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REVIEW ARTICLE

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Identification of risk in cutaneous melanoma patients: Prognostic and predictive markers

David M. Hyams MD[1] | Robert W. Cook PhD[2] | Antonio C. Buzaid MD[3,4]

[1]Desert Surgical Oncology, Eisenhower Medical Center, Rancho Mirage, California

[2]R&D and Medical Affairs, Castle Biosciences, Inc, Friendswood, Texas

[3]Oncology Center, Hospital Israelita Albert Einstein, São Paulo, Brazil

[4]Centro Oncológico Antonio Ermírio de Moraes, Beneficência Portuguesa de São Paulo, São Paulo, Brazil

Correspondence
David M. Hyams, MD, Desert Surgical Oncology, Eisenhower Medical Center, Suite K-207, 39000 Bob Hope Drive, Rancho Mirage, CA 92270.
Email: dhyams.md@me.com

New therapeutic modalities for melanoma promise benefit in selected individuals. Efficacy appears greater in patients with lower tumor burden, suggesting an important role for risk-stratified surveillance. Robust predictive markers might permit optimization of agent to patient, while low-risk prognostic markers might guide more conservative management. This review evaluates protein, gene, and multiplexed marker panels that may contribute to better risk assessment and improved management of patients with cutaneous melanoma.

KEYWORDS
gene expression profile, gene signature, melanoma, predictive biomarkers, prognostic biomarkers, protein biomarkers

1 | INTRODUCTION

More than 91 000 people were diagnosed with cutaneous melanoma in the United States (US) in 2018. In the same period, melanoma was associated with over 9000 deaths.[1] Although cutaneous melanoma currently represents 1.5% of all cancer deaths, the NCI/SEER database notes a 50% increase in US melanoma incidence during the past 20 years, from 15 per 100 000 population per year to 22.8 per 100 000 per year.[2]

Ten-year survival is now greater than 95% for those with thin nonulcerated melanomas and negative nodes. However, by the time the disease becomes increasingly penetrative of the skin and/or develops local ulceration, 10-year survival rates may be as low as 40%, even with negative nodes.[3] Although the clinicopathologic features of melanoma provide useful information about overall risk, they provide more limited information about outcomes in individual patients.

The recent development of effective systemic drug therapy for melanoma has significantly changed patient management and outcomes, particularly in more advanced disease.[4,5] Several studies of stage IV disease demonstrate greater efficacy of immune checkpoint inhibitors (ICIs) and MAP-kinase directed drugs in patients with lower tumor burden. This suggests that early detection of recurrence or distant metastasis may have a unique value in melanoma treatment and outcome.[4,6-8]

Individual assessment of risk may be relevant to migration of these new therapeutic agents into the adjuvant setting. Enriching a clinical trial population for the stage I and II patients destined to recur minimizes overall trial size and may make otherwise prohibitively expensive studies feasible. Although adjuvant therapies are approved for stage IIIA disease, some of these node-positive patients may have favorable risk profiles that do not justify adjuvant therapy. In clinical practice, the identification of individual early stage patients with uniquely high recurrence risk could facilitate access to life-saving adjuvant therapies. Alternatively, the identification of truly low-risk early stage patients, with little likelihood of recurrence, would help such individuals avoid the burdens and costs of unnecessary treatment.

In this review, we will explore the development and validation of a variety of putative prognostic tumor biomarkers and biomarker panels in cutaneous melanoma. We will explore the issues of prognosis and prediction, and will highlight the importance of validation in the assessment of any biomarker assay. Finally, we will review the level of evidence (LOE) that might support the clinical incorporation of any of these tests into standard clinical practice.

TABLE 1 Conventional risk factors of primary early stage cutaneous melanoma

Risk factor	Evidence summary	References
Breslow thickness	Consistent evidence supports correlation of BT with recurrence and poor survival; key histologic factor for staging	Gershenwald et al[3] Balch et al[12]
Ulceration	Consistent evidence supports correlation of ulceration with recurrence and poor survival; key histologic factor for staging	Gershenwald et al[3] Balch et al[12]
Mitotic rate	Consistent evidence supports correlation of MR with recurrence and poor survival; inconsistent evidence for independent value of MR and dichotomization (led to removal as a key histologic factor for staging)	Gershenwald et al[3] Balch et al[12]
Lymphovascular invasion	Consistent evidence supports association with SLN positivity and poor survival outcomes; inconsistent evidence to support independent predictive value for outcomes	Namikawa et al[15] Egger et al[16]
Positive deep margins	Consistent evidence to support association of PDM with increased risk and consideration of SLNBx for T1a patients	Mills et al[18] Koshenkov et al[17]
Tumor infiltrating lymphocytes	Inconsistent evidence associating TILs with good prognosis and negative SLN; several studies have reported no independent prognostic value	Tas et al[20] Weiss et al[21]
Clark level	Consistent evidence supports association with recurrence and SLN positivity; lacks independent prognostic value (led to removal as a key histologic factor)	Eriksson et al[13] Balch et al[12]
Vertical growth phase	Limited evidence in support of VGP as an independent prognostic feature; VGP is associated with SLN positivity in thin melanomas	Appleton et al[22] Eriksson et al[13]
Regression	Inconsistent evidence in support of prognostic value of regression; limited evidence for association with lower SLN positivity rates and better outcomes	Gualano et al[23] Ribero et al[24]
Perineural invasion	Weak evidence to support PNI as an independent prognostic factor, other than in the desmoplastic subtype	Namikawa et al[15] Frydenlund et al[25]

Abbreviations: SLN, sentinel lymph node; SLNBx, sentinel lymph node biopsy; TIL, tumor infiltrating lymphocyte; VGP, viral protein genome.

2 | CLINICOPATHOLOGIC FEATURES FOR RISK ASSESSMENT

Pathologic features have been the basis of cutaneous melanoma prognosis and staging for nearly 40 years.[9] Clark and Breslow each described features of primary melanoma invasion in the early 1970s that became incorporated into the American Joint Committee on Cancer (AJCC) 1st Edition Staging Manual, published in 1977.[10,11] Additional features were added over the years, with modification of various T-stage cutoffs. The Clark levels were ultimately discarded from AJCC staging and have become of largely historic interest.[3,12,13] The AJCC 8th Edition Staging Manual now assesses early stage disease based only on Breslow thickness, ulceration, and lymph node status. The most recent National Comprehensive Cancer Network (NCCN) Guidelines document identifies elements of a primary lesion that must be included in the pathology report. These are Breslow thickness (mm), ulceration, and microsatellite metastasis. Additional factors of prognostic relevance recommended for inclusion in the pathology report are dermal mitotic rate (per mm^2), lymphovascular invasion, and peripheral and deep margins (Table 1).[14-18]

The American Academy of Dermatology (AAD) suggests additional prognostic features that may be utilized in pathology reporting including gross appearance, Clark level (only in tumors <1 mm in the absence of mitotic rate), angiolymphatic/lymphovascular invasion, histologic subtype, neurotropism/perineural invasion, desmoplasia status, regression, tumor-infiltrating lymphocytes, and vertical growth phase.[13,19-25] Some of these recommendations are derived from older AJCC staging requirements, some are based on isolated reports, and others are based on uncertain behavioral tendencies of specific subtypes.[19]

There are several staging changes in the latest *AJCC 8th Edition Staging Manual* that will likely migrate into future guidelines of organizations like NCCN and AAD. Many of these changes were derived from information gleaned from the contemporary International Melanoma Database and Discovery Platform (IMDDP). Analyses were limited to patients diagnosed with Stage I-III cutaneous melanoma since 1998, representing patients managed with modern assessment tools such as sentinel lymph node (SLN) biopsy (SLNBx). Using evidence-based analyses from the IMDDP, only tumor thickness thresholds of 0.8, 1.0, 2.0, and 4.0 mm, with or without ulceration, were found to contribute to primary T-staging. Multivariate analyses did not suggest that binary reporting of any of the other features present in NCCN or AAD guidelines added significantly to prognosis as measured by melanoma specific survival (MSS).[3]

One such feature, the binary assessment of mitotic rate, was dropped entirely from the AJCC 8th Edition staging. Nonetheless, the individual tumor mitotic rate, when explored across its full range, remained a prognostic feature in their analyses. This suggests that quantitative mitotic rate might be incorporated into future dynamic algorithmic staging systems.[3]

In an effort to increase prognostic accuracy, at a cost of increased complexity, microsatellitosis was incorporated into N-staging and new categories of M staging were created. With these and other changes, the AJCC 8th edition now has 80 possible permutations of T- and N-stage for stage I-III cutaneous melanoma with a

documented primary site. Another eight combinations of T- and N-stage are possible just for patients with an unknown primary.

