## Title

Prognostic Value of BAP1 and Preferentially Expressed Antigen in Melanoma (PRAME) Immunohistochemistry in Uveal Melanomas



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**Prognostic value of BAP1 and PRAME immunohistochemistry in uveal melanomas**

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**Running title:** BAP1 and PRAME stains in Uveal Melanomas

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**ABSTRACT**

Uveal melanoma (UM) is the most common primary intraocular tumor in adults and despite excellent local control, more than 50% of patients develop and die from metastatic disease. Loss of BAP1 nuclear staining, a surrogate marker of BAP1 mutation, and PRAME (PReferentially expressed Antigen in MElanoma) mRNA overexpression, as assessed by qPCR, were shown to correlate with increased metastasis rate in UM. In this study, we demonstrated UM can be successfully risk stratified using a combination of BAP1 and PRAME immunohistochemical (IHC) stains. We retrospectively reviewed 318 uveal melanoma cases with sufficient tissue and performed BAP1 and PRAME IHC to stratify them as BAP1+/PRAME- (group 1, n=135), BAP1+/PRAME+ (group 2, n=43), BAP1-/PRAME- (group 3, n=94) and BAP1-/PRAME+ (group 4, n=46). Increasing study risk group based on loss of BAP1 expression and positive PRAME staining is associated with higher rate of metastasis and disease-specific death, and lower metastasis-free (MFS) and disease-specific survival (DSS). Among tumors with loss of BAP1 staining, PRAME positivity is associated with shorter MFS (p=0.018) and shows a trend towards shorter DSS (p=0.061). Among tumors with retained BAP1 staining, PRAME positivity is associated with shorter MFS and DSS (p=0.001 and p=0.021, respectively). In summary, a combination of BAP1 and PRAME immunohistochemistry can be used for risk stratification of uveal melanomas.

## INTRODUCTION

Uveal melanoma (UM) is the most common primary intraocular malignancy in adults and is associated with poor prognosis and frequent metastasis. [1,2] UM accounts for 3-4% of all melanomas with an incidence of 5-7 per million individuals per year in the western world. [1,3-5] Although most cases can be managed aggressively for local control, approximately 50% of patients develop metastatic disease within the first 15 years of diagnosis, most frequently to the liver. [1-4] The 5-year overall survival is 69-80%; however, following metastasis, the median survival significantly reduces to approximately 6 months to 1 year. [1-3,6,7]

Treatment of primary UM involves radiotherapy and/or surgery and a large randomized trial has shown that the long-term survival was not associated with the treatment modality. [8] Unfortunately, despite significant improvements in diagnosis and understanding of the genetic and epigenetic alterations in UM, there is still no durable cure for metastatic disease. Risk stratification of patients with UM is critical for determining the frequency and length of surveillance for systemic metastasis as patients with high-risk tumors are screened more aggressively and frequently for metastatic disease. [9,10]

Numerous risk factors associated with metastasis and disease-related death have been described ranging from patient demographics to histologic and genomic features of the tumors. Older age at diagnosis, ciliary body involvement, large tumor size, extraocular extension, and epithelioid histology are among the most robust features associated with poor prognosis, many of which are included in the pathologic tumor/node/metastasis (pTNM) classification and the

American Joint Committee on Cancer (AJCC) tumor staging, 8th edition. [11-17] More recently, with advances in molecular techniques, risk stratification based on the genomic landscape of the tumor including presence of certain mutations, chromosomal copy number changes and gene expression profiling has become fairly routine, at least in the developed countries, although access to these modalities maybe more limited world-wide.

Mutually exclusive mutations in the *GNAQ* and *GNA11* genes are an early event in the pathogenesis, followed by additional driver mutations involving *BAP1*, *SF3B1* and *EIF1AX*, and/or chromosomal alterations including monosomy 3.[18-25] Identification of these additional molecular alterations is becoming increasingly important for prognostication and identifying the patients who might benefit from enrolling in an adjuvant clinical trials.[20-25] Loss of BAP1 staining on immunohistochemistry has been associated with mutations and higher metastatic risk and has been proposed as a surrogate marker.[26,27] Alternatively, a clinically validated gene expression profiling (GEP) test using a 15-gene array, has been validated as a prognostic test that assigns tumor samples to Class 1 (low-risk) or Class 2 (high-risk) categories, predicting the propensity for metastasis.[28] Class 2 tumors are strongly associated with inactivation of the *BAP1* tumor suppressor gene and carry a 72% risk of metastasis in 5 years.[27,29,30] Class 1 tumors can be further subdivided into Class 1A (low-risk) and Class 1B (intermediate-risk) with *EIF1AX* and *SF3B1* mutations, with 5-year metastasis rates of 2% and 21%, respectively.[10,21]

Recent studies have highlighted PRAME (PReferentially expressed Antigen in MElanoma) as an independent biomarker for metastasis.[31-33] Overexpression of PRAME was associated with

higher risk of metastasis among Class 1 tumors, and with earlier metastasis among Class 2 tumors.[32,33] Furthermore, PRAME is a neoantigen with limited expression in normal tissue, making it a potential target for immunotherapy.[34,35] However, in all these studies, PRAME expression was assessed by qPCR evaluating mRNA expression, which is currently only available by a commercial lab and its world-wide application might be limited due to access and cost. To the best of our knowledge, there are no studies evaluating the prognostic value of PRAME immunohistochemistry (IHC). The purpose of this study is to evaluate the role of BAP1 and PRAME immunohistochemistry in the prognostic stratification of uveal melanomas.

## MATERIALS AND METHODS

### Case Selection

This study was conducted as a retrospective cohort study in accordance with the Declaration of Helsinki and Good Clinical Practice Guidelines, under the approval of the University of California San Francisco (UCSF) Institutional Review Board with a waiver of patient consent (#20-30753). We searched the Ophthalmic Pathology archives at University of California San Francisco for UMs diagnosed on surgical resection specimens, including iridocyclectomy, enucleation or exenteration, between January 2000 and December 2020, and the cytopathology archives for UMs diagnosed on fine needle aspiration (FNA) biopsy between January 2015 and December 2020. After confirmation of the diagnosis, 357 tumors (318 surgical specimens and 39 FNA cell blocks) from 354 patients with sufficient pathology material were included in the study. Three patients had both cytology and surgical material with concordant results for BAP1 and PRAME. Clinical and molecular data were retrieved from electronic medical records. Histopathologic

features including the tumor size, involvement of ocular structures, presence of extraocular extension, tumor growth pattern, histologic type, mitotic rate, and margin status, were obtained from the pathology report, and/or by review of all available material by a study pathologist (MP).

