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Validation of the Prognostic Usefulness of the Gene Expression Profiling Test in Patients with Uveal Melanoma

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Abstract

Purpose: To validate the prognostic usefulness of gene expression profile (GEP) testing in patients with uveal melanoma. To determine whether combining tumor size with the GEP classification provides additional prognostic value.

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Conception and design: Miguez, Sznol, Kluger, Goldbaum, Francis, Pointdujour-Lim, Bakhoum

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HUMAN SUBJECTS: Human subjects were not included in this study. This was a retrospective chart review study that adhered to the tenets of the Declaration of Helsinki and was conducted with approval obtained from the Internal Review Board (IRB) of Yale University (IRB # 2000031254), University of California San Diego (UCSD) (IRB #190665CX), and Memorial Sloan Kettering Cancer Center (MSKCC) (IRB #16-1464). A waiver of informed consent was obtained from the internal review board of each institution.

No animal subjects were included in this study.

Design: Retrospective analysis.

Participants: Patients with a diagnosis of choroidal melanoma examined at Yale New Haven Hospital; University of California, San Diego; and Memorial Sloan Kettering Cancer Center.

Methods: Patients' demographic and clinical data and tumor characteristics were collected. Univariate and multivariate Cox hazard regression analysis were used to assess the association between tumor characteristics and GEP classification with metastasis as an outcome.

Main Outcome Measures: Metastasis-free survival (MFS).

Results: Of the 337 individuals included in the study, 87 demonstrated metastases. The mean follow-up time was 37.2 (standard deviation [SD], 40.2) months for patients with metastases and 55.0 (SD, 49.3) months for those without metastases. Tumors of larger thickness and GEP class 2 (vs. class 1) were associated significantly with increased risk of metastasis. Tumor thickness showed better prognostic usefulness than GEP classification (Wald statistic, 40.7 and 24.2, respectively). Class 2 tumors with a thickness of 7.0 mm or more were associated with increased risk of metastasis than tumors with a thickness of < 7.0 mm (hazard ratio [HR], 3.23; 95% confidence interval [CI], 1.61–6.51), whereas class 1 tumors with a thickness of 9.0 mm or more were associated with increased risk of metastasis than tumors with a thickness of < 9.0 mm (HR, 2.07; 95% CI, 0.86–4.99). No difference in MFS was found between patients with class 1A tumors compared with those with class 1B tumors ($P = 0.8$). Patients with class 2 tumors showed an observed 5-year MFS of 47.5% (95% CI, 36.0%–62.8%).

Conclusions: Tumor size was the most significant predictor of metastasis and provided additional prognostic value independent of GEP classification. In addition, rates of metastasis for class 2 tumors were lower than estimates reported by Castle Bioscience, and no difference in rates of metastasis were found between class 1A and 1B tumors. This indicates that tumor size should be accounted for when relying on GEP for prognostication and that patients with GEP class 1A or 1B tumors may benefit from the same metastatic surveillance protocols.

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Keywords

Gene expression profile; Ocular oncology; Prognostication; Uveal melanoma; Metastasis

Uveal melanoma (UM) is the most common primary intraocular cancer in adults. It arises from melanocytes within the uveal tract (choroid, ciliary body, and iris).[1–3] Approximately one third of patients with UM will demonstrate metastatic disease within 5 years, which usually is fatal within 1 year of clinical onset.[2,4] Uveal melanomas that tend to metastasize often have cells of epithelioid morphologic features with enlarged nuclei, and they frequently harbor mutations in the *BAP1* (BRCA1-associated protein-1) gene or exhibit genomic copy loss of chromosome 3.[1,5,6] Additionally, highly metastatic tumors can be distinguished from their indolent counterparts by their gene expression profile (GEP).[1,7,8] In clinical practice, biopsy samples are obtained commonly at the time of treatment and are analyzed to assign a prognostic class to the tumor with the goal of providing an accurate estimate of the metastatic risk. Two main molecular prognostic tests are commercially

available and used commonly. The first is a DNA-based test (offered by Impact Genetics) that analyzes the tumor's chromosomal number alterations, mainly chromosomes 3, 6, and 8, and mutations in the *BAP1, EIF1AX (Eukaryotic Translation Initiation Factor 1A X-Linked)*, and *SF3B1* (Splicing Factor 3b Subunit 1) genes. The other is an RNA-based test (DecisionDx; Castle Biosciences), which assesses the expression levels of 12 discriminant genes to stratify tumors into 3 different prognostic groups.[8–11]

Although these molecular tests generally provide accurate prognostication, evidence from retrospective cohorts suggests that tumor size contributes to the risk of metastasis independent of molecular classification. For instance, combining the American Joint Committee on Cancer (AJCC) staging, which accounts for both tumor thickness and diameter,[12] with The Cancer Genome Atlas (TCGA) molecular classification, based on chromosomal analysis, provides additional prognostic usefulness.[13] Tumor size has been incorporated into most prognostic DNA-based tests (such as Impact Genetics, the Liverpool Uveal Melanoma Prognosticator Online tool,[14] and TCGA[13]) to enhance their prognostic accuracy. The GEP test, however, is thought to provide prognostic prediction independent of size by stratifying tumors into classes 1A, 1B, and 2, which are associated with a 5-year metastatic risk of 2%, 21%, and 72%, respectively.[8–10] Integrative molecular analysis of UM tumors based on TCGA demonstrated that nearly all tumors with a GEP class 2 signature exhibit evidence of chromosome 3 loss.[1] This is consistent with transcriptional analysis of tumor single cells, which demonstrated a strong correlation between monosomy 3 and GEP class 2 signatures.[15] Therefore, if tumor size is a modifier of metastatic risk for monosomy 3 tumors, it also should be a modifier of metastatic risk for GEP class 2 tumors. However, tumor size has yet to be incorporated into the GEP clinical test (DecisionDx), in which rates of metastasis are reported solely based on molecular classification. Gene expression profile class 2 UMs often are larger than their GEP class 1 counterparts,[16–18] and larger UMs have higher metastatic tendencies, but does tumor size provide additional prognostic information in the context of GEP classification? Prior studies have reached conflicting conclusions, with some demonstrating that incorporating tumor size information into GEP does not offer any additional prognostic information.[19–21] Other researchers have shown that incorporating tumor diameter but not thickness[22–26]; thickness, but not diameter[27]; or either thickness and diameter[28,29] provides additional prognostic usefulness. However, even for these studies that have demonstrated additional prognostic usefulness for tumor thickness or diameter, GEP classification remained by far the most significant predictor of metastasis.

