastatin	8-year disease-free survival Stage I melanomas: diffuse melastatin 100%; melastatin loss 77% Stage II melanomas: diffuse melastatin 90%; melastatin loss 51%
Alonso <i>et al.</i> (2004) ⁶	60 vertical growth phase	Bcl6	6-year overall survival Bcl-6 negative: 74%; Bcl-6 positive: 0%
Massi <i>et al.</i> (2006) ⁴⁸	45	LVDpt	~1,800-day survival Low LVDpt: 74%; high LVDpt: 38%
Dadras <i>et al.</i> (2003) ⁴⁹	37	LVA	~100-month survival Low LVA: 100%; medium LVA: 52%; high LVA: 16%
Straume <i>et al.</i> (2003) ⁵¹	202 nodular	LVDit LVDpt	5-year overall survival High LVDit 74%; low LVDit 53% High LVDpt 77%; low LVDpt 49%
Korabiowska <i>et al.</i> (2002) ⁴⁰	106	GADD153	5-year survival GADD153-positive: 50%; GADD153-negative: 0%
Straume and Akslen (2002) ⁴²	202	bFGF	~120-month survival bFGF-positive: 55%; bFGF-negative: 35%
Florenes <i>et al.</i> (2001) ⁴⁵	110 superficial spreading	Cyclin A	~160-month relapse-free survival Cyclin A <5%: 83%; Cyclin A ≥5%: 36%
Ostmeier <i>et al.</i> (2001) ²¹	452	HLA-DQ	~120-month metastasis-free survival HLA-DQ <3%: 63%; HLA-DQ ≥3%: 31%

Abbreviations: Bcl6, B cell lymphoma 6; bFGF, basic fibroblast growth factor; CEACAM1, carcinoembryonic-antigen-related cell adhesion molecule 1; CXCR4, CXC chemokine receptor type 4; HIF2α, hypoxia-inducible factor 2 α; HLA-DQ, human leukocyte antigen DQ; HNK1, human natural killer 1; LVA, lymphatic vessel area; LVD, lymphatic vessel density; LVDit, lymphatic vessel density intratumoral; LVDpt, lymphatic vessel density peritumoral; MAP2, microtubule-associated protein 2; MCAM, melanoma cell adhesion molecule; MCM, minichromosome maintenance complex protein; mRNA, messenger RNA; MT, metallothioneins; NCOA3, nuclear receptor coactivator 3; PUMA, p53 upregulated modulator of apoptosis

expression.⁵⁴ Thus, such a panel of markers might prove useful in the detection of micrometastatic lymph node disease.

Kuo *et al.* evaluated lymph nodes in patients with melanoma; 37 were identified as positive and 40 were identified as negative on histological analysis.⁶³ RT-PCR was performed for tyrosinase, MART-1, TRP-1, and TRP-2 (thioredoxin-related proteins 1 and 2). Both nevi and melanomas stained positively for tyrosinase.⁶⁴ TRPs inhibit tyrosinase-induced apoptosis in melanocytes,⁶⁵ and in this study 53% of the patients with lymph nodes negative for this marker had recurrence of their melanoma. In total, 22 patients expressed at least one marker, 10 patients expressed two or more markers, and 4 patients expressed three or more markers. Tyrosinase and MART-1 expression correlated with tumor thickness as did the presence of two or more positive markers. In a univariate analysis, TRP-1, MART-1, and tyrosinase were significantly associated with poor survival.⁶³ Multivariate analysis controlled for sex, age, site, Breslow thickness, and histologic presence of SLN metastases revealed that tyrosinase and MART-1 were both independently associated with a worse prognosis.⁶³ Staining for two or more markers was observed in 2 of 19 (11%) histologically negative lymph nodes without recurrence, and 8 of 21 (38%) negative nodes with recurrence. Histologically negative lymph nodes that expressed two or more markers were associated with a worse prognosis, as shown by 72-month survival reported for only 17% of these patients compared with 47% of the patients with fewer than two markers; this result was not independent of the variables listed above.⁶³ Gene arrays that include tyrosinase and MART-1 along with other markers of independent prognostic value might help to detect micrometastatic lymph node disease. Although the above studies are small, we hope that these results will be validated in the large, prospective, Multicenter Selective Lymphadenectomy Trial-II (MSLT-II), which is currently underway.⁶⁶

Gradilone *et al.* used RT-PCR to examine expression of survivin, bcl-2, bcl-X, and bax in 36 lymph nodes.⁶⁷ These four proteins are involved in apoptosis. Only survivin was associated with a decreased prognosis. Survivin has been implicated in the conversion of normal human melanocytes to proliferating melanocytes, such as those seen in nevi that might go on to become melanoma.⁶⁸ Survivin is expressed in melanomas as well as benign nevi, but not in regular

skin melanocytes.⁶⁸ No recurrences were noted in patients whose tumors lacked survivin expression after a 53-month follow-up, whereas 62% of patients whose tumors did express survivin experienced recurrent disease.⁶⁷ This small study will also need to be validated, but survivin could also be a candidate for an array of lymph node markers used to detect micrometastatic disease.

