15-Gene Expression Profile and *PRAME* as Integrated Prognostic Test for Uveal Melanoma: First Report of Collaborative Ocular Oncology Group Study No. 2 (COOG2.1)

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ABSTRACT

PURPOSE Validated and accurate prognostic testing is critical for precision medicine in uveal melanoma (UM). Our aims were to (1) prospectively validate an integrated prognostic classifier combining a 15-gene expression profile (15-GEP) and *PRAME* RNA expression and (2) identify clinical variables that enhance the prognostic accuracy of the 15-GEP/*PRAME* classifier.

MATERIALS AND METHODS This study included 1,577 patients with UM of the choroid and/or ciliary body who were enrolled in the Collaborative Ocular Oncology Group Study Number 2 (COOG2) and prospectively monitored across 26 North American centers. Test results for 15-GEP (class 1 or class 2) and *PRAME* expression status (negative or positive) were available for all patients. The primary end point was metastasis-free survival (MFS).

RESULTS 15-GEP was class 1 in 1,082 (68.6%) and class 2 in 495 (31.4%) patients. *PRAME* status was negative in 1,106 (70.1%) and positive in 471 (29.9%) patients. Five-year MFS was 95.6% (95% CI, 93.9 to 97.4) for class 1/*PRAME*(−), 80.6% (95% CI, 73.9 to 87.9) for class 1/*PRAME*(+), 58.3% (95% CI, 51.1 to 66.4) for class 2/*PRAME*(−), and 44.8% (95% CI, 37.9 to 52.8) for class 2/*PRAME*(+). By multivariable Cox proportional hazards analysis, 15-GEP was the most important independent predictor of MFS (hazard ratio [HR], 5.95 [95% CI, 4.43 to 7.99]; $P < .001$), followed by *PRAME* status (HR, 1.82 [95% CI, 1.42 to 2.33]; $P < .001$). The only clinical variable demonstrating additional prognostic value was tumor diameter.

CONCLUSION In the largest prospective multicenter prognostic biomarker study performed to date in UM to our knowledge, the COOG2 study validated the superior prognostic accuracy of the integrated 15-GEP/*PRAME* classifier over 15-GEP alone and clinical prognostic variables. Tumor diameter was found to be the only clinical variable to provide additional prognostic information. This prognostic classifier provides an advanced resource for risk-adjusted metastatic surveillance and adjuvant trial stratification in patients with UM.

ACCOMPANYING CONTENT

Appendix

Data Sharing Statement

Data Supplement

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INTRODUCTION

Uveal melanoma (UM) is the most common primary malignancy of the eye and has a strong propensity for metastasis.[1] Despite improvements in primary tumor treatment, there has been no survival increase,[2] due at least in part to early subclinical micrometastasis.[3,4] Tebentafusp recently became the first drug showing a survival benefit in UM patients with metastatic disease restricted to HLA:A02-01.[5-7] It is likely that such therapies will have even greater benefit in the adjuvant or early metastatic setting when disease burden is low.[8-12] As such, there is a critical need for validated, standardized, and highly accurate prognostic testing to tailor surveillance to metastatic risk and to identify high-risk patients for enrollment in adjuvant clinical trials.[1] Such testing can also reduce surveillance in low-risk patients,

CONTEXT

Key Objective

The key objective of this study was to prospectively evaluate the 15-gene expression profile (15-GEP) and *PRAME* RNA expression status as an integrated prognostic tool in 1,577 patients with uveal melanoma (UM) enrolled in the Collaborative Ocular Oncology Study Number 2 (COOG2).

Knowledge Generated

PRAME status significantly enhanced the prognostic accuracy of 15-GEP in patients with UM. Tumor diameter made a small additional contribution to prognostic accuracy.

Relevance (G.K. Schwartz)

The addition of *PRAME* RNA expression to the 15-GEP offers an enhanced diagnostic tool to predict clinical outcome in patients with primary UM.*

*Relevance section written by JCO Associate Editor Gary K. Schwartz, MD, FASCO.

which may decrease test-related anxiety and cost to the health care system.

While there are numerous clinical, histopathologic, and molecular factors that have been proposed as prognostic variables in UM,[1] gene expression profile (GEP) has been shown to provide prognostic accuracy superior to other factors.[13-17] Accordingly, a standardized 15-GEP was developed using targeted cDNA amplification, microfluidics qPCR technology, and machine learning to classify primary UMs from a biopsy sample.[18] The 15-GEP has undergone analytic optimization for use on fine needle and formalin-fixed samples.[19] The 15-GEP was prospectively validated by the Collaborative Ocular Oncology Group Study Number 1 (COOG1) [17] and numerous subsequent studies.[19-23] It is included in National Comprehensive Cancer Network guidelines and is widely used in routine clinical practice.[1,20]

In subsequent studies, RNA expression of the cancer-testis antigen Preferentially Expressed Antigen in Melanoma (*PRAME*) was found to provide additional prognostic information independent of 15-GEP, being associated with increased metastatic risk in both class 1 and class 2 tumors.[24-27] Although the initial COOG1 study did not identify any clinical factors that provided prognostic information independent of 15-GEP, subsequent retrospective studies have suggested that tumor diameter may enhance the accuracy of 15-GEP.[21,28-31] In this first report of the Collaborative Ocular Oncology Group Number 2 (COOG2), to our knowledge, the largest prospective biomarker study performed to date in UM, we evaluate the prognostic value of 15-GEP, *PRAME*, and clinical prognostic factors in developing an integrated prognostic classifier suitable for routine clinical practice and clinical trial stratification.

MATERIALS AND METHODS

Patient Enrollment

Between January 2017 and April 2020, COOG2 prospectively enrolled 1,687 patients with UM involving the choroid, ciliary body and/or iris across 26 ocular oncology centers in the United States and Canada (Appendix Table A1, online only). Informed consent was obtained from each patient. Primary treatment was performed according to the standard at each center. Federal Wide Assurance from the Office of Human Research Protections and Institutional Review Board (IRB) or Ethics Committee approval was obtained in accordance with policies at each center. Noninclusion criteria included patient age <18 years, diagnosis of a uveal tumor other than UM (eg, metastatic cancer), prior radiotherapy, and patient withdrawal from the study. Fifty-one patients who met entry criteria were not included because their tumor sample was inadequate to allow reverse transcription of RNA and/or amplification of cDNA for GEP and *PRAME* testing. Prior photodynamic therapy or transpupillary thermotherapy was allowed if there was evidence of tumor regrowth. No participants were excluded on the basis of sex, ethnicity, or race. A data lock was performed on March 9, 2023, and patients with primary iris melanoma (n = 101) or metastatic UM at baseline (n = 9) were excluded, resulting in 1,577 patients included in this report. Given the published distribution and metastatic rates of class 1A, class 1B, class 2, *PRAME*-, and *PRAME*+ in UM,[24,25,27,32] a sample size of approximately 1,500 was estimated to yield sufficient patients with discordant *PRAME* versus 1A/1B results to allow detection of a relative risk of >3.0 in 5-year metastatic rates between *PRAME*+/1A compared with *PRAME*-/1B with >80% power.