The DecisionDx test is used in clinical practice to inform patients of their risk of metastases developing, which often are lethal. Now it has been incorporated into national guidelines such as those of the National Comprehensive Cancer Network, in which GEP classification is used to estimate the risk of metastasis and to guide providers in determining the frequency of metastatic surveillance tests. This impacts patient management and patients' expectations of disease progression. Therefore, it is crucial to validate the test's findings in actual clinical settings using large cohorts and long-term follow-up data. Since its implementation, the test's prognostic usefulness has been validated in multiple studies. However, incorporating tumor size as an independent factor along with GEP classification to predict metastasis onset has not been studied extensively. Additionally, no studies have validated the prognostic usefulness of subclassifying GEP class 1 tumors into class 1A and 1B tumors.[8,16] Herein,

we report outcomes of patients with a diagnosis of UMs from 3 different United States institutions and with long-term follow-up data. We compared metastatic outcomes between patients with GEP class 1A and 1B tumors. We also determined whether tumor size provides additional prognostic value to GEP classification.

Methods

Study Design and Ethics Statement

This was a retrospective chart review study that adhered to the tenets of the Declaration of Helsinki and was conducted with approval obtained from the internal review board of Yale University (identifier, 2000031254); the University of California, San Diego (identifier, 190665CX); and Memorial Sloan Kettering Cancer Center (identifier, 16–1464).

Data Collection

A retrospective chart review of medical records within the electronic health record systems of Yale New Haven Hospital; University of California, San Diego; and Memorial Sloan Kettering Cancer Center was performed. A waiver of informed consent was obtained from the internal review board of each institution. Inclusion criteria included individuals with a diagnosis of posterior UM (not including iris melanoma) and who were examined between 2010 and 2021 (Yale University), 2006 and 2020 (University of California, San Diego), and 2005 and 2020 (Memorial Sloan Kettering Cancer Center). We excluded those with missing necessary information such as tumor size or location. We collected patients' demographic data including age at primary diagnosis, sex, ethnicity, and race, along with clinical data including date of diagnosis for the primary tumor, primary treatment method (transpupillary thermotherapy, cryoablation, radiation therapy [iodine plaque or proton beam therapy], or enucleation), and dates of metastasis and death, if applicable. Tumor characteristics including apical thickness, largest basal diameter, and results from GEP (DecisionDx) also were recorded.

Statistical Analysis

We report descriptive statistics (mean and standard deviation) for continuous variables and frequency distributions (number and percentage) for categorical variables. Statistical significance was assessed using the Student *t* test for continuous variables and Pearson's chi-square test for categorical variables. Univariate Cox hazard regression analysis was used to determine the relationship between tumor characteristics, including thickness, diameter, AJCC stage, GEP classification, and ciliary body involvement, and patient demographics, including age at diagnosis and sex, with risk of metastasis. Hazard ratios (HRs) with 95% confidence intervals (CIs) were calculated. The HR and CI values for continuous values are reported in increments of 5 mm for tumor thickness and diameter and of 5 years for patient age. Multivariate Cox hazard regression analysis was used to assess the association of significant covariates with metastasis as an outcome. Time-dependent receiver operator characteristic (ROC) curves were generated to compare the prognostic value of tumor thickness and diameter. Area under the ROC curve (AUC) was calculated for each variable to compare its association with metastasis at 5 years. The 5-year rate of metastasis-free survival (MFS) was calculated using Kaplan–Meier curves. From the ROC curve of

continuous variables, the Youden index was calculated to determine the optimal cutoff point. Metastatic rates were plotted using Kaplan–Meier curves, and differences between groups were analyzed using the log-rank Mantel–Cox test. Statistical analyses and graphs were generated using GraphPad Prism version 8.0.0 software and R version 4.0 software (R Foundation for Statistical Computing). The following packages were used to generate Kaplan–Meier curves and ROC curves, respectively: ggplot2 and survivalROC.[30,31]

Results

We identified 410 individuals with a diagnosis of primary UM. Seventy-three patients had incomplete records and were excluded from the analysis. Demographics and baseline characteristics for the study cohort (n = 337) are summarized in Table 1. The mean ± standard deviation age at diagnosis was 61.8 ± 15.6 years. One hundred eighty-seven patients (55.5%) were men, and 150 patients (44.5%) were women. The average tumor thickness was 6.17 ± 4.0 mm, and the average tumor largest basal diameter was 12.5 ± 4.9 mm. Based on AJCC Criteria, 95 tumors (28.2%), 100 tumors (29.7%), 102 tumors (30.3%), and 40 tumors (11.9%) were classified as stages T1, T2, T3, and T4, respectively. Gene expression profile information was available for 241 patients (71.5%). Of those, 106 patients (44.0%) had GEP class 1A tumors, 50 patients (20.7%) had GEP class 1B tumors, and 85 patients (35.3%) had GEP class 2 tumors. Ciliary body involvement was noted in 74 patients (22.0%). A total of 87 patients demonstrated metastases. The mean time from primary diagnosis to metastasis was 37.2 ± 40.2 months. Among patients who did not demonstrate metastases, the average time from primary diagnosis to last follow-up was 55.0 ± 49.3 months.

We interrogated the relationship between metastasis and tumor characteristics, including tumor thickness, diameter, AJCC T stage, GEP classification, ciliary body involvement, and patients' clinical characteristics, including age at diagnosis and sex, using univariate Cox proportional hazards regression analysis. Tumor thickness, tumor diameter, GEP class 2 classification (compared with GEP class 1), ciliary body involvement, and age at diagnosis were associated significantly with metastasis with HRs of 2.24 (95% CI, 1.74–2.86), 1.69 (95% CI, 1.39–2.04), 3.83 (95% CI, 2.28–6.53), 2.33 (95% CI, 1.47–3.62), and 1.02 (95% CI, 1.00–1.03), respectively, whereas patient sex and a GEP class 1B classification (compared with class 1A) were not ($P = 0.61$ and $P = 0.75$, respectively; Table S2, available at www.aaojournal.org). Tumors with AJCC T stages T3 and T4 (compared with stage T1) were associated significantly with metastasis with HRs of 3.46 (95% CI, 1.86–6.85) and 6.18 (95% CI, 3.11–12.75), respectively, whereas T2 tumors were not ($P = 0.43$; Table S2).