**Immunohistochemistry**

Immunohistochemical analysis of BAP1 and PRAME was performed using formalin-fixed and paraffin-embedded (FFPE) sections on a combination of tissue microarray (TMA) and whole slide sections. TMAs were created using 2 mm cores in duplicate from areas of viable tumor from cases with sufficient material (n=274). Surgical cases with stains performed as part of clinical care (n=10), cases with limited viable tumor tissue insufficient to create TMA punches (n=34) and all FNA cell blocks (n=39) were included as whole slide sections. Four-micron sections were stained with standard techniques using a mouse monoclonal antibody against BAP1 (clone C-4, 1:100 dilution; Santa Cruz Biotechnology, Dallas, TX), and rabbit monoclonal antibody against PRAME (clone EPR20330, 1:50 dilution; Abcam, Waltham, MA). A uveal melanoma with known PRAME expression (tested by qPCR in a commercial laboratory, Castle Biosciences, Phoenix, AZ), adult testis and cerebral cortex tissues were used as positive controls for PRAME stain. A uveal melanoma with molecularly confirmed truncating *BAP1* mutation is used as negative control, and endothelial cells within all tumors are used as internal positive control for BAP1 stain.

Immunohistochemical stains were scored independently by two authors (LMH, MP) and discrepant cases were re-reviewed until a consensus was reached. Nuclear staining for BAP1

was scored as “lost” if more than 95% of the tumor cells were negative and scored as “retained” if there was staining in 5% or more of tumor cells. Representative examples are provided in Figure 1A and 1B. Only cases with appropriate internal positive control staining (endothelial cells and inflammatory cells) were scored and included in the study. Nuclear staining for PRAME was scored for intensity as negative (0), weak (1+), moderate (2+), and strong (3+) and for proportion in a scale from 0 to 3 (see below). Representative examples are provided in Figures 1 C-F). A total staining score is calculated as sum of the intensity and proportion scores (modified Allred score), and only cases with a total score of 4 or more (any proportion of strong staining, at least patchy moderate staining, and any intensity of diffuse staining) were considered positive for PRAME. Proportion of PRAME staining was scored as follows:

- Negative (0): Negative (n=176 surgical and 23 FNA)
- Focal (1): Staining in less than 10% of tumor cells (n=31 surgical and 5 FNA)
- Patchy (2): Staining in 10-90% of tumor cells (n=77 surgical and 8 FNA)
- Diffuse (3): Staining in greater than 90% or more of tumor cells (n=34 surgical and 3 FNA)

Tumors are grouped based on their BAP1 and PRAME status into four groups as follows:

Risk group 1: BAP1-retained and PRAME-negative (n=135 surgical and 27 FNA)

Risk group 2: BAP1-retained and PRAME-positive (n=43 surgical and 7 FNA)

Risk group 3: BAP1-lost and PRAME-negative (n=94 surgical and 4 FNA)

Risk group 4: BAP1-lost and PRAME-positive (n= 46 surgical and 1 FNA)

**Statistical Analysis**

Statistical analysis was performed in Stata Version 16 (StataCorp, College Station, Texas, USA). Age was the only continuous variable with normal distribution; therefore, descriptive analyses were provided as mean +/- standard deviation for age, and median (range) for all other variables. Comparison of clinical and histopathologic features of risk groups were performed using Pearson Chi-square for categorical variables and using Kruskal-Wallis tests for continuous variables as appropriate. Local recurrence-free survival (LRFS), metastasis-free survival (MFS) and disease-specific survival (DSS) periods were calculated from the time of initial diagnosis to local recurrence or progression that required additional treatment, metastasis documented on imaging, or disease-related death, respectively. Cox proportional hazards univariate models were used to determine whether clinical or histopathologic features, or the risk groups based on BAP1 and PRAME results were associated with LRFS, MFS or DSS. Variables statistically significant for MFS were included in the multivariate model for MFS, excluding variables used to generate other variables in the model (i.e. greatest basal diameter and tumor height were not included as they determine the pTNM stage). Survival curves stratified by pTNM stage or study risk group were generated by Kaplan-Meier method. Patients with surgical and FNA samples were analyzed separately, given the significantly shorter follow-up and fewer numbers of outcome events in patients with FNA material. All *p*-values less than 0.05 were considered statistically significant.

## RESULTS

### Surgical cohort

There were 318 surgical specimens, which can be successfully grouped using BAP1 and PRAME stains and included in the study. BAP1 stain was retained in 178 and lost in 140 cases. Sequencing studies capturing *BAP1* gene were available for 33 tumors (21 *BAP1*-altered) which correlated with BAP1 stain results in 32 tumors. One UM with *SF3B1* mutation lacking a detectable *BAP1* mutation showed loss of BAP1 staining. Gene expression profiling results were available for a different group of 33 tumors (10 Class 1a, 5 Class 1b and 18 Class 2) and all Class 1 tumors showed retained BAP1 staining, and all but one Class 2 tumor showed BAP1 loss.

PRAME was considered positive in 89 (28%) cases, with diffuse-strong (n=27), diffuse-moderate (n=6), diffuse-weak (n=1), patchy-strong (n=23) and patchy-moderate (n=32) staining. Of these 89 cases, 66 (74%) showed staining in 75% or more of the tumor cells with a median of 95% (range 75-99%). The staining ranged from 20% to 60% in the remaining 23 cases (9 BAP1-retained and 14 BAP1-lost). PRAME was completely negative in 176 tumors and show only focal-weak staining in 29, focal-moderate staining in 2, and patchy-weak staining in 22 tumors. PRAME qPCR results were available only for four Class 1 tumors with concordant PRAME stain results in three cases (2 positive and 1 negative). Two PRAME qPCR-positive cases showed 50% and 95% staining in PRAME IHC. One Class 1a UM with positive PRAME qPCR was completely negative on PRAME immunohistochemistry.

Primary treatment was surgical in 225 cases (71%), radiotherapy in 88 cases (28%) and cryotherapy, transpupillary thermotherapy or unknown in 5 cases. Patients who received a primary non-surgical treatment underwent subsequent surgical resection either due to recurrent disease or due to blind painful eye or other complications. Detailed results for each clinical and pathologic feature are summarized in Table 1 and Table 2, respectively.

Briefly, the patients with BAP1-retained tumors (risk groups 1 and 2) were younger than patients with BAP1-negative tumors (risk groups 3 and 4, p=0.0005). BAP1-retained tumors were thinner (p=0.0001) and more likely to be treated with radiation as primary treatment (p=0.021) in comparison to tumors with BAP1-loss. Group 1 (BAP1-retained and PRAME-negative) tumors have smaller basal diameter (p=0.0001) and are less likely to involve choroid (p=0.003), which correlates with the higher frequency of iridectomy and iridocyclectomy (p=0.004) performed in these smaller anterior segment tumors with low pTNM stage (p=0.0001). They are also more likely to have spindle cell morphology (p<0.0001) and low mitotic count (p=0.0001).

