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Gene Expression Profiling and *PRAME* Status Versus Tumor-Node-Metastasis Staging for Prognostication in Uveal Melanoma

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Abstract

Purpose: To compare the prognostic accuracy of gene expression profiling (GEP) combined with *PRAME* status versus the clinical Tumor-Node-Metastasis (TNM) staging in patients with uveal melanoma (UM).

Design: Retrospective cohort study.

Methods: The study included 240 consecutive patients with UM. Tumors were assessed for GEP status (Class 1 or Class 2) using a validated 15-gene assay, and *FRAME* expression status using quantitative PCR. TNM staging was according to the American Joint Committee on Cancer (AJCC) 8th edition. Statistical analysis included univariate and multivariate Cox proportional hazard models. Metastasis was the primary endpoint.

Results: GEP was Class 1 in 128 (53.3%) cases, and Class 2 in 112 (46.7%) cases. *PRAME* status was negative in 157 (65.4%) cases and positive in 83 (34.6%) cases. TNM was stage I in 26 (10.8%) cases, IIA in 67 (27.9%) cases, IIB in 50 (20.8%) cases, IIIA in 59 (24.6%) cases and IIIB in 38 (15.8%) cases. Metastatic disease was detected in 59 (24.6%) cases after median follow-up of 29 months (mean 42 months; range 1–195 months). Variables associated with metastasis included (in order of decreasing significance): GEP class (P=1.5 × 10^{-8}), largest basal tumor diameter (P=2.5 × 10^{-6}), *PRAME* status (P=2.6 × 10^{-6}), and TNM stage (P=3.7 × 10^{-6}). The prognostic accuracy of an optimized 3-category GEP/*PRAME* model (P = 8.6 × 10^{-14}) was superior to an optimized TNM model (P = 1.3 × 10^{-5}).

Conclusions: In UM, molecular prognostic testing using GEP and *FRAME* provides prognostic accuracy that is superior to TNM staging.

INTRODUCTION

Uveal melanoma (UM) is the most common primary malignancy of the eye and leads to fatal metastasis in up to half of patients.[1] Despite ongoing improvements in the diagnosis and

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management of UM, survival rates have not improved as a result of micrometastasis occurring prior to treatment of the primary tumor.[2,3] Consequently, preemptive treatment of micrometastatic disease in the adjuvant setting may be required to improve the survival rate in UM. Indeed, there are an increasing number of clinical trials designed to evaluate adjuvant therapy in patients with high risk UM.[4,5] However, in order to utilize adjuvant therapy most effectively, an accurate method is needed to distinguish high risk patients who may benefit from adjuvant therapy from low risk patients who do not require such therapy.

The American Joint Committee on Cancer (AJCC) Tumor-Node-Metastasis (TNM) staging system has been used for a variety of cancers to stratify patients according to metastatic risk.[6] The TNM system divides solid tumor types into 4 stages based on the assumption that cancer progresses temporally from primary tumor to local invasion, regional lymphatic extension, and distant metastasis.[7] However, anatomic staging systems such as the TNM are now being re-examined in light of evolving knowledge, such as new prognostic cancer biomarkers.[7–9] Further, there is growing recognition that many cancers do not progress in the stepwise manner stipulated by the TNM formula, such as the lack of lymph node dissemination (the “N” component of the TNM) in UM. Additionally, the inherent complexity of the TNM methodology limits its precision, reproducibility, and ease of use in the clinical setting.[10,11]

As an alternative, molecular prognostic testing based on gene expression profiling (GEP) has been shown to yield superior prognostic accuracy compared to clinical, histopathologic and chromosomal features.[12–15] An optimized GEP test for routine clinical use has been developed using a 15-gene array on a microfluidics quantitative PCR platform, allowing accurate analysis of very small needle biopsy samples.[12] The test uses a machine learning algorithm and an annotated training set to assign tumor samples to Class 1 (low metastatic risk) versus Class 2 (high risk),[16] and it is the only such test for UM to be validated in a prospective, multicenter study.[12] Further, Class 1 tumors have been shown to harbor mutations in the translation elongation factor EIF1AX and the splicing factor SF3B1, whereas Class 2 tumors are strongly associated with mutations in the tumor suppressor gene BAP1.[17–20] More recently, the cancer-testis antigen *PRAME* (Preferentially Expressed Antigen in Melanoma) was found to represent an independent biomarker providing an additional layer of prognostic precision to the Class 1/Class 2 GEP system.[21–23] The presence of *PRAME* mRNA, which can be assessed from the sample biopsy sample as the GEP, is associated with increased metastatic risk in both Class 1 and Class 2 UMs, although the optimal use of *FRAME* status as a complement to the GEP has not been established.

