Anatomy and Pathology/Oncology

PRiMeUM: A Model for Predicting Risk of Metastasis in Uveal Melanoma

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PURPOSE. To create an interactive web-based tool for the Prediction of Risk of Metastasis in Uveal Melanoma (PRiMeUM) that can provide a personalized risk estimate of developing metastases within 48 months of primary uveal melanoma (UM) treatment. The model utilizes routinely collected clinical and tumor characteristics on 1227 UM, with the option of including chromosome information when available.

METHODS. Using a cohort of 1227 UM cases, Cox proportional hazard modeling was used to assess significant predictors of metastasis including clinical and chromosomal characteristics. A multivariate model to predict risk of metastasis was evaluated using machine learning methods including logistic regression, decision trees, survival random forest, and survival-based regression models. Based on cross-validation results, a logistic regression classifier was developed to compute an individualized risk of metastasis based on clinical and chromosomal information.

RESULTS. The PRiMeUM model provides prognostic information for personalized risk of metastasis in UM. The accuracy of the risk prediction ranged between 80% (using chromosomal features only), 85% using clinical features only (age, sex, tumor location, and size), and 85% (clinical and chromosomal information). Kaplan-Meier analysis showed these risk scores to be highly predictive of metastasis (P < 0.0001).

CONCLUSIONS. PRiMeUM provides a tool for predicting an individual’s personal risk of metastasis based on their individual and tumor characteristics. It will aid physicians with decisions concerning frequency of systemic surveillance and can be used as a criterion for entering clinical trials for adjuvant therapies.

Keywords: uveal melanoma, metastasis, prediction model, prognostic features, ocular cancer

Uveal melanoma (UM) is an aggressive ocular tumor associated with loss of vision and high morbidity.1 The population-based mortality rate is quoted as high as 50%2 within an interval of 4 to 5 years, primarily a result of metastasis to the liver. However, the personal risk for metastasis varies for each individual because of the fact that all tumors carry unique combinations of features. Our previous studies, and those of others, have shown that clinical characteristics, including male sex, older age, larger tumor diameter and thickness, and ciliary body involvement can increase the risk of metastasis.3–9 Risk is also increased by the presence of chromosome 3 monosomy, loss of chromosome 1p or 8p, or gain of 8q, or decreased by the presence of chromosome 3 monosomy, loss of chromosome 1p or 8p, or gain of 8q, or decreased by the gain of 6p in the tumors.4–9,16 Dogrusöz et al.17 have recently shown that the prognostic value of the American Joint Committee on Cancer (AJCC) Tumor-Node-Metastasis staging system18 can be improved by adding chromosome 3 and 8q status. Somatic mutations in BAP1 and SF3B1, and PRAME expression have also been associated with increased metastatic risk, whereas mutations in EIF1AX have been shown to be protective.19–21

Models designed to predict either survival or risk of metastasis following treatment for UM based on gene expression profiling (GEP),22–26 or clinical and tumor characteristics, and cytogenetic risk factors27–29 have been described. The GEP model uses the expression profile of a 12-gene panel in combination with tumor diameter to classify UM into low (class 1A and 1B) or high (class 2) risk of metastasis.22–26 Using this GEP model, the 5-year actuarial metastasis-free survival estimate for class 1 tumors was 97% or 90%, depending on whether the basal diameter was less or greater than 12 mm. For class 2 tumors, the survival estimate was 90% for tumors having a basal diameter less than 12 mm and 30% for those greater than 12 mm.25 An alternative model, Liverpool Uveal Melanoma Prognosticator Online (LUMPO)27,28 utilizes a set of clinical, histological and chromosomal features to estimate relative overall survival in individuals with UM relative to an age and sex matched British population and adjusted for risk from death due to all other causes.20–29 This model has recently been validated in an independent cohort of 390 UM individuals from University of California-San Francisco (C-index = 0.72).29

The aim of this study was to develop a web based tool titled Predicting Risk of Metastasis in Uveal Melanoma (PRiMeUM), which provides an individualized prediction of risk of metastasis from UM within 48 months following treatment.
When compared with previously established models, this risk prognostication integrates clinical and tumor characteristics that are routinely obtained as part of the clinical work-up and can be refined by chromosome 3, 1p, 6, and 8 copy number status when known. This personalized estimate of risk of developing metastases within 48 months of treatment can be generated with or without chromosomal copy number information and does not require extrinsic population-based survival statistics. PRiMeUM output provides information that is important for the patient and helps physicians decide on a more individualized plan of care and treatment.