A review of all the changes to AJCC staging and the evidence supporting them is beyond the scope of this study. Several available publications more fully discuss the evolution and supportive evidence for current staging systems.[3,26]

3 | RISK STRATIFICATION AND TREATMENT SELECTION

Since the early 1900s, surgery has been the mainstay of melanoma treatment.[9] Primary resection is currently designed to achieve local control, while lymph node biopsy and lymphadenectomy are reserved for risk assessment and improved local/regional control. In the era before more widely effective systemic therapy, metastasectomy of limited distant disease was of variable value only, without clearly demonstrated benefit in the majority of patients.[27]

Surgical resection of melanoma was largely ineffective until wider margins were proposed in localized early disease. Handley,[28] a disciple of Halsted, first recognized the value of basing the extent of resections on a minimal proposed distance from a primary lesion. The choice of margins was a compromise between the distance needed to achieve low recurrence rates at the resection site and the recognition that patients with disease recurring beyond a certain resection distance were also likely to have more aggressive disease with distant micrometastasis. Choosing 4 to 5 cm resection margins acknowledged the importance of biologic risk and long-term prognosis in selecting therapy.[29]

Decisions regarding extent of surgery currently depend upon primary disease characteristics associated with increased recurrence rates.[30] Margin recommendations have been developed based on Breslow thickness and are now codified in the NCCN guidelines.[14] For lesion up to 1 mm in thickness, 1.0 cm has been recommended. Margins between 1.0 and 2.0 cm are advised for lesions over 1.0 cm and up to 2 mm in thickness. For lesions over 2 mm, 2 cm margins are recommended. How much more of a role precise biomarker assessment of risk might play in determining optimal margins is uncertain at this time.

Elective lymphadenectomy in melanoma of clinically negative regional nodes was historically practiced to improve survival by attempting to outrun the stepwise spread of metastatic disease, using the approach proposed by Halsted in breast cancer.[31] However, several prospective randomized studies, as well as a meta-analysis, have failed to show value for this approach.[32-35] The concomitant advent of the sentinel lymph node biopsy (SLNBx) technique eliminated elective lymph node dissection for all but proven disease in regional lymphatic beds.

The Multicenter Selective Lymphadenectomy Trial (MSLT)-I confirmed the value of the SLNBx technique in identifying patients with pathologically positive, but clinically occult regional lymph nodes.[36] Compared to the observation arm, there was no difference in MSS for all study participants based on treatment arm allocation. However biopsy-based management improved the 10-year rate of distant disease-free survival (hazard ratio [HR] for distant metastasis, 0.62; $P = 0.02$) and the 10-year rate of MSS (HR for death from melanoma, 0.56; $P = 0.006$) for 255 evaluable patients with intermediate-thickness (1.2-3.5 mm) melanomas and nodal metastases.[36]

For patients with intermediate-thickness melanomas (defined as 1.2-3.5 mm in the study), the 10-year MSS rate was 63.1% ± 4.2% among those with metastasis versus 85.7% ± 1.4% for those without metastasis (HR for death from melanoma, 2.32, 95% CI [1.62, 3.32], $P < 0.0001$). Reflecting the increased incidence of node-negative patients in this study population, nearly twice as many patients with intermediate-thickness melanomas and node-negative disease died compared with those having node-positive disease (98 of 784 versus 50 of 152). This appears to mirror findings in the general population and suggests that a negative SLNBx is insufficient to exclude a risk of melanoma-specific mortality in patients with early stage melanoma.[37]

The MSLT-I identified a false negative rate of less than 4% when SLNBx was utilized. As a result, current NCCN guidelines do not recommend SLNBx if the risk of a positive node is less than 5%, as this approaches the false negative rate.[14] Although risk assessment based on primary pathologic features may be helpful, it is not sufficient to identify many low-risk patients with intermediate thickness lesions who do not meet criteria for SLNBx. Many of these patients could be spared lymph node surgery with more precise risk profiling.

A second international trial, MSLT-II, evaluated completion lymphadenectomy versus observation for patients with positive SLNBx. This study found no difference in MSS or distant metastasis-free survival (DMFS) between patients in either of the randomized treatment arms.[38] There was a slight but significant improvement in disease free survival (DFS) at 3 years (68% ± 1.7% and 63% ± 1.7%, respectively; $P = 0.05$ by the log-rank test), and DFS in regional nodes (92% ± 1.0%, and 77% ± 1.5%, respectively; $P < 0.001$ by the log-rank test). Based on the information available, there is no evidence that recognized prognostic indicators affected these findings.

4 | BIOMARKERS

4.1 | Biomarkers as prognostic and predictive tools

Over the last 40 years, molecular and protein biomarkers have provided important prognostic information about tumor outcome and important predictive information about tumor response to therapy. Such tests provide no clinical utility if they are not reproducible or unreliable. However, a direct link between the validity of an initial prognostic or predictive assay and any subsequent modification of that assay is necessary if analytic validity is to be maintained over evolving iterations of assay development.

In a number of diseases including breast, colon, prostate, lung, leukemia, and melanoma, a variety of individual proteins and genetic/genomic features have been proposed as prognostic and/or predictive markers with clinical utility. Multiplexed panels of markers have also been proposed and commercialized with broad adoption

and, in some cases, even incorporation into the AJCC Staging Manual.[39] However, the levels of evidence used to validate these markers have varied enormously.

Simon et al have described an approach to validation that is based on types of study information and the presence or absence of confirmatory studies.[40] Level IA is the highest LOE. Such validation studies are prospective, purposefully designed trials with randomization if varied treatments might be indicated based on risk group assignment. Level IIB may consist of a single prospective study design, using a previously conducted prospective study, with a new biomarker question evaluated with a contemporary standard analytic procedure (SAP). Level IIC consists of two confirmatory prospective registry or case series studies with prospective enrollment and tissue collection, using contemporary SAP. Level IIIC is a single case series study.

The development of two widely marketed breast cancer multiplexed gene expression panels has included studies with Level IA evidence.[41,42] However, these types of very large studies are not practical for most assay development. The ability to conduct even Level IIB studies may be limited, as such studies depend on the availability of appropriately archived and accessible tissue from previously conducted prospective trials. In melanoma, such studies are rare, and tissue availability is even rarer. At best, evidence in melanoma biomarker studies has come from individual prospective registry studies in which prospective design and contemporary SAPs have been utilized. In the absence of large prospective trials, evaluation of an assay across several studies with consistent results may have to suffice in providing reasonable, if imperfect, early validation.

4.2 | Prognostic biomarkers in melanoma

Estimating the risk of metastasis and/or survival associated with early stage melanoma is a clinical challenge. As described above, two out of three early-stage patients who die from melanoma are initially diagnosed with stage I or II disease.[36] Biomarkers for early stage melanoma prognosis and prediction span protein, nucleic acid, and metabolic molecules, but the molecular markers that have been successfully translated to the clinic are primarily nucleic acids, likely due to rapid and reproducible results. The development of robust methods for purifying RNA from FFPE tumor tissue, the standard preservation method implemented for primary melanoma tumors, has allowed for the discovery of multiplexed markers associated with prognostication of sentinel lymph node status, locoregional recurrences, distant metastases, and survival. Gene expression profiling (GEP) has subsequently advanced to the clinical setting to inform patient management decisions. This section reviews the single and multimarker assays that have been developed for the purpose of identifying patients who have low-risk disease according to standard staging criteria, but potentially harbor more aggressive tumors.