In this study, we hypothesized that the prognostic accuracy of the *GEP/PRAME* molecular prognostic system is non-inferior to the AJCC 8th edition TNM staging system for UM. To test this hypothesis, we compared GEP and *FRAME* to the TNM clinical staging system in 240 patients with UM treated by a single surgeon. Further, we optimized a method for combining GEP and *PRAME* into a simple 3-category prognostic system.

METHODS

Clinical Data Collection

This retrospective cohort study was approved by the Institutional Review Board of the University of Miami School of Medicine. Patient information was accessed with proper informed consent and in accordance with the Health Insurance Portability and Accountability Act (HIPAA). The study included 240 patients with primary UMs arising from the choroid and/or ciliary body from the ocular oncology practice of JWH. The ocular pathology laboratory routinely provided cytologic verification of fine needle biopsy samples in patients treated with plaque radiotherapy, and by histopathologic analysis in those treated with enucleation. Patients with primary iris melanomas and those who presented with metastasis were excluded. Collected data included age at diagnosis, sex, largest basal diameter (LBD), tumor thickness, ciliary body involvement, extraocular extension, node status, primary treatment modality, first detection of metastasis, date and cause of death, and date of last follow up. LBD was measured using ultrasonography and indirect ophthalmoscopy, and the larger value of the two was used. Extraocular extension was assessed by ultrasonography in patients undergoing plaque radiotherapy and by histopathologic analysis in patients undergoing enucleation. Tumors were staged according to the AJCC 8th edition TNM staging manual.[24] Since most tumors were treated by I-125 plaque radiotherapy, where the histopathologic classification could not be applied, we used only the clinical classification for all patients. GEP class status (Class 1 versus Class 2) was determined with a prospectively validated 15-gene expression profile available as the DecisionDX-UM™ test.[25] The GEP test also sub-classifies Class 1 tumors into Class 1A (low metastatic risk) and Class 1B (intermediate risk).[26] However, this sub-classification was not used here since *PRAME* was used to sub-classify both Class 1 and Class 2 tumors, as described in the Results. RNA expression of *PRAME* was determined by quantitative PCR and categorized as *PRAME+* or *PRAME-,* as previously described.[21]

Statistical Analysis

Progression free survival (PFS) was measured as the time interval between diagnosis of UM and first detection of metastatic disease. In patients who did not develop metastasis, survival was censored at last follow up. Kaplan-Meier survival curves were used to analyze associations between prognostic factors and PFS. Differences in PFS among prognostic groups were analyzed for statistical significance using the log rank test. Prognostic variables were evaluated using Cox proportional hazards regression (using both simultaneous and stepwise methods). Statistical analysis was performed with MedCalc software (version 18; Ostend, Belgium).

RESULTS

Among 240 consecutive patients diagnosed with UM arising from the choroid and/or ciliary body (**Table 1**), primary treatment consisted of I-125 plaque radiotherapy in 165 (68.8%) cases, enucleation in 74 (30.8%) cases, and observation in 1 case (0.4%). Tumor sample was obtained by fine needle aspiration biopsy in 166 (69.2%) and by post-enucleation needle biopsy in 74 (30.8%). GEP was Class 1 in 128 (53.3%) cases and Class 2 in 112 (46.7%)

cases. *PRAME* was positive in 83 (34.6%) cases, including 38 (15.8%) Class 1 cases and 45 (18.8%) Class 2 cases. After a median follow up of 29 months (mean 42 months; range 1–195 months), metastasis was detected in 59 (24.6%) cases (**Supplemental Material at AJO.com**).

First, we used univariate Cox proportional hazards analysis to identify variables that were significantly associated with metastasis. For this initial step, we did not discretize continuous variables (age, LBD, thickness) in order to avoid arbitrary cutoff intervals. The factors demonstrating the strongest association with metastasis included: GEP class ($P = 1.5 \times 10^{-8}$), LBD ($P = 2.5 \times 10^{-6}$), *PRAME* status ($P = 2.6 \times 10^{-6}$), and TNM stage ($P = 3.7 \times 10^{-6}$) (**Table 2**). We then used multivariate Cox proportional hazards analysis to identify prognostic variables that provided significant independent prognostic information. These variables included: GEP ($P = 2.8 \times 10^{-6}$), *PRAME* ($P = 2.3 \times 10^{-4}$) and TNM ($P = 7.1 \times 10^{-4}$) (**Table 3**). We then analyzed the TNM clinical variables independently. This multivariate analysis revealed that LBD was the only TNM clinical variable that contributed prognostic information that was independent of GEP and *PRAME* (**Table 3**).