**METHODS**

**UM Cohort**

The dataset consisted of 1227 UM cases managed by the Ocular Oncology Service at Wills Eye Hospital, Philadelphia, Pennsylvania between December 1985 and March 2016. Cases included in this study were drawn from those submitted to the Genetic Diagnostic Laboratory, University of Pennsylvania. Inclusion was based on the availability of complete clinical information and information on whether metastases had occurred within a known follow-up period. The Wills Eye Hospital Ocular Oncology Service sees approximately 9 to 10 new patients per week, or 475 per year. Based on the estimate of 2000 new UM cases per year in the United States, this number represents approximately 24% of new cases each year. Approximately 57% of the cases included in the dataset used to create the PRiMeUM model are regional to Philadelphia (PA, NJ, NY, MD, and DE). 40% are national (largely from VA, TX, FL, NC, MI, SC, GA, and IL), and 3% are international. This dataset reflects closely the geographic distribution of patients seen by the Ocular Oncology Service. Because UM is a rare tumor, there are a limited number of specialty ocular oncology centers where most of the affected cases are treated. In our dataset, in comparing cases with less than and greater than 48 months follow-up, there is no significant difference in the distribution of cases based on regional, national, or international residential addresses (P = 0.23). More than 50% of the cases in this study come from a large radius along the east coast of the United States, suggesting a bias in terms of the residential addresses. Hence, although we note that there is a regional bias, it will likely be the same for every specialty center that treats UM.

Archived and fresh tumor samples collected following enucleation or fine-needle aspiration biopsy (FNAB) were submitted to the Genetic Diagnostic Laboratory for chromosomal copy number analysis. Information on age at time of UM diagnosis, sex, tumor location and size, and information on UM metastasis and follow-up time was obtained by a retrospective review of medical charts for all individuals. Tumor dimensions were determined at the time of initial diagnosis. Informed consent for the use of excess tissue and relevant information for research purposes was obtained from all individuals who submitted samples for chromosomal testing. This research was approved by the Institutional Review Board of the University of Pennsylvania.

**DNA Extraction and Determination of Chromosomal Alterations**

Genomic DNA was isolated from archived or fresh-frozen tumor samples and FNABs, and whole genome copy number status determined as previously described. Chromosome 3 copy number was available for 1158 tumors, whereas chromosome 1p, 6, and 8 status were available for a subset of 688 tumors. Tumors having a pattern of mixed chromosome disomy and monosomy likely as a result of tumor heterogeneity were categorized as mosaic.

**Data for Analysis**

Tumors were labeled “metastasis free” when there were no metastases within 48 months of primary UM treatment (n = 593, 48%), and “metastasis positive” (n = 204, 17%) for those cases where systemic tumors were identified within 48 months of treatment. Cases with less than 48 months follow-up were considered “unlabeled” (n = 430, 35%). Because our goal was to create a predictive model for metastasis within 48 months, this third group was considered unlabeled data for the classification task.

Data available for all 1227 cases included the following features: age and sex, tumor location (choroidal [CH], ciliary body [CB], ciliochoroidal [CB-CH], iris, iris-CB, and iris CB-CH), diameter, and thickness (mm). Chromosome 3 status (disomy, monosomy, partial monosomy, or mosaic) was available for 1158 tumors. The copy number status of 1p, 6p, 6q, 8p, and 8q (disomy, gain, or loss) was available for a subset of 688 tumors. Based on the availability of chromosome information, all tumors (labeled and unlabeled) were divided into three categories and were used to generate three related models (Table 1): SET1 tumors had clinical and tumor characteristics but no chromosome information (N = 1227 total), SET2 had SET1 features plus chromosome 3 status (n = 1158), and SET3 had SET2 features plus chromosome 1p, 6p, 6q, 8p, and 8q data (n = 688). SET0 used chromosomal information only to demonstrate the improvement in the accuracy of the prognostication provided by inclusion of clinical data (n = 688).

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**Table 1. Clinical, Tumor, and Chromosomal Features Used in the Three Datasets Used to Train the PRiMeUM Models**

<table>
<thead>
<tr>
<th>Model</th>
<th>Variables Used</th>
<th>Total Number of UM Cases Available to Train Model</th>
<th>Labeled UM Cases*</th>
<th>Unlabeled UM Cases†</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>SET0: chromosome 3, 1p, 6, and 8 copy number</td>
<td>688</td>
<td>381</td>
<td>307</td>
</tr>
<tr>
<td>1</td>
<td>SET1: age and sex, tumor location, diameter and thickness (mm)</td>
<td>1227</td>
<td>797</td>
<td>430</td>
</tr>
<tr>
<td>2</td>
<td>SET2: age and sex, tumor location, diameter and thickness (mm) plus chromosome 3 copy number</td>
<td>1158</td>
<td>757</td>
<td>401</td>
</tr>
<tr>
<td>3</td>
<td>SET3: age and sex, tumor location, diameter and thickness (mm) plus chromosome 3, 1p, 6 and 8 copy number</td>
<td>688</td>
<td>381</td>
<td>307</td>
</tr>
</tbody>
</table>