4.3 | Protein biomarkers

Although a great amount of research effort has been focused on the discovery of proteins associated with melanoma prognosis (Table 2), the translation of most findings to clinical practice has not been achieved. There are several protein candidates significantly associated with worse survival, including lactate dehydrogenase (LDH). The association of LDH with poor outcomes is well documented and has led to its inclusion as an AJCC staging criterion for categorizing metastasis (M), but clinical utility of the marker is primarily limited to stage IV disease.[3] C-reactive protein and S100B are also late-stage protein serum markers that are associated with recurrence and/or survival.[43-45]

Kashani-Sabet et al[46] have reported a multiprotein marker for melanoma prognosis that includes NCOA3, SPP1, and RGS1. The panel was discovered using tissue microarray analysis of 395 tumors and validated in an independent cohort of 141 patients, and was a significant predictor of both disease-specific survival and SLN status when compared to standard clinical factors in Cox multivariate regression analysis. The prognostic accuracy of the three-protein marker was subsequently validated in a cohort of 248 patients enrolled in the Eastern Cooperative Oncology Group 1690 (E1690) clinical trial.[47] Although protein expression significantly correlated with recurrence and survival and all high-risk patients had events within 5 years, the E1690 cohort was primarily composed of high-risk tumors with five-year survival rates that approached 50% among low-risk patients. Similar to other panels that have been reported, the clinical validity and utility of the multiprotein marker in an early stage melanoma population remains to be determined.[48]

TABLE 2 Single protein and molecular prognostic markers in cutaneous melanoma

Prognostic marker	Evidence summary	References
LDH	Included as AJCC staging criteria based on association with metastatic melanoma	Gershenwald et al[3]
S100B	Reported independent prognostic marker for melanoma metastasis, but not currently included in AJCC staging system	Wevers et al[45] Weide et al[44]
C-reactive protein	Reported independent prognostic marker for melanoma metastasis, but not currently included in AJCC staging system	Deichmann et al[43]
NCOA3/SPP1/RGS1	Significantly separates risk group	Kashani-Sabet et al[47] Kashani-Sabet et al[46]
Immunoscore	Limited evidence supporting application to melanoma	Galon et al[50]

Abbreviations: AJCC, American Joint Committee on Cancer; LDH, lactate dehydrogenase.

TABLE 3 Multiplexed prognostic gene expression profile tests in cutaneous melanoma

Prognostic marker	Evidence summary	Evidence type	References
DecisionDx-Melanoma (31-GEP)	Consistent evidence supports independent prognostic value across multiple prospective and retrospective validation studies; utility for impacting patient management in prospective and retrospective studies; robust analytic validity	CV, CU, AV	Gastman et al[71] Greenhaw et al[69] Dillon et al[73] Hsueh et al[70] Cook et al[68] Berger et al[72]
Melagenix (9-GEP)	Limited evidence supports prognostic value in single retrospective study; lacks clinical utility evidence	CV	Brunner et al[55]
53-Gene immune GEP	Limited evidence supports prognostic value in single retrospective study; lacks clinical utility evidence	CV	Sivendran et al[58]
ITLP group	Limited evidence supports prognostic value for informing SLN status in single retrospective study; lacks clinical utility evidence	CV	Meves, et al[57]

Abbreviations: GEP, gene expression profiling; SLN, sentinel lymph node.

Because melanomas are among the most immunoreactive of human malignancies, cluster of differentiation (CD) antigens and other molecules expressed on or within T-cells have been molecular targets of interest. The immunoscore, a tool that has been successfully applied for prognosis in early stage colorectal cancer, has also been evaluated for prediction of survival in melanoma.[49,50] Initial reports suggested that CD3, CD8 and CD20 were differentially expressed in lymphadenectomy tissue from stage III melanoma patients. Additionally, in exploratory analysis, CD2 was shown to be an independent predictor of disease recurrence and overall survival in multivariate regression analysis of 90 stage II-III patients, with higher expression of CD2 correlated with tumor infiltrating lymphocytes and better outcomes.[51] However, broader validation of these markers, particularly for early stage melanoma, has not been achieved.

4.4 | Single gene biomarkers

Perhaps due to the ease with which multimarker gene panels can be constructed and evaluated, and the benefits of monitoring multiple cellular pathways concurrently, recent literature supporting the clinical use of independent gene markers for melanoma prognosis and survival is limited. While numerous studies have suggested the correlation of the mutation status and/or expression level of individual genes and microRNAs with melanoma outcomes, few have been validated to show consistent results across melanoma subgroups.[52-54] Unlike other cancer types, there are no independent genomic markers that are currently recommended for melanoma prognosis by the NCCN or included as part of the AJCC 8th Edition staging system.

4.5 | Multiplexed prognostic biomarkers

GEP technology is well suited for prognosis of early stage melanoma. RT-PCR technology is an analytically robust and reproducible platform able to simultaneously evaluate many genes representing multiple cellular pathways. Four prognostic GEP signatures have been reported in the literature, with varying degrees of evidence supporting their utility for informing decisions about patient management (Table 3).[55-58] The first to appear in published literature was a nine-gene signature from Brunner and colleagues, discovered from analysis of 92 candidate genes previously shown by microarray analysis to be differentially expressed in correlation with overall survival.[55] Of note, fresh-frozen primary tumor tissue was used to develop and validate the gene signature. Study authors reassessed the candidate genes using 38 specimens from a training cohort of 91 patients and found that 11 genes correlated with overall survival. The prognostic value of the 11 genes was then examined in the expanded training set (n = 91), leading to the removal of two genes that did not satisfy significance parameters in univariate analysis. A predictive risk score was calculated by summing the weighted expression data for each gene, with weighting based on the regression coefficients obtained from multivariate Cox regression analysis. For this particular training cohort, the risk score was dichotomized with a cutoff of 1.46 (range 0-3.85), and samples with a score equal to or above the cutoff were classified as high risk. Although Kaplan-Meier survival curves were only shown for the training cohort, the authors assert that the risk score was validated in a cohort of 44 patients. Within that cohort, 10 patients had a high-risk score while 34 had a low-risk score, with misclassification rates of 29%.

The authors have presented unpublished reports that indicate a reduction to include only eight genes in the current GEP panel and a change in risk score cutoffs that define low and high-risk groups.[59] New validation will be required to show the accuracy of the test for early stage melanoma patients, and clinical utility of the signature needs to be thoroughly demonstrated. Nonetheless, the eight-gene signature for prognosis, branded as MelaGenix, is commercially available through NeraCare.

A second GEP signature reported by Sivendran and colleagues included 53 genes with an observed association with melanoma nonprogression.[58] To determine the 53-gene panel, study authors utilized 40 FFPE primary stage II-III melanoma tumors to evaluate 446 immune- or melanoma-related genes. Cross-validation and bootstrapping methods applied to the 40-sample training set resulted in strong area under the curve (AUC) values (>0.75). Independent validation of the signature in a cohort of 48 stage II-III melanomas achieved an AUC = 0.79 for prediction of progression, and the

53-gene panel and ulceration were independent predictors of disease progression in multivariate logistic regression models. Of note, all 53 genes were upregulated in tumors that did not progress to metastasis, reflecting an increased immune profile in the primary tumor microenvironment. Further validation studies have not been published, and evidence supporting clinical use of the panel is limited at this time.

The ability to better predict who is at elevated risk for SLN positivity would be clinically impactful for reducing surgical procedures (only 15%-20% of patients with intermediate thickness melanoma who undergo the procedure have a positive SLN) and increasing the yield of positive SLN outcomes.[36,60] To that end, Meves et al[57] performed next-generation sequencing (NGS) of primary melanoma tumors to identify genes that discriminated metastatic from nonmetastatic primary lesions. NGS results were validated with a quantitative RT-PCR platform. Eight control genes and 54 experimental cell adhesion-related genes were assessed. The study found that in a cohort 360 primary tumors (74 with SLN positive disease), age, ulceration and Breslow thickness were predictors of SLN status in a multivariate analysis of SLN status. Additionally, classification and regression tree modeling and logic regression modeling identified *ITGB3*, *TP53*, *LAMB1*, and *PLAT* as genes able to discriminate patients with or without nodal disease. Based on these findings, authors developed the "ITLP group" by combining expression levels of the four prognostic genes and aimed to determine the value added by the ITLP group to clinical factors in a logistic regression model of SLN positivity. An increase in accuracy was observed when comparing the clinical model (AUC = 0.78) and the model that included the ITLP group (AUC = 0.89). The latter was further validated with primary tumors from 146 melanoma patients and resulted in a false positivity rate of 22%, a false-negative rate of 0% and an AUC = 0.93, substantially better than observed for the clinical model alone (AUC = 0.68).