We then sought to determine the relative contribution of these factors to metastasis. First, to choose the best measure of tumor size as a predictor of metastasis, we used time-dependent ROC analysis for thickness and diameter and used metastasis as the outcome. Tumor thickness was found to have a slightly higher AUC value (0.71) compared with tumor diameter (AUC, 0.68; Fig S1A, B, available at www.aaojournal.org). Thereafter, we used tumor thickness as a surrogate of tumor size. We analyzed the association between metastasis and covariates that were associated significantly with metastasis in the univariate model, including GEP classification (class 2 vs. 1), thickness or AJCC staging, ciliary body

involvement, and age at diagnosis, using multivariate Cox proportional hazards regression analyses. Given that both tumor thickness and AJCC staging are measures of tumor size, we analyzed their association with metastases using 2 independent models. In a model in which thickness was used as a measure of tumor size, both GEP class 2 classification (vs. class 1) and tumor thickness were associated significantly with metastasis, with an HR of metastasis of 3.22 (95% CI, 1.85–5.67) and 1.98 (95% CI, 1.46–2.67), respectively (Fig 2A). In this model, ciliary body involvement and age at diagnosis were not associated significantly with increased risk of metastasis ($P = 0.87$ and $P = 0.80$, respectively; Fig 2A). Similarly, in an alternative model in which AJCC staging was used as a surrogate of tumor size, an AJCC stage of T4 and T3 (vs. T1) and GEP class 2 (vs. GEP class 1) were associated significantly with metastasis, with an HR of metastasis of 4.79 (95% CI, 1.83–13.99), 3.32 (95% CI, 1.44–9.00), and 3.17 (95% CI, 1.83–5.56), respectively (Fig 2B). In this model, an AJCC stage of T2 (vs. T1), ciliary body involvement, and age at diagnosis were not associated significantly with higher odds of metastasis ($P = 0.26$, $P = 0.52$, and $P = 0.48$, respectively; Fig 2B).

Next, to identify the minimum variables that are most predictive of metastasis, we applied backward stepwise regression in which the least significant covariates were omitted in a stepwise fashion to identify a reduced model in which only the most significant covariates are included. Tumor size, measured by thickness or AJCC staging, and a GEP class 2 phenotype remained associated significantly with metastasis regardless of inclusion of other covariates, whereas ciliary body involvement, age at primary diagnosis, and GEP class 1B were not (Table 3). We compared the predictive value of the different models using the Wald statistic test. Models including tumor thickness, AJCC staging, or GEP classification had Wald statistic values of 40.7, 37.0, and 24.2, respectively. Combining GEP with tumor thickness or AJCC staging resulted in Wald statistic values of 42.3 and 37.7, respectively (Table 3).

To visualize the difference in the incidence of metastasis and to identify 5-year MFS rates, we compared MFS between subgroups using Kaplan–Meier analysis. We found no difference in odds of metastasis between GEP class 1A and 1B tumors ($P = 0.82$; Fig 3A). The 5-year MFS rates for GEP class 1A and 1B tumors were 90.0% (95% CI, 83.6%–96.9%) and 86.9% (95% CI, 76.7%–98.5%), respectively, and the 7-year MFS rates were 79.9% (95% CI, 69.6%–91.8%) and 86.9% (95% CI, 76.7%–98.5%), respectively (Table S4, available at www.aaojournal.org). Gene expression profile class 2 tumors showed higher odds of metastasis than GEP class 1 tumors ($P < 0.0001$; Fig 3B), with 3-year, 5-year, and 7-year MFS rates of 55.2% (95% CI, 44.2%–68.9%), 47.5% (95% CI, 36.0%–62.8%), and 47.5% (95% CI, 36.0%–62.8%), respectively (Table S4).

Given that tumor size, assessed by thickness or AJCC staging, and GEP class 2 were independent predictors of metastasis in the multivariate model, we sought to determine whether tumor size could stratify GEP class 1 and 2 tumors further into additional prognostic subgroups. First, to determine the optimal cutoff value for thickness as a predictor of metastasis, we analyzed the relationship between metastasis and different cutoff values of tumor thickness using a time-dependent ROC curve. The optimal cutoff points corresponding with the largest AUC value were 9.0 mm and 7.0 mm for GEP class 1 and

2 tumors, respectively (Table S5, available at www.aaojournal.org). Accordingly, GEP class 2 tumors with a thickness of 7.0 mm or more showed higher odds of metastasis than GEP class 2 tumors with a thickness of < 7.0 mm (HR, 3.23; 95% CI, 1.61–6.51; Fig 3C), and GEP class 1 tumors with a thickness of 9.0 mm or more were associated with higher odds of metastasis than GEP class 1 tumors with a thickness of < 9.0 mm (HR, 2.07; 95% CI, 0.86–4.99; Fig 3D).

Finally, we present the 5-year MFS rates for tumors stratified by GEP classification (class 1 vs. class 2) and tumor thickness or AJCC staging. The overall 5-year MFS rates for GEP class 1 and 2 tumors, respectively, were 88.9% (95% CI, 83.4%–94.9%) and 47.5% (95% CI, 36.0%–62.8%). For GEP class 1 tumors, the 5-year MFS rates were 92.7% (95% CI, 87.5%–98.1%) and 73.4% (95% CI, 56.4%–95.4%) for tumors with thickness less than or more than 9.0 mm, respectively. For GEP class 2 tumors, the 5-year MFS rates were 64.4% (95% CI, 49.2%–84.3%) and 26.7% (95% CI, 13.5%–52.9%) for tumors less than or more than 7.0 mm, respectively (Fig 4). For GEP class 1 tumors, the 5-year MFS rates for T1 or T2 tumors and T3 or T4 tumors were 93.3% (95% CI, 87.8%–99.2%) and 81.2% (95% CI, 70.0%–94.2%), respectively, and for GEP class 2 tumors, the 5-year MFS rates for T1 or T2 tumors and T3 or T4 tumors were 60.1% (95% CI, 43.1%–83.9%) and 36.2% (95% CI, 22.7%–57.8%), respectively (Fig 4).

Discussion

We analyzed outcomes from a retrospective cohort of patients with UM from 3 different United States institutions with long-term follow-up data. The main findings were (1) that tumor size was the most significant predictor of metastasis and provided additional prognostic value independent of GEP classification, (2) that no difference existed in the odds of metastasis between GEP class 1A and 1B tumors over the duration of this study, and (3) that the rates of metastasis for GEP class 2 tumors were lower than reported in DecisionDx.