* Labeled UM cases had either developed metastases within 48 months or were metastasis free for at least 48 months following treatment.
† Unlabeled cases were metastasis free, but with a follow-up time of less than 48 months following treatment.
Continuous features (age and tumor diameter and thickness) were compared using Mann-Whitney U test, and discrete features (sex, tumor location, and chromosome copy number) using Fisher exact tests (vassarstats.net). Cox univariate and multivariate proportional hazard regression was used to determine association with metastasis (SPSS 24, IBM, New York, NY, USA). When building the models, discrete attributes were turned into binary features, whereas age, diameter, and thickness were scaled and used as continuous variables. The information content describes the mutual information between a discrete feature (e.g., metastasis yes/no) and a dichotomized continuous feature for a given threshold (e.g., tumor diameter). The P value was computed using a two-sided Fisher exact test.

Classification Algorithm

For the classification task, both linear (logistic regression) and nonlinear models were tested (see Supplementary Methods), and the simpler logistic regression model was chosen to build the prognostic classifier. It was evaluated using 10-fold cross validation repeated 10 times using random permutation of the case cohort. The logistic regression linear model was fit by coordinate descent using the Liblinear package. This allowed only 797 labeled samples to be used for both training and testing. We also utilized the 430 unlabeled samples by training the algorithm in a two-step approach using the cross entropy loss function (see Supplementary Methods). The algorithm was then retrained using both the labeled data and the predicted (probabilistic) labels for the unlabeled data. Including the 430 unlabeled cases with the two-step approach resulted in a slight improvement in the accuracy. The accuracy of the model was estimated using the area under the curve (AUC).

To develop a personalized risk score (PRS) based on the individual’s clinical characteristics and chromosomal features of the tumor, we transformed the logistic regression output (termed ‘raw model score’ throughout) using a local positive prediction value approach based on the labeled cases only. For this purpose, we defined a score’s neighborhood to include the scores ±5% around the given score’s percentile. Thus, for each individual, we considered all other raw model scores within ±5% of that individual. The PRS was then calculated as the fraction of metastasis in those individuals and used as the individualized risk score for that individual. The individual risk of metastasis is therefore influenced by the individual’s raw model score and by the rate of metastasis for individuals in our cohort with a similar raw model score. For example, if an individual’s score was at the 78th percentile of the scores in the population, we computed the expected PRS based on the scores in the 73rd to 83rd percentiles. The Kaplan-Meier survival method was used to visualize metastasis-free survival with five equal sized categories of individualized risk scores (SPSS 24, IBM).

Webtool Implementation

The PRiMeUM webtool is located on Galaxy server free to access at https://primeum.biociphers.org/. Users can enter clinical and chromosomal data (when available) to generate a personalized risk estimate based on the PRS (Figs. 1A, 1B). Estimates of the standard error of the individual risk score were based on the 10 iterations of 10-fold cross validation described above.

RESULTS

UM Individual and Tumor Characteristics

The cohort used to train and validate the PRiMeUM prognostication model comprised 1227 samples described in Table 2. These included 593 individuals without metastases within 48 months of diagnosis based on the input features. PRiMeUM can be accessed at https://primeum.biociphers.org/.

Continuous features (age and tumor diameter and thickness) were compared using Mann-Whitney U test, and discrete features (sex, tumor location, and chromosome copy number) using Fisher exact tests (vassarstats.net). Cox univariate and multivariate proportional hazard regression was used to determine association with metastasis (SPSS 24, IBM, New York, NY, USA). When building the models, discrete attributes were turned into binary features, whereas age, diameter, and thickness were scaled and used as continuous variables. The information content describes the mutual information between a discrete feature (e.g., metastasis yes/no) and a dichotomized continuous feature for a given threshold (e.g., tumor diameter). The P value was computed using a two-sided Fisher exact test.
Table 2. Cox Univariate and Multivariate Regression Analysis Correlating the Incidence of Metastasis in 1227 UM Cases (SET1) With Clinical and Tumor Features Plus Chromosome 3, 1p, 6, and 8 Copy Number Status When Known