While the ITLP profile for SLN positivity is promising and biologically well founded, expanded validation studies have not been published to date. Clinical utility of the signature will be required to address whether the ITLP group will have an impact on the number of SLNBx performed or lead to better positivity rates among those patients who are assessed with the SLNBx procedure. Additionally, given that current AJCC and NCCN guidelines already recommend SLNBx for patients with greater than 5% positivity risk, the clinical use of the ITLP profile as a rule-in test may not further contribute to clinical utility.

The prognostic GEP that has been most widely reported in the literature is DecisionDx-Melanoma (Castle Biosciences, Friendswood, TX), a 31-gene (31-GEP) signature reported by Gerami et al[56] in 2015. Genes included in the 31-GEP were distilled from a comparative review of multimarker studies performed between 2000 and 2011, from which over 150 genes associated with recurrence were identified.[61-65] The test includes three control genes and 28 prognostic genes that have been validated in three multicenter retrospective cohorts, two prospective studies, and an analytic analysis that reported robust reproducibility of the test (98% technical success rate in 8244 clinically tested specimens).[56,66-70] Unlike the tests described above, the 31-GEP uses radial basis machine (RBM) pattern recognition modeling to compare the GEP of a test sample to a training set containing 164 melanoma tumors with known outcomes. Four subclasses of patients are reported: class 1A (low risk of metastasis within 5 years of diagnosis), class 1B and 2A (intermediate-risk) and class 2B (high-risk).

In a series of additional retrospective and prospective studies, the 31-GEP has consistently segregated risk for patients with stage I, II or III tumors.[69,70] A recent publication analyzed the accuracy of the test in 690 early stage melanoma patients. The study included 393 patients with stage I-IIA tumors, a group for whom national guidelines currently recommend only low intensity follow up and monitoring. Yet the 5-year relapse free survival (RFS), DMFS, and MSS for class 2B patients in this group were 60.9% (95% CI, 48.3%-76.7%), 75.8% (95% CI, 64.3%-89.4%), and 85.9% (95% CI, 76.0%-97.1%) respectively. The 31-GEP proved to be an independent predictor of RFS and DMFS in a multivariate analysis that included thickness, ulceration, and mitotic rate. The difference in MSS between patients with class 1 and class 2 tumors, having stage I to IIA disease, was significant (HR, 6.13; 95% CI, 1.07-35.24; $P = 0.04$). The class 2 association with high-risk disease has been further validated in both a single-center prospective study that reported a 5-year RFS rate of 68.7% for class 2 patients, and a multicenter prospective analysis that reported class 2 RFS rates of 77% in a cohort 1.5 years median follow up.[69-71]

In addition to clinical and analytic validity studies, the clinical utility of the 31-GEP has been assessed in prospectively and retrospectively designed studies.[72-74] An important utility of the test is its use in risk-stratifying follow up frequency and imaging intensity. The accurate and early selection of high-risk patients, followed by appropriate surveillance of high-risk disease, could lead to earlier identification of recurrence or metastasis. Studies of contemporary anti-melanoma therapies suggest greater efficacy of these drugs in patients with lower tumor burden.[4,6,8] For patients with low-risk disease, avoidance of unnecessary physician visits, laboratory tests, and imaging studies decreases cost, anxiety, and time lost from work and family.

The 31-GEP may also be useful in identifying patients unlikely to have SLN metastasis. When combined with Breslow thickness and age, the test may identify a group of patients who could avoid SLNBx. A study of 1421 patients reported by Vetto and colleagues at the 2018 American Academy of Dermatology Annual Meeting found that patients 55 years of age or older who had T1-T2 thickness tumors and class 1A results had SLN positivity rates under 5%. NCCN guidelines do not currently recommend SLNBx for patients who fall within the 4% to 5% false negative threshold of the SLNBx procedure. These data also suggest that the 31-GEP could increase the yield of actionable SLN-positive outcomes, which are currently only in the 15%-20% range for intermediate thickness tumors, and lower for thin tumors.

4.6 | Predictive biomarkers in melanoma

An exciting area of exploration in melanoma has been the identification of predictive biomarkers that may guide appropriate

TABLE 4 Predictive biomarkers and biomarker panels in cutaneous melanoma

Prognostic marker	Evidence summary	References
BRAF mutation status	Correlated with response to *BRAF*-targeted therapies has led to FDA approval of amplification and sequencing technologies, and multiple laboratory tests to assess *BRAF* mutation status	Long et al[4]
NRAS mutation status	Correlated with response to MEK inhibitors; association with response to anti-PD-1 therapy reported	Johnson, 2015[99]
Microsatellite instability	Approved for tissue agnostic analysis to identify patients with high mutation rates in areas of microsatellites; application to melanoma questionable due to predominance of MSI-low type	Bonneville et al[94] Kubeček and Kopecký[93]
Tumor mutation burden	TMB usually high in melanoma. Correlation with increased neoantigen production reported; independent validation of response prediction for ICIs in melanoma is questionable	Morrison, 2018[100]

Abbreviations: ICI, immune checkpoint inhibitor; MSI, microsatellite instability; TMB, tumor mutation burden.

administration of contemporary treatment. Given the potential adverse events associated with targeted and immune-associated treatments, the ability to make an informed decision about therapeutic interventions is critical. Efforts toward this end have been focused on the identification of actionable genetic alterations in cancer drivers of cell-cycle regulation, PI3K/AKT, MAPK, and other pathways.

4.7 | Protein biomarkers

Identification of the programmed cell death-1 (PD1)/PD ligand-1 (PD-L1) pathway as an inhibitor of T-cell response to cancer has transformed the management of patients with melanoma. The success of the PD1 inhibitors nivolumab and pembrolizumab in extending RFS in resected elevated-risk stage III melanoma patients has led to FDA approval of nivolumab, and current regulatory evaluation of pembrolizumab, for first line adjuvant treatment.[5,75] Clinical trials are in development to assess adjuvant use in earlier stage II disease. However, PD1 inhibitors are associated with grade 3, or higher, adverse event rates of approximately 15%.[8,76-78] The substantial costs and real toxicities associated with each of these agents have sparked a search for markers of response to the immunomodulators.

Expression of PD-L1 on tumor cells reflects a biological mechanism through which melanoma can evade the host immune system. Thus, the development of IHC-based assays to identify levels of the marker has been pursued with great interest. However, confounding the predictive utility of PD-L1 detection are patients who have low levels of the marker yet respond to immunomodulators, and those with high levels who do not. There are inconsistencies associated with the detection of PD-L1 protein, as well, including expression of the ligand on other cell types in the tumor microenvironment.[79,80] Additionally, results from the Checkmate-238 and EORTC 1325 studies show that regardless of PD-L1 status, patients benefited more from treatment with nivolumab or pembrolizumab compared to the CTLA-4 inhibitor ipilimumab or placebo, respectively.[77,78] Regardless of the limitations of PD-L1 protein as a marker for therapy response, two studies have undertaken systematic reviews and meta-analyses to evaluate the predictive value of PD-L1 and found that the marker is significantly associated with clinical response in patients.[81,82] Of note, among 4230 patients evaluated as part of the first study and 1987 cases in the second, highly correlating odds ratios of 2.14 and 2.04 were reported for PD-L1 association with objective response rates.

4.8 | Single gene predictive biomarkers

Advances in genomic sequencing technologies, coupled with the development of effective melanoma therapies, have identified genes with specific driver mutations as predictors of response. The best-studied example in melanoma is the *BRAF* V600 mutation that promotes constitutive activation of the MAPK pathway and is present in approximately 50% of melanoma tumors.[83,84] To date, two PCR-based companion diagnostics (CDx), the Cobas 4800 (Roche Diagnostics, Indianapolis, IN) and the THxID kit (BioMerieux, Marcy-l'Étoile, France), have been FDA approved for the detection of the V600E and/or V600K *BRAF* mutations, allowing for the appropriate use of BRAF and MEK inhibitors in patients with mutations.[85,86] It should also be noted that NGS detection of BRAF and NRAS driver mutations is widely available through other laboratory developed test technologies that do not require FDA approval.