Next, we directly compared the ability of the TNM versus *GEP/PRAME* to stratify metastatic risk. To optimize the TNM, we used Kaplan-Meier analysis to perform pairwise comparisons between TNM stages (except stage IV) to identify and combine prognostically redundant categories (**Figure 1, Top left**). There was no significant difference between stages I versus IIA, I versus IIB, I versus IIIA, IIA versus IIB, IIB versus IIIA, or IIIA versus IIIB (**Figure 1, Middle left**). Consequently, stages IIA + IIB and stages IIIA + IIIB were combined to form three categories with modestly improved statistical significance ($P = 1.3 \times 10^{-5}$) (**Figure 1, Bottom left**). A similar procedure was performed for *GEP/PRAME* (**Figure 1, Top right**). The four *GEP/PRAME* categories provided statistically significant separation between survival curves ($P = 3.3 \times 10^{-13}$), that was superior to TNM survival curves ($P = 6.1 \times 10^{-5}$). All *GEP/PRAME* categories were non-redundant except Class 1^{PRAME+} and Class 2^{PRAME-}, which were then combined (**Figure 1, Middle right)** to form three *GEP/PRAME* categories: Class 1^{PRAME-}, Class 1^{PRAME+} or Class 2^{PRAME-}, and Class 2^{PRAME+} (**Figure 1, Bottom right**). The prognostic accuracy of the optimized *GEP/PRAME* categories ($P = 8.6 \times 10^{-14}$) was superior to the optimized TNM categories ($P = 1.3 \times 10^{-5}$). At every follow up point, the *GEP+PRAME* model maintained a greater separation between metastatic risk groups than did the TNM. For patients without metastasis after ≥5 years follow-up (n=45), a false positive “high risk” result would have been given in 16 (35.6%) patients using the TNM, compared to 3 (6.7%) using *GEP/PRAME.* To further investigate this tendency for increased false positives with the TNM, we performed a sub-analysis of tumors with LBD ≥12 mm (n=180). The TNM classified all of these larger tumors as being at increased metastatic risk **(Figure 2, Left)**, whereas *GEP/PRAME* identified 52/180 (29%) of these tumors as having low metastatic risk, only 3 (6%) of which gave rise to metastasis **(Figure 2, Right)**.

DISCUSSION

In this study, we confirmed that GEP, *PRAME* and TNM stage were each prognostic of metastasis in UM. Individually, GEP and *PRAME* both demonstrated prognostic accuracy

that was superior to the TNM staging system. Combining GEP and *PRAME* into a 3-category model further enhanced the prognostic accuracy of this molecular classification system.

There are several limitations to the use of the TNM system for prognostication in UM. First, the TNM assigns increased metastatic risk to all UMs with LBD > 12 mm, yet almost a third of these "large tumors" have low metastatic risk based on their molecular profile. This could result in false positive classification of UMs as having a high metastatic risk, leading to over-management of such patients. Second, several variables used in the clinical "T" stage provide redundant prognostic information. For example, increased tumor thickness is related to increased LBD and ciliary body involvement. This may explain why the prognostic accuracy of LBD alone was similar to the entire clinical TNM staging system in this study and others.[10] Third, the assessment of "T" variables, such as measuring tumor dimensions and determining ciliary body involvement, is not standardized and may vary from center to center.[27,28]. Fourth, some variables that were included in the TNM system for UM to conform to the standard TNM template are of little or no value in UM. For example, it is usually possible to assess extraocular tumor extension only in eyes treated by enucleation (which is performed in a minority of cases). Nodal involvement - the "N" component - does not occur in UM, thereby rendering this dimension of the TNM system irrelevant. Perhaps most importantly, the dependence of TNM staging on anatomic and morphologic features fails to accommodate new scientific understanding of cancer behavior, including powerful molecular prognostic biomarkers such as GEP and *PRAME*.[29]