CUTANEOUS MELANOMA BIOMARKERS ASSOCIATED WITH PROGNOSIS

Many studies have found associations between one or more biomarkers and melanoma prognosis; however, these associations were not significant when variables such as thickness were excluded in a multivariate analysis.^{6,28,69–85} Since most of these markers are associated with thickness, they will have limited value in the prediction of aggressive behavior in thin melanomas. Nonetheless, as these markers are likely to be involved in melanoma progression, future therapeutic investigations that target the pathways of these markers may be useful, some of which are shown in Table 3.

CONCLUSIONS

Currently, the single best indicator of prognosis for patients with invasive melanoma is the tumor stage at first clinical presentation. This staging is reliant on clinical and histologic findings: tumor depth, the presence or absence of ulceration, the presence or absence of microscopic metastases, and the number of positive regional lymph nodes. Although such a staging system is able to predict outcomes accurately for the vast majority of melanoma patients, subsets of patients exist who have early-stage melanoma that will go on to develop metastases, or who will die of their disease. The identification of meaningful tumor markers that could accurately identify individuals with early-stage disease who are at risk for metastasis is necessary for appropriate upfront treatment and cure. We have discussed many biomarkers for early identification of aggressive melanomas and detection of micrometastatic disease in SLNs. Although no single markers can consistently predict which patients will experience disease progression, several melanoma biomarkers have been associated with significant survival differences. Those biomarkers associated with a greater than 35% increase in survival are MAP2, MCAM, dysadherin, metallothioneins, NCOA3, HNK-1, melastatin, Bcl-6, lymphatic vascular area (LYVE-1), GADD153, cyclin A, β_3 integrin, p16, HIF2 α , CXCR4, and CEACAM1 (Tables 1 and 2).

Table 3 Summary of cutaneous biomarkers with non-independent prognostic associations.

Reference	Number of patients	Markers studied	Clinical outcome
Winnepenninckx <i>et al.</i> (2006) ²⁸	176	MCM3, KPNA2, geminin	4-year overall survival MCM3-negative: 76%; MCM3-positive: 67% KPNA2-negative: 85%; KPNA2-positive: 66%
Li <i>et al.</i> (2004) ⁶⁹	104	Skp2	10-year survival ≤20% Skp2 cytoplasmic intensity: 86% >20% Skp2 cytoplasmic intensity: 38%
Jørgensen <i>et al.</i> (2006) ⁷⁰	94	JNK	~400-month survival JNK-negative: 74%; JNK-positive: 40%
Bachmann <i>et al.</i> (2006) ⁷¹	202	EZH2	5-year survival EZH2-negative: 71%; EZH2-positive: 48%
Straume and Akslen (2005) ⁷²	119	ID1	~200-month survival ID1<4: 64%; ID1 ≥4: 40%
Andersen <i>et al.</i> (2004) ⁷³	159	S100A4 E-cadherin	~200-month disease-free survival S100A4-negative: 71%; S100A4-positive: 53% E-cadherin-positive: 72%; E-cadherin-negative: 49%
Streit <i>et al.</i> (2006) ⁷⁴	159	FGFR4	~125-month survival FGFR4-negative: 51%; FGFR4-positive: 28%
Piras <i>et al.</i> (2005) ⁷⁵	152	CD8 HLA-DR	~10-year survival High CD8: 78%; moderate CD8: 44%; low CD8: 17% High HLA-DR: 40%; low HLA-DR: 17%
Thies <i>et al.</i> (2001) ⁷⁷	100	MC	Metastasis frequency MC-positive: 69%; MC-negative: 25%
Ricaniadis <i>et al.</i> (2001) ⁷⁸	40	HSP-70	7-year survival HSP-70-positive: 70%; HSP-70-negative: 27%
Henrique <i>et al.</i> (2000) ⁷⁹	84	Ki-67	~84-month survival Ki-67 ≤14%: 78%; Ki-67 >14%: 43%
McDermott <i>et al.</i> (2000) ⁸⁰	145	Nm23	Overall survival with a 5–12 year follow-up Nm23 ≥50% of cells: 76%; Nm23 10–50% of cells: 0%
Florenes <i>et al.</i> (2000) ⁸¹	172 ^a	Cyclin D3	~180-month overall survival; superficial spreading, nodular Cyclin D3 <5%: 67%, 67%; Cyclin D3 >5%: 58%, 41%
Karjalainen <i>et al.</i> (1999) ⁸²	369	p21 p53	5-year overall survival p21 <1%: 91%; p21 1–10%: 84%; p21 10–20%: 85%; p21 >20%: 84% p53 index 0: 94%; p53 index ≤1: 88%; p53 index 1–10: 82%; p53 index >10: 68%
Chana <i>et al.</i> (1998) ⁸³	25 ^b	Nuclear c-myc	~Overall 20-month survival Low c-myc: 57%; high c-myc: 24%
Dietrich <i>et al.</i> (1997) ⁸⁴	92	CD44	~5-year survival CD44 low: 91%; CD44 high: 72%
Strebhardt <i>et al.</i> (2000) ⁸⁵	175	PLK1	10-year survival rate PLK1 moderate: 93.6%; PLK1 high expression: 84.9%