<table>
<thead>
<tr>
<th>Variable</th>
<th>Total (Frequency)</th>
<th>Unlabeled</th>
<th>Labeled</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex</td>
<td></td>
<td>No. Metastases, Follow-Up Period</td>
<td>No. Metastases Within 48 Months Following Treatment</td>
</tr>
<tr>
<td>Female</td>
<td>621 (0.51)</td>
<td>192 (0.45)</td>
<td>299 (0.50)</td>
</tr>
<tr>
<td>Male</td>
<td>606 (0.49)</td>
<td>238 (0.55)</td>
<td>294 (0.50)</td>
</tr>
<tr>
<td>Age (months, median, mean ± SD, range)</td>
<td>59.0, 57.1 ± 14.7, 7–95</td>
<td>59.4, 57.6 ± 16.5, 10–95</td>
<td>57.0, 55.6 ± 13.2, 7.0–87</td>
</tr>
<tr>
<td>Basal diameter (median, mean ± SD, range, mm)</td>
<td>12.0, 11.6 ± 4.4, 1.5–50</td>
<td>12.0, 11.5 ± 4.2, 3.5–24.0</td>
<td>10.0, 10.4 ± 3.6, 1.5–20.0</td>
</tr>
<tr>
<td>Thickness (median, mean ± SD, range, mm)</td>
<td>4.4, 5.4 ± 3.5, 0.5–40</td>
<td>4.4, 5.4 ± 3.1, 0.6–29.0</td>
<td>3.6, 4.6 ± 2.7, 1.0–14.0</td>
</tr>
<tr>
<td>Location of tumor Ch</td>
<td>960 (0.782) 340 (0.79) 495 (0.84) 125 (0.61)</td>
<td>Reference</td>
<td>Reference</td>
</tr>
<tr>
<td>CB</td>
<td>25 (0.02)</td>
<td>6 (0.014)</td>
<td>15 (0.025)</td>
</tr>
<tr>
<td>CB-CH</td>
<td>170 (0.14)</td>
<td>50 (0.12)</td>
<td>54 (0.091)</td>
</tr>
<tr>
<td>Iris</td>
<td>35 (0.029)</td>
<td>17 (0.040)</td>
<td>15 (0.025)</td>
</tr>
<tr>
<td>Iris-CB and Iris-CB-CH</td>
<td>37 (0.031)</td>
<td>17 (0.040)</td>
<td>14 (0.024)</td>
</tr>
</tbody>
</table>

Bold text indicates significance P < 0.05. HR, hazard ratio.

* Median interval metastasis free in 430 UM with follow-up < 48 months (mean ± SD, range) = 22.0 (23.2 ± 13.2, 1–47 months).
† Median interval metastasis free follow-up time in 593 UM (mean ± SD, range) = 63.0 (68.7 ± 18.8, 48–191 months).
‡ Median time to metastases for 204 UM (mean ± SD, range) = 19 (21.5 ± 13.3, 0–48 months).
§ P value derived from Cox proportional hazards models using clinical and tumor features plus chromosome 3 (Table 3), 1p, 6q, 8p, and 8q (Table 4) copy number status.
¶ Features that are significant in both the univariate regression analysis and information content analysis shown in Figure 2 and Supplementary Figure S1.
TABLE 3. Cox Univariate and Multivariate Regression Analyses Correlating the Incidence of Metastasis in 1158 UM Cases (SET2) for Which Chromosome 3 Copy Number Status Was Available

<table>
<thead>
<tr>
<th>Variable</th>
<th>Total (Frequency)</th>
<th>Unlabeled</th>
<th>Labeled</th>
<th>Univariate Logistic Regression HR (95% CI), P Value</th>
<th>Multivariate Logistic Regression HR (95% CI), P Value*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N = 1158</td>
<td>N = 401</td>
<td>N = 558</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Chromosome 3</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Disomy</td>
<td>543 (0.47)</td>
<td>197 (0.49)</td>
<td>313 (0.56)</td>
<td>33 (0.17) Reference</td>
<td>Reference</td>
</tr>
<tr>
<td>Monosomy</td>
<td>398 (0.34)</td>
<td>130 (0.32)</td>
<td>131 (0.23)</td>
<td>137 (0.69) 7.26 (4.96–10.62), &lt;0.001 2.32 (1.35–3.98) 0.002</td>
<td></td>
</tr>
<tr>
<td>Partial monosomy</td>
<td>108 (0.095)</td>
<td>38 (0.095)</td>
<td>51 (0.091)</td>
<td>19 (0.099) 3.10 (1.76–5.45), &lt;0.001 2.10 (0.89–4.97), 0.092</td>
<td></td>
</tr>
<tr>
<td>Mosaic</td>
<td>109 (0.094)</td>
<td>36 (0.090)</td>
<td>63 (0.11)</td>
<td>10 (0.050) 1.53 (0.75–3.10), 0.24 4.07 (1.17–14.13), 0.027</td>
<td></td>
</tr>
</tbody>
</table>

Bold text indicates significance P < 0.05.
* P value derived from Cox proportional hazards models using clinical and tumor features (Table 2) and chromosome 3 copy number status.