We acknowledge several limitations of this study. First, this was a single center retrospective study, whereas we prefer prospective multi-center validation of prognostic markers.[12] Second, while the sample size was adequate for the intended purpose of this study, we would prefer a larger number of subjects to provide statistical power for detailed sub-analyses (such as the role of LBD in the *GEP/PRAME* prognostic system). Third, the median follow-up was relatively short (29 months), whereas we would prefer longer follow-up to minimize effects of lead time bias. However, a sub-analysis of patients with at least 5 years follow-up yielded results that were consistent with the overall findings. These limitations will each be addressed by the Collaborative Ocular Oncology Group Study Number 2 (COOG2), an ongoing prospective, multi-center clinical study funded by the National Cancer Institute. This study will also formally compare the new *GEP/PRAME* model described here to the existing Class 1A/1B/2 system, as well as mutations in BAP1, SF3B1 and EIF1AX and chromosomal copy number changes.

In conclusion, this study confirmed that both GEP and *PRAME* were individually superior to TNM in predicting a patient's risk of developing metastasis from UM. Moreover, GEP could be combined with *PRAME* to create an even more efficient and simplified 3-category molecular prognostic model. These findings continue to support the superior prognostic accuracy of these molecular biomarkers over anatomic features. Despite the deficiencies of the TNM system for personalized management of individual patients, it continues to be valuable for grouping patients with similar extent of disease into discrete "bins" for purposes of clinical, epidemiologic and health policy research.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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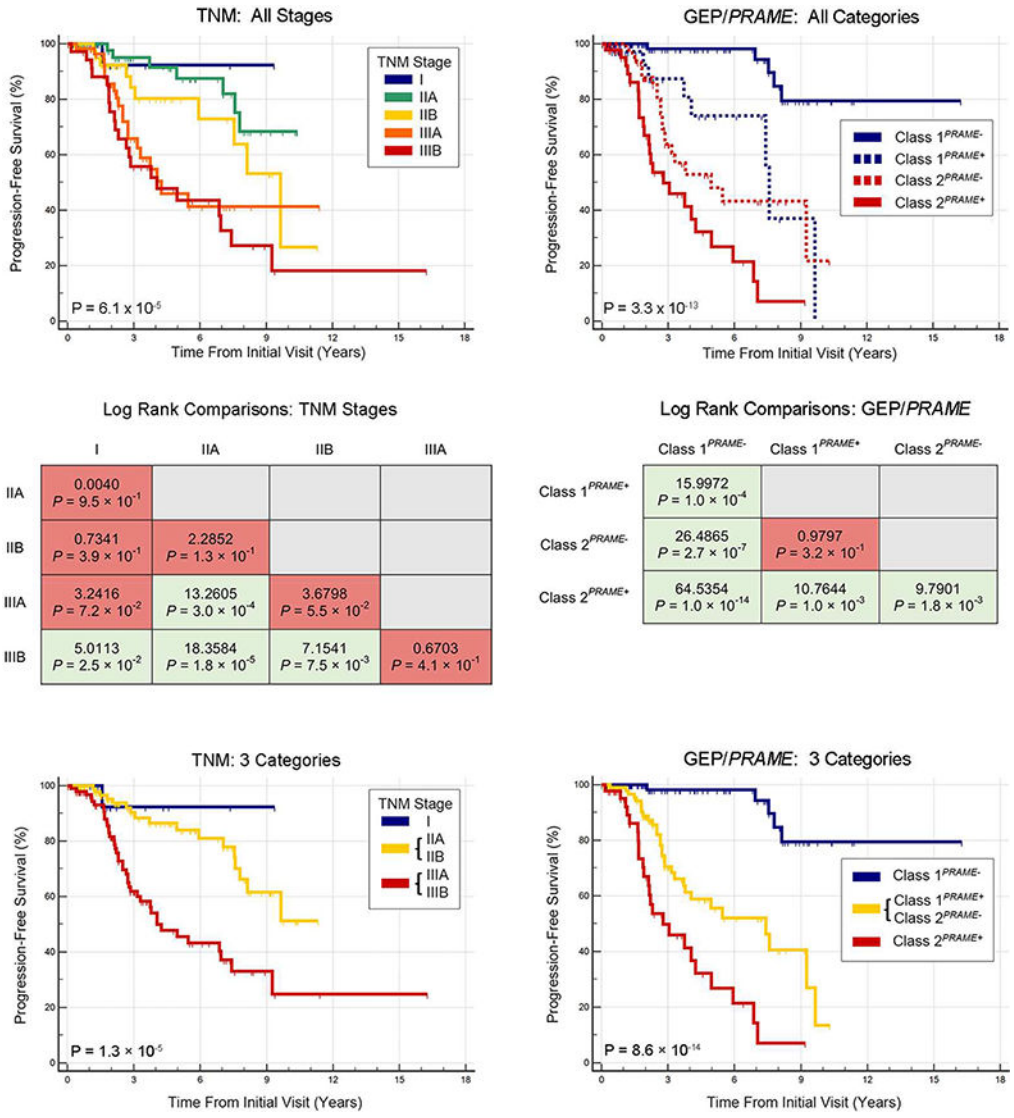