Clinical follow-up providing data about recurrence and metastasis was available for a subset of patients (n=204 and n=194, respectively). Overall, 34.3% of the cases showed local recurrence requiring additional treatment, and there was no association between the study risk groups and the rate of recurrence or local recurrence-free survival (Tables 1 and 3). However, increasing study risk group based on loss of BAP1 expression and positive PRAME staining was associated with higher rate of metastasis and disease-specific death, and lower metastasis-free and

disease-specific survival (Table 3, Figures 2 and 3). Other clinical and histologic features associated with worse metastasis-free survival include primary surgical resection, history of local recurrence, epithelioid or mixed histologic type, increased mitotic activity, larger tumor size, ciliary body involvement, and higher pTNM stage (Table 4). 5-year metastasis-free survival rates for study risk groups were 93%, 73%, 57% and 28%, respectively. 5-year and 10-year metastasis-free survival and disease-specific survival results for study risk groups and for pTNM stages are also provided in Figures 2 and 3, respectively. The association between the study risk groups and metastasis-free survival remained significant in multivariate analysis, albeit it was largely driven by the BAP1 results (Table 4).

Pairwise comparisons of metastasis-free and disease-specific survival rates between study risk groups and pTNM stages were also performed and details are reported in Figures 2 and 3. Among tumors with retained BAP1 expression (which would largely correspond to Class 1 tumors in GEP), those with PRAME staining have worse MFS (HR of 6.1, p=0.001, Figure 2B) and DSS (HR of 8.3, p=0.021, Figure 3B). Among tumors with loss of BAP1 expression (largely corresponding to Class 2 tumors in GEP), those with PRAME staining have worse MFS (HR of 2.4, p=0.018, Figure 2B) and show a trend towards worse DSS (HR of 2.2, p=0.061, Figure 3B). There was no statistically significant difference for MFS and DSS between tumors with pTNM stages 1 and 2, and between tumors with pTNM stages 3 and 4 (Figures 2D-F and 3D-F).

**Cytology Cohort**

To demonstrate the feasibility of the risk stratification using immunohistochemical stains on cytology specimens, we have repeated the analysis in a small cohort of fine needle aspiration specimens with cell block material. Given the shorter follow-up time and lower event rates among cases with cytology specimen only, these were analyzed separately, and the results are reported in Table 5.

There were 39 cytology cases included in this study, of which 34 showed retained BAP1 staining, and 5 showed loss of BAP1 staining. Gene expression profiling results were available for 5 cases (2 Class 1a, 1 Class 1b and 2 Class 2), all of which correlated with BAP1 staining results. PRAME was positive in eight (21%) cases with patchy-moderate staining in four, diffuse-strong-diffuse staining in three and patchy-strong staining in one. Remaining 31 (80%) cases were PRAME-negative. PRAME qPCR results were not available for any of the cytology cases. Three patients had a subsequent surgical specimen, and risk groups based on BAP1 and PRAME were identical on cytology and surgical cases (one of each from groups 2, 3, and 4).

Median age at diagnosis was 64 years (range 35-86), which is comparable to the median age of 62 years (range 20-94) in surgical cohort. There is a trend towards higher clinical stage, higher rate of surgical treatment and higher recurrence as risk group based on BAP1 and PRAME stains worsens; however, we did not perform further statistical analyses given the small sample size and limited follow-up. Three patients who had both cytology and surgical material and both BAP1 and PRAME stains showed concordant results.

**DISCUSSION**

In this study, we demonstrated that uveal melanomas can be risk-stratified using BAP1 and PRAME immunohistochemical stains. These stains can also be applied to cytologic materials; therefore, this method can also be used in cases where the intended primary treatment is non-surgical.

Prior gene expression profiling (GEP) studies of uveal melanomas have shown that most Class 2 tumors correlate with the inactivation mutation of BAP1.[27-29] These studies have illustrated that GEP Class 2 tumors have a stronger association with metastasis within 3 years of diagnosis and melanoma-specific mortality than other adverse prognostic factors including patient age, ciliary body involvement, large tumor diameter and thickness, epithelioid cell type, and monosomy 3.[27-29] Prior studies have also shown that tumors with *BAP1* gene mutations have worse outcomes, and BAP1 immunohistochemistry demonstrating loss of staining correlates with increased risk of metastasis.[20,24-26,29] Our study showed similar results confirming that tumors with loss of BAP1 immunostaining are associated with a higher rate of metastasis and disease-related death compared to those with retained BAP1 staining. BAP1 loss (as seen in risk groups 3 and 4) remained to be prognostic in multivariate analysis. We have also redemonstrated that loss of BAP1 staining correlates with presence of a damaging/deleterious BAP1 mutation, which has been shown to correlate with Class 2 gene expression profile.[24,26,27,29,30] While we have only limited number of cases with known GEP results, they largely correlate with the risk groups in this study where all Class 1 tumors have retained BAP1 staining. Therefore, our results further

support that BAP1 protein expression as measured by immunohistochemistry is an excellent surrogate biomarker to predict prognosis in uveal melanoma.

PRAME has been discovered as an antigen in cutaneous melanoma whereby increased expression, which is expected to promote tumorigenesis, can be used to differentiate malignant melanoma from benign nevi.[36-38] More recently, studies evaluating the role of PRAME expression in uveal melanoma demonstrated that PRAME mRNA expression is an independent prognostic indicator, and PRAME expression was associated with increased risk for metastasis among Class1 tumors, and earlier metastasis among Class 2 tumors.[31-33,39] In this study we have demonstrated that a similar prognostic classification can be achieved by immunohistochemical stains, that BAP1-retained/PRAME-negative cases carry a low risk for metastasis and disease related death. Like previous studies using GEP and PRAME mRNA expression, we have also demonstrated that pairwise comparison of risk groups provides a better prediction of metastasis-free and disease-specific survival than the pTNM stage alone.[39] As evident by the 5-year and 10-year metastasis-free and disease-specific survival rates among tumors with retained BAP1, positive PRAME staining can be used to identify cases with intermediate risk of metastasis, who may undergo more frequent surveillance.[39] Also evident from 5-year and 10-year metastasis-free survival rates among tumors with BAP1 loss, positive PRAME staining can be used to identify cases with highest risk of metastasis with shortest metastasis-free survival.