Feature Analysis

In line with previous studies,3–9,11 Cox univariate analysis showed that male sex (P = 0.007), older age (P < 0.001), tumor basal diameter and thickness (both P < 0.001), ciliochoroidal location (CB-CH, P < 0.001), and chromosome 3 monosomy or partial monosomy (P < 0.001) were all significantly associated with increased incidence of metastases (Tables 2 and 3). Copy number statuses of chromosomes 1p, 6, and 8 were included because of their previously described association with metastasis in UM1,10–12 as follows: 1p loss (P < 0.001), 6p loss (0.026), 6q loss (P < 0.001), 8p loss (P < 0.001), and 8q

months of treatment (48%), 204 with metastases within this time period (17%), and 430 with no known metastases (35%), but with follow-up time less than 48 months. The total number of tumors originally considered for analysis included 250 that had metastasized, 204 (82%) within 48 months following initial treatment. To maximize the number of labeled metastasis-free cases while ensuring greater accuracy in the prognostic model, 48 months was chosen as the cut-off for defining the metastasis-free label. The remaining 46 (18%) metastasized between 49 and 133 months and were not included in this study (see Supplementary Methods).

TABLE 4. Cox Univariate and Multivariate Regression Analyses Correlating the Incidence of Metastasis in 688 UM Cases (SET3) for Which Chromosomes 3, 1p, 6, and 8 Copy Number Status Was Available

<table>
<thead>
<tr>
<th>Variable</th>
<th>Total (Frequency)</th>
<th>Unlabeled</th>
<th>Labeled</th>
<th>Univariate Logistic Regression HR (95% CI), P Value</th>
<th>Multivariate Logistic Regression HR (95% CI), P Value*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N = 688</td>
<td>N = 307</td>
<td>N = 153</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Chromosome 1p</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Disomy</td>
<td>571 (0.830)</td>
<td>269 (0.88)</td>
<td>199 (0.87)</td>
<td>103 (0.67) Reference</td>
<td>Reference</td>
</tr>
<tr>
<td>Loss</td>
<td>117 (0.170)</td>
<td>38 (0.12)</td>
<td>29 (0.13)</td>
<td>50 (0.35) 2.75 (1.96–3.85), &lt;0.001 1.51 (1.05–2.18), 0.028</td>
<td></td>
</tr>
<tr>
<td>Chromosome 6p</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Disomy</td>
<td>490 (0.712)</td>
<td>214 (0.70)</td>
<td>157 (0.69)</td>
<td>119 (0.78) Reference</td>
<td>Reference</td>
</tr>
<tr>
<td>Gain</td>
<td>196 (0.285)</td>
<td>93 (0.30)</td>
<td>71 (0.31)</td>
<td>32 (0.21) 0.66 (0.45–0.97), 0.036 0.49 (0.29–0.81), 0.006</td>
<td></td>
</tr>
<tr>
<td>Loss</td>
<td>2 (0.003)</td>
<td>0</td>
<td>0</td>
<td>2 (0.013) 4.89 (1.20–19.86), 0.026 0.51 (0.09–2.85), 0.44</td>
<td></td>
</tr>
<tr>
<td>Chromosome 6q</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Disomy</td>
<td>544 (0.791)</td>
<td>249 (0.81)</td>
<td>186 (0.82)</td>
<td>109 (0.71) Reference</td>
<td>Reference</td>
</tr>
<tr>
<td>Gain</td>
<td>24 (0.035)</td>
<td>9 (0.029)</td>
<td>13 (0.057)</td>
<td>2 (0.013) 0.32 (0.08–1.30), 0.11 0.69 (0.16–3.08), 0.63</td>
<td></td>
</tr>
<tr>
<td>Loss</td>
<td>120 (0.174)</td>
<td>49 (0.16)</td>
<td>29 (0.13)</td>
<td>42 (0.28) 1.91 (1.34–2.72), &lt;0.001 1.47 (0.94–2.32), 0.093</td>
<td></td>
</tr>
<tr>
<td>Chromosome 8p</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Disomy</td>
<td>493 (0.717)</td>
<td>245 (0.80)</td>
<td>180 (0.79)</td>
<td>68 (0.44) Reference</td>
<td>Reference</td>
</tr>
<tr>
<td>Gain</td>
<td>74 (0.108)</td>
<td>25 (0.081)</td>
<td>27 (0.11)</td>
<td>22 (0.14) 2.21 (1.36–3.57), 0.001 1.14 (0.67–1.94), 0.63</td>
<td></td>
</tr>
<tr>
<td>Loss</td>
<td>121 (0.176)</td>
<td>37 (0.12)</td>
<td>21 (0.092)</td>
<td>63 (0.41) 5.49 (3.88–7.75), &lt;0.001** 2.14 (1.43–3.22), 0.001</td>
<td></td>
</tr>
<tr>
<td>Chromosome 8q</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Disomy</td>
<td>355 (0.513)</td>
<td>183 (0.60)</td>
<td>144 (0.63)</td>
<td>26 (0.17) Reference</td>
<td>Reference</td>
</tr>
<tr>
<td>Gain</td>
<td>355 (0.487)</td>
<td>124 (0.40)</td>
<td>84 (0.37)</td>
<td>127 (0.83) 6.42 (4.21–9.80), &lt;0.001** 1.14 (1.23–3.73), 0.007</td>
<td></td>
</tr>
</tbody>
</table>