In clinical practice, patients with GEP class 2 tumors are given a 5-year risk of metastasis of 72%, corresponding to an MFS rate of 28%.[7,8,10,11] These numbers are supported by the initial Ocular Oncology Group Report, a prospective validation of the GEP test, which reported a 3-year MFS of 35%.[19] However, in this cohort, the overall 5-year MFS of patients with GEP class 2 tumors was 47.5% (95% CI, 36.0%–62.8%). Our findings are strikingly in line with a recent report that analyzed outcomes of patients with UMs from 2 different United States institutions who reported an observed 5-year MFS rate of 47% (95% CI, 37%–61%) for patients with GEP class 2 tumors.[29] These rates also are consistent with the meta-analysis of published studies examining the MFS of patients with GEP class 2 tumors.[29]

Molecular tests other than the GEP are used in clinical practice to estimate MFS for individuals with UMs, including tumor cytogenetic (chromosomal) analysis, which preceded the GEP. Loss of chromosome 3 is associated with worse outcomes, with a 3-year MFS of 50%.[32] Additional chromosomal copy number variations, such as 6p gain or 8q gain, can stratify disomy and monosomy 3 UMs further into 2 additional subgroups with distinct prognoses. The transcriptional-based (RNA) stratification (GEP test) was thought to be

superior to the DNA-based methods (chromosome 3 status).[19] However, integrative analysis of the transcriptome and chromosome copy number variations in TCGA demonstrated that monosomy 3 UMs exhibit a GEP class 2 signature.[3] This was confirmed by analyzing the tumor at the single-cell level, which demonstrated a significant correlation between a transcriptional signature of monosomy 3 UMs and the GEP class 2 signature.[15] Hence, one would expect that the MFS rates for patients with monosomy 3 UMs would be similar to those of patients with GEP class 2 UMs. Indeed, patients with GEP class 2 tumors in this cohort showed MFS rates of 55.2% (95% CI, 44.2%–68.9%) at 3 years and 47.5% (95% CI, 36.0%–62.8%) at 5 years, which were very similar to published MFS rates of patients with monosomy 3 UMs.[13,32–36] In summary, historical survival data and molecular analysis support that monosomy 3 and a GEP class 2 signature provide equal prognostic usefulness.

Although molecular tests initially stratified UMs into 2 major risk groups, low risk (disomy 3 or GEP class 1 tumors) and high risk (monosomy 3 or GEP class 2 tumors), approximately 15% to 20% of patients in the low-risk group will demonstrate metastasis, and up to half of those in the high-risk group will not demonstrate metastasis. This prompted the development of additional subclassifications to obtain more accurate prognostication. For instance, TCGA analysis demonstrated that each major group can be subdivided further into 2 subgroups with different outcomes, and this has been validated using independent clinical datasets.[1,3,36] Similarly, for the DecisionDx GEP test, class 1 tumors are subdivided into class 1A and 1B tumors, with low and intermediate risk of metastasis, respectively.[8,16] Higher Preferentially Expressed Antigen in Melanoma expression also has been shown to correlate with higher odds of metastasis in GEP class 1 tumors and subsequently was incorporated into the DecisionDx clinical test.[37] However, we found no difference in MFS between GEP class 1A and 1B tumors at 3, 5, or 7 years. The 5-year MFS rates for patients with GEP class 1A and 1B tumors were 90.0% and 86.9%, respectively, and the 7-year MFS rates for GEP class 1A and 1B tumors were 79.9% and 86.9%, respectively. This finding is in contrast with the initial report of the 5-year metastatic risk of 2% and 21% for GEP class 1A and 1B tumors, respectively.[8,16] A study that has validated the difference in metastasis rates at 5 years between GEP class 1A and 1B tumors has not been published. Demirci et al[23] demonstrated 3-year MFS rates of 99% (95% CI, 94%–99%) and 90% (95% CI, 77%–96%) for GEP class 1A and 1B tumors, respectively. However, their analysis was limited by the short follow-up period, with a mean follow-up period of 26 months, compared with 55.7 months in the present cohort. The distinction between GEP class 1A and 1B tumors relies on analyzing the expression levels of *CDH1* (Cadherin 1) and *RAB31* (Member RAS Oncogene Family), both of which are GEP class 2 markers.[16] However, the exact technical detail of the clinical test is not available, which limits its validation using orthogonal methods. Although GEP class 1 or disomy 3 UMs can be stratified further into subgroups with distinct prognosis based on gene expression or chromosomal analysis,[1,3,13] our findings indicate no difference in the rates of metastasis between GEP class 1A and 1B tumors.

Until recently, it was thought that high-risk and low-risk UMs are fundamentally distinct disease subtypes. However, using single-cell RNA sequencing of enucleated UM tumors, we previously demonstrated that individual tumors harbor clones from both prognostic classes.[15] Therefore, prognostic tests that rely solely on classifying tumors into 2 or 3 prognostic groups may not capture this heterogeneity and thus can lead to misclassification.[6]

This intratumoral heterogeneity also raises potential concerns about sampling errors.[15] In clinical practice, biopsy samples often are obtained using fine-needle aspiration to determine a tumor's metastatic potential. This contrasts with earlier studies that established the predicted metastatic risk based on analysis of large specimens from enucleated tumors, specifically for the GEP test.[7] Tests that assess tumor heterogeneity, rather than classifying tumors into distinct groups, may offer more accurate prognostication. The use of DNA methylation to analyze tumor heterogeneity further may be considered, which could demonstrate the preponderance of aggressive subclones within a tumor. We recently demonstrated that methylation of a single locus in the *BAP1* gene provides accurate prognostic information.[6]

Increasing evidence suggests that tumor size contributes to the risk of metastasis independent of molecular classification. This has been studied extensively and demonstrated in the context of chromosomal analysis.[13] However, the GEP test initially was thought to provide prognostic prediction independent of size.[19–21] Yet recent studies have demonstrated that incorporating tumor size may provide additional prognostic usefulness.[22–29] We found that incorporating tumor thickness into the GEP classification provides additional prognostic usefulness. In fact, tumor size (based on thickness or AJCC staging) provided better prediction of metastasis than GEP classification. These findings are in contrast to prior studies that demonstrated a significant but minimal benefit to incorporating tumor size into GEP classification.[22–27] Two factors may explain the discrepancy between our findings and those of prior studies. First, some studies compared GEP classification and tumor size using a cutoff value for tumor thickness or diameter to stratify tumors into binary subgroups.[23,26] Given that tumor size is a continuous variable, stratifying tumors into 2 groups based on a cutoff value reduces the prognostic predictive value of the test. Accordingly, Shields et al[4] analyzed outcomes of 7256 eyes with choroidal melanoma and demonstrated a strong correlation between tumor thickness as a continuous variable and metastasis. In our models, we analyzed tumor thickness and diameter as continuous variables. Second, most studies included both thickness and diameter in a single multivariate model.[19,20,25,26] Because both are measures of tumor size, including both variables in a single model attenuates their corresponding predictive value. We included tumor thickness only in the multivariate analysis because it was a better predictor of metastasis than diameter based on higher AUC values in the ROC analysis. We demonstrated that, for both GEP class 1 and 2 tumors, thickness could be used to stratify tumors further into prognostically distinct groups, albeit using different thickness thresholds. It is not surprising that tumor thickness may be a better predictor of metastasis than diameter given that thickness measurements may be less prone to interobserver variability than tumor diameter, which is measured using the tumor's arc length. Additionally, the tumor's peripheral edges may be less defined on ultrasonography, and measurements of the tumor's diameter may be influenced by the eye's curvature. The Collaborative Ocular Melanoma Study reported that diameter estimates by ultrasonography showed only a 58% correlation within 2 mm of histopathologic measurements. In contrast, tumor thickness measured using ultrasonography showed a 90% correlation with histopathologic measurements.[38] In summary, we demonstrated a remarkable benefit to incorporating tumor size into GEP classification.