In a small study cohort of Class 1 tumors, Field et al evaluated PRAME expression by quantitative PCR and correlated these findings to immunohistochemical stains and found that

PRAME mRNA expression correlated with nuclear protein staining.[31] The group further found that metastatic events were found more frequently in Class 1 PRAME-positive tumors compared to Class 1 PRAME-negative tumors.[31] These Class 1 PRAME-positive tumors also had a meaningful difference in survival compared to Class 2 tumors.[31] In this study, we have shown similar findings in an expanded cohort of uveal melanomas, using PRAME immunohistochemistry, which is more widely available than a commercial qPCR test. Overall, our results support that PRAME protein expression as measured by immunohistochemistry is an excellent surrogate biomarker to predict prognosis in uveal melanoma.

We have also analyzed a small cohort of uveal melanomas which were diagnosed on FNA and had available cell blocks for immunohistochemical stains. The results of the survival analysis for the cytology cohort do not reach statistical significance, due rare events (metastasis and disease-related death) in this cohort. This might be partly due to relatively smaller tumors in this cohort, which are more amenable to globe-sparing radiotherapy, as evident by overall lower pTNM stage and lower enucleation rates as primary treatment. Furthermore, our follow-up period, especially for cytology cases are short with many cases being lost to follow-up hampering the statistical analyses. However, the primary objective to include this cohort in the study was to provide feasibility data that cell block material can be used for risk stratification by immunohistochemistry, and we achieved this goal. Larger, prospective FNA cohorts can be further studied to verify the role of BAP1 and PRAME immunohistochemistry in prognostication of uveal melanomas.

Majority of the studies in the literature evaluating PRAME expression in melanocytic tumor focus on its diagnostic value to differentiate benign nevi from melanoma, and studies propose various scoring schemes with various sensitivity and specificity, some using 75% as cut-off and some including intensity in their score.[40,41] While the mechanism of PRAME (over)expression in uveal melanoma is not fully studied, it is expected to be a continuum and we believe both the percentage of PRAME-positive cells and the intensity of staining in each cell would collectively contribute to the overall expression levels. Therefore, we have used a scoring scheme that included both quantity and intensity, and considered patchy staining positive, if the intensity was reliably high. While we only have a few cases with known PRAME mRNA results, there is at least one case in our cohort which showed PRAME staining in only 50% of the cells but had positive PRAME mRNA results. A previous study comparing the PRAME IHC results with PRAME mRNA expression in uveal melanoma showed that six out of seven tumors with patchy staining had positive results on RT-PCR, supporting our findings.[42]

This is a retrospective cohort with variable availability of clinical data, which is one the main limitations of this study. We do not have molecular and GEP data for all patients in our cohort; therefore, we were unable to perform a more in-depth analysis of how these features correlate with each other and with outcome. Prospective cohorts and/or clinical trials including immunohistochemistry as one of the risk stratification methods can be considered to confirm our findings.

The most important contribution of this study to the scientific literature is the verification that risk assessment of uveal melanoma based on BAP1 and PRAME expression status can be performed by immunohistochemistry. The main benefit of utilizing immunohistochemistry is the accessibility of these stains in most routine pathology laboratories world-wide. Risk stratification using immunohistochemistry would not require specialized, commercial molecular pathology laboratories to perform DNA sequencing, quantitative PCR, or gene expression profiling. While we did not perform a cost analysis in this study, it is generally accepted that immunohistochemistry can be a cost-effective and fast surrogate marker for various molecular alterations. [43] Therefore, a combination of BAP1 and PRAME immunohistochemistry may be a cheaper and faster surrogate for sequencing and gene expression for risk stratification of uveal melanomas due to low cost and turnaround time.

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**Conflict of Interest**

The authors of this manuscript have no competing financial interests in relation to the work described.

**Ethics Approval and Consent to Participate**

This study was conducted as a retrospective, cross-sectional study in accordance with the Declaration of Helsinki and Good Clinical Practice Guidelines, under the approval of the University of California San Francisco (UCSF) Institutional Review Board with a waiver of patient consent (#20-30753).

**Author Contributions**:

M.P. performed study concept and design; L.M.H., K.W.L. and M.P. performed development of methodology and writing of the paper; G.U., M.S. A.A. and M.M.B provided additional review and revision of the paper; L.M.H., K.W.L., G.U., M.M.B. and M.P. provided acquisition, analysis and interpretation of data; M.P. performed statistical analysis; G.U. M.S. and A.A. provided technical and material support. All authors read and approved the final paper.

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**Data Availability Statement**

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

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**Table 1: Clinical features of cases with surgical specimens**

| | All patients n=318 | Group 1 (BAP1+/PRAME-) n=135 | Group 2 (BAP1+/PRAME+) n=43 | Group 3 (BAP1-/PRAME-) n=94 | Group 4 (BAP1-/PRAME+) n=46 | p |
|---|---|---|---|---|---|---|
| Age (years) | | | | | | |
| Median (range) | 62 (20-94) | 59 (20-91) | 58 (32-94) | 65 (31-92) | 66 (35-92) | **0.0005*** |
| Mean +/- standard deviation | 61.9 +/- 14.9 | 58.6 +/- 15.2 | 58.5 +/- 15.1 | 66 +/- 14.2 | 66.1 +/- 12.1 | |
| Laterality | | | | | | **0.013 #** |
| Left (%) | 152 (47.8%) | 54 (40%) | 28 (65.1%) | 43 (45.7%) | 27 (58.7%) | |
| Right (%) | 166 (52.2%) | 81 (60%) | 15 (34.9%) | 51 (54.3%) | 19 (41.3%) | |
| Primary treatment (surgical versus nor | | | | | | **0.021 #^** |
| Surgery, n (%) | 225 (70.8%) | 90 (66.7%) | 29 (67.4%) | 71 (75.6%) | 35 (76.1%) | |
| Local resection, n (%) | 66 (20.8%) | 42 (31.1%) | 5 (11.6%) | 15 (16%) | 4 (8.7%) | |
| Enucleation, n (%) | 156 (49.1%) | 48 (35.6%) | 24 (55.8%) | 55 (58.5%) | 29 (63%) | |
| Exenteration, n (%) | 3 (0.9%) | - | - | 1 (1.1%) | 2 (4.4%) | |
| Radiation, n (%) | 88 (27.7%) | 42 (31.1%) | 14 (32.6%) | 22 (23.4%) | 10 (21.8%) | |
| Plaque brachytherapy, n (%) | 14 (4.4%) | 9 (6.7%) | 2 (4.7%) | 2 (2.1%) | 1 (2.2%) | |
| Proton beam, n (%) | 69 (21.7%) | 30 (22.2%) | 12 (27.9%) | 18 (19.2%) | 9 (19.6%) | |
| External beam, n (%) | 5 (1.6%) | 3 (2.2%) | - | 2 (2.1%) | - | |
| Other/Unknown, n (%) | 5 (1.6%) | 3 (2.2%) | - | 1 (1.1%) | 1 (2.2%) | |
| Recurrence , n (%) | 70 (34.3%) | 34 (36.6%) | 11 (42.3%) | 18 (30.5%) | 7 (26.9%) | 0.582 # |
| Metastasis, n (%) | 52 (26.8%) | 8 (8.8%) | 8 (30.8%) | 22 (40.7%) | 14 (60.9%) | **<0.0001 #** |
| Disease-related death, n (%) | 32 (10.1%) | 2 (1.5%) | 3 (7%) | 18 (19.2%) | 9 (19.6%) | **<0.0001 #** |
| Follow up (months), median (range) | 17.8 (0-1459.4) | 41.1 (0-1459.4) | 22.6 (0-234.9) | 13 (0-144.9) | 4.3 (0-103.6) | **0.0001*** |