Tumor Sample Analysis

All patients underwent testing of their primary UM sample with DecisionDx-UM (class 1A, class 1B or class 2) and DecisionDx-*PRAME* (negative or positive), as previously described.[18,24] This testing was performed by the Castle Biosciences College of American Pathologists–accredited, Clinical Laboratory Improvement Amendments–certified laboratory, as per standard of care.[19,33] For 369 (22%) of the patients, molecular analysis was completed on residual clinical samples collected between May 2014 and December 2016, before enrollment in COOG2, after which they continued to be monitored prospectively. The median time from sample collection to COOG2 initiation was 11 months (0.1 to 32 months).

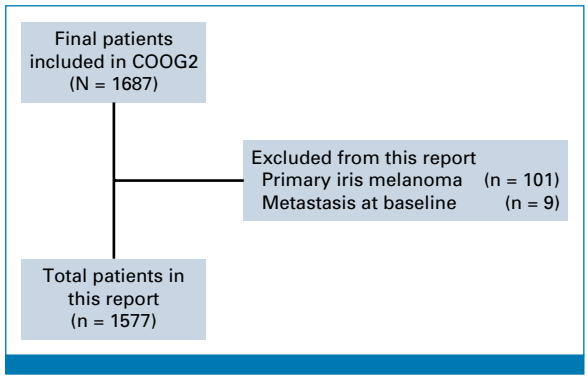

FIG 1. Overview of Collaborative Ocular Oncology Group Study 2 (COOG2) and patients included in this report.

Data Management

REDCap, a secure HIPAA compliant application,[35] was used for electronic data management. Each center was given restricted access by key study personnel and were issued unique research ID numbers to assign study patients. Deidentified clinical data were entered by each center at baseline and subsequent follow-up intervals. Baseline data included date of enrollment, date and method of biopsy, cytology result (if available), date and method of primary tumor treatment, patient age at study entry, sex, self-reported race and ethnicity, iris color (blue/green, intermediate, or brown), tumor diameter, tumor thickness, ciliary body involvement, and metastatic status. The American Joint Committee on Cancer (AJCC) 8th edition[36] was used for tumor staging. Follow-up data included local tumor recurrence (tumor regrowth in the eye or orbit after radiotherapy or in the orbit after enucleation), metastatic status, date and location of initial metastasis, systemic status at last follow-up, and date and cause of death. Molecular test results were entered into REDCap by Castle Biosciences, which was masked to other REDCap data. Each center was masked to data entered by other centers and by Castle Biosciences. Only the coordinating center and COOG2 Data Committee had access to all data (Data Supplement, online only).

Baseline and follow-up ophthalmic visits were performed as per standard of care at each center but typically included a comprehensive ophthalmic examination, fundus photography, optical coherence tomography, and ultrasonography performed at least every 3-4 months for the first year after treatment, every 4-6 months for the second year, and every 6-12 months thereafter. Baseline systemic imaging was typically performed with computed tomography (CT) of the chest, abdomen, and pelvis. Subsequent systemic surveillance typically included imaging of the liver with CT, magnetic resonance imaging, or ultrasound at least twice a year, along with chest CT or chest x-ray at least once a year.

Statistical Analysis

Statistical analysis was performed using SAS 9.4 (SAS Institute, Cary, NC). The Chi-square test was used to compare categorical variables, and Wilcoxon signed-rank test was used for comparing continuous variables. All statistical tests were two-sided, and statistical significance was defined as $P < .05$. Differences in metastasis-free survival (MFS, time from primary tumor treatment to first radiographic detection of metastatic disease) and melanoma-specific survival (MSS, time from primary tumor treatment to death due to melanoma metastasis) associated with a given factor were evaluated using Kaplan-Meier (KM) survival curves and the log-rank test. Cox regression was used to assess the contribution of multiple factors influencing metastatic risk. Univariable and multivariable Cox models were constructed to assess the impact of variables both separately and in combination. Model performance was evaluated by the concordance statistic (C-statistic), which measures the ability of a model to accurately rank individuals by their predicted risk.

RESULTS

Patient Enrollment

Of 1,687 total patients enrolled in COOG2, 101 patients with primary iris melanoma and nine patients with metastatic UM at baseline were excluded, resulting in 1,577 patients included in this report (Fig 1). Baseline demographic and clinical information are summarized in Table 1. The median follow-up was 43.6 months. Metastatic disease was detected in 269 (17.1%) patients, and the median time to metastasis among patients with an event was 22.6 months (range, 0.1-92.9). Local tumor recurrence was identified in 68 (4.3%) patients with a median time of 23.9 months (range, 3.5-82.2 months) after biopsy/primary enucleation, with 30 (44.1%) of these subsequently developing metastatic disease. The study included 530 (33.6%) AJCC T1 tumors, compared with 81 (17.6%) in COOG1[17] and 0 (0%) in The Cancer Genome Atlas (TCGA) cohort[37] (Appendix Table A2). Conversely, 161 (10.2%) of patients in this study were treated with primary enucleation, compared with 92 (20.0%) in COOG1 and 77 (96.3%) in TCGA. The median follow-up time was 43.6 months for COOG2 compared with 17.4 months for COOG1 and 26.2 months for TCGA.