Uveal melanoma is characterized by a striking latency in distant metastasis formation, which can manifest years after the primary treatment. The latency of metastasis has raised an important question: does local primary treatment reduce the odds of metastasis? The ability of molecular analysis to stratify tumors into distinct prognostic groups led to the hypothesis that prognosis is set in stone. However, presence of intratumor heterogeneity suggests otherwise; low-risk tumors may harbor high-risk cells and, in theory, can progress to acquire more metastatic tendencies.[15,39] We recently demonstrated that tumor cells may evolve from a low-risk to high-risk phenotype through the loss of a key epigenetic regulator, polycomb repressive complex 1.[15] These findings suggest that untreated UM with a good prognosis may evolve over time to acquire a more aggressive phenotype. Although the impact of primary treatment on metastasis has not been evaluated using a randomized controlled study, post hoc analysis of cohorts of patients who declined treatment of the primary tumor indicate that they had worse outcomes.[40,41] The fact that tumor size is a predictor of metastasis independent of molecular classification is consistent with the hypothesis that tumors may evolve from an indolent to a more aggressive phenotype[15] and that treatment of smaller tumors may have an impact on metastatic rates. However, better outcomes for patients with smaller tumors in part may be the result of lead time bias.

Limitations of the study are inherent to the retrospective design and inclusion of patients from 3 different institutions. Tumor measurements were subject to interobserver variability, and biopsy techniques may vary among physicians. However, this scenario reflects UM management in clinical settings. Nevertheless, patient demographic features and tumor characteristics, including size, GEP subtype distribution, ciliary body involvement, and metastatic rates, largely were consistent with those of other published studies.[4,22,28,29,42]

In conclusion, we demonstrated (1) that tumor size was the most significant predictor of metastasis and provided additional prognostic value independent of GEP classification, (2) that no difference exists in odds of metastasis between GEP class 1A and 1B tumors, and (3) that the rates of metastasis for GEP class 2 tumors were lower than what is reported in a commonly used clinical test (DecisionDx).

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Abbreviations and Acronyms:

AJCC	American Joint Committee on Cancer
AUC	area under the receiver operator characteristic curve
CI	confidence interval
GEP	gene expression profile
HR	hazard ratio
MFS	metastasis-free survival
ROC	receiver operator characteristic
TCGA	The Cancer Genome Atlas
UM	uveal melanoma

References

1. Bakhoum MF, Esmaeli B. Molecular characteristics of uveal melanoma: insights from the Cancer Genome Atlas (TCGA) project. Cancers (Basel). 2019;11(8):1061. Available at: https://pubmed.ncbi.nlm.nih.gov/31357599/. [PubMed: 31357599]
2. Jager MJ, Shields CL, Cebulla CM, et al. Uveal melanoma. Nat Rev Dis Primers. 2020;6(1):24. [PubMed: 32273508]
3. Robertson AG, Shih J, Yau C, et al. Integrative analysis identifies four molecular and clinical subsets in uveal melanoma. Cancer Cell. 2017;32(2):204–220 e15. [PubMed: 28810145]
4. Shields CL, Furuta M, Thangappan A, et al. Metastasis of uveal melanoma millimeter-by-millimeter in 8033 consecutive eyes. Arch Ophthalmol. 2009;127(8):989–998. [PubMed: 19667335]
5. Callender G Malignant melanotic tumors of the eye: a study of histopathologic types in 111 cases. American Academy Ophthalmology. 1931;36:131–142.
6. Bakhoum MF, Curtis EJ, Goldbaum MH, Mischel PS. BAP1 methylation: a prognostic marker of uveal melanoma metastasis. NPJ Precis Oncol. 2021;5(1):89. [PubMed: 34593944]
7. Harbour JW, Chen R. The DecisionDx-UM gene expression profile test provides risk stratification and individualized patient care in uveal melanoma. PLoS Curr. 2013. 10.1371/currents.eogt.af8ba80fc776c8f1ce8f5dc485d4a618.
8. Field MG, Harbour JW. Recent developments in prognostic and predictive testing in uveal melanoma. Curr Opin Ophthalmol. 2014;25(3):234–239. [PubMed: 24713608]
9. Schefler AC, Koca E, Bernicker EH, Correa ZM. Relationship between clinical features, GEP class, and PRAME expression in uveal melanoma. Graefes Arch Clin Exp Ophthalmol. 2019;257(7):1541–1545. [PubMed: 31065847]
10. Onken MD, Worley LA, Ehlers JP, Harbour JW. Gene expression profiling in uveal melanoma reveals two molecular classes and predicts metastatic death. Cancer Res. 2004;64(20):7205–7209. [PubMed: 15492234]
11. Onken MD, Worley LA, Tuscan MD, Harbour JW. An accurate, clinically feasible multi-gene expression assay for predicting metastasis in uveal melanoma. J Mol Diagn. 2010;12(4):461–468. [PubMed: 20413675]
12. Shields CL, Kaliki S, Furuta M, et al. American Joint Committee on Cancer classification of posterior uveal melanoma (tumor size category) predicts prognosis in 7731 patients. Ophthalmology. 2013;120(10):2066–2071. [PubMed: 23664467]