% values are percentages in each column;
* p value for continuous variables calculated by Kruskal-Wallis test
# p values for categorical variables are calculated by Pearson Chi-square test
^ analysis compares the overall rate of primary surgical versus non-surgical treatment

**Table 2: Pathologic findings of cases with surgical specimens**

| | All patients n=318 | Group 1 (BAP1+/PRAME-) n=135 | Group 2 (BAP1+/PRAME+) n=43 | Group 3 (BAP1-/PRAME-) n=94 | Group 4 (BAP1-/PRAME+) n=46 | p #* |
|---|---|---|---|---|---|---|
| **Surgical specimen included in the study** | | | | | | **0.004 #** |
| Iridectomy/iridocyclectomy, n (%) | 64 (20.1%) | 41 (30.4%) | 5 (11.6%) | 13 (13.8%) | 5 (10.9%) | |
| Enucleation, n (%) | 244 (76.7%) | 90 (66.7%) | 35 (81.4%) | 80 (85.1%) | 39 (84.8%) | |
| Extenteration, n (%) | 10 (3.2%) | 4 (2.9%) | 3 (7%) | 1 (1.1%) | 2 (4.4%) | |
| **Greatest basal diameter (mm), median (range)** | 12.1 (1-33) | 10 (1-27) | 15 (1-33) | 13.3 (3.2-22.9) | 13 (3-24) | **0.0001** * |
| **Greatest tumor height (mm), median (range)** | 7.2 (0.5-22) | 6 (0.5-17.1) | 6.5 (1-19) | 8.2 (1-22) | 8.5 (1.5-18.5) | **0.0001** * |
| **Tumor involvement of** | | | | | | |
| Choroid, n (%) | 280 (88.3%) | 108 (80.6%) | 40 (93%) | 88 (93.6%) | 44 (95.7%) | **0.003 #** |
| Ciliary body, n (%) | 138 (44.4%) | 52 (39.4%) | 20 (47.6%) | 41 (44.6%) | 25 (55.6%) | 0.285 # |
| Iris, n (%) | 58 (18.8%) | 30 (22.9%) | 5 (11.9%) | 15 (16.7%) | 8 (17.8%) | 0.385 # |
| Sclera, n (%) | 142 (45.4%) | 57 (43.2%) | 16 (38.1%) | 47 (50.5%) | 22 (47.8%) | 0.519 # |
| Extraocular extension, n (%) | 37 (11.9%) | 10 (7.8%) | 6 (14%) | 14 (15.1%) | 7 (15.2%) | 0.312 # |
| **Growth pattern** | | | | | | **0.040 #** |
| Diffuse/flat, n (%) | 61 (19.3%) | 37 (27.6%) | 10 (23.3%) | 11 (11.8%) | 3 (6.5%) | |
| Dome-shaped, n (%) | 185 (58.5%) | 71 (53%) | 25 (58.1%) | 56 (60.2%) | 33 (71.7%) | |
| Endophytic/solid, n (%) | 32 (10.1%) | 10 (7.5%) | 3 (7%) | 14 (15.1%) | 5 (10.9%) | |
| Mushroom, n (%) | 38 (12%) | 16 (11.9%) | 5 (11.6%) | 12 (12.9%) | 5 (10.9%) | |
| **Histologic subtype** | | | | | | **<0.0001 #** |
| Spindle, n (%) | 62 (19.5%) | 45 (33.3%) | 7 (16.3%) | 8 (8.5%) | 2 (4.4%) | |
| Mixed, n (%) | 224 (70.4%) | 83 (61.5%) | 32 (74.4%) | 76 (80.9%) | 33 (71.7%) | |
| Epithelioid, n (%) | 32 (10.1%) | 7 (5.2%) | 4 (9.3%) | 10 (10.6%) | 11 (23.9%) | |
| **Mitotic figures (per 40 HPFs), median (range)** | 4 (0-51) | 2 (0-43) | 7 (0-51) | 4 (0-48) | 6 (0-40) | **0.0001*** |
| Less than 4 mitoses per 40 HPFs, n (%) | 158 (49.7%) | 87 (64.4%) | 11 (25.6%) | 44 (46.8%) | 16 (34.8%) | **<0.0001 #** |
| 4 or more mitoses per 40 HPFs, n (%) | 160 (50.3%) | 48 (35.6%) | 32 (74.4%) | 50 (53.2%) | 30 (65.2%) | |
| **Margin** Positive, n (%) | 81 (25.5%) | 35 (25.9%) | 11 (25.6%) | 23 (24.5%) | 12 (26.1%) | 0.995 # |
| **Prior treatment, n (%)** | 73 (23%) | 36 (26.7%) | 13 (30.2%) | 17 (18.1%) | 7 (15.2%) | 0.262 # |
| **pTNM stage** | | | | | | **0.0001 #** |
| pT1 | 62 (19.5%) | 40 (29.6%) | 4 (9.3%) | 12 (12.8%) | 6 (13%) | |
| pT2 | 74 (23.3%) | 38 (28.2%) | 13 (30.2%) | 16 (17%) | 7 (15.2%) | |
| pT3 | 113 (35.5%) | 38 (28.2%) | 14 (32.6%) | 39 (41.5%) | 22 (47.8%) | |
| pT4 | 69 (21.7%) | 19 (14.1%) | 12 (27.9%) | 27 (28.7%) | 11 (23.9%) | |

% values are percentages in each column;
* p value for continuous variables calculated by Kruskal-Wallis test;
# p values for categorical variables are calculated by Pearson Chi-square test