TABLE 1. Summary of COOG2 Cohort of 1,577 Patients With Posterior Uveal Melanoma

Characteristic	Value
Age at study entry, years	
Median (range)	64 (18-99)
Mean (SD)	62 (13.5)
Male sex, No. (%)	809 (51.3)
Ethnicity, No. (%)	
Non-Hispanic or Latino	1,494 (94.7)
Hispanic or Latino	55 (3.5)
Not specified	28 (1.8)
Race, No. (%)	
White	1,518 (96.3)
Black	12 (0.8)
Asian	7 (0.4)
Native American/Alaskan	3 (0.2)
Native Hawaiian/Pacific Islander	2 (0.1)
More than one race	5 (0.3)
Not specified	30 (1.9)
Eye, right, No. (%)	801 (50.8)
Iris color, No. (%)	
Blue/green	613 (38.9)
Brown	238 (15.1)
Intermediate	127 (8.1)
Not specified	599 (38.0)
Ciliary body involvement, No. (%)	250 (15.9)
Melanocytosis, No. (%)	75 (4.7)
Cell type, No. (%)	
Spindle	252 (16.0)
Mixed	234 (14.8)
Epithelioid	76 (4.8)
Other	309 (19.6)
Not performed or not specified	706 (44.8)
Tumor diameter, mm	
Median (range)	12 (2-32)
Mean (SD)	12.1 (±4.0)
Tumor thickness, mm	
Median (range)	4.1 (0.5-18)
Mean (SD)	5.1 (±3.1)
AJCC T-category, No. (%)	
T1	530 (33.6)
T2	564 (35.8)
T3	360 (22.8)
T4	123 (7.8)
Primary tumor treatment	
^{125}I plaque brachytherapy	1,265 (80.2)
Enucleation	161 (10.2)
Proton beam radiotherapy	115 (7.3)
External beam radiotherapy	11 (0.7)
Laser therapy	8 (0.5)
Other or unspecified	17 (1.1)
Type of biopsy, No. (%)	
Transvitreal FNAB	699 (44.3)
Transscleral FNAB	638 (40.5)
Transcameral FNAB	4 (0.3)
Vitrectomy biopsy	67 (4.3)
Incisional biopsy	6 (0.4)
Not specified	163 (10.3)
15-GEP test results, No. (%)	
Class 1	1,082 (68.6)
Class 1A	693 (43.9)
Class 1B	389 (24.7)
Class 2	495 (31.4)
PRAME, No. (%)	
Negative (−)	1,106 (70.1)
Positive (+)	471 (29.9)
Distant metastasis, No. (%)	269 (17.1)
Time to metastasis/last follow-up, months	
Median (range)	43.6 (0-104.4)
Mean (SD)	45.2 (±22.4)
Local recurrence, No. (% of all patients)	68 (4.3)
Primary tumor treatment, No. (% of 68 local recurrences)	
^{125}I plaque brachytherapy	48 (70.6)
Proton beam radiotherapy	12 (17.6)
Enucleation	5 (7.4)
Other	3 (4.4)
Time to local recurrence, months	
Median (range)	23.9 (3.5-82.2)
Mean (SD)	28.4 (19.6)

Abbreviations: 15-GEP, 15-gene expression profile test; AJCC, American Joint Committee on Cancer; FNAB, fine-needle aspiration biopsy; SD, standard deviation.

15-GEP and *PRAME*

Molecular test results are summarized in Table 1. The 15-GEP result was class 1A in 693 patients (43.9%), class 1B in 389 (24.7%), and class 2 in 495 (31.4%). KM survival analysis and the log-rank test demonstrated no significant difference in MFS or MSS between class 1A and 1B cases (Appendix Fig A1). Therefore, these groups were combined and reported as one category (class 1) for all subsequent analyses. The development of metastatic disease was noted in 64 (5.9%) of 1,082 patients with a class 1 tumor versus 205 (41.4%) of 495 patients with a class 2 tumor ($P < .001$). Class 1 tumors were associated with superior actuarial survival compared with class 2 tumors, with 5-year MFS of 92.3% (95% CI, 90.2 to 94.4) for class 1 versus 52.1% (95% CI, 47.0 to 57.8) for class 2, and 5-year MSS of 97.4% (95% CI, 96.2 to 98.7) for class 1 versus 68.8% (95% CI, 63.6 to 74.5) for class 2 (Figs 2A and 2B; Appendix Table A3).