13. Gelmi MC, Bas Z, Malkani K, et al. Adding the Cancer Genome Atlas chromosome classes to American Joint Committee on Cancer system offers more precise prognostication in uveal melanoma. Ophthalmology. 2022;129(4):431–437. [PubMed: 34793831]
14. Eleuteri A, Taktak AFG, Coupland SE, et al. Prognostication of metastatic death in uveal melanoma patients: a Markov multi-state model. Comput Biol Med. 2018;102:151–156. [PubMed: 30278339]
15. Bakhoum MF, Francis JH, Agustinus A, et al. Loss of polycomb repressive complex 1 activity and chromosomal instability drive uveal melanoma progression. Nat Commun. 2021;12(1):5402. [PubMed: 34518527]
16. Plasseraud KM, Wilkinson JK, Oelschlager KM, et al. Gene expression profiling in uveal melanoma: technical reliability and correlation of molecular class with pathologic characteristics. Diagn Pathol. 2017;12(1):59. [PubMed: 28778171]
17. Correa ZM, Augsburger JJ. Sufficiency of FNAB aspirates of posterior uveal melanoma for cytologic versus GEP classification in 159 patients, and relative prognostic significance of these classifications. Graefes Arch Clin Exp Ophthalmol. 2014;252(1):131–135. [PubMed: 24270974]
18. Berry D, Seider M, Stinnett S, et al. Relationship of clinical features and baseline tumor size with gene expression profile status in uveal melanoma: a multi-institutional study. Retina. 2019;39(6):1154–1164. [PubMed: 29578940]
19. Onken MD, Worley LA, Char DH, et al. Collaborative Ocular Oncology Group report number 1: prospective validation of a multi-gene prognostic assay in uveal melanoma. Ophthalmology. 2012;119(8):1596–1603. [PubMed: 22521086]
20. Aaberg TM, Covington KR, Tsai T, et al. Gene expression profiling in uveal melanoma: five-year prospective outcomes and meta-analysis. Ocul Oncol Pathol. 2020;6(5): 360–367. [PubMed: 33123530]
21. Worley LA, Onken MD, Person E, et al. Transcriptomic versus chromosomal prognostic markers and clinical outcome in uveal melanoma. Clin Cancer Res. 2007;13(5):1466–1471. [PubMed: 17332290]
22. Correa ZM, Augsburger JJ. Independent prognostic significance of gene expression profile class and largest basal diameter of posterior uveal melanomas. Am J Ophthalmol. 2016;162:20–27 e1. [PubMed: 26596399]
23. Demirci H, Niziol LM, Ozkurt Z, et al. Do largest basal tumor diameter and the American Joint Committee on Cancer's cancer staging influence prognostication by gene expression profiling in choroidal melanoma. Am J Ophthalmol. 2018;195: 83–92. [PubMed: 30081017]
24. Cai L, Paez-Escamilla M, Walter SD, et al. Gene expression profiling and PRAME status versus tumor-node-metastasis staging for prognostication in uveal melanoma. Am J Ophthalmol. 2018;195:154–160. [PubMed: 30092184]
25. Kujala E, Makitie T, Kivela T. Very long-term prognosis of patients with malignant uveal melanoma. Invest Ophthalmol Vis Sci. 2003;44(11):4651–4659. [PubMed: 14578381]
26. Walter SD, Chao DL, Feuer W, et al. Prognostic implications of tumor diameter in association with gene expression profile for uveal melanoma. JAMA Ophthalmol. 2016;134(7):734–740. [PubMed: 27123792]
27. Plasseraud KM, Cook RW, Tsai T, et al. Clinical performance and management outcomes with the DecisionDx-UM gene expression profile test in a prospective multicenter study. J Oncol. 2016;2016:5325762. [PubMed: 27446211]
28. Binkley EM, Bena JF, Davanzo JM, et al. Gene expression profiling prognostication of posterior uveal melanoma: does size matter? Ophthalmol Retina. 2020;4(6):620–629. [PubMed: 32081600]
29. Singh AD, Binkley EM, Wrenn JM, et al. Predicted vs observed metastasis-free survival in individuals with uveal melanoma. JAMA Ophthalmol. 2022;140(9):847–854. [PubMed: 35862032]
30. Wickham H ggplot2: Elegant Graphics for Data Analysis. Springer-Verlag New York; 2016. version 3.3.3.
31. Heagerty PJ, Saha-Chaudhuri PBP. survivalROC: Time-dependent ROC curve estimation from censored survival data. R package version 1.0.3:2013.

32. Bornfeld N, Prescher G, Becher R, et al. Prognostic implications of monosomy 3 in uveal melanoma. Lancet. 1996;347(9010):1222–1225. [PubMed: 8622452]
33. Thornton S, Coupland SE, Olohan L, et al. Targeted nextgeneration sequencing of 117 routine clinical samples provides further insights into the molecular landscape of uveal melanoma. Cancers (Basel). 2020;12(4):1039. [PubMed: 32340176]
34. Dogrusoz M, Bagger M, van Duinen SG, et al. The prognostic value of AJCC staging in uveal melanoma is enhanced by adding chromosome 3 and 8q status. Invest Ophthalmol Vis Sci. 2017;58(2):833–842. [PubMed: 28159971]
35. Wierenga APA, Brouwer NJ, Gelmi MC, et al. Chromosome 3 and 8q aberrations in uveal melanoma show greater impact on survival in patients with light iris versus dark iris color. Ophthalmology. 2022;129(4):421–430. [PubMed: 34780841]
36. Shields CL, Mayro EL, Bas Z, et al. Ten-year outcomes of uveal melanoma based on The Cancer Genome Atlas (TCGA) classification in 1001 cases. Indian J Ophthalmol. 2021;69(7): 1839–1845. [PubMed: 34146040]
37. Field MG, Decatur CL, Kurtenbach S, et al. PRAME as an independent biomarker for metastasis in uveal melanoma. Clin Cancer Res. 2016;22(5):1234–1242. [PubMed: 26933176]
38. Collaborative Ocular Melanoma Study Group. Comparison of clinical, echographic, and histopathological measurements from eyes with medium-sized choroidal melanoma in the collaborative ocular melanoma study: COMS report no. 21. Arch Ophthalmol. 2003;121(8):1163–1171. [PubMed: 12912695]
39. Shain AH, Bagger MM, Yu R, et al. The genetic evolution of metastatic uveal melanoma. Nat Genet. 2019;51(7):1123–1130. [PubMed: 31253977]
40. Straatsma BR, Diener-West M, Caldwell R, et al. Mortality after deferral of treatment or no treatment for choroidal melanoma. Indian J Ophthalmol. 2018;66(10):1395–1400. [PubMed: 30249822]
41. Bowen RC, Hansell S, Raval V, et al. Uveal melanoma: refusal of treatment. Ocul Oncol Pathol. 2021;7(5):361–367. [PubMed: 34722493]
42. Shields CL, Sioufi K, Robbins JS, et al. Large uveal melanoma (>/=10 mm thickness): clinical features and millimeter-by-millimeter risk of metastasis in 1311 cases. The 2018 Albert E. Finley Lecture. Retina. 2018;38(10):2010–2022. [PubMed: 29528980]