The emergence of NGS technologies has allowed for the detection of *BRAF* mutations in combination with other predictive markers for melanoma (Table 4). The US FDA premarket approval of CDx from FoundationOne allows for more accurate determination of *BRAF* mutation status. Although the approval specifies the indication for *BRAF* targeted therapy in melanoma, CDx also assesses mutations in 324 genes, gene rearrangements, as well as microsatellite instability and tumor mutation burden (TMB). Included in the 324-gene panel are *NRAS* and *NF1* which, along with *BRAF*, have been identified as integral melanoma markers during the genomic evaluation of primary and metastatic melanoma conducted as part of The Cancer Genome Atlas project.[84]

Detection of activating mutations in *KIT* can also have predictive value for identifying tumors with activated receptor tyrosine kinase (RTK) pathways, although mutations are only observed in 2% of

melanomas.[87,88] Studies have shown that patients with mucosal, acral or chronically sun-damaged melanomas and *KIT* mutations have previously demonstrated better objective response rates following treatment with the RTK inhibitors, imatinib and nilotinib, compared to those who had amplifications of the gene.[89] While low objective response rates have been observed (15%-26% with nilotinib), *KIT* mutation status is still used to guide patient treatment with both RTK inhibitors.[90]

4.9 | Multiplexed predictive biomarkers

Modern technologies allow for the concurrent detection of large numbers of genomic aberrations. As a result, predictive biomarker panels associated with patient response to ICIs have been reported. Most markers are reflective of the cellular processes that stimulate, or are downstream from, the infiltration of T cells to the tumor microenvironment. This section will review several of the multi-marker tests that are available for clinical use or have shown promise as candidates for informing decisions about therapeutic intervention for melanoma patients.

GEP has been widely implemented to identify markers indicative of tumor-immune cell interactions that might act as predictors of response to ICIs. Genes associated with the inflammatory interferon-γ (INF-γ) pathway are among those that have been evaluated. INF-γ appears to function as a driver of PD-L1 expression in several cell types within a tumor's microenvironment, leading to activation of PD-1 signaling. Of interest for potential clinical use, Ayers and colleagues identified a 28-gene GEP associated with INF-γ signaling, inflammation, T cell markers and other immune-related pathways.[91] The group demonstrated the significant correlation of the GEP with best overall response rate and progression-free survival in patients enrolled in the KEYNOTE studies who were treated with pembrolizumab.[92] The group further refined the GEP to 18 genes that were shown to predict treatment response across several other tumor types, but melanoma was not included in the analysis of the refined GEP, leaving unclear the clinical utility of the INF-γ signature for response prediction in melanoma.

Microsatellite instability (MSI) is defined by an increased mutation rate in regions of the genome marked by nucleotide repeats. These mutations can result from disruptions in the functions of the mismatch repair (MMR) genes that are responsible for repairing erroneously placed DNA bases during the process of replication or from ultraviolet radiation-induced damage. Both processes increase the mutation rate (likely with concurrent development of neoantigens), which can be quantified by determining the number of variable alleles in genomic loci with microsatellites. Tumors demonstrating increased MSI have been shown to respond more favorably to ICIs. This has resulted in the approval of pembrolizumab for treatment of solid tumors with high rates of microsatellite instability (MSI-high), also referred to as MMR deficient (Table 4). Reports suggest that MSI is detected in nearly a third of primary melanoma tumors.[93] However, MSI is further divided into MSI-high and MSI-low categories. The majority of melanoma tumors are expected to be MSI-low, as a recent report found that only 0.64% of tumors were MSI-high.[94] Thus, the clinical application for melanoma patients is still in question.

Like MSI, TMB is a measure of genomic instability that can be measured using NGS technology. TMB reflects the number of somatic mutations per megabase of genomic DNA and is positively correlated with the number of neoantigens produced by the tumor.[95,96] TMB has also been associated with response of melanoma patients to ICI, although increased accuracy is required in order for TMB to be widely embraced for clinical use. A significant number of patients who are identified with high TMB do not respond to CTLA-4 or PD-1 therapies.

NGS detection methods are also being applied to liquid biopsies that capture circulating tumor cells and cell-free DNA extracted from blood, potentially providing predictive information about treatment response and the opportunity to screen patients to determine metastatic burden and response to therapies in real time. Liquid biopsies offer several advantages over tumor biopsies, including a noninvasive method for tissue collection, the potential for simplified collection of serial samples during the course of a patient's management, with no requirement for formalin fixation and paraffin embedding processes that may impact the NGS sequencing. Disadvantages of liquid biopsy techniques include limited sensitivity and of the fact that only a subset of the molecular markers from a primary or metastatic lesion are captured. Nonetheless continuing improvements in technology will likely make this approach a preferred tool for molecular profiling of solid tumors in the near future. Currently, a number of NGS-based assays are available for clinical analysis of both traditionally sourced solid tumors as well as liquid biopsies. These tests have potential application to breast, ovarian, prostate, colorectal, pancreatic, and numerous other types of cancer.

The most accurate determination of treatment response in the era of ICIs will most likely include the concurrent detection of protein and nucleic acid biomarkers on multiple platforms. To that end, companies such as OmniSeq and Nanostring already offer testing to simultaneously evaluate T cell and other immune markers, large-scale gene expression signatures, TMB and/or MSI to obtain a complete picture of the tumor microenvironment and better inform therapy decisions. Given the recent advances in neoantigen and microbiome detection, it is likely that future multiplexed predictive tests will assess larger numbers of biomarkers. The question remaining to be answered is whether these technologies can be used to guide treatment opportunities for melanoma patients.

5 | DISCUSSION

It has become clear that the simple classification of tumor clinicopathologic features of past years is no longer adequate to assess and determine risk. The expansion of subcategories of T-stage, N-stage, and overall tumor stage suggests that the assessment of outcome based purely on phenotypic features will require ever more complex sub categorizations and computational algorithms to make an outcome assessment possible.

AJCC has recognized the biologic and practical limitations of ordinal staging systems. There is interest in the facilitation of personalized probabilistic predictions using accurate risk models or calculators.[97] However, as has become clear in other solid tumor types, models based solely on clinicopathologic variables may fall short in assessing individual tumor prognosis, and are even more unlikely to be predictive of response to individual therapeutic agents. This has led to an interest in biomarker identification and pathway network integration that might provide an alternative window into relevant tumor cell biology and behavior.[98]

Beyond complexity, the primary dependence on Breslow thickness is a significant limiting feature of AJCC staging. It is estimated that more than 20% of melanomas are diagnosed from shave biopsies with involved deep biopsy margins, making accurate Breslow thickness impossible. Additional patients are noted to have significant regression in these tumors, making the Breslow thickness uncertain. Although regression may be independently assessed as a binary risk variable, standard reporting of Breslow thickness is reported on the available tumor, rather than on what may be the true tumor thickness. As a result, nearly a third of cutaneous melanoma patients may be inaccurately staged, and these inaccurate stages entered into national databases.

The combination of increasing complexity of staging, the limitation in assessing Breslow thickness on the initial tumor biopsy, and the recognition that phenotype does not always represent biology in individual tumors, provide a strong rationale for validated biomarker assessments. The ability to interrogate the intrinsic biology of cancer cells could provide an important adjunct to clinicopathologic assessment for more accurate clinical decision-making.

AJCC staging has been used to assess risk and direct surgical therapy such as planned resection margins, as well as to determine who should have a SLNBx. However, validated gene expression panels such as the 31-GEP test have the potential to do better. Data presented in this review highlight the low risk (<5%) that class I patients have to develop a positive SLN when they are 55 years or older. With additional validation data, such an assay may allow large populations of patients to avoid unnecessary surgical intervention.