FIGURE 1.

Kaplan-Meier survival analysis in 240 patients with uveal melanoma using Tumor-Node-Metastasis staging and gene expression profiling/*PRAME* classification. (**Top left**) Survival curves are shown using all Tumor-Node-Metastasis stages except stage IV. (**Middle left**) Comparisons of log-rank statistics are shown between Tumor-Node-Metastasis stages. (**Bottom left**) Survival curves are shown using optimized Tumor-Node-Metastasis categories. **(Top right)** Survival curves are shown using all gene expression profiling/*PRAME* categories. **(Middle right)** Comparisons of log-rank statistics are shown between gene expression profiling/*PRAME* categories. **(Bottom right)** Survival curves are shown using optimized gene expression profiling/*PRAME* categories.

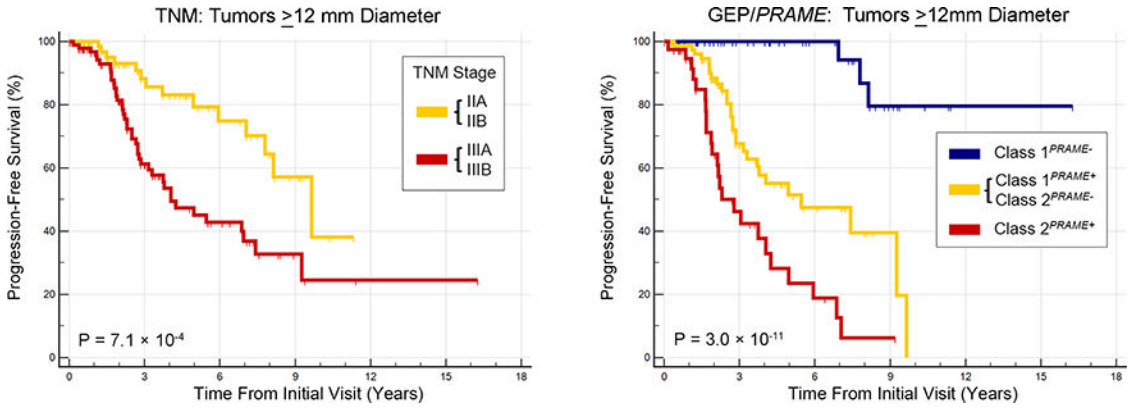

FIGURE 2.

Kaplan-Meier survival analysis in 180 patients with uveal melanoma with largest basal diameter ≥12 mm. **(Left)** Survival curves are shown using optimized Tumor-Node-Metastasis categories. **(Right)** Survival curves are shown using gene expression profiling/ *PRAME* categories.

Table 1.

Summary of clinicopathologic and molecular features in 240 patients with uveal melanoma

Variable	Summary Data (N=240)
Age at diagnosis (years)	
Mean	62.4
Median	64 (14 to 93)
Sex	
Female	121 (50.4%)
Male	119 (49.6%)
Largest basal diameter (mm)	
Mean	14.6
Median (range)	15.0 (3 to 24)
No. of tumors with LBD < 12	60 (25.0%)
No. of tumors with LBD ≥ 12	180 (75.0%)
Thickness (mm)	
Mean	6.9
Median (range)	6.4 (1.2 to 16.4)
Ciliary body involvement	
Yes	104 (43.3%)
No	136 (56.7%)
Extraocular extension	
Yes	17 (7.1%)
No	51 (21.3%)
Unable to be assessed	172 (72.0%)
Gene expression profile	
Class 1	128 (53.3%)
Class 2	112 (46.7%)
PRAME status	
PRAME (−)	157 (65.4%)
PRAME (+)	83 (34.6%)
Gene expression profile and *PRAME* status	
Class 1 *PRAME* (−)	90 (37.5%)
Class 1 *PRAME* (+)	38 (15.8%)
Class 2 *PRAME* (−)	67 (27.9%)
Class 2 *PRAME* (+)	45 (18.8%)
TNM stage	
I	26 (10.8%)
IIA	67 (27.9%)
IIB	50 (20.8%)
IIIA	59 (24.6%)