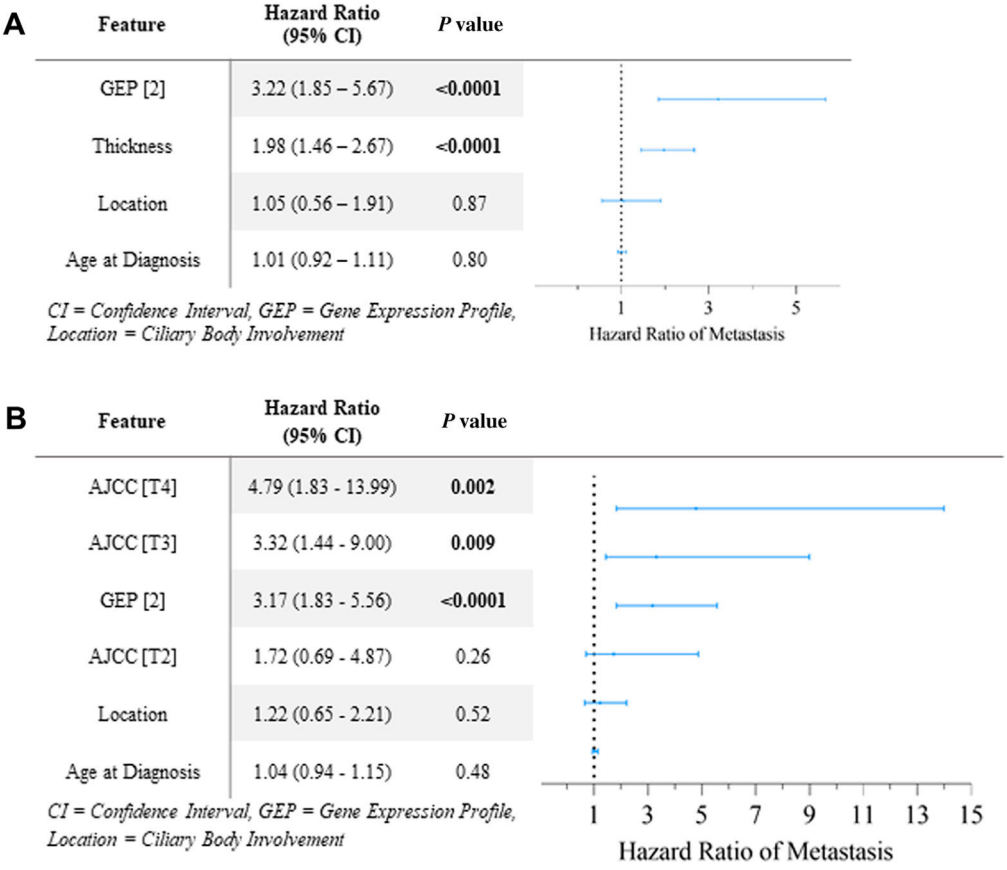

Figure 2.

Forest plots showing hazard ratios for metastasis. Cox proportional hazard regression models were used to identify risk of metastasis. Covariates included (**A**) age at diagnosis, ciliary body involvement, gene expression profile (GEP) class, and tumor thickness or (**B**) American Joint Committee on Cancer (AJCC) staging. Error bars represent 95% confidence intervals (CIs). The dotted line indicates a hazard ratio of 1. P values of statistical significance are bolded.

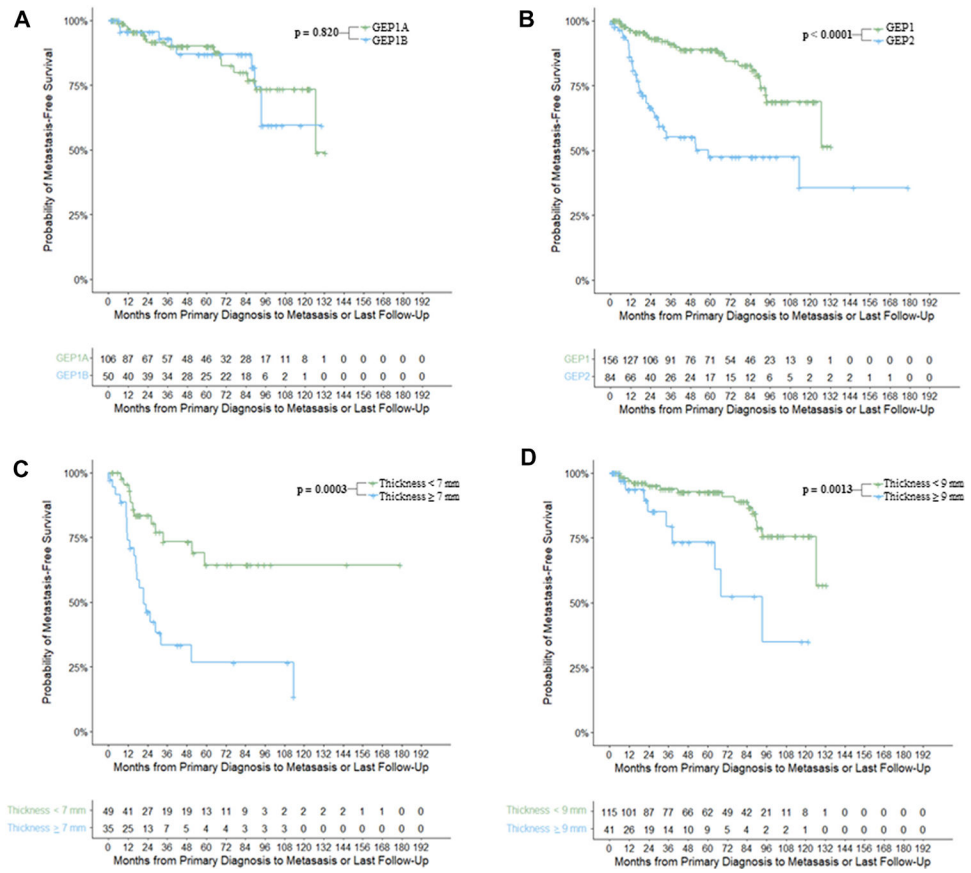

Figure 3.

Graphs showing probability of survival stratified based on (**A**) gene expression profiling (GEP) classes 1A and 1B, (**B**) GEP classes 1 and 2, (**C**) thickness in GEP class 2 tumors using a thickness threshold of 7.0 mm, and (**D**) thickness in GEP class 1 tumors using a thickness threshold of 9.0 mm. Statistical significance determined using 2-sided log-rank test.

