Clinical Cancer Research

Validation of Melanoma Immune Profile (MIP), a Prognostic Immune Gene Prediction Score for Stage II–III Melanoma

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Abstract

Purpose: Biomarkers are needed to stratify patients with stage II–III melanoma for clinical trials of adjuvant therapy because, while immunotherapy is protective, it also confers the risk of severe toxicity. We previously defined and validated a 53-immune gene melanoma immune profile (MIP) predictive both of distant metastatic recurrence and of disease-specific survival (DSS). Here, we test MIP on a third independent population.

Experimental Design: A retrospective cohort of 78 patients with stage II–III primary melanoma was analyzed using the NanoString assay to measure expression of 53 target genes, and MIP score was calculated. Statistical analysis correlating MIP with DSS, overall survival, distant metastatic recurrence, and distant metastasis-free interval was performed using ROC curves, Kaplan–Meier curves, and standard univariable and multivariable Cox proportional hazards models.

Results: MIP significantly distinguished patients with distant metastatic recurrence from those without distant metastatic recurrence using ROC curve analysis (AUC = 0.695; P = 0.008). We defined high- and low-risk groups based on the cutoff defined by this ROC curve and find that MIP correlates with both DSS and overall survival by ROC curve analysis (AUC = 0.719; P = 0.004 and AUC = 0.698; P = 0.004, respectively). Univariable Cox regression reveals that a high-risk MIP score correlates with DSS (P = 0.015; HR = 3.2).

Conclusions: MIP identifies patients with low risk of death from melanoma and may constitute a clinical tool to stratify patients with stage II–III melanoma for enrollment in clinical trials.

Introduction

Melanoma is an aggressive cancer with an estimated incidence of 91,270 cases in the United States in 2018 (1, 2). Patients with melanoma are most frequently diagnosed with early-stage disease and treated with surgical resection. Unfortunately, many patients already have undetected micrometastases at the time of surgery and these patients are at high risk of death from melanoma (3). Overall, patients diagnosed with stage II or III melanoma have a risk of subsequent death from melanoma ranging from approximately 8% to 25% for stage II disease and 12% to 86% for stage III disease depending on substage (4, 5). Although the American Joint Committee on Cancer (AJCC) Tumor Node Metastasis (TNM) staging system is used clinically to assess prognosis, there is considerable heterogeneity within each clinical stage (3, 5, 6). In addition, lymph node surgeries are required to distinguish between stage II and stage III disease, but these have not been shown to improve survival in studies and, depending on institutional practices, patients may therefore choose to forgo a complete staging evaluation (7–10). Furthermore, official guidelines currently do not consistently recommend SLNB or CLND (11, 12). Therefore, this population of patients with melanoma is faced with considerable uncertainty.

Adjuvant immunotherapy remains controversial in early-stage melanoma due to concerns for rare and in some cases lethal toxicities. Furthermore, the long-term impacts of immunotherapy on quality-of-life have not been studied (13–17). Although many melanoma clinicians recommend immunotherapy for stage IIIC–D disease, the role of immunotherapy in stage II–IIIB disease remains debatable, despite recent findings that adjuvant pembrolizumab, adjuvant ipilimumab, and adjuvant nivolumab each independently show a recurrence-free survival (RFS) benefit in stage III disease (18–20). Meanwhile, combination inhibition of BRAF and MEK has shown modest overall survival (OS) advantage in a recent phase III study. However, treatment with dual tyrosine kinase inhibitor therapy is difficult for some patients to tolerate, with 26% of patients discontinuing treatment due to toxicity, 38% requiring a dose reduction, and 66% requiring a dose interruption (21). The AJCC committee itself has recognized

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Translational Relevance

Immunotherapy has revolutionized the treatment of metastatic melanoma, a disease which previously led to almost certain fatality. Yet, adjuvant immunotherapy for patients with stage II–III melanoma remains controversial, as clinicians weigh the risk of recurrence against the risk of serious immune-related and other adverse events associated with these therapies. Biomarkers are therefore needed to determine which patients with early-stage disease are at significant risk of death from melanoma and likely to benefit from adjuvant immunotherapy or BRAF inhibition. The melanoma immune profile (MIP) presented in this article has previously been tested in two retrospective populations and is now validated in a third one. Patients with a favorable MIP have low risk of death from melanoma and may represent a population that could be spared the toxicity of treatment and excluded from adjuvant immunotherapy trials. Further study in a prospective setting is warranted and MIP may add to existing prognostic tools in early-stage melanoma.

that novel and more accurate tools for risk assessment are urgently needed to identify those patients who are at highest risk for recurrence and death from melanoma, and who are therefore most likely to benefit from adjuvant therapy (5).