**Table 3: Associations of study risk group and local recurrence-free, metastasis-free, and disease specific survival in cases with surgical specimens**

| | | | | Local recurrence-free survival | | | | | Metastasis-free survival | | | | | Disease specific survival | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Recurrence (%) | Median LRFS Months (95% CI) | HR | 95% CI | p | Metastasis (%) | Median MFS Months (95% CI) | HR | 95% CI | p | Disease specific death (%) | Median DSS Months (95% CI) | HR | 95% CI | p |
| **STUDY GROUP** | | | | | | | | | | | | | | | | |
| **Group 1** | BAP1+/PRAME- | 36.6% | 124.6 (78.7 - 267.8) | ref | | | 8.8% | NA (NA) | ref | | | 1.9% | NA (NA) | ref | | |
| **Group 2** | BAP1+/PRAME+ | 42.3% | 127.4 (25.7 - NA) | 1.39 | 0.70 - 2.77 | 0.343 | 30.8% | 109 (35.4 - NA) | **6.11** | **2.19 - 16.99** | **0.001** | 11.1% | NA (107.6 - NA) | **8.27** | **1.37 - 49.78** | **0.021** |
| **Group 3** | BAP1-/PRAME- | 30.5% | 61.8 (26.4 - NA) | 1.74 | 0.95 - 3.19 | 0.073 | 40.7% | 63.4 (27.2 - NA) | **10.83** | **4.32 - 27.18** | **<0.0001** | 30.5% | 106.6 (49.5 - NA) | **33.86** | **7.63 - 150.23** | **<0.0001** |
| **Group 4** | BAP1-/PRAME+ | 26.9% | 98.3 (53.8 - NA) | 1.63 | 0.67 - 3.99 | 0.282 | 60.9% | 31.8 (6.9 - NA) | **26.26** | **9.58 - 72.01** | **<0.0001** | 36.0% | 58.2 (24.4 - NA) | **73.93** | **15.02 - 363.93** | **<0.0001** |
| **ALL** | | 34.3% | 99.3 (73.17 - 157.6) | | | | 26.8% | 217.4 (144.9 - NA) | | | | 14.8% | NA (NA) | | | |

NA: Not available
HR: Hazard ratio; 95%CI: 95%confidence interval

**Table 4: Associations of clinical and pathologic features and metastasis-free survival in cases with surgical specimens**

| | n* (column %) | % metastasis | Median Metastasis-Free Survival (95% CI) | Univariate analysis | | | Multivariate analysis | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | HR | 95% CI | p | HR | 95% CI | p |
| **Age** | | | | 0.99 | 0.98 - 1.01 | 0.629 | NA | | |
| **Primary treatment** | | | | | | | | | |
| Nonsurgical | 87 (45.8%) | 19.5% | NA (168.2 - NA) | ref | | | ref | | |
| Surgical | 103 (51.2%) | 34.2% | 106.2 (50.6 - 228.1) | **2.71** | **1.52 - 4.85** | **0.001** | 1.16 | 0.44 - 3.03 | 0.762 |
| **Margin** | | | | | | | NA | | |
| Negative | 159 (82.8%) | 26.3% | 228.1 (144.9 - NA) | ref | | | | | |
| Positive | 33 (17.2%) | 34.2% | 124.9 (104.2 - NA) | 1.09 | 0.56 - 2.12 | 0.804 | | | |
| **Local recurrence** | | | | | | | | | |
| No | 118 (64.5%) | 30.1% | 168.2 (104.2 - NA) | ref | | | ref | | |
| Yes | 65 (35.5%) | 20% | 217.4 (124.9 - NA) | **0.48** | **0.26 - 0.91** | **0.024** | **0.27** | **0.09 - 0.82** | **0.022** |
| **Histologic type** | | | | | | | | | |
| Spindle | 44 (22.9%) | 12.5% | NA (NA) | ref | | | ref | | |
| Mixed | 130 (67.7%) | 31.7% | 168.2 (106.2 - NA) | **2.63** | **1.11 - 6.20** | **0.028** | 2.97 | 0.87 - 10.18 | 0.083 |
| Epithelioid | 18 (9.4%) | 36.8% | 144.9 (23.8 - NA) | **3.75** | **1.26 - 11.18** | **0.018** | 2.82 | 0.65 - 12.24 | 0.166 |
| * **Mitotic figures (per 40 HPFs)** | - | - | - | **1.05** | **1.03 - 1.07** | **<0.0001** | NA | | |
| Less than 4 | 105 (54.7%) | 18.8% | NA (168.2 - NA) | ref | | | ref | | |
| 4 or more | 87 (45.3%) | 38.1% | 104.2 (35.4 - NA) | **3.21** | **1.81 - 5.67** | **<0.0001** | **2.26** | **1.06 - 4.82** | **0.035** |
| **Greatest basal diameter (mm)** | - | - | - | **1.11** | **1.07 - 1.16** | **<0.0001** | NA | | |
| **Greatest tumor height (mm)** | - | - | - | **1.16** | **1.09 - 1.23** | **<0.0001** | NA | | |
| **Ciliary body involvement** | | | | | | | NA | | |
| Absent | 118 (61.8%) | 19.7% | NA (168.2 - NA) | ref | | | | | |
| Present | 73 (38.2) | 39.5% | 107.2 (50.6 - NA) | **2.21** | **1.28 - 3.81** | **0.004** | | | |
| **Extraocular extension** | | | | | | | NA | | |
| Absent | 172 (90.5%) | 26.6% | NA (109 - NA) | ref | | | | | |
| Present | 18 (9.5%) | 39.1% | 217.4 (24.6 - NA) | 1.29 | 0.58 - 2.87 | 0.526 | | | |
| **pT stage** | | | | | | | | | |
| pT1 | 42 (21.9%) | 10.9% | NA (124.9 - NA) | ref | | | ref | | |
| pT2 | 48 (25%) | 12.5% | NA (NA) | 1.23 | 0.38 - 4.04 | 0.732 | 1.42 | 0.31 - 6.38 | 0.651 |
| pT3 | 68 (35.4%) | 36% | 63.4 (38.5 - NA) | **5.98** | **2.25 - 15. 94** | **<0.0001** | 2.67 | 0.68 - 10.59 | 0.161 |
| pT4 | 34 (17.7%) | 50% | 75. 5 (21.8 - 217.4) | **7.15** | **2.63 - 19.45** | **<0.0001** | **6.61** | **1.58 - 27.69** | **0.01** |
| **BAP1 staining** | | | | | | | NA | | |
| Retained | 107 (60.4%) | 13.6% | NA (217.4 - NA) | ref | | | | | |
| Loss | 70 (39.6%) | 47.4% | 50.6 (27.2 - 107.2) | **7.41** | **3.79 - 14. 47** | **<0.0001** | | | |
| **PRAME staining** | | | | | | | NA | | |
| Negative | 131 (74%) | 21% | NA (228.1 - NA) | ref | | | | | |
| Positive | 46 (26%) | 46% | 55.5 (25.5 - 168.2) | **3.39** | **1.90 - 6.05** | **<0.0001** | | | |
| **STUDY GROUP** | | | | | | | | | |
| Group 1 BAP1+/PRAME- | 82 (46.3%) | 8.8% | NA (NA) | ref | | | ref | | |
| Group 2 BAP1+/PRAME+ | 25 (14.1%) | 30.8% | 109 (35.4 - NA) | **6.11** | **2.19 - 16.99** | **0.001** | 2.59 | 0.79 - 8.45 | 0.115 |
| Group 3 BAP1-/PRAME- | 49 (27.7%) | 40.7% | 63.4 (27.2 - NA) | **10.83** | **4.32 - 27.18** | **<0.0001** | **5.74** | **2.03- 16.27** | **0.001** |
| Group 4 BAP1-/PRAME+ | 21 (11.9%) | 60.9% | 31.8 (6.9 - NA) | **26.26** | **9.58 - 72.01** | **<0.0001** | **14.12** | **4.41 - 45.23** | **<0.001** |