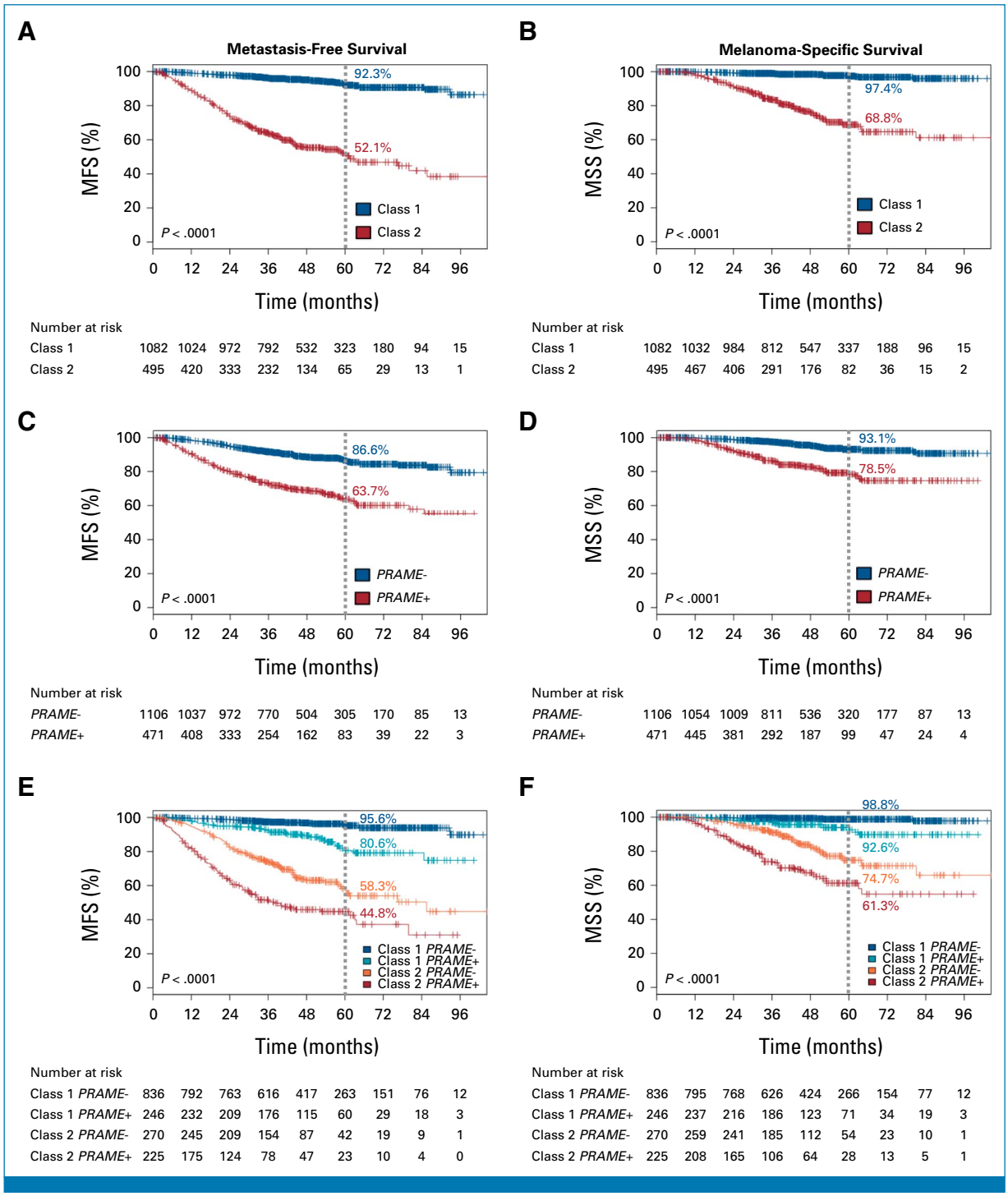

FIG 2. Metastasis-free survival (left panels) and melanoma-specific survival (right panels) stratified by (A) and (B) 15-gene expression profile (15-GEP) class, (C) and (D) *PRAME* RNA expression status, and (E) and (F) integrated 15-GEP/*PRAME* subclassifications. Five-year point estimates are indicated by the gray dotted line. *P* value, log-rank test, and number of patients at risk at each 12-month interval are shown. Censoring events are marked with a vertical hash across the survival curve (See Appendix Table A3 for complete 3-year and 5-year outcomes with 95% CIs, along with total number of metastasis/death events on study). MFS, metastasis-free survival; MSS, melanoma-specific survival.

PRAME status was negative (*PRAME*−) in 1,106 patients (70.1%) and positive (*PRAME*+) in 471 (29.9%). *PRAME*− status was associated with better survival, with a 5-year MFS of 86.6% (95% CI, 84.2 to 89.1), compared with 63.7% (95% CI, 58.5 to 69.3) for *PRAME*+ tumors. Similarly, 5-year MSS was 93.1% (95% CI, 91.2 to 95.0) for *PRAME*− tumors compared with 78.5% (95% CI, 73.9 to 83.3) for *PRAME*+ tumors (Figs 2C and 2D; Appendix Table A3). Furthermore, *PRAME*+ status was associated with worse survival in both class 1 and class 2 tumors, with 5-year MFS of 95.6% (95% CI, 93.9 to 97.4) in class 1/*PRAME*−, 80.6% (95% CI, 73.9 to 87.9) in class 1/*PRAME*+, 58.3% (95% CI, 51.1 to 66.4) in class 2/*PRAME*−, and 44.8% (95% CI, 37.9 to 52.8) in class 2/*PRAME*+ (Figs 2E and 2F; Appendix Table A3). The median time to metastasis for patients with a metastatic event was 31.3 months for class 1/*PRAME*−, 34.9 months for class 1/*PRAME*+, 24.7 months for class 2/*PRAME*−, and 16.5 months for class 2/*PRAME*+ (Table 2). Overall, class 2/*PRAME*+ patients had a greatly increased risk of metastasis compared with class 1/*PRAME*− patients (hazard ratio [HR], 22.06 [95% CI, 14.86 to 32.76]; $P < .001$; Appendix Table A4).

TABLE 2. Summary of Enrolled Patients by 15-GEP/*PRAME* Status

Characteristic	Class 1 *PRAME* (−)	Class 1 *PRAME* (+)	Class 2 *PRAME* (−)	Class 2 *PRAME* (+)
No. (% of 1,577 in total cohort)	836 (53.0)	246 (15.6)	270 (17.1)	225 (14.3)
Age at study entry, years				
Median (range)	62 (19-94)	61 (18-99)	66 (32-98)	66 (22-95)
Mean (SD)	61 (13.7)	60 (14.1)	65 (12.2)	66 (12.0)
Male sex, No. (%)	437 (52.3)	128 (52.0)	136 (50.4)	108 (48.0)
Eye, right, No. (%)	434 (51.9)	117 (47.6)	135 (50.0)	115 (51.1)
Ciliary body involvement, No. (%)	83 (9.9)	37 (15.0)	55 (20.4)	75 (33.3)
Tumor diameter, mm				
Median (range)	10.2 (2-26)	13 (4-32)	13.6 (4-28.7)	15 (5-28.9)
Mean (SD)	10.5 (3.5)	13.3 (3.9)	13.4 (3.6)	15.0 (3.8)
Tumor thickness, mm				
Median (range)	3.4 (0.5-17)	4.6 (1-14.4)	5.3 (1-16)	6.1 (1.6-18)
Mean (SD)	4.2 (2.5)	5.4 (3.0)	6.1 (3.4)	6.9 (3.5)
AJCC T-category, No. (%)				
T1	390 (46.7)	64 (26.0)	51 (18.9)	25 (11.1)
T2	304 (36.4)	89 (36.2)	100 (37.0)	71 (31.6)
T3	124 (14.8)	66 (26.8)	93 (34.4)	77 (34.2)
T4	18 (2.2)	27 (11.0)	26 (9.6)	52 (23.1)
Primary enucleation, No. (%)	47 (5.6)	28 (11.4)	30 (11.1)	56 (24.9)
15-GEP 1A/B subclass, No. (%)				
1A	545 (65.2)	148 (60.2)	NA	NA
1B	291 (34.8)	98 (39.8)	NA	NA
Time to metastasis or last follow-up, median, months	47.9	46.1	39.4	26.9
Time to last follow-up, months[a]				
Median (range)	49.2 (0-103.2)	48.5 (0.5-100.3)	45.9 (1.1-104.4)	44.0 (1.1-95.1)
Mean (SD)	51.6 (21.3)	50.2 (20.5)	48.1 (18.7)	44.7 (20.3)
Patients with metastatic event on study, No. (%)	32 (3.8)	32 (13.0)	93 (34.4)	112 (49.8)
Time to metastasis, months[b]				
Median (range)	31.3 (6.3-92.9)	34.9 (3.6-84.7)	24.7 (2.2-85.6)	16.5 (0.1-79.9)
Mean (SD)	35.3 (20.7)	34.3 (20.6)	28.5 (16.5)	19.5 (14.4)
Local recurrence, No. (%)	23 (2.8)	12 (4.9)	18 (6.7)	15 (6.7)
Time to local recurrence, months[b]				
Median (range)	18.7 (3.5-82.2)	28.9 (9.2-82.0)	21.1 (3.5-62.8)	27.4 (6.8-58.6)
Mean (SD)	26.9 (24.0)	32.7 (19.8)	26.6 (17.2)	29.3 (15.4)

Abbreviations: 15-GEP, 15-gene expression profile; AJCC, American Joint Committee on Cancer; NA, not applicable; NS, not statistically significant; SD, standard deviation.

[a]Excludes patients with metastatic events or death from any cause.

[b]Includes only patients with an indicated event recorded.