Tumor-infiltrating lymphocytes (TIL) have been proposed as markers of a favorable tumor-immune microenvironment, and it has been shown that patients with very high numbers of TILs have a favorable prognosis (22). However, only 3%–5% of patients with stage I or II melanoma fall into this category (22, 23). The immunoscore was proposed as a more quantitative metric for T-cell infiltration but has not been successfully applied to primary melanoma (24–27). More recently, a gene signature consisting of genes implicated in the epithelial-to-mesenchymal transition (EMT) has been analyzed on 534 patients with resectable melanoma, of which 50% were stage I patients, 18% stage II patients, and 33% stage III patients (28). The EMT signature was shown to have 85% sensitivity and 64% specificity for melanoma-specific survival (28). However, it is applied inconsistently in clinical practice, as it is insufficiently precise for higher-risk patients to safely avoid adjuvant therapy. In addition, many of the patients included had stage I disease, for which the risk of death is under 5% and for whom adjuvant therapy is not recommended because of risks of toxicity. Furthermore, the EMT signature does not include immune factors known to contribute significantly to melanoma progression and may potentially be improved through inclusion of immune parameters.

Previously, we characterized a 53-immune gene transcriptomic signature score, which we call melanoma immune profile (MIP), associated with lack of disease progression (or distant metastatic recurrence, DMR) using NanoString transcriptomic profiling (29). A group of 446 immune-related candidate genes were identified from established literature and RNA from a training set of 40 patients was quantified. With random forest and elastic net analysis MIP added significantly to the predictive power of standard clinicopathologic features for RFS and disease-specific survival (DSS; ref. 29). MIP was also more enriched compared with the original 446 genes in a coexpression network constructed with genes from unbiased network analysis of 46 primary melanomas from the GEO database (29). Bayesian analysis of the coexpression network identified driver genes with functions in lymphocyte aggregation and activation, including *Ccr5*, *Cd8*, and *Cd3* (29). These driver genes are implicated in the crucial mechanism of Th1 immune surveillance represented by the gene panel and are taken into consideration to predict the disease progression (29–31). The predictive value of MIP was further validated using a set of 48 patients using AUC analysis (AUC = 0.787; $P < 0.01$; ref. 29).

In this work, we validate MIP, using the identical equation, for a second time and in a third, larger independent cohort of 78 patients. We find again that MIP correlates with non-DMR. A favorable signature score correlates with DSS and is an independent predictor of DSS when other clinical features are taken into account.

Materials and Methods

Patient selection

This study was approved by Columbia University Irving Medical Center's (CUIMC) Institutional Review Board (IRB). This study was determined by CUIMC's IRB to not require written consent from subjects, as it is retrospective and involves minimal risk. This study was conducted in accordance with the ethical guidelines outlined by the Declaration of Helsinki. A patient database of patients with stage II–III melanoma at CUIMC was created retrospectively by searching dermatopathology and surgical pathology records from 2000 to 2014 for "melanoma." After reviewing 1,352 patients, we identified 786 patients with stage II–III who had primary melanoma samples available at CUIMC (Fig. 1). Complete pathologic staging was not available on all patients as some patients declined sentinel lymph node biopsy (SLNB) or completion lymph node dissection (CLND); however, pathology information was included when available. BRAF status was not included as the vast majority of these melanomas were diagnosed before BRAF testing was standard. Of these 786 remaining patients, 235 had available survival data, defined as known date of death and/or 24 months of documented clinical follow-up. Hematoxylin and eosin slides were cut and reviewed with a dermatopathologist, and 209 patients had confirmed melanoma, whereas 26 patients did not have melanoma in the residual specimen. Second resections and wide local excision samples ($n = 42$) were excluded because of concerns for altered immune infiltration following the initial biopsy. In addition, 6 blocks were missing upon request, leaving 161 primary biopsy specimens for study. Of these specimens, 75 patients had no available clinical data to determine whether DMR occurred over a minimum 24-month follow-up period, due to the fact that, although their specimens were evaluated in the pathology department at CUIMC, clinical care was not provided at CUIMC, leaving 86 patients with sufficient follow-up for analysis. During the RNA extraction process, pathology specimens from 8 patients contained insufficient RNA for extraction and analysis, thus leaving a final total of 78 patients with successfully extracted and analyzable RNA (Table 1).