***** Total number of cases changes for each variable based on the data availibility.
Number of cases in this table differs from the overall study population since table is limited to patients with data for metastasis status

**Table 5: Clinical and pathologic findings in cases with fine needle aspiration biopsy material**

| | All patients n=39 | Group 1 BAP1+/PRAME- n=27 | Group 2 BAP1+/PRAME+ n=7 | Group 3 BAP1-/PRAME- n=4 | Group 4 BAP1-/PRAME+ n=1 |
|---|---|---|---|---|---|
| **Age (years)** | | | | | |
| Median (range) | 64 (35 - 86) | 65 (43 - 86) | 60 (35 - 72) | 59 (53 - 81) | 74 |
| Mean +/- standard deviation | 63.5 +/- 12.5 | 64.9 +/- 12.3 | 56.7 +/- 13 | 63 +/- 13.3 | 74 |
| **Laterality** | | | | | |
| Left (%) | 23 (59%) | 17 (63%) | 4 (57.1%) | 2 (50%) | 1 (100%) |
| Right (%) | 16 (41%) | 10 (37%) | 3 (42.9%) | 2 (50%) | - |
| **Histologic subtype** | | | | | |
| Spindle, n (%) | 15 (46.9%) | 10 (50%) | 3 (42.9%) | 2 (50%) | - |
| Mixed, n (%) | 11 (34.4%) | 6 (30%) | 4 (57.1%) | - | 1 (100%) |
| Epithelioid, n (%) | 6 (18.8%) | 4 (20%) | - | 2 (50%) | - |
| **cTNM stage^** | | | | | |
| 1 | 10 (34.5%) | 8 (42.1%) | 2 (33.3%) | - | - |
| 2 | 11 (37.9) | 7 (36.8%) | 3 (50%) | 1 (33.3%) | - |
| 3 | 5 (17.2%) | 2 (10.5%) | 1 (16.7%) | 2 (66.7%) | - |
| 4 | 3 (10.4%) | 2 (10.5%) | - | - | 1 (100%) |
| **Primary treatment** | | | | | |
| Endoresection, n (%) | 4 (10.3%) | 2 (7.4%) | - | 2 (50%) | - |
| Enucleation, n (%) | 2 (5.1%) | - | - | 1 (25%) | 1 (100%) |
| Plaque brachytherapy, n (%) | 29 (74.4%) | 21 (77.8%) | 7 (100%) | 1 (25%) | - |
| Unknown, n (%) | 4 (10.3%) | 4 (14.8%) | - | - | - |
| **Local recurrence , n (%)** | 5 (13.9%) | 3 (12%) | 1 (14.3%) | 1 (25%) | - |
| **Local recurrence free survival (HR; 95%CI)** | | ref | 1.17 (0.12 - 11.29) | 1.96 (0.20 - 18.83) | - |
| **Metastasis, n (%)** | 2 (5.6%) | 1 (4%) | 1 (14.3%) | - | - |
| **Metastasis-free survival (HR; 95% CI)** | | ref | 4.44 (0.27 - 73.26) | NA | NA |
| **Disease-related death, n (%)** | 2 (5.1%) | 1 (3.7%) | 1 (14.3%) | - | - |
| **Disease-specific survival (HR; 95%CI)** | | ref | 4.05 (0.25 - 65.80) | NA | |
| **Follow up (months), median (range)** | 39.4 (0-224.6) | 31.1 (0 - 224.6) | 39.4 (3.1 - 92.5) | 50.1 (9.1 - 62.7) | NA |

% values are percentages in each column

^ Clinical stage (cTNM) data were available for 29 tumors

HR: Hazard ratio; 95%CI: 95% confidence interval; ref: reference group for Cox regression analysis, NA: Not available