TABLE 3. Univariate and Multivariate Cox Regression Analyses of Metastasis-Free Survival

Risk Factor	Univariate HR (95% CI)	*P*	Multivariate HR (95% CI)	*P*
15-GEP class 2	9.77 (7.36 to 12.95)	<.001	5.95 (4.43 to 7.99)	<.001
PRAME (+)	3.31 (2.60 to 4.20)	<.001	1.82 (1.42 to 2.33)	<.001
Ciliary body involvement	2.88 (2.22 to 3.74)	<.001		
Tumor diameter	1.25 (1.21 to 1.28)	<.001	1.13 (1.09 to 1.17)	<.001
Tumor thickness	1.24 (1.20 to 1.27)	<.001	1.07 (1.03 to 1.11)	<.01
Age, years	1.02 (1.01 to 1.03)	<.001		

NOTE. Binary variables: 15-GEP, class 2 (*v* class 1), *PRAME*+ (*v PRAME*–), and ciliary body involvement, yes (*v* no). Continuous variables: tumor diameter, tumor thickness, and patient age.
Abbreviations: 15-GEP, 15-gene expression profile; HR, hazard ratio; NS, not statistically significant.

15-GEP/*PRAME* Classifier Versus AJCC Staging

To determine the performance of the integrated 15-GEP/*PRAME* classifier across different tumor size categories, survival outcomes were assessed on the basis of the AJCC T-category.[38] The integrated 15-GEP/*PRAME* system robustly stratified patients by MFS in each AJCC T-category, and it revealed prognostic deficiencies in the AJCC system, which both overestimated and underestimated metastatic risk. While the 5-year MFS for T1 tumors overall was excellent at 94.4% (95% CI, 92.1 to 96.8), 76/530 (14.3%) T1 tumors were classified as class 2/*PRAME*– or class 2/*PRAME*+, and the 5-year MFS for these subgroups was only 77.6% and 69.5%, respectively. On the other hand, the 5-year MFS for T4 tumors overall was poor at 36.2% (95% CI, 26.2 to 50.0). However, 18/123 (14.6%) of patients with a T4 tumor were classified as class 1/*PRAME*–, of which only one patient went on to develop metastasis (Appendix Fig A2; Appendix Table A3).

Prognostic Model Optimization

Cox regression was performed to identify clinical factors that may enhance the 15-GEP/*PRAME* model by providing independent prognostic information. Univariate analysis revealed the following variables to be significantly associated with MFS: 15-GEP class 2 (HR, 9.77, $P < .001$), *PRAME*+ status (HR, 3.31, $P < .001$), ciliary body involvement (HR, 2.88, $P < .001$), increased tumor diameter (HR, 1.25, $P < .001$), increased tumor thickness (HR, 1.24, $P < .001$), and increased patient age (HR, 1.02, $P < .001$; Table 3). Sex and iris color were not significantly associated with MFS.

Multivariate models were constructed on the basis of the stepwise addition of each variable according to their relative importance in the univariable analysis. When 15-GEP and *PRAME* were added to the model, age (HR, 1.01, $P = .114$) and ciliary body involvement (HR, 1.11, $P = .46$) were no longer significant, whereas tumor diameter (HR, 1.13, $P < .001$) and tumor thickness (HR, 1.07, $P = .01$) remained significant, albeit with diminished hazard ratios. To assess the clinical value of tumor diameter and tumor thickness, we calculated the concordance statistic (C-statistic) when these variables are included in the model (Table 4). The 15-GEP performed well on its own (C-statistic = 0.77), and the addition of *PRAME* status provided a substantial improvement (C-statistic = 0.81). The addition of tumor diameter to the 15-GEP + *PRAME* model provided a further improvement (C-statistic = 0.85). However, the addition of tumor thickness did not further increase the C-statistic. We next evaluated the performance of a model incorporating the 15-GEP + *PRAME* model with AJCC T-category, which includes both tumor diameter and thickness. The predictive performance of the 15-GEP + *PRAME* model was improved by the addition of T-category to a lesser extent (C-statistic = 0.84) than tumor diameter alone (Table 4).

DISCUSSION

To our knowledge, COOG2 is the largest multicenter prospective biomarker study to date in UM, with longer follow-up and more representative, real-world distribution of tumor size, ciliary body involvement, and AJCC tumor stage than COOG1 or TCGA[17,37] and more similar to a large international database encompassing the full spectrum of UM[36] (Appendix Table A2). This may explain, at least in part, the more favorable outcomes in COOG2. Key findings include (1) prospective validation of 15-GEP and *PRAME* as independent prognostic biomarkers in UM, (2) superiority of *PRAME* status over the 1A/1B system for class 1 tumors, (3) establishment of a new 4-group 15-GEP/*PRAME* system, and (4)

TABLE 4. Predictive Performance of Models With Stepwise Addition of Variables to the 15-GEP

Model	C-Statistic (95% CI)
15-GEP	0.77 (0.75 to 0.79)
15-GEP + *PRAME*	0.81 (0.79 to 0.83)
15-GEP + *PRAME* + diameter	0.85 (0.83 to 0.87)
15-GEP + *PRAME* + diameter + thickness	0.85 (0.83 to 0.87)
15-GEP + *PRAME* + AJCC T-category	0.84 (0.82 to 0.86)

Abbreviations: 15-GEP, 15-gene expression profile; AJCC, American Joint Committee on Cancer.

validation of tumor diameter as the only clinical factor that improves the accuracy of 15-GEP/*PRAME*.

The 1A/1B system was an early attempt to subdivide class 1 UM on the basis of prognosis into low (1A) versus intermediate (1B) metastatic risk using the differential expression of two genes in the 15-GEP (*CDH1* and *RAB31*).[19] While initial reports found this system to discriminate metastatic risk among class 1 UMs,[21] subsequent studies did not corroborate these findings.[26,32] A transcriptome-wide search identified *PRAME* as the most significant biomarker for identifying class 1 tumors with increased metastatic risk.[25] A subsequent study showed that *PRAME* may also identify class 2 tumors with shorter time to metastasis.[24] The method for determining *PRAME* positivity was analytically validated and incorporated into the workflow of the 15-GEP platform,[39] such that both are assessed from a single sample. In this study, there was no significant difference in outcome between class 1A and class 1B, whereas *PRAME* status demonstrated significant predictive value independent of 15-GEP. Thus, we propose that *PRAME* should supersede the 1A/1B system and be integrated with 15-GEP to subdivide UMs into four prognostically significant subgroups: class 1/*PRAME*−, class 1/*PRAME*+, class 2/*PRAME*−, and class 2/*PRAME*+. Interestingly, *PRAME* was recently shown to induce aneuploidy in UM,[40] suggesting that it is not simply a biomarker but likely a driver of metastasis. Although a so-called TCGA classification system has been proposed for UM,[41] TCGA performed a one-time multiomics analysis of 80 large, enucleated UMs, which was not adequate (nor intended) to form the basis for a prognostic test. Nevertheless, TCGA did confirm the fundamental 4-group molecular landscape of UM described by 15-GEP/*PRAME* (Fig 2E).[37]