RNA isolation and nCounter assay

For each patient in the cohort, formalin-fixed paraffin-embedded (FFPE) primary melanoma specimen blocks were measured and then cut in 10-μm sections to provide 250 mm^2 of tissue. RNA extraction was performed with miRNeasy FFPE kit (Qiagen) following kit protocol and quantitated by Agilent Bioanalyzer

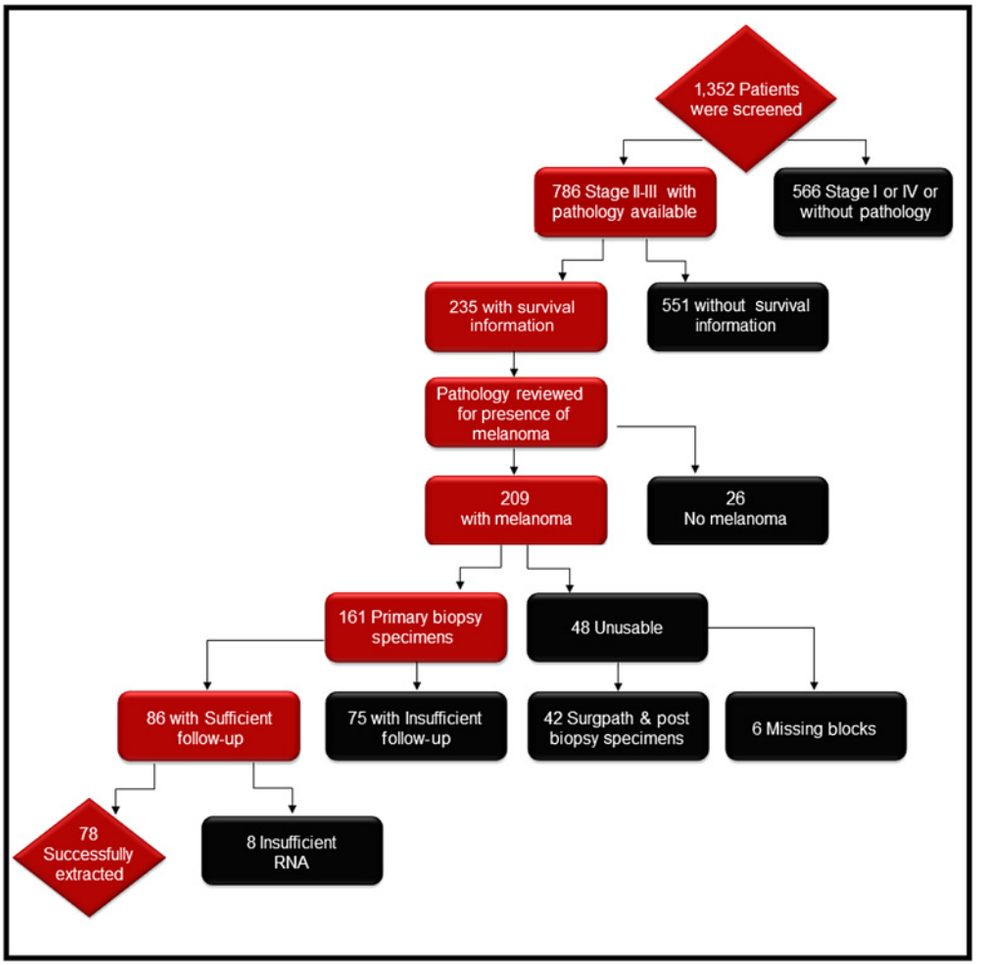

Figure 1.
Patient selection flowchart.

with RNA Nano chip assay, then stored at −80°C. Eight patients were excluded because of insufficient RNA (Fig. 1).

The nanoString assay performed measures expression of 53 target and 17 housekeeping genes. The controls in the assay include (i) a 6-point linear titration of exogenous *in vitro*–transcribed RNA targets and corresponding probes covering an approximately 1000-fold RNA concentration range (0.125–8 fM; positive control probes); (ii) an exogenous probe set lacking homology to human RNA sequences (negative control probes); and (iii): a set of PAGE-purified DNA oligos corresponding to the 100 nt probe-binding site on the 70 targets mRNAs (reference control samples).

RNA samples that passed quality and concentration standards were hybridized in a multiplexed manner to target-specific probes (probes A and B) and assay controls in a single tube for 20 hours at 65°C using 100–400 ng of RNA. A standard run contained 10 randomly positioned samples plus duplicate reference controls in each cartridge. Following hybridization, the target–probe complexes were purified and immobilized on the nCounter prep station. Digital counts for each gene-specific target RNA were then acquired on the nCounter detection analyzer and normalized in nSolver software (NanoString) to account for slight differences in assay efficiency such as hybridization, purification, and binding. The results from nCounter software were then used to apply a MIP score, computed by an investigator blinded to the clinical information (SP).