Conversely, the ability to identify a biologically indolent tumor, even with a positive SLNBx, may allow some patients with Stage IIIA disease to avoid the toxicity and cost of systemic adjuvant therapy. Since the approval trials for checkpoint inhibitors did not include this patient subset, and no value for their use in this population has been proven, a more robust tool to assess individual risk would be for selecting therapy. Although BRAF and MEK-pathway targeted drugs are approved for stage IIIA disease, not all patients may have a true risk that justifies their use.

For patients who have node-negative disease after SLNBx and fall in the Stage I and Stage II range, having an additional biologic prognosticator, to be used with or without further probabilistic predictors, may allow both the development and more rational use of new adjuvant drugs in the subset of high-risk node-negative patients. This population gives rise to the majority of melanoma deaths among patients presenting with early-stage disease.

Clearly there needs to be more development and integration of predictive biomarkers and/or biomarker panels for therapeutic decision-making in early stage melanoma. The ability to precisely assess and target risk of recurrence is at the core of precision medicine and is critical to take full advantage of the evolving tools to treat malignant cutaneous melanoma.

6 | CONCLUSIONS

Robust biomarker panels offer an opportunity to better understand the underlying biology of early stage melanomas leading to better treatment decisions and outcomes. Such biomarker panels may also help patients avoid unnecessary anxiety, treatment toxicity, and cost. To date, there are few drug/biomarker combinations that provide sufficient predictive value to allow individualized choice of treatment, but this is beginning to change. Biomarker discovery and panel validation must become a standard part of investigative study conduct and should lead to increasing clinical utility as part of the standard clinical care of early and late stage cutaneous melanoma in the years to come.

CONFLICTS OF INTEREST

DMH, Castle Biosciences (Speakers' Bureau), Genomic Health (Speakers' Bureau, Consultant), Exact Sciences (Consultant); RC, Castle Biosciences (Employee, Shareholder); AB, there are no conflicts of conflicts of interest.

ORCID

David M. Hyams http://orcid.org/0000-0002-5680-9488
Robert W. Cook http://orcid.org/0000-0003-1166-4796
Antonio C. Buzaid http://orcid.org/0000-0002-1674-4579

REFERENCES

1. Siegel RL, Miller KD, Jemal A. Cancer statistics. *CA Cancer J Clin*. 2018;68(1):7-30.
2. Noone AM, Howlader N, Krapcho M, et al. *SEER Cancer Statistics Review, 1975-2015*. Bethesda, MD: National Cancer Institute; 2018.
3. Gershenwald JE, Scolyer RA, Hess KR, et al. Melanoma staging: evidence-based changes in the American Joint Committee on Cancer eighth edition cancer staging manual. *CA Cancer J Clin*. 2017; 67(6):472-492.
4. Long GV, Hauschild A, Santinami M, et al. Adjuvant dabrafenib plus trametinib in stage III BRAF-mutated melanoma. *N Engl J Med*. 2017;377(19):1813-1823.
5. Weber J, Mandala M, Del Vecchio M, et al. Adjuvant nivolumab versus ipilimumab in resected stage III or IV melanoma. *N Engl J Med*. 2017;377(19):1824-1835.
6. Andtbacka RHI, Ross M, Puzanov I, et al. Patterns of clinical response with talimogene laherparepvec (T-VEC) in patients with melanoma treated in the OPTiM phase III clinical trial. *Ann Surg Oncol*. 2016;23(13):4169-4177.
7. Joseph RW, Elassaiss-Schaap J, Kefford RF, et al. Baseline tumor size is an independent prognostic factor for overall survival in patients with melanoma treated with pembrolizumab. *Clin Cancer Res*. 2018; 24(20):4960-4967.
8. Ribas A, Hamid O, Daud A, et al. Association of pembrolizumab with tumor response and survival among patients with advanced melanoma. *JAMA*. 2016;315(15):1600-1609.

9. Rebecca VW, Sondak VK, Smalley KS. A brief history of melanoma: from mummies to mutations. *Melanoma Res*. 2012;22(2):114-122.
10. Breslow A. Thickness, cross-sectional areas and depth of invasion in the prognosis of cutaneous melanoma. *Ann Surg*. 1970;172(5):902-908.
11. Clark WH Jr, Jr., From L, Bernardino EA, Mihm MC. The histogenesis and biologic behavior of primary human malignant melanomas of the skin. *Cancer Res*. 1969;29(3):705-727.
12. Balch CM, Gershenwald JE, Soong S, et al. Final version of 2009 AJCC melanoma staging and classification. *J Clin Oncol*. 2009; 27(36):6199-6206.
13. Eriksson H, Frohm-Nilsson M, Järås J, et al. Prognostic factors in localized invasive primary cutaneous malignant melanoma: results of a large population-based study. *Br J Dermatol*. 2015;172(1): 175-186.
14. Coit DG, Thompson JA, Albertini MR, et al. *Melanoma, Version 3.2018. NCCN Clinical Practice Guidelines in Oncology*. Plymouth Meeting, PA: NCCN; 2018.
15. Namikawa K, Aung PP, Gershenwald JE, Milton DR, Prieto VG. Clinical impact of ulceration width, lymphovascular invasion, microscopic satellitosis, perineural invasion, and mitotic rate in patients undergoing sentinel lymph node biopsy for cutaneous melanoma: a retrospective observational study at a comprehensive cancer center. *Cancer Med*. 2018;7(3):583-593.
16. Egger ME, Gilbert JE, Burton AL, et al. Lymphovascular invasion as a prognostic factor in melanoma. *Am Surg*. 2011;77(8):992-997.
17. Koshenkov VP, Shulkin D, Bustami R, Chevinsky AH, Whitman ED. Role of sentinel lymphadenectomy in thin cutaneous melanomas with positive deep margins on initial biopsy. *J Surg Oncol*. 2012;106(4):363-368.
18. Mills JK, White I, Diggs B, Fortino J, Vetto JT. Effect of biopsy type on outcomes in the treatment of primary cutaneous melanoma. *Am J Surg*. 2013;205(5):585-590. discussion 590.
19. Bichakjian CK, Halpern AC, Johnson TM, et al. Guidelines of care for the management of primary cutaneous melanoma. American Academy of Dermatology. *J Am Acad Dermatol*. 2011;65(5):1032-1047.
20. Tas F, Erturk K. Tumor infiltrating lymphocytes (TILs) may be only an independent predictor of nodal involvement but not for recurrence and survival in cutaneous melanoma patients. *Cancer Invest*. 2017;35(8):501-505.
21. Weiss SA, Han SW, Lui K, et al. Immunologic heterogeneity of tumor-infiltrating lymphocyte composition in primary melanoma. *Hum Pathol*. 2016;57:116-125.
22. Appleton SE, Fadel Z, Williams JS, Bezuhly M. Vertical growth phase as a prognostic factor for sentinel lymph node positivity in thin melanomas: a systematic review and meta-analysis. *Plast Reconstr Surg*. 2018;141(6):1529-1540.
23. Gualano MR, Osella-Abate S, Scaioli G, et al. Prognostic role of histological regression in primary cutaneous melanoma: a systematic review and meta-analysis. *Br J Dermatol*. 2018; 178(2):357-362.
24. Ribero S, Gualano MR, Osella-Abate S, et al. Association of histologic regression in primary melanoma with sentinel lymph node status: a systematic review and meta-analysis. *JAMA Dermatol*. 2015;151(12):1301-1307.
25. Frydenlund N, Mahalingam M. Desmoplastic melanoma, neurotropism, and neurotrophin receptors-what we know and what we do not. *Adv Anat Pathol*. 2015;22(4):227-241.
26. Abdel-Rahman O. Evaluation of the eighth American Joint Committee on Cancer staging system for malignant melanoma of the skin. *Future Oncol*. 2018;14(5):471-481.
27. Wollina U, Brzezinski P. The value of metastasectomy in stage IV cutaneous melanoma. *Wien Med Wochenschr*. 2018;1-8. https://doi.org/10.1007/s10354-018-0630-6. [Epub ahead of print].
28. Handley WS. The Bunterian Lectures onthe pathology of melanotic growths in relation to their operative treatment. *Lancet*. 1907; 169(4362):927-933.
29. Balch CM, Soong SJ, Smith T, et al. Long-term results of a prospective surgical trial comparing 2 cm vs. 4 cm excision margins for 740 patients with 1-4 mm melanomas. *Ann Surg Oncol*. 2001;8(2):101-108.
30. Lens MB, Dawes M, Goodacre T, Bishop JA. Excision margins in the treatment of primary cutaneous melanoma: a systematic review of randomized controlled trials comparing narrow vs wide excision. *AArch Surg*. 2002;137(10):1101-1105.
31. Halsted WS. The results of operations for the cure of cancer of the breast performed at the Johns Hopkins Hospital from June, 1889, to January, 1894. *Ann Surg*. 1894;20(5):497-555.
32. Balch CM, Soong S, Ross MI, et al. Long-term results of a multi-institutional randomized trial comparing prognostic factors and surgical results for intermediate thickness melanomas (1.0 to 4.0 mm). Intergroup Melanoma Surgical Trial. *Ann Surg Oncol*. 2000; 7(2):87-97.
33. Cascinelli N, Morabito A, Santinami M, MacKie R, Belli F. Immediate or delayed dissection of regional nodes in patients with melanoma of the trunk: a randomised trial. WHO Melanoma Programme. *Lancet*. 1998;351(9105):793-796.
34. Lens MB, Dawes M, Goodacre T, Newton-Bishop JA. Elective lymph node dissection in patients with melanoma: systematic review and meta-analysis of randomized controlled trials. *AArch Surg*. 2002;137(4):458-461.
35. Veronesi U, Adamus J, Bandiera DC, et al. Inefficacy of immediate node dissection in stage 1 melanoma of the limbs. *N Engl J Med*. 1977;297(12):627-630.
36. Morton DL, Thompson JF, Cochran AJ, et al. Final trial report of sentinel-node biopsy versus nodal observation in melanoma. *N Engl J Med*. 2014;370(7):599-609.
37. Chen J, Xu Y, Zhou Y, Wang Y, Zhu H, Shi Y. Prognostic role of sentinel lymph node biopsy for patients with cutaneous melanoma: A retrospective study of surveillance, epidemiology, and end-result population-based data. *Oncotarget*. 2016;7(29):45671-45677.
38. Faries MB, Thompson JF, Cochran AJ, et al. Completion dissection or observation for sentinel-node metastasis in melanoma. *N Engl J Med*. 2017;376(23):2211-2222.
39. Hyams DM, Schuur E, Angel Aristizabal J, et al. Selecting post-operative adjuvant systemic therapy for early stage breast cancer: A critical assessment of commercially available gene expression assays. *J Surg Oncol*. 2017;115(6):647-662.
40. Simon RM, Paik S, Hayes DF. Use of archived specimens in evaluation of prognostic and predictive biomarkers. *JNCI*. 2009;101(21):1446-1452.
41. Cardoso F, Piccart-Gebhart M, Van't Veer L, Rutgers E, Consortium T. The MINDACT trial: the first prospective clinical validation of a genomic tool. *Mol Oncol*. 2007;1(3):246-251.
42. Sparano JA, Gray RJ, Makower DF, et al. Adjuvant chemotherapy guided by a 21-gene expression assay in breast cancer. *N Engl J Med*. 2018;379(2):111-121.
43. Deichmann M, Kahle B, Moser K, Wacker J, Wüst K. Diagnosing melanoma patients entering American Joint Committee on Cancer stage IV, C-reactive protein in serum is superior to lactate dehydrogenase. *Br J Cancer*. 2004;91(4):699-702.
44. Weide B, Elsässer M, Büttner P, et al. Serum markers lactate dehydrogenase and S100B predict independently disease outcome in melanoma patients with distant metastasis. *Br J Cancer*. 2012;107(3):422-428.
45. Wevers KP, Kruijff S, Speijers MJ, Bastiaannet E, Muller Kobold AC, Hoekstra HJ. S-100B: a stronger prognostic biomarker than LDH in stage IIIB-C melanoma. *Ann Surg Oncol*. 2013;20(8):2772-2779.
46. Kashani-Sabet M, Venna S, Nosrati M, et al. A multimarker prognostic assay for primary cutaneous melanoma. *Clin Cancer Res*. 2009;15(22): 6987-6992.
47. Kashani-Sabet M, Nosrati M, Miller JR, 3rd, et al. Prospective validation of molecular prognostic markers in cutaneous melanoma:

a correlative analysis of E1690. *Clin Cancer Res*. 2017;23(22): 6888-6892.
48. Romaine ST, Wells-Jordan P, de Haro T, et al. A small multimarker panel using simple immunohistochemistry methods is an adjunct to stage for cutaneous melanoma prognosis. *Melanoma Res*. 2016;26(6):580-587.
49. Pagès F, Mlecnik B, Marliot F, et al. International validation of the consensus Immunoscore for the classification of colon cancer: a prognostic and accuracy study. *Lancet*. 2018;391(10135):2128-2139.
50. Galon J, Fox BA, Bifulco CB, et al. Immunoscore and Immunoprofiling in cancer: an update from the melanoma and immunotherapy bridge 2015. *J Transl Med*. 2016;14:273.
51. Harcharik S, Bernardo S, Moskalenko M, et al. Defining the role of CD2 in disease progression and overall survival among patients with completely resected stage-II to -III cutaneous melanoma. *J Am Acad Dermatol*. 2014;70(6):1036-1044.
52. Brożyna AA, Guo H, Yang SE, et al. TRPM1 (melastatin) expression is an independent predictor of overall survival in clinical AJCC stage I and II melanoma patients. *J Cutan Pathol*. 2017;44(4):328-337.
53. Jakob JA, Bassett RL, Jr., Ng CS, et al. NRAS mutation status is an independent prognostic factor in metastatic melanoma. *Cancer*. 2012;118(16):4014-4023.
54. Mumford SL, Towler BP, Pashler AL, Gilleard O, Martin Y, Newbury SF. Circulating MicroRNA biomarkers in melanoma: tools and challenges in personalised medicine. *Biomolecules*. 2018;8(2):21.
55. Brunner G, Reitz M, Heinecke A, et al. A nine-gene signature predicting clinical outcome in cutaneous melanoma. *J Cancer Res Clin Oncol*. 2013;139(2):249-258.
56. Gerami P, Cook RW, Wilkinson J, et al. Development of a prognostic genetic signature to predict the metastatic risk associated with cutaneous melanoma. *Clin Cancer Res*. 2015;21(1):175-183.
57. Meves A, Nikolova E, Heim JB, et al. Tumor cell adhesion as a risk factor for sentinel lymph node metastasis in primary cutaneous melanoma. *J Clin Oncol*. 2015;33(23):2509-2515.
58. Sivendran S, Chang R, Pham L, et al. Dissection of immune gene networks in primary melanoma tumors critical for antitumor surveillance of patients with stage II-III resectable disease. *J Invest Dermatol*. 2014;134(8):2202-2211.
59. Brunner G, Schmitz J, Borowski M. *Impact of a Gene Expression Profiling Risk Score and Web-based Melanoma Outcome Calculator on the Precision of AJCC-based Prognostic Assessment*. Chicago, IL: American Society of Clinical Oncology Annual Meeting; 2018.
60. Han D, Zager JS, Shyr Y, et al. Clinicopathologic predictors of sentinel lymph node metastasis in thin melanoma. *J Clin Oncol*. 2013; 31(35):4387-4393.
61. Bittner M, Meltzer P, Chen Y, et al. Molecular classification of cutaneous malignant melanoma by gene expression profiling. *Nature*. 2000;406(6795):536-540.
62. Jaeger J, Koczan D, Thiesen HJ, et al. Gene expression signatures for tumor progression, tumor subtype, and tumor thickness in laser-microdissected melanoma tissues. *Clin Cancer Res*. 2007;13(3):806-815.
63. Mauerer A, Roesch A, Hafner C, et al. Identification of new genes associated with melanoma. *Exp Dermatol*. 2011;20(6):502-507.
64. Winnepenninckx V, Lazar V, Michiels S, et al. Gene expression profiling of primary cutaneous melanoma and clinical outcome. *JNCI*. 2006;98(7):472-482.
65. Scatolini M, Grand MM, Grosso E, et al. Altered molecular pathways in melanocytic lesions. *Int J Cancer*. 2010;126(8):1869-1881.
66. Gerami P, Cook RW, Russell MC, et al. Gene expression profiling for molecular staging of cutaneous melanoma in patients undergoing sentinel lymph node biopsy. *J Am Acad Dermatol*. 2015;72(5):780-785 e783.
67. Zager JS, Gastman BR, Leachman S, et al. Performance of a prognostic 31-gene expression profile in an independent cohort of 523 cutaneous melanoma patients. *BMC Cancer*. 2018;18(1):130.
68. Cook RW, Middlebrook B, Wilkinson J, et al. Analytic validity of DecisionDx-Melanoma, a gene expression profile test for determining metastatic risk in melanoma patients. *Diagn Pathol*. 2018;13(1):13.
69. Greenhaw BN, Zitelli JA, Brodland DG. Estimation of prognosis in invasive cutaneous melanoma: an independent study of the accuracy of a gene expression profile test. *Dermatol Surg*. 2018;44(12):1494-1500.
70. Hsueh EC, DeBloom JR, Lee J, et al. Interim analysis of survival in a prospective, multi-center registry cohort of cutaneous melanoma tested with a prognostic 31-gene expression profile test. *J Hematol Oncol*. 2017;10(1):152.
71. Gastman BR, Gerami P, Kurley SJ, Cook RW, Leachman S, Vetto JT. Identification of patients at risk of metastasis using a prognostic 31-gene expression profile in subpopulations of melanoma patients with favorable outcomes by standard criteria. *J Am Acad Dermatol*. 2018.
72. Berger AC, Davidson RS, Poitras JK, et al. Clinical impact of a 31-gene expression profile test for cutaneous melanoma in 156 prospectively and consecutively tested patients. *Curr Med Res Opin*. 2016;32:1-6.
73. Dillon LD, Gadzia JE, Davidson RS, et al. Prospective, multicenter clinical impact evaluation of a 31-gene expression profile test for management of melanoma patients. *SKIN J Cutan Med*. 2018;2(3):111-121.
74. Schuitevoerder D, Heath M, Cook RW, et al. Impact of gene expre ssion profiling on decision-making in clinically node negative melanoma patients after surgical staging. *J Drugs Dermatol*. 2018;17(2):196-199.
75. Eggermont AMM, Blank CU, Mandala M, et al. Adjuvant pembrolizumab versus placebo in resected stage III melanoma. *N Engl J Med*. 2018;378(19):1789-1801.
76. Robert C, Long GV, Brady B, et al. Nivolumab in previously untreated melanoma without BRAF mutation. *N Engl J Med*. 2015;372(4):320-330.
77. Robert C, Schachter J, Long GV, et al. Pembrolizumab versus Ipilimumab in Advanced Melanoma. *N Engl J Med*. 2015;372(26):2521-2532.
78. Weber JS, D'Angelo SP, Minor D, et al. Nivolumab versus chemotherapy in patients with advanced melanoma who progressed after anti-CTLA-4 treatment (CheckMate 037): a randomised, controlled, open-label, phase 3 trial. *Lancet Oncol*. 2015;16(4):375-384.
79. Kondo A, Yamashita T, Tamura H, et al. Interferon-gamma and tumor necrosis factor-alpha induce an immunoinhibitory molecule, B7-H1, via nuclear factor-kappaB activation in blasts in myelodysplastic syndromes. *Blood*. 2010;116(7):1124-1131.
80. Liu J, Hamrouni A, Wolowiec D, et al. Plasma cells from multiple myeloma patients express B7-H1 (PD-L1) and increase expression after stimulation with IFN-{gamma} and TLR ligands via a MyD88-, TRAF6-, and MEK-dependent pathway. *Blood*. 2007;110(1):296-304.
81. Gandini S, Massi D, Mandalà M, Massi D, Mandala, M. PD-L1 expression in cancer patients receiving anti PD-1/PD-L1 antibodies: A systematic review and meta-analysis. *Crit Rev Oncol Hematol*. 2016;100:88-98.
82. Khunger M, Jain P, Rakshit S, et al. Safety and efficacy of PD-1/PD-L1 inhibitors in treatment-naive and chemotherapy-refractory patients with non-small-cell lung cancer: a systematic review and meta-analysis. *Clin Lung Cancer*. 2018;19(3):e335-e348.
83. Long GV, Menzies AM, Nagrial AM, et al. Prognostic and clinicopathologic associations of oncogenic BRAF in metastatic melanoma. *J Clin Oncol*. 2011;29(10):1239-1246.
84. Cancer Genome Akbani R, Akdemir KC, Aksoy BA, et al. Genomic classification of cutaneous melanoma. *Cell*. 2015;161(7):1681-1696.
85. Chapman PB, Hauschild A, Robert C, et al. Improved survival with vemurafenib in melanoma with BRAF V600E mutation. *N Engl J Med*. 2011;364(26):2507-2516.
86. Hauschild A, Grob JJ, Demidov LV, et al. Dabrafenib in BRAF-mutated metastatic melanoma: a multicentre, open-label,