In COOG1, no clinical features were found to provide prognostic information independent of the 15-GEP.[17] However, subsequent retrospective studies suggested that tumor diameter could potentially improve the prognostic accuracy of the 15-GEP.[21,28-31,42] Recently, it has been suggested that AJCC clinical staging needs to be combined with 15-GEP.[42] Here, we investigated whether any clinical variables, including those in the AJCC system, enhance the accuracy of the 15-GEP/*PRAME* classifier. As expected, patient age, ciliary body involvement, tumor diameter, and thickness were significantly associated with MFS by univariate analysis (Table 3). However, when 15-GEP and *PRAME* were added to a multivariate model, age and ciliary body involvement were no longer significant, and the hazard ratios for tumor diameter and thickness were reduced. Using the C-statistic to assess predictive accuracy, tumor diameter provided a slight improvement over 15-GEP + *PRAME*, whereas tumor thickness provided no further improvement (Table 4). Furthermore, AJCC T-category, which incorporates both tumor diameter and tumor thickness, improved the performance of the 15-GEP + *PRAME* to a lesser extent than tumor diameter alone, consistent with previous work.[43] Consequently, our findings support a parsimonious prognostic model that includes 15-GEP/*PRAME* plus tumor diameter, but not the AJCC T-category or other clinical variables. A method for incorporating tumor diameter into a clinically practical integrated model will be published separately.

Among the various methods that have been proposed for prognostication in UM, including chromosomal analysis, mutation profiling, and immunohistochemistry (IHC),[37,44] only 15-GEP/*PRAME* achieves the highest level of evidence (level I) in the National Comprehensive Cancer Network Tumor Marker Utility Grading System, which requires prospective validation.[45] Most of these alternative methods have only been evaluated in single institution retrospective studies using platforms that are not standardized across centers and that achieve no better than level III evidence. A direct prospective comparison showed the 15-GEP to be superior to monosomy 3,[17] which is the most important chromosomal prognostic marker. While mutations in *BAP1*, *SF3B1*, and *EIF1AX* are the most important prognostic mutations in UM,[46-48] they are not as accurate as 15-GEP for predicting metastasis.[49] In the case of *BAP1*, whose mutational inactivation is strongly associated with class 2 GEP,[46] mutations may include large deletions and other alterations that can be difficult to detect even with whole-exome sequencing.[50] IHC for *BAP1* and *PRAME* have been proposed as surrogates for 15-GEP/*PRAME*.[44] However, aside from the inferior sensitivity, specificity, dynamic range, and analytical precision of IHC compared with quantitative PCR,[45,51,52] IHC requires archival tissue from eyes that have been enucleated, which is performed in <20% of patients in modern ocular oncology practice.[53,54] The 15-GEP/*PRAME* is covered by many third-party payers in the United States and is available internationally, thereby providing a standardized platform that can be compared across centers worldwide. A multicenter prospective study is warranted to compare the analytical performance, prognostic accuracy, cost-effectiveness, and clinical utility of 15-GEP/*PRAME* to the best alternative methods to establish an international standard for adjuvant trial design.

In conclusion, this report provides prospective multicenter validation, the highest level of biomarker evidence,[45] for integrating 15-GEP with *PRAME* expression status into a 4-group prognostic classification system. This integrated prognostic tool is a uniquely valuable resource to establish standardized entry criteria for high-risk adjuvant clinical trials, and it provides a gold standard for evaluating other prognostic biomarkers.

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AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

15-Gene Expression Profile and *PRAME* as Integrated Prognostic Test for Uveal Melanoma: First Report of Collaborative Ocular Oncology Group Study No. 2 (COOG2.1)

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APPENDIX

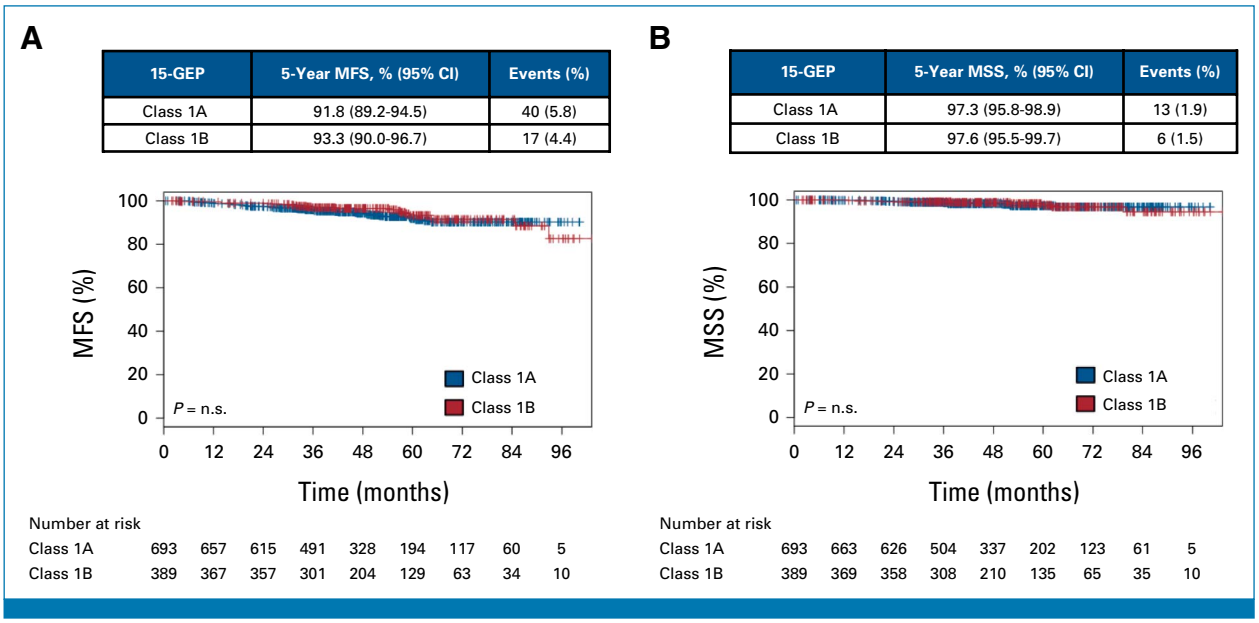
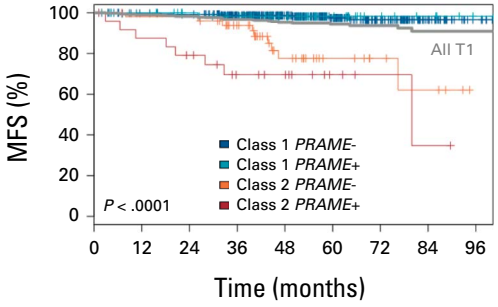


FIG A1. Kaplan-Meier survival curves for patients with class 1 15-gene expression profile results, separated by subclass 1A and 1B. (A) MSS and (B) MFS. Five-year point estimates and 95% CIs, along with the number of events (detection of distant metastasis or uveal melanoma-related death) are shown in the tables above each curve. *P* value, log-rank test. Number of patients at risk at each 12-month time point is shown below the plots. Censoring events are marked with a vertical hash across the survival curve. 15-GEP, 15-gene expression profile; MFS, metastasis-free survival; MSS, melanoma-specific survival; n.s., not statistically significant.