Statistical analysis

The MIP score was calculated using a proprietary algorithm with the same gene coefficients and equation as in the original publication (29). Survival time was defined from the time of biopsy. Patients who died of melanoma were classified as dead for DSS and OS. Patients who died of other documented causes or lived for at least 24 months without recurrence and died of unknown causes were censored at date of death for DSS and classified as dead for OS. DMR was defined as development of systemic metastasis (stage IV disease) or, if this was not documented, as death from melanoma. Distant metastasis-free interval (DMFI) was defined as time from biopsy to development of first metastasis or, if this was not documented, as date of death from melanoma. Analysis was completed with R Studio version 1.1.453 (CRAN) and GraphPad Prism Version 7.02. Statistical significance

Table 1. Patient characteristics in MIP validation cohort

Patient characteristics of the validation cohort ($n = 78$)	
Clinical characteristics	
Gender, *n* (%)	
Male	59 (75.6)
Female	19 (24.4)
Age	
Median, *n* (range)	67 (22–96)
Location of tumor, *n* (%)	
Trunk	45 (57.7)
Extremity	32 (41.0)
Unknown	1 (1.3)
Stage, *n* (%)	
II	63 (80.8)
III	15 (19.2)
Pathologic characteristics	
Depth (mm)	
Median, *n* (range)	2.7 (0.7–26)
Ulceration, *n* (%)	
Absent	30 (38.5)
Present	44 (56.4)
Unknown	4 (5.1)
Outcome characteristics	
Patient follow-up (months)	
Median, *n* (range)	60.5 (7–187)
Systemic recurrence, *n* (%)	
Yes	24 (30.8)
Known date	21
Unknown date	3
No	49 (62.8)
Local recurrence only	6
No local recurrence	43
Unknown	5 (6.4)
OS (months), *n* (%)	
Alive (at least 2 years)	50 (64.1)
Dead	28 (35.9)
DSS (months), *n* (%)	
Alive or NED at death	59 (75.6)
Dead with melanoma	19 (24.4)

Abbreviation: NED, no evidence of disease.

was defined as $P \leq 0.05$. The effect of prediction score on survival was analyzed by ROC curve analyses using package "plotROC," Kaplan–Meier (KM) curves, and standard univariable and multivariable Cox proportional hazards model using package "survival" and "survminer" in RStudio. *P* values for ROC curve analyses were calculated using Wilcoxon signed-rank test and KM curve *P* values were calculated using log-rank (Mantel–Cox) test in R version 3.4.4 (CRAN). Comparison of the "discovery," "test," and validation cohorts was done in R using package "tableone," where Pearson χ^2 test with continuity correction was performed for all categorical variables and ANOVA was performed for continuous variables.

Results

Patient population

The validation cohort consisted of 78 patients, all of whom had available DSS data, defined as known cause and date of death and/or documented clinical follow-up of at least 24 months (Table 1). This cohort consisted of more males ($n = 59$) than females ($n = 19$). In keeping with higher incidence rates of stage II relative to stage III melanoma, the cohort had more stage II ($n = 63$) than stage III ($n = 15$) patients. In addition, stage correlated significantly with DSS ($P = 0.002$) in this population using Cox regression. Thus, our validation population was similar in most respects to the populations of patients with melanoma used to generate staging criteria and was generally similar to other populations of patients with melanoma in the United States (3, 32). Median patient age was 67 years and median tumor depth was 2.7 mm. 57.7% of tumors were located on the trunk or head and 56.4% of tumors were ulcerated. In this cohort, 19 patients were confirmed to have died of melanoma in the medical record and 28 patients died of any cause. Clinical records were analyzed to determine whether patients had documented local and/or systemic recurrence, finding that 24 patients developed distant metastases and 6 patients developed local recurrence only. Median time of follow-up was 60.5 months. As this study is a validation, we next performed statistical analyses to compare the populations from our original study (29) to our current validation cohort (Supplementary Table S1). Referring to our original study, "discovery" defines our discovery population and "test" is the validation population from the original publication (29). The patients in our original study were treated at the Icahn School of Medicine at Mount Sinai (ISMMS), New York University Medical Center (NYUMC), and Geisinger Medical Center (GMC). The CUIMC population contained less female patients than did the populations from our initial study ($P = 0.04$). In addition, CUIMC patients were generally less clinically advanced than the patients in the earlier study, with significantly lower frequency of stage III disease ($P = 0.001$). In addition, significantly longer follow-up was available for the CUIMC cohort (68.6 months, $P = 0.007$). Consistent with less advanced stage at diagnosis, distant recurrence was less common in the CUIMC patients ($P = 0.04$) although DSS was similar ($P = 0.3$). There was no difference between the previously studied patients and CUIMC patients in terms of depth, ulceration, age, gender, or anatomic tumor location. Thus, in summary, the cohort included in this study was not dissimilar from most patient cohorts within the United States.