Bold text indicates significance P < 0.05.
* P value derived from Cox proportional hazards models using clinical and tumor features (Table 2) and chromosome 3 copy number status.
gain \((P < 0.001)\) were associated with increased risk of metastasis, whereas 6p gain was associated with a decreased risk \((P = 0.036; \text{Table 4})\). These results were largely recapitulated using an alternative approach using information content analysis to assess statistical enrichment for any of these variables with metastatic outcome (two-sided Fisher exact test, see nodes in Fig. 2). Sex was the only variable that did not replicate the findings from the univariate Cox models (Table 2). We also assessed the statistical enrichment between all pairs of variables (see edges in Fig. 2). There was a significant association between tumor thickness, diameter, and location. Indeed, tumor thickness had the highest number of significant associations \((n = 6)\) with other variables, including chromosome 3 monosomy. This finding was consistent when we considered the additional chromosomal features in Set 3 (Supplementary Fig. S1). In general, chromosomal features were highly associated with one another, as indicated by thicker edges in Figure 2 and Supplementary Figure S1, whereas tumor thickness had many weak associations with clinical and chromosomal features (thin lines). For example, we observe a significant association between monosomy-3 and chromosome 8q gain \((P = 0.002)\). Given these complex interactions in our feature space, we focused on developing a multivariate model.

We then evaluated the concept that interaction of multiple features contribute to risk of metastasis with a Cox multivariate model (Table 2), which included all 11 features. Male sex \((P < 0.001)\), tumor diameter \((P < 0.001)\), chromosome 3 monosomy \((P = 0.027)\), 1p loss \((P = 0.028)\), 8p loss \((P = 0.001)\), 8q gain \((P = 0.007)\), and 6p gain (protective, \(P = 0.006)\) remained significantly associated with metastasis after adjusting for the effects of all other features. Iris-CB or iris CB-CH \((P = 0.008)\) tumor location and a mosaic chromosome 3 pattern \((P = 0.027)\) were also significant despite not being significant in the univariate analysis. These 11 features were therefore used in building an algorithm to yield precise personalized prediction of risk of metastasis within 48 months following initial treatment.

**Development and Implementation of the PRIMeUM Model**

To develop a multivariate prognostic classifier, we next evaluated machine learning algorithms. We tested a number of alternative models, including a mixture of decision trees, logistic regression, standard Cox survival models, and random survival trees \(^{34}\) (see Supplementary Methods). We found that for our dataset, boosting over decision trees \(^{35}\) and logistic regression had similar performance, whereas the other models suffered from lower accuracy or higher variability (data not
shown). We therefore selected the simpler logistic regression model to build our prognostic classifier.

Our basic model that used clinical and tumor features had an accuracy of 83% for predicting risk of metastasis at 48 months following diagnosis (Fig. 3A, SET1). Adding chromosome 3 information to the basic model increased its accuracy to 85% (SET2). Adding chromosomes 1p, 6, and 8 data (SET3) did not increase the accuracy beyond 85% despite all features being informative. This was probably a result of the smaller number of cases used to train the latter model. Figure 2A also demonstrated that, relative to an accuracy of 79.6% for SET0 features (chromosomal information only), a 3% to 6% increase in the accuracy of the prediction of risk of metastasis was achieved by adding clinical (age and sex) and tumor (size and location) information to chromosomal information (SET2 and SET3, respectively).

As seen in Figure 3B, the PRS based on the 797 labeled cases corresponded well with the raw model score (red line) generated by the logistic regression model. For example, individuals with a raw model score of 0.13 were in the 40% to 45% percentile group, where 4 of 38 individuals developed metastasis. This translated to a predicted risk of metastasis of 10.9% within 48 months. This PRS approach captured the large training cohort available for our study and enabled clear clinical interpretation of the model output compared to using the output of the logistic regression model alone.

Critical, the individualized PRS were highly predictive of metastasis as shown by Kaplan-Meier analysis in Figure 4. Cases were split into five groups based on their individualized risk score, which resulted in significant discrimination for risk of metastasis \( P < 0.0001 \). These results indicated that 50% of patients fell in the lowest risk group (0%-20%) and were unlikely to develop metastasis within 48 months, whereas a risk score >60% was associated with a particularly poor prognosis.