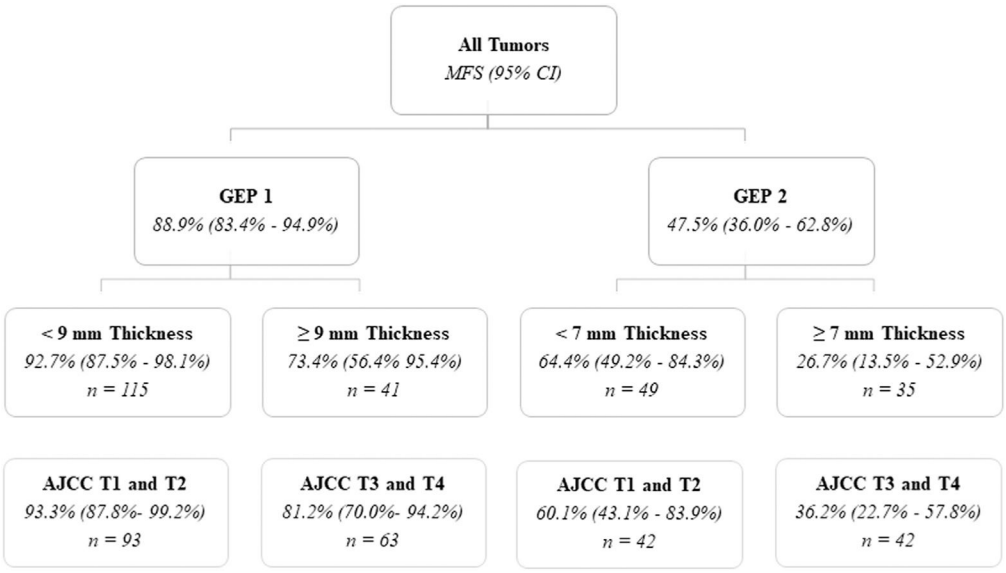

Figure 4.

Flow diagram showing rate of metastasis-free survival (MFS) at 5 years with 95% confidence interval (CI) for patients with tumors stratified based on gene expression profile (GEP) classification and either tumor thickness or American Joint Committee on Cancer (AJCC) staging.

Table 1.

Demographics and Clinical Characteristics of the Study Cohort

Variable	No.	Statistics
Age at diagnosis (yrs), mean ± SD	337	61.8 ± 15.6
Sex, no. (%)		
Male		187, (55.5)
Female		150, (44.5)
Time from primary diagnosis to		
Metastasis		37.2 ± 40.2, 21.4
Last follow-up		55.0 ± 49.3, 43.8
Tumor size (mm), mean ± SD, median		
Thickness (height)		6.17 ± 4.0, 5.0
Largest basal diameter		12.5 ± 4.9, 12
T staging AJCC criteria, no. (%)		
T1		95, (28.2)
T2		100, (29.7)
T3		102, (30.3)
T4		40, (11.9)
GEP classification, no. (%)	241	
GEP 1A		106, (44.0)
GEP 1B		50, (20.7)
GEP 2		85, (35.3)
Primary treatment, no. (%)	320	
Radiation therapy		239, (74.7)
Enucleation		81, (25.3)
Ciliary body involvement, no. (%)		
Yes		74, (22.0)
Metastasis, no.		
Yes		87
Institution, no. (%)		
Yale		191, (56.7)
UCSD		96, (28.5)
MSKCC		50, (14.8)

AJCC = American Joint Committee on Cancer; GEP = gene expression profile; MSKCC = Memorial Sloane Kettering Cancer Center; SD = standard deviation; UCSD = University of California San Diego.

Table 3.

Backward Stepwise Regression Selection Models Evaluating the Relationship between Metastasis and Patients and Tumor Characteristics

Wald Statistic *P* value	**GEP [2]**	**GEP [1B]**	**Thickness**	**AJCC T2**	**AJCC T3**	**AJCC T4**	**Age at Diagnosis**	**Ciliary Body Involvement**
43.15 < 0.0001	3.35 (1.81–6.47) **0.0002**	1.31 (0.55–2.97) 0.529	1.86 (1.35–2.53) **0.0001**				1.01 (0.99–1.03) 0.488	1.15 (0.61–2.10) 0.657
42.88 < 0.0001	3.42 (1.86–6.56) **0.0001**	1.31 (0.55–2.97) 0.524	1.89 (1.40–2.55) < **0.0001**				1.01 (0.99–1.03) 0.526	
42.32 < 0.0001	3.56 (1.97–6.76) < **0.0001**	1.28 (0.54–2.90) 0.559	1.88 (1.39–2.53) < **0.0001**					
40.67 < 0.0001			2.24 (1.74–2.86) < **0.0001**					
38.30 <0.0001	3.31 (1.80–6.39) **0.0002**	1.14 (0.48–2.59) 0.756		1.72 (0.69–4.86) 0.268	3.29 (1.43–8.95) **0.010**	4.79 (1.83–14.00) **0.002**	1.01 (0.99–1.03) 0.468	1.22 (0.65–2.20) 0.527
38.12 <0.0001	3.41 (1.87–6.54) **0.0001**	1.15 (0.48–2.62) 0.739		1.73 (0.70–4.90) 0.261	3.38 (1.47–9.16) **0.008**	5.2 (2.00–14.69) **0.001**	1.01 (0.99–1.03) 0.533	
37.68 <0.0001	3.53 (1.95–6.71) **<0.0001**	1.12 (0.47–2.52) 0.790		1.81 (0.73–5.07) 0.222	3.42 (1.49–9.26) **0.007**	5.11 (2.00–14.65) **0.001**		
36.95 <0.0001				1.33 (0.66–2.77) 0.426	3.46 (1.86–6.85) **0.0002**	6.18 (3.11–12.75) < **0.0001**		
24.23 < 0.0001	3.92 (2.18–7.42) < **0.0001**	1.14 (0.48–2.58) 0.750						

Hazard ratio with 95% confidence intervals and Wald test statistic reported for each model. Boldface indicates statistical significance.

A backward stepwise regression of cox-proportional hazards model evaluating the relationship between metastasis and GEP classification, tumor thickness, AJCC T stage, subject age at diagnosis, and ciliary body involvement. Includes Wald test statistic and associated *P* values for the model as a whole, and hazard ratios, 95% confidence intervals, and *P* values for each factor included in the model.

AJCC = American Joint Committee on Cancer; GEP = gene expression profile.