A

T1, n = 530	5-Year MFS, % (95% CI)	Events (%)
Class 1 *PRAME*-	97.5 (95.5-99.5)	7 (1.8)
Class 1 *PRAME*+	98.2 (94.9-100)	1 (1.6)
Class 2 *PRAME*-	77.6 (64.5-93.3)	8 (15.7)
Class 2 *PRAME*+	69.5 (52.9-91.4)	7 (28.0)

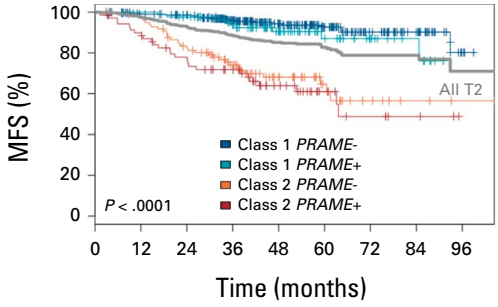


Number at risk

Class 1 *PRAME*-	390	376	369	310	219	136	79	33	7
Class 1 *PRAME*+	64	63	57	49	32	17	8	5	1
Class 2 *PRAME*-	51	49	49	38	20	10	6	4	0
Class 2 *PRAME*+	25	21	18	12	10	4	2	1	0

B

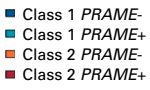
T2, n = 564	5-Year MFS, % (95% CI)	Events (%)
Class 1 *PRAME*-	92.7 (89.1-96.5)	16 (5.3)
Class 1 *PRAME*+	87.0 (78.2-96.9)	8 (9.0)
Class 2 *PRAME*-	64.7 (54.2-77.4)	29 (29.0)
Class 2 *PRAME*+	61.1 (49.6-75.3)	24 (33.8)



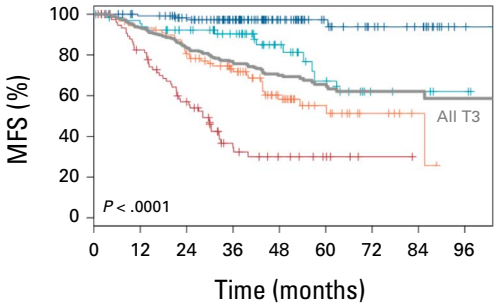
Number at risk

Class 1 *PRAME*-	304	287	276	217	145	93	53	29	3
Class 1 *PRAME*+	89	85	81	70	48	25	15	8	0
Class 2 *PRAME*-	100	94	77	57	34	17	7	3	1
Class 2 *PRAME*+	71	62	51	41	25	12	7	3	0

C



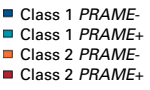
T3, n = 360	5-Year MFS, % (95% CI)	Events (%)
Class 1 *PRAME*-	97.2 (94.2-100)	3 (2.4)
Class 1 *PRAME*+	67.1 (51.7-87.2)	12 (18.2)
Class 2 *PRAME*-	55.2 (44.1-69.1)	33 (35.5)
Class 2 *PRAME*+	30.0 (20.2-44.6)	46 (59.7)



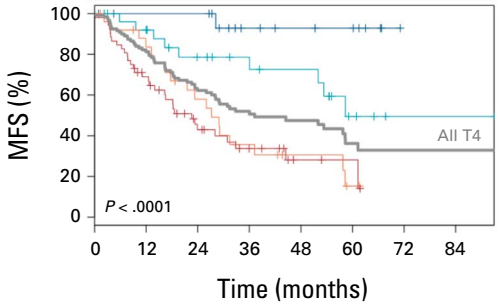
Number at risk

Class 1 *PRAME*-	124	113	102	80	46	28	19	14	2
Class 1 *PRAME*+	66	61	55	44	24	14	5	4	2
Class 2 *PRAME*-	93	82	70	52	29	14	6	2	0
Class 2 *PRAME*+	77	60	39	16	9	5	1	0	0

D



T4, n = 123	5-Year MFS, % (95% CI)	Events (%)
Class 1 *PRAME*-	92.9 (80.3-100)	1 (5.6)
Class 1 *PRAME*+	49.5 (29.3-83.6)	9 (33.3)
Class 2 *PRAME*-	15.3 (4.78-49.0)	18 (69.2)
Class 2 *PRAME*+	28.1 (16.0-49.4)	31 (59.6)



Number at risk

Class 1 *PRAME*-	18	16	16	9	7	6	0	0
Class 1 *PRAME*+	27	23	16	13	11	4	1	1
Class 2 *PRAME*-	26	20	13	7	4	1	0	0
Class 2 *PRAME*+	52	32	16	9	3	2	0	0

FIG A2. Five-year metastasis-free survival by AJCC 8th edition T stage, subclassified by GEP/*PRAME* groups. (A) T1, (B) T2, (C) T3, and (D) T4. *P* value, log-rank test. Number of patients at risk at each 12-month time point is shown below the plots. Censoring events are marked with a vertical hash across the survival curve. 15-GEP, 15-gene expression profile; AJCC, American Joint Committee on Cancer; MFS, metastasis-free survival.