**PRiMeUM Web-Based Tool**

To translate the metastasis prediction model into a tool for clinical use, we developed the PRiMeUM web tool. The PRiMeUM model was trained using the entire cohort of 1227 UM to maximize accuracy as opposed to the 10-fold cross validation approach described above. Based on a Galaxy server, PRiMeUM allows clinicians to input clinical and/or chromosomal data and provides the PRS score as an estimate of the individualized risk of metastasis within 48 months. The input page requires the individual’s age and sex and tumor diameter, thickness, and location. Optional fields include chromosome 3 plus 1p, 6p, 6q, 8p, and 8q status, if available (Fig. 1A). The output from executing PRiMeUM is presented in a downloadable and printable document for clinicians to easily interpret and share with their patients (Fig. 1B). Table 5 shows examples of PRiMeUM’s PRS for different combinations of features, indicating how changing sex, tumor size, or location or the addition of chromosome 3, 1p, 6, and 8 statuses can change the predicted metastatic risk score. Profiles presented in rows 1 to 3 show that adding chromosome 3, 1p, 8p, and 8q information to a small, choroidal tumor increased the risk score from 12% to 51%. Alternatively, in rows 5 to 7 it can be seen...
that a large choroid tumor with ciliary body involvement had a risk of metastasis of >75%, but adding information about chromosomal status decreased the risk to 24%. The profiles in rows 4 and 8 are similar to those shown in rows 3 and 7, respectively, but with different metastatic outcomes reflecting the model’s inaccuracy rate of 15%, and suggesting the existence of additional risk factors. PRiMeUM provides a tool that simultaneously takes into account a large set of features previously known to contribute individually to the risk of metastasis in a single, combined personalized risk score for metastatic risk.

### DISCUSSION

The aim of precision medicine in cancer is to use clinical profiles of individuals and genetic profiles of tumors for accurate prognostic classification and to facilitate best clinical management paradigms. In the field of UM management, multiple tumor classification models have been adopted that include clinical and tumor characteristics, chromosomal profiles, and/or gene expression profiles. Two recent publications report combining two traditional predictors of metastasis to provide models with increased prognostic value: AJCC staging with chromosome 3 and 8q status17 and GEP with tumor diameter.25

In UM, the overall population risk of metastases is projected to be as high as 50%;2 however, this population risk does not provide a personalized risk for the individual that could be considerably less (or greater) than 50%. In one survey of individuals who underwent UM testing, 97% of participants reported that they would like to receive prognostic information even when no prophylactic adjuvant therapies were available.27 Even if the prognostic result suggested an increased risk of metastasis, the participants indicated a heightened sense of control and reduction in uncertainty and accompanying anxiety.37–39

The estimated risk of metastasis calculated based on the PRiMeUM model is reported in the range between 15% and 75% and takes into account the observed misclassification rate of approximately 15%. The main advantage is that this is an individualized risk estimate, which is different from population-based risk estimates.

In addition to developing a model for individualized risk score, a further motivation for developing PRiMeUM is that in many cases the chromosomal and clinical features are not always correlated. From our experience, there can be small tumors with disomy 3 that metastasize soon after diagnosis, whereas large, monosomy 3 tumors do not.20 These findings can be partially explained by the presence of tumor heterogeneity.40–42 Both PRiMeUM and GEP24 use information gathered from a single-site FNAB or tumor biopsy. Data from Ewens et al.4 and Augsburger et al.43 indicate 10% to 15% discordance between FNAB samples from the same tumor, thus it is likely that tumor heterogeneity may explain a significant proportion of the misclassification rates for both models. Other contributing factors could include additional prognostic factors that are not captured by the model including both genetic and epidemiological factors. For LUMPO,27,28 information derived from cytology, including the cellular nature of the sample and the mitotic index, are included. Because of the small number of cells collected during the FNAB procedure, it was not feasible to collect this information for the current data series and it was not included in the PRiMeUM model.

Another limitation of most prognostic models, including the PRiMeUM, is that not all risk factors have been identified. An increasing number of studies are showing that mutations in genes such as SF3B1, EIF1AX, and expression of PRAME29–44 are associated with increased or decreased risk of metastases. Whole-genome, whole-exome, and cancer panel studies48,49 are also likely to identify additional potential risk (or protective) genes. Although the UM survival rate was not shown to improve between 1973 and 2008, it has been acknowledged that prognostication in UM is evolving as better markers are identified.50,51

The benefits of such a prognostic model are obvious. It empowers the individual with additional, accurate information concerning their future prognosis, can aid the medical oncologist with decisions concerning frequency of systemic surveillance and can be used as a criterion for entering clinical trials for adjuvant therapies. The utility of the PRiMeUM prognostic model, in comparison with the GEP molecular classification model25,26 is that it can be run using data routinely collected at the time of UM treatment and does not require the input of either chromosome copy number status or