Validation that MIP correlates with distant metastatic recurrence

To test whether MIP can classify patients into those who developed metastatic disease and those who did not, we used identical criteria to define patients in terms of DMR status (previously defined as progression in our original publication; ref. 29), whereby patients were classified as having distant metastatic recurrence if they developed stage IV metastatic disease ($n = 24$). Patients with no recurrence of melanoma were defined as lacking DMR ($n = 43$) and patients with local recurrence only were excluded ($n = 6$). Using these criteria, we find that MIP significantly predicted DMR using ROC curve analysis (AUC = 0.695; $P = 0.008$; Fig. 2A). As a next step, to include the full cohort, we included patients who had a local recurrence only ($n = 6$) in the non-DMR group (metastasis cohort; Supplementary Table S2). Again, MIP identified absence of DMR with significant accuracy (AUC = 0.691; $P = 0.008$; Supplementary Fig. S1).

A heatmap of the 53 genes from MIP is shown in Fig. 2B ($n = 78$). The corresponding gene list is shown in Supplementary Fig. S2. Similar to what was observed in our prior publication, we found that patients with DMR had an overall lower expression of immune genes. Thus, consistent with prior findings, immune gene expression was decreased in patients who progressed to metastatic disease, validating that MIP is able to identify patients who progress to metastatic disease in a third independent cohort.

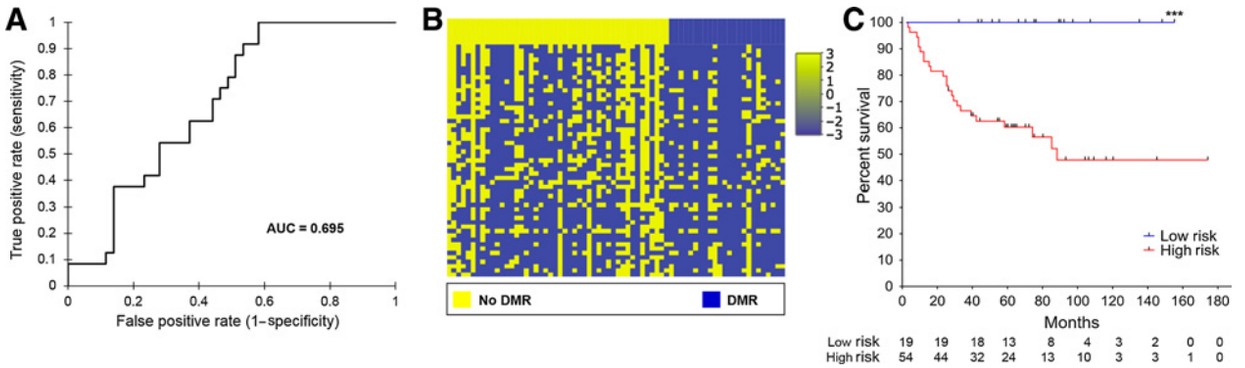

Figure 2.

MIP correlates with lack of DMR. **A,** ROC curve analysis for progression as defined in original population, excluding patients with local recurrence only ($n = 67$; AUC = 0.695; $P = 0.008$). **B,** Heatmap showing relative levels of mRNA expression for each gene. Each column represents a patient, and each row represents one of the 53 genes. Patients with DMR are labeled in blue; those without DMR are labeled in yellow. Yellow indicates higher expression, and blue indicates lower expression of each gene in the color scale. **C,** KM curve for distant metastasis-free interval (DMFI; $P = 0.0009$) created using AUC cutoff from ROC curve shown in **A** (cutoff = −1720.205). Statistical comparison for DMFI KM curve performed using log-rank (Mantel–Cox) test. Values are significant at $P \leq 0.05$ (*, $P \leq 0.05$; **, $P \leq 0.01$; ***, $P \leq 0.001$).

MIP correlates with DMFI, DSS, and OS

We further evaluated MIP as a predictor of DMFI, DSS, and OS. To test the ability of the score to classify patients based on risk, we defined high- and low-risk groups based on the MIP score cutoff defined by the DMR ROC curve (−1720.205; Fig. 2A) and used this cutoff for all survival curves. Of note, this cutoff was the same for DSS and DMFI ROC curve analysis (Table 2). We found that MIP-defined low-risk patients had a significantly longer DMFI than did high-risk patients ($P = 0.0009$; Fig. 2C).

DSS is a key endpoint for prognostic studies and thus, an important evaluation of MIP. Using ROC curve analysis we found that MIP correlates with DSS (AUC = 0.719; $P = 0.004$; Fig. 3A) and patients with low-risk MIP had significantly longer DSS ($P = 0.003$; Fig. 3B). For these same patients we evaluated OS, finding that a low-risk MIP indicated good OS using ROC curve (AUC = 0.698; $P = 0.004$; Supplementary Fig. S3A) and KM curve ($P = 0.003$; Supplementary Fig. S3B) analyses. Next, in view of potential clinical application, we tested the sensitivity and the specificity of MIP classification for DMFI, DSS, and OS (Table 2). MIP was highly sensitive (100%) for DMFI and DSS but not for OS (75%). Specificity was lower, with a value of 39% for DMFI, 37% for DSS, and 62% for OS.