TABLE A1. COOG2 Participating Sites

COOG2 Study Sites
Associated Retinal Consultants Michigan
Colorado Retina Associates
Duke University
Emory University
Hartford Hospital
Retina Consultants of Texas
Massachusetts Eye and Ear Infirmary
Oregon Health & Science University
Retina Associates of Arizona
Retina Consultants of Alabama
Retina Consultants of Sacramento
Retina Specialists of Michigan
Stanford University
Tennessee Retina
Texas Retina Associates
Tufts Medical Center
Tumori Foundation
University of Alberta
University of Cincinnati
University of Colorado
University of Miami
University of Michigan
University of Texas MD Anderson Cancer Center
University of Virginia
University of Wisconsin-Madison
Washington University in St Louis

TABLE A2. Comparison of Patient Cohorts Included in the Present Study (COOG2) Versus COOG1, AJCC, and TCGA

	Study (year)			
Variable	COOG2 (2024)	COOG1[17] (2012)	TCGA[37] (2017)	AJCC[36] (2015)
Patients, No.	1,577	459	80	3,217
Centers, No.	26	12	6	10
Study type	Prospective	Prospective	Retrospective	Retrospective
Age at study entry, years				
Median (range)	64 (18-99)	61 (NR)	62 (22-86)	NR
Mean	62	62	61	
Male sex, No. (%)	809 (51.3)	235 (51.2)	45 (56.3)	NR
Ciliary body involvement, No. (%)	250 (15.9)	139 (30.3)	16 (20.0)	459 (14.3)
Tumor diameter, mm				
Median (range)	12 (2-32)	12.7 (1.3-24.0)	16.8 (10-23.6)	11.8 (2-30)
Mean	12.1	12.8	16.2	11.7
Thickness, mm				
Median (range)	4.1 (0.5-18)	5.5 (1.0-17.5)	11 (4.4-16)	4.7 (1.1-23)
Mean	5.1	6.3	10.8	5.4
AJCC tumor stage, No. (%)	n = 1,577	n = 425	n = 80	n = 3,217
T1	530 (33.6)	81 (19.1)	0	1,115 (34.7)
T2	564 (35.8)	170 (40.0)	14 (17.5)	1,128 (35.0)
T3	360 (22.8)	140 (32.9)	32 (40.0)	789 (24.5)
T4	123 (7.8)	34 (8.0)	34 (42.5)	185 (5.8)
Primary enucleation, No. (%)	161 (10.2)	92 (20.0)	77 (96.3)	NR
15-GEP test results, No. (%)				
Class 1	1,082 (68.6)	276 (61.9)	NR	NR
Class 2	495 (31.4)	170 (38.1)		
Patients with metastatic event on study, No. (%)	269 (17.1)	47 (10.2)	26 (32.5)	325 (10.1)
Time to metastasis/last follow-up, months				
Median (range)	43.6 (0-104.4)	17.4	26.2 (0-85.4)	38.4 (1.0-151.3)
Mean	45.2	18.0	26.9	NR

Abbreviations: 15-GEP, 15-gene expression profile; AJCC, American Joint Committee on Cancer; NR, not reported; TCGA, The Cancer Genome Atlas.

TABLE A3. Summary of Survival Statistics Across Patient Subsets by 15-GEP Class, *PRAME*, Integrated 15-GEP/*PRAME*, and AJCC T-Stage in Patients With Posterior Uveal Melanoma

		MFS, % (95% CI)		Events, No.	MSS, % (95% CI)		Events, No.
Subgroup	No. (%)	3-Year	5-Year	5-Year	3-Year	5-Year	5-Year
All patients	1,577 (100)	86.1 (84.4 to 87.9)	79.9 (77.5 to 82.3)	253	94.1 (92.9 to 95.4)	88.9 (86.9 to 90.9)	126
15-GEP							
Class 1A	693 (43.9)	95.9 (94.4 to 97.5)	91.8 (89.2 to 94.5)	40	98.7 (97.8 to 99.6)	97.3 (95.8 to 98.9)	13
Class 1B	389 (24.7)	96.9 (95.0 to 98.7)	93.3 (90.0 to 96.7)	17	99.2 (98.3 to 100)	97.6 (95.5 to 99.7)	6
Class 1 (A + B)	1,082 (68.6)	96.2 (95.1 to 97.4)	92.3 (90.2 to 94.4)	57	98.9 (98.2 to 99.5)	97.4 (96.2 to 98.7)	19
Class 2	495 (31.4)	63.9 (59.6 to 68.5)	52.1 (47.0 to 57.8)	196	83.4 (80.0 to 87.0)	68.8 (63.6 to 74.5)	107
PRAME							
Negative (−)	1,106 (70.1)	91.7 (90.0 to 93.4)	86.6 (84.2 to 89.1)	115	97.4 (96.4 to 98.4)	93.1 (91.2 to 95.0)	51
Positive (+)	471 (29.9)	72.9 (68.8 to 77.3)	63.7 (58.5 to 69.3)	138	86.2 (82.9 to 89.6)	78.5 (73.9 to 83.3)	75
15-GEP/*PRAME*							
Class 1/(−)	836 (53.0)	97.4 (96.2 to 98.5)	95.6 (93.9 to 97.4)	27	99.5 (99.0 to 100)	98.8 (97.9 to 99.7)	7
Class 1/(+)	246 (15.6)	92.4 (89.0 to 96.0)	80.6 (73.9 to 87.9)	30	96.8 (94.5 to 99.2)	92.6 (88.3 to 97.1)	12
Class 2/(−)	270 (17.1)	74.2 (68.9 to 79.8)	58.3 (51.1 to 66.4)	88	90.9 (87.3 to 94.6)	74.7 (68.0 to 82.2)	44
Class 2/(+)	225 (14.3)	51.0 (44.5 to 58.5)	44.8 (37.9 to 52.8)	108	73.8 (67.8 to 80.4)	61.3 (53.5 to 70.2)	63
AJCC T-category							
T1	530 (33.6)	96.8 (95.3 to 98.4)	94.4 (92.1 to 96.8)	23	99.6 (99.1 to 100)	98.1 (96.5 to 99.7)	6
T2	564 (35.8)	88.6 (85.9 to 91.4)	82.8 (79.1 to 86.6)	77	96.3 (94.6 to 97.9)	90.5 (87.5 to 93.7)	36
T3	360 (22.8)	76.4 (71.9 to 81.3)	65.5 (59.4 to 72.2)	94	89.1 (85.7 to 92.7)	79.0 (73.4 to 85.1)	50
T4	123 (7.8)	52.0 (43.0 to 62.7)	36.2 (26.2 to 50.0)	59	70.7 (62.0 to 80.7)	60.7 (50.6 to 72.8)	34

Abbreviations: 15-GEP, 15-gene expression profile; AJCC T-stage; American Joint Committee on Cancer tumor stage (8th edition); MFS, metastasis-free survival; MSS, melanoma-specific survival.

TABLE A4. Cox Regression Analysis of Metastasis-Free Survival by 15-Gene Expression Profile/*PRAME* Status

Risk Factor	Univariate HR (95% CI)	*P*
Class 1/*PRAME*−	Reference	
Class 1/*PRAME*+	3.66 (2.24 to 5.98)	<.001
Class 2/*PRAME*−	11.33 (7.57 to 16.94)	<.001
Class 2/*PRAME*+	22.06 (14.86 to 32.76)	<.001

Abbreviation: HR, hazard ratio.