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### TABLE 5. Examples of PRiMeUM Individualized UM Metastatic PRS

<table>
<thead>
<tr>
<th>Row</th>
<th>Sex</th>
<th>Age</th>
<th>Location</th>
<th>Diameter, mm</th>
<th>Thickness, mm</th>
<th>Chr 3</th>
<th>Chr 1p</th>
<th>Chr 6p</th>
<th>Chr 6q</th>
<th>Chr 8p</th>
<th>Chr 8q</th>
<th>Personalized Risk of Metastasis Within 48 Months (± STD)</th>
<th>Metastatic Outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Male</td>
<td>72</td>
<td>Choroid</td>
<td>8.0</td>
<td>2.5</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>12% (± 3%) No metastases at 49 months</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>Male</td>
<td>72</td>
<td>Choroid</td>
<td>8.0</td>
<td>2.5</td>
<td>Monosomy</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>22% (± 3%) 49 months</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>Male</td>
<td>72</td>
<td>Choroid</td>
<td>8.0</td>
<td>2.5</td>
<td>Monosomy</td>
<td>Disomy</td>
<td>Disomy</td>
<td>Disomy</td>
<td>Loss</td>
<td>Gain</td>
<td>51% (± 8%) Metastasis at 25 months</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>Male</td>
<td>40</td>
<td>Choroid</td>
<td>8.0</td>
<td>3.3</td>
<td>Monosomy</td>
<td>Disomy</td>
<td>Disomy</td>
<td>Loss</td>
<td>Gain</td>
<td>Gain</td>
<td>45% (± 8%) 60 months</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>Male</td>
<td>62</td>
<td>CH-CB</td>
<td>20.0</td>
<td>13.3</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>&gt; 75% No metastases at 60 months</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>Male</td>
<td>62</td>
<td>CH-CB</td>
<td>20.0</td>
<td>13.3</td>
<td>Disomy</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>59% (± 3%) 60 months</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>Male</td>
<td>62</td>
<td>CH-CB</td>
<td>20.0</td>
<td>13.3</td>
<td>Disomy</td>
<td>Disomy</td>
<td>Gain</td>
<td>Gain</td>
<td>Disomy</td>
<td>Disomy</td>
<td>24% (± 6%) 60 months</td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>Male</td>
<td>65</td>
<td>CH-CB</td>
<td>19.0</td>
<td>13.3</td>
<td>Disomy</td>
<td>Disomy</td>
<td>Gain</td>
<td>Loss</td>
<td>Disomy</td>
<td>Disomy</td>
<td>37% (± 4%) Metastasis at 23 months</td>
<td></td>
</tr>
</tbody>
</table>

Rows 1 to 3 and 5 to 7 present information from case records and indicate the increase or decrease in the PRS depending on whether SET1, 2, or 3 features are included in the model; rows 4 and 8 present case information similar to those in rows 3 and 7, respectively, but with different metastatic outcomes.

* Feature information not included in PRiMeUM input to demonstrate how the risk score changes depending on what information is input into model.
gene expression values. In addition, PRiMeUM is designed to predict the risk of metastasis within 48 months of treatment rather than melanoma-specific mortality that necessitates comparison with the appropriate population-based survival statistics.\textsuperscript{28,29} The results of PRiMeUM are easily interpretable, and it is openly available to physicians and genetic counselors. For the future, the availability of larger data sets with additional labeled individuals and longer follow-up times will further increase the accuracy of the model. However, in trying to achieve this goal, one is limited by the lack of current follow-up information because of the loss of individuals who are followed by their local providers and the observations that lower risk individuals may not return to their original surgical oncologists.

Prognostic models are only as accurate as the data on which they are based and may be collected differently in different centers. They require frequent reassessment, recalibration, and may be collected differently in different types of models for classification were evaluated, including both linear and nonlinear regression. The model was designed such that as additional informative features are identified and as the science of UM evolves, it can be updated to accommodate this new information. It is also possible that with additional features and a larger dataset we could improve the model by revisiting other model designs or trying other nonlinear regression-based approaches. Although the current version of PRiMeUM provides an accuracy rate of approximately 85%, this rate will likely increase in future iterations as additional UM cases having longer follow-up times are incorporated into the training set. Furthermore, the model can easily be extended to incorporate new risk factors that are identified.

In summary, PRiMeUM provides a tool to allow physicians and genetic counselors to estimate an individual’s risk of metastasis with 48 months with an accuracy of 83% when only clinical and tumor characteristics are used and 85% when chromosomal copy number status is known as well. Given that 97% of individuals with UM responded that they wanted prognostic information at the time of treatment,\textsuperscript{37} the general availability of PRiMeUM will provide an important source of information on which to base decisions of UM management and surveillance.

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