MIP is an independent predictor of death from melanoma

To further evaluate the robustness and potential clinical application of the prediction score, we performed univariable and multivariable Cox regression for DSS and DMFI. Univariable Cox regression for DMFI revealed that a high-risk MIP score specified poor prognosis ($P = 0.016$; HR = 2.7; Fig. 4A). In evaluation of other clinical variables, including stage, gender, age, location, depth, and ulceration, only stage, a standard predictor of prognosis, was found to be significant ($P = 0.014$; HR = 2.8; Fig. 4A). Multivariable Cox regression for DMFI was most significant when MIP, ulceration, and stage were combined ($P = 0.002$; Fig. 4A).

For DSS, we found that a high-risk MIP indicated poor prognosis ($P = 0.015$; HR = 3.2; Fig. 4B). Among other clinical parameters in this cohort, including stage, gender, age, location, depth, and ulceration, only stage was found to be significant ($P = 0.002$; HR = 4.2; Fig. 4B). Multivariable Cox regression for DSS was most significant when MIP, ulceration, and stage were combined ($P = 0.001$; Fig. 4B).

Discussion

In this work, we validate in a third independent patient cohort that MIP correlates with risk of DMR and risk of death from melanoma in patients with stage II–III disease. In addition, we demonstrate that risk classification using MIP defines two groups that correlate with DMFI, DSS, and OS by KM curve analysis. Furthermore, using Cox regression we find that a favorable MIP score is an independent predictor of prolonged survival. Finally, we find that a favorable MIP correlates with low risk of death from melanoma, with none of 22 patients in the low-risk group dying of melanoma and 20 of 56 (36%) patients in the high-risk group dying of melanoma. The identification of a low-risk group has potential clinical implications, as exclusion of patients in the low-risk group from clinical trials would enrich for high-risk patients and thereby decrease the enrollment needed to achieve a

Table 2. Comparison of ROC curves for DMR, DMFI, DSS, and OS

Variable	AUC	Cutoff	Sensitivity	Specificity	*P*
DMR (local recurrence only excluded)	0.695	−1,720.205	1.000	0.419	0.006
DMR (local recurrence only included)	0.691	−1,720.205	1.000	0.388	0.006
DMFI	0.667	−1,720.205	1.000	0.388	0.024
DSS	0.719	−1,720.205	1.000	0.373	0.002
OS	0.698	−1,122.597	0.750	0.620	0.004

Note: Values are significant at $P \leq 0.05$.

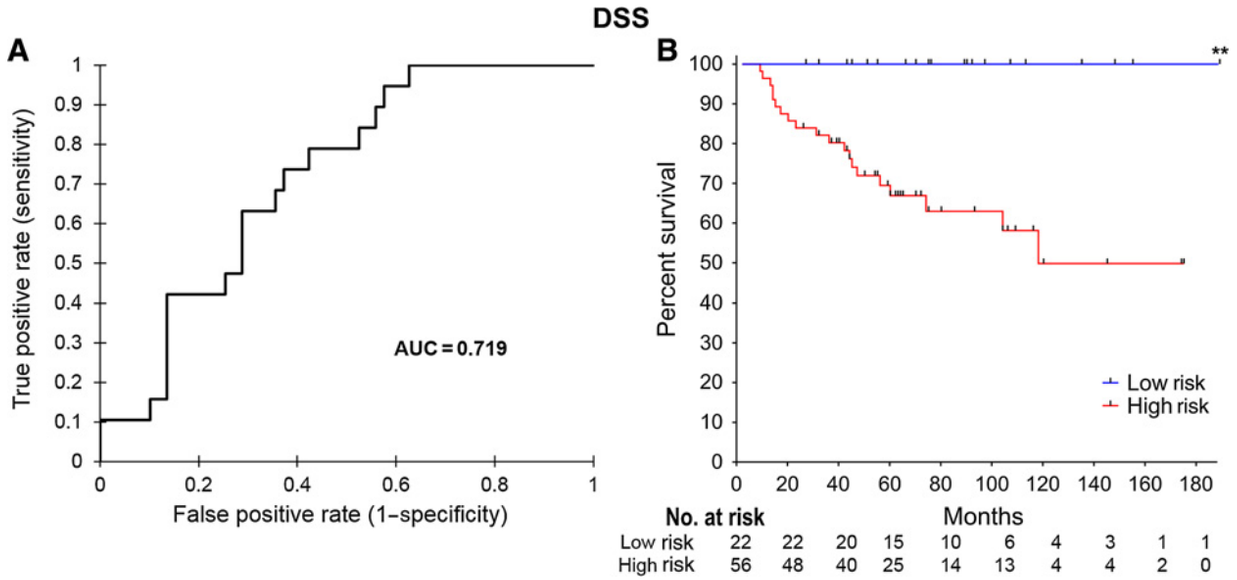

Figure 3.