^a110 superficial and spreading, 62 nodular tumors. ^bScalp melanomas. Abbreviations: EZH2, enhancer of zeste homolog 2; FGFR4, fibroblast growth factor receptor 4; HLA-DR, human leukocyte antigen DR; HSP-70, heat shock protein 70; ID1, inhibitor of differentiation-1; JNK, c-Jun N-terminal kinase 1; KPNA2, karyopherin subunit alpha-2; MC, microvascular channels; MCM3, minichromosome maintenance complex 3; PLK1, polo-like kinase 1

Studies of these markers have demonstrated that they have independent prognostic significance. This factor is critical to their potential utility in the identification of thin, aggressive melanomas.

Only MAP2, however, has been shown to distinguish primary tumors with a significant prognostic survival difference independent of other variables.⁸⁰ Some melanoma biomarkers provide

improved prognostic value compared to Breslow thickness, and these include MMP2,⁷ c-myc (in acral melanomas only),⁸⁶ MCAM, NCOA3, and lymphangiogenesis. Unfortunately, the majority of these studies have been small and have looked at only tens or hundreds of patients. Similarly for lymph nodes, several small studies have been completed and tyrosinase, survivin, and MART-1 were independently associated with poor prognosis.

Given the current status of biomarkers in melanoma and the above early data on biomarkers with potential prognostic significance, what can patients with melanoma expect with regard to their clinical implementation? Although the above-mentioned studies are reason for optimism, large-scale studies must be performed to confirm the clinical utility of biomarkers expected to be used in clinical practice. The good news might be that one reason we have had such difficulty with the identification of clinically meaningful biomarkers for melanoma is that our current staging system is so accurate. Despite the lack of biomarker assessments in melanoma staging, the American Joint Committee on Cancer criteria predict the likelihood of tumor progression reasonably well in the majority of patients. Although we hope that meaningful tumor biomarkers will improve prognostic accuracy, such information will be of little benefit without the identification of useful adjuvant therapies with proven efficacy. Clearly melanoma biomarkers and new therapies must be developed in tandem to effect a meaningful change in patients' outcomes. We further note that advances in the definition of genetically distinct subsets of melanomas might also be relevant to the identification of useful melanoma biomarkers. Indeed, skin sites of melanoma primary tumors (i.e. acral, mucosal, cutaneous) and levels of sun exposure associated with melanoma primary tumors (none, constant, intermittent) seem to indicate that different genetic pathways are involved in melanoma development,²⁴ which might be characterized by different prognostic biomarkers.

We conclude that, although much work has been done to identify biomarkers of clinical utility in melanoma, such studies have been hampered by the failure of individual biomarkers to provide significant prognostic information beyond the simple clinical and histologic criteria that form the basis of our current staging system. As additional information about the molecular defects associated with melanoma development and progression becomes available, additional biomarkers of prognostic significance will also be identified.

Ultimately, we expect that large-scale studies will allow us to determine which single or groups of biomarkers will provide significant prognostic information for further risk-stratification of patients with melanoma beyond our current standards. As new, targeted therapies are developed for melanoma, such stratification of patients will become critically important for early identification of high-risk patients who will benefit from upfront adjuvant targeted therapies.

KEY POINTS

- Current melanoma staging relies solely on clinical and histologic criteria; the majority of melanomas that are diagnosed at the earliest stages can be cured by surgery
- Early stage melanomas occasionally progress to advanced disease despite adequate surgical management; however, no current biomarkers are available to identify such high-risk primary tumors
- Several melanoma biomarkers have been evaluated for their prognostic utility with promising early results; however, to date, none has been proven to be clinically useful in large-scale studies
- A major need exists to identify biomarkers with prognostic significance in melanoma, which would ultimately be expected to guide management of patients and result in new, therapeutic interventions

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Competing interests

RM Alani declared she has associations with the following organization: Johns Hopkins University. See the article online for full details of the relationship. The other authors declared no competing interests.