Variable	Summary Data (N=240)
IIIB	38 (15.8%)
Metastasis	
Yes	59 (24.6%)
No	181 (75.4%)
Last status	
Alive without metastasis	175 (72.9%)
Alive with metastasis	23 (9.6%)
Melanoma specific mortality	36 (15.0%)
Non-melanoma specific mortality	6 (2.5%)
Treatment	
Observation	1 (0.4%)
Plaque brachytherapy	165 (68.8%)
Enucleation	74 (30.8%)
Follow-up (months)	
Mean	42
Median (Range)	29 (1 to 195)

Abbreviations: TNM, Tumor-Node-Metastasis; *PRAME,* preferentially expressed antigen in melanoma.

Table 2.

Univariate Cox proportional hazards analysis of clinicopathologic and molecular prognostic variables in 240 patients with uveal melanoma

Covariate	Regression Coefficient, β (SE)	Wald Statistic	*P*-value	Hazard Coefficient, Exp(b) (95% CI)
Gene expression profile	1.8230 (0.3218)	32.10	1.5×10^{-8}	6.1907 (3.2948 to 11.632)
Largest basal diameter	0.1595 (0.0339)	22.21	2.5×10^{-6}	1.1729 (1.0976 to 1.2534)
PRAME status	1.2500 (0.2660)	22.09	2.6×10^{-6}	3.4902 (2.0724 to 5.8781)
TNM stage	0.5384 (0.1163)	21.42	3.7×10^{-6}	1.7132 (1.3639 to 2.1520)
Tumor thickness	0.1168 (0.0341)	11.78	6.0×10^{-4}	1.1239 (1.0514 to 1.2015)
Ciliary body involvement	0.8256 (0.2756)	8.97	0.0027	2.2832 (1.3302 to 3.9188)
Male gender	0.6345 (0.2694)	5.54	0.019	1.8860 (1.1124 to 3.1978)
Age	0.01806 (0.00987)	3.35	0.067	1.0182 (0.9987 to 1.0381)
Extraocular extension	0.4271 (0.3731)	1.31	0.25	1.5328 (0.7378 to 3.1848)

Abbreviations: SE, standard error; CI, confidence interval; TNM, Tumor-Node-Metastasis; *PRAME,* Preferentially Expressed Antigen in Melanoma

Table 3.

Multivariate Cox proportional hazards analysis of clinicopathologic and molecular prognostic variables in 240 patients with uveal melanoma

Clinical variables incorporated into TNM stage				
Covariate	**Regression Coefficient, β (SE)**	**Wald Statistic**	***P*-value**	**Hazard Coefficient, Exp(b) (95% CI)**
Gene expression profile	1.5594 (0.3329)	21.95	2.8×10^{-6}	4.7559 (2.4768 to 9.1319)
PRAME status	10061(0.2732)	13.56	2.3×10^{-4}	2.7349 (1.6009 to 4.6720)
TNM stage	0.4136 (0.1221)	11.47	7.1×10^{-4}	1.5122 (1.1903 to 1.9213)
Male gender[a]				
Clinical variables analyzed separately				
Covariate	**Regression Coefficient, β (SE)**	**Wald Statistic**	***P*-value**	**Hazard Coefficient, Exp(b) (95% CI)**
Gene expression profile	1.6787 (0.3276)	26.26	3.0×10^{-7}	5.3484 (2.8195 to 10.1833)
Largest basal diameter	0.1340 (0.0391)	11.76	6.0×10^{-4}	1.1434 (1.0591 to 1.2344)
PRAME status	0.7989 (0.2784)	8.23	0.0041	2.2230 (1.2881 to 3.8364)
Tumor thickness[a]				
Ciliary body involvement[a]				
Male gender[a]				

[a]Excluded by the Cox multivariate model due to lack of significant independent prognostic value

Abbreviations: SE, standard error; CI, confidence interval; *PRAME,* preferentially expressed antigen in melanoma; TNM, Tumor-Node-Metastasis