MIP correlates with DSS. KM curves created using AUC cutoff from DMR (cutoff = −1720.205). **A,** AUC curve for DSS, excluding patients without known disease status at last follow-up or death ($n = 78$; AUC = 0.719; $P = 0.004$). **B,** KM curve for DSS ($P = 0.003$). Statistical comparison for DSS KM curve performed using log-rank (Mantel–Cox) test. Values are significant at $P \leq 0.05$ (*, $P \leq 0.05$; **, $P \leq 0.01$; ***, $P \leq 0.001$).

statistically significant benefit. Furthermore, these patients in the low-risk group could be spared exposure to potentially more toxic immunotherapy.

The fact that an immune gene signature, such as MIP, can define a low-risk subset of patients is consistent with data showing that high numbers of tumor-infiltrating lymphocytes (TIL) confer a favorable prognosis (22). Unfortunately, TILs have been difficult to integrate into clinical care because of interobserver variability and thus are not standardly used for prognosis by clinicians (22, 23). Furthermore, additional information can be obtained using gene expression profiling because TILs may represent B cells as well as a variety of T-cell phenotypes (33). In-depth analysis of immune infiltrates may expand on the value of TILs. The immunoscore, consisting of a more precise quantification of $CD8^+$ T cells within the tumor microenvironment, has been proposed as a biomarker in multiple tumor types (34–36). Recently, we have found that the ratio of $CD8^+$ T cells to $CD68^+$ macrophages in the stroma confers a favorable prognosis in a single patient cohort of stage II–III melanoma patients (37). Histopathologic as well as genomic assessments of the tumor immune microenvironment are likely to provide useful prognostic information in early-stage melanoma, and a combined biomarker including a more quantitative assessment of TILs will likely have application in the clinical setting.

The utility of a biomarker such as MIP should be interpreted within the clinical context of stage II–III melanoma. Both immunotherapy and combined MEK and BRAF inhibition have shown benefit in terms of DFMI in stage IIIB–D disease, whereas combined MEK and BRAF inhibition, but not adjuvant immunotherapy, has established OS benefit in stage III disease for patients bearing classic BRAF mutations (21). However, OS benefit in this study was difficult to assess based on the fact that patients received divergent therapies postrecurrence and that it was not documented whether patients in the placebo arm ever received combination tyrosine kinase inhibition (21). There is therefore no consensus currently in the field as to whether adjuvant immunotherapy or targeted therapy is superior and both are associated with toxicities (11). A more accurate assessment of recurrence risk, and particularly identification of a low-risk group that could be spared the toxicities of adjuvant therapy, would therefore benefit patients.

MIP can be distinguished from other genomic signatures such as the Castle Biosciences signature (28) because it focuses specifically on stage II and III melanoma where risk is the highest. Note that treating patients with stage I melanoma with immunotherapy would require a very high degree of certitude of adverse outcome. In addition, MIP is an immune-based assay; markers included in the signature are implicated in Th1 signaling pathways consistent with the immune surveillance hypothesis. Notably, as presented in the earlier publication, network analysis showed that genes included in MIP are part of a larger network of genes with the most important node in the network being *Ccl5*, known to be important for Th1 responses (29–31). Furthermore, because immunotherapy plays such a critical role in management of patients with melanoma, an immune-based signature, such as MIP, may ultimately have application both as a predictive and as a prognostic biomarker. Notably, infiltrating $CD8^+$ T cells and IFN-related signature scores have both been previously proposed as predictors of response to checkpoint blockade (38, 39). Thus, developing both prognostic and predictive biomarkers assessing the tumor–immune microenvironment in stage II–III melanoma is likely to yield tools of clinical utility.

MIP was developed to predict progression to metastatic disease at a distant site rather than recurrence *per se*. This is because local resectable recurrence does not necessarily indicate an aggressive biology in melanoma, whereas distant metastasis almost