phase 3 randomised controlled trial. *Lancet*. 2012;380(9839):358-365.
87. Lovly CM, Dahlman KB, Fohn LE, et al. Routine multiplex mutational profiling of melanomas enables enrollment in genotype-driven therapeutic trials. *PLOS One*. 2012;7(4):e35309.
88. Beadling C, Jacobson-Dunlop E, Hodi FS, et al. KIT gene mutations and copy number in melanoma subtypes. *Clin Cancer Res*. 2008;14(21):6821-6828.
89. Hodi FS, O'Day SJ, McDermott DF, et al. Improved survival with ipilimumab in patients with metastatic melanoma. *N Engl J Med*. 2010;363(8):711-723.
90. Guo J, Carvajal RD, Dummer R, et al. Efficacy and safety of nilotinib in patients with KIT-mutated metastatic or inoperable melanoma: final results from the global, single-arm, phase II TEAM trial. *Ann Oncol*. 2017;28(6):1380-1387.
91. Ayers M, Lunceford J, Nebozhyn M, et al. IFN-gamma-related mRNA profile predicts clinical response to PD-1 blockade. *J Clin Invest*. 2017;127(8):2930-2940.
92. Robert C, Ribas A, Wolchok JD, et al. Anti-programmed-death-receptor-1 treatment with pembrolizumab in ipilimumab-refractory advanced melanoma: a randomised dose-comparison cohort of a phase 1 trial. *Lancet*. 2014;384(9948):1109-1117.
93. Kubeček O, Kopecký J. Microsatellite instability in melanoma: a comprehensive review. *Melanoma Res*. 2016;26(6):545-550.
94. Bonneville R, Krook MA, Kautto EA, et al. Landscape of microsatellite instability across 39 cancer types. *JCO Precis Oncol*. 2017;2017:1-15.
95. McGranahan N, Furness AJS, Rosenthal R, et al. Clonal neoantigens elicit T cell immunoreactivity and sensitivity to immune checkpoint blockade. *Science*. 2016;351(6280):1463-1469.
96. Spranger S, Luke JJ, Bao R, et al. Density of immunogenic antigens does not explain the presence or absence of the T-cell-inflamed tumor microenvironment in melanoma. *Proc Natl Acad Sci USA*. 2016;113(48):E7759-E7768.
97. Kattan MW, Hess KR, Amin MB, et al. American Joint Committee on Cancer acceptance criteria for inclusion of risk models for individualized prognosis in the practice of precision medicine. *CA Cancer J Clin*. 2016;66(5):370-374.
98. Kim YA, Cho DY, Przytycka TM. Understanding genotype-phenotype effects in cancer via network approaches. *PLOS Comput Biol*. 2016;12(3):e1004747.
99. Johnson DB, Lovly CM, Flavin M, et al. Impact of NRAS mutations for patients with advanced melanoma treated with immune therapies. *Cancer Immunol Res*. 2015;3(3):288-295.
100. Morrison C, Pabla S, Conroy JM, et al. Predicting response to checkpoint inhibitors in melanoma beyond PD-L1 and mutational burden. *J Immunother Cancer*. 2018;6(1):32.

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