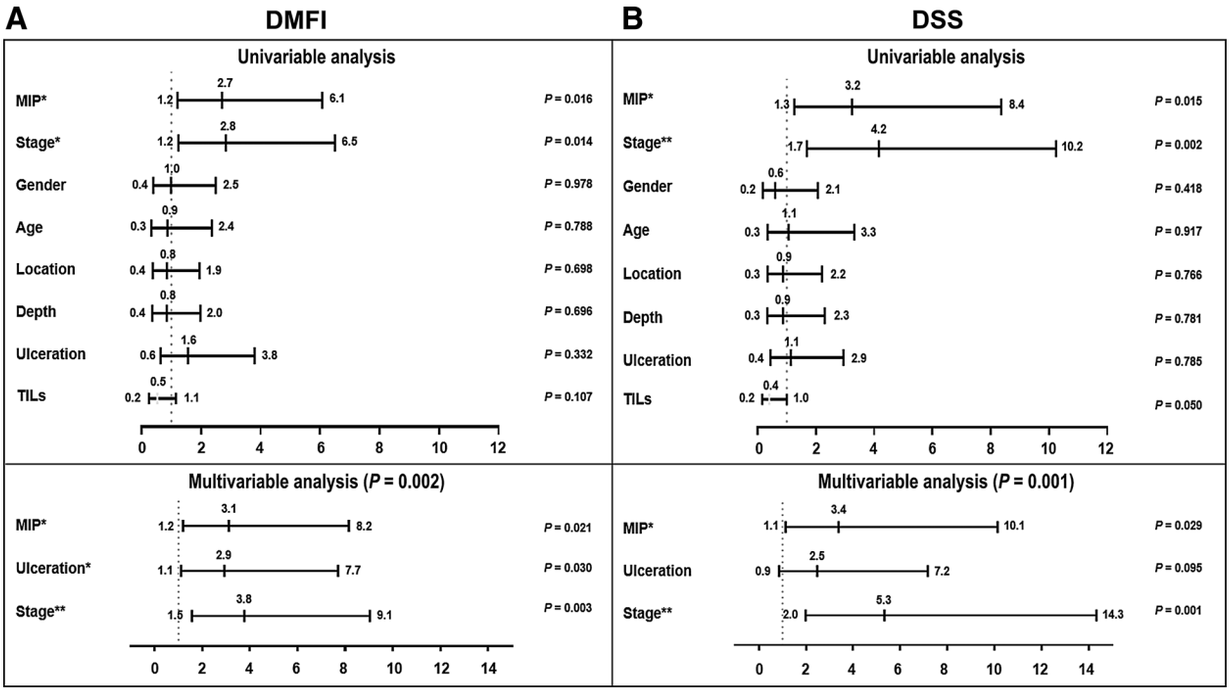

Figure 4.

MIP correlates with DMFI and DSS using Cox regression analysis. **A,** Univariable and multivariable Cox analysis of DMFI. **B,** Univariable and multivariable Cox analysis of DSS. Values are significant at $P \leq 0.05$ (*, $P \leq 0.05$; **, $P \leq 0.01$; ***, $P \leq 0.001$).

invariably does. Thus, in one recent study of patients who developed limited local resectable recurrence between 2005 and 2014, only 31% developed distant metastasis while 29% died of melanoma during the follow-up period (40). This finding has been reproduced in several other studies (41, 42). This is not dissimilar from the death rate from stage III primary melanoma (40). Although the reasons why patients with a resected local recurrence often do not develop metastatic disease are not understood, this may be reflective of a protective immune response. Distant metastatic disease portends very poor prognosis in the absence of therapy and is a valuable endpoint in that it minimizes the impact of improvements in therapy in the metastatic setting on data consistency over time. The introduction of novel and effective therapies would be expected to improve DSS and OS curves over time, thereby limiting the ability to accurately validate a biomarker using these metrics over time and this is a limitation of our method.

Limitations to our work include that while MIP has been tested in three independent patient datasets from different institutions, all three have been modest in size, with this last one being the largest, at 78 patients. In addition, while Cox regression has shown that MIP is an independent predictor of DSS in all three populations, further study is required to validate the findings presented in this article in a prospective setting. Furthermore, although median follow-up time was 60.5 months, our work may have been biased by the inclusion of patients with only 24 months of clinical follow-up who remain at risk for poor outcomes. Nonetheless, this work demonstrates that MIP merits prospective validation and has potential clinical utility in prognostication for patients with stage II–III melanoma, a population in which there is urgent need for better biomarkers. We have known for generations that the immune system modulates melanoma growth and MIP presents a quantitative metric to translate this knowledge to patient care. Further studies in well-curated cohorts from cooperative group samples and prospective validation are warranted.

Disclosure of Potential Conflicts of Interest

M.C. Ernstroff is a consultant/advisory board member for OmniSeq, Celyad, Iovance, and Alkermes, and reports receiving commercial research support from Alter BioScience, Bristol-Myers Squibb, Alkermes, Merck, and Merrimack. S. Pabla is an employee of and has ownership interests (including patents) at OmniSeq. Y.M. Saenger reports receiving commercial research grants from Amgen, Intensity Therapeutics, Omniseq, and Valeant. No potential conflicts of interest were disclosed by the other authors.

Disclaimer

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