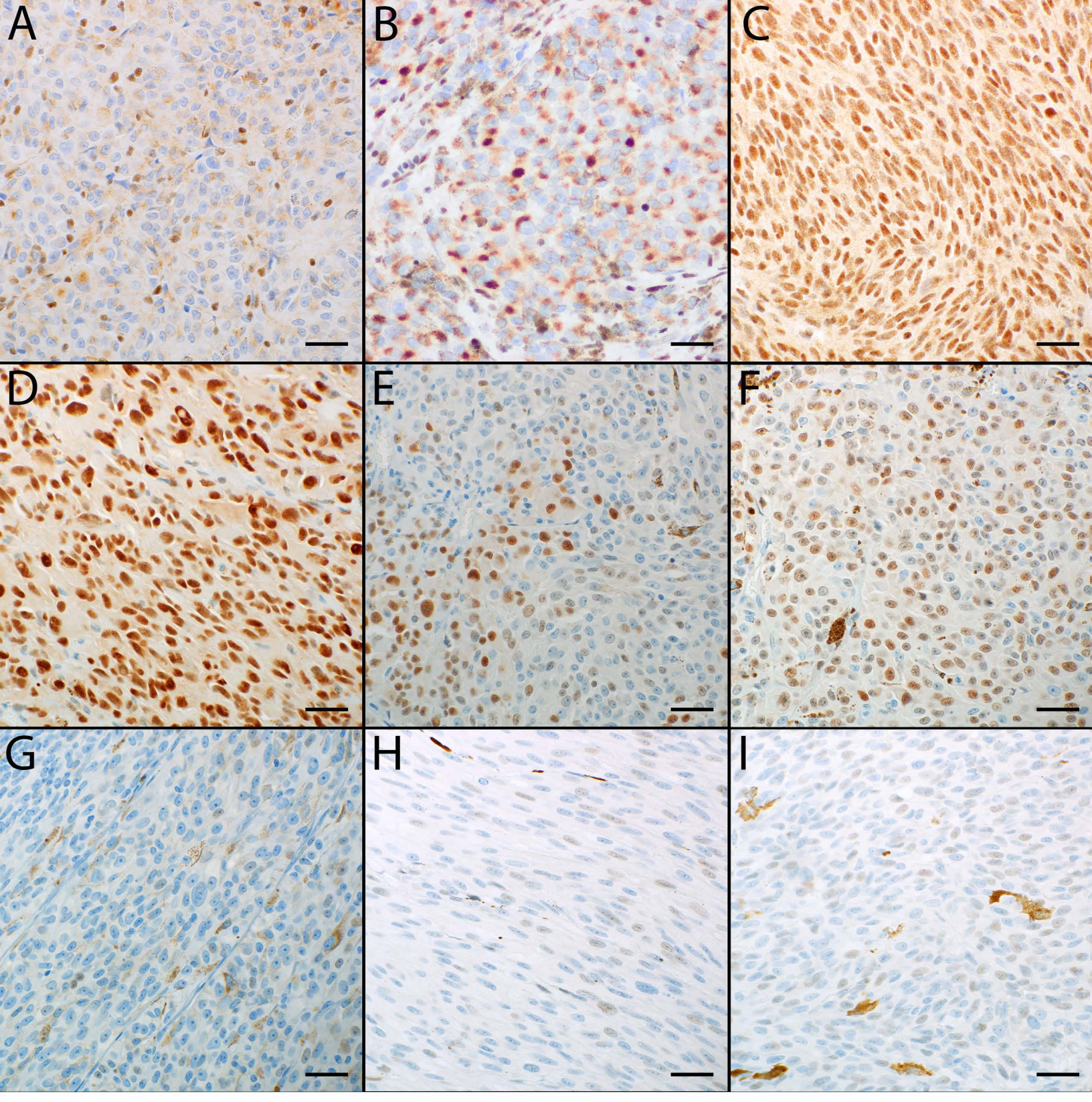
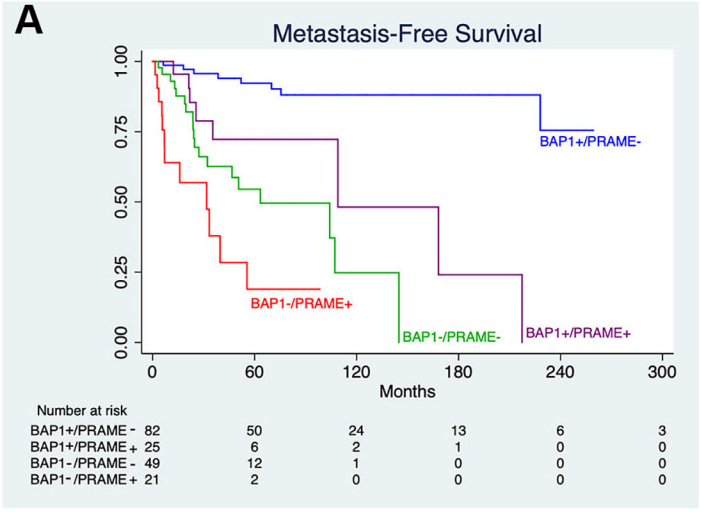
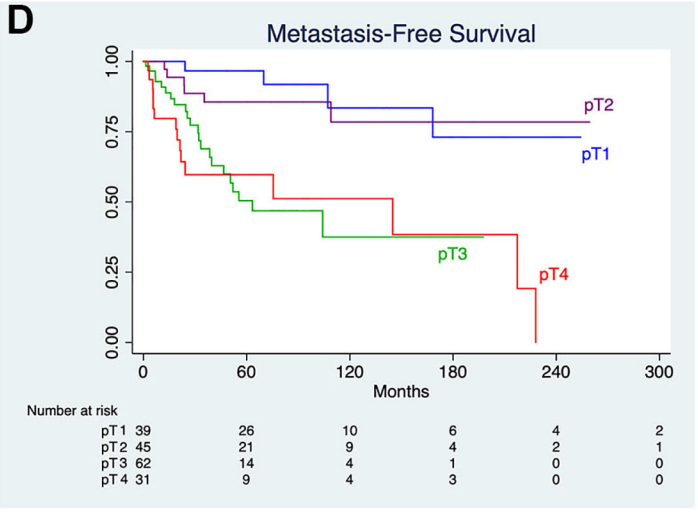



**Figure 1: Representative images of BAP1 and PRAME immunohistochemistry scores**
A-B) Representative examples of tumors with loss of nuclear BAP1 staining. Note that endothelial and inflammatory cells serve as the internal positive control. In panel B, there is cytoplasmic staining of BAP1, which might be seen in select cases. C) Representative example of a tumor with retained BAP1 nuclear staining. D-F) Representative examples of tumors with positive PRAME staining. The stains were scored as strong diffuse (D), strong patchy (E) and moderate diffuse (F). G-I) Representative examples of tumors with negative PRAME staining. Rare weak positive cells (G) and weak focal and patchy staining that cannot be reliably differentiated from nonspecific blush (H and I) were considered negative for the study purposes. All images are 400x magnification and the ruler measures 20 microns.




**B**

Pairwise comparison of study groups based on BAP1 and PRAME results

| | BAP1+ / PRAME- | BAP1+ / PRAME+ | BAP1- / PRAME- |
|---|---|---|---|
| BAP1+ / PRAME+ | HR: 6.1 p=0.001 | | |
| BAP1- / PRAME- | HR: 10.8 p<0.0001 | HR: 1.8 p=0.185 | |
| BAP1- / PRAME+ | HR: 26.3 p<0.0001 | HR: 4.3 p=0.002 | HR: 2.4 p=0.018 |

**E**

Pairwise comparison of TNM stages

| | pT1 | pT2 | pT3 |
|---|---|---|---|
| pT2 | HR: 1.2 p=0.732 | | |
| pT3 | HR:5.98 p<0.0001 | HR: 4.9 p=0.001 | |
| pT4 | HR: 7.2 p<0.0001 | HR: 5.8 p<0.0001 | HR: 1.2 p=0.581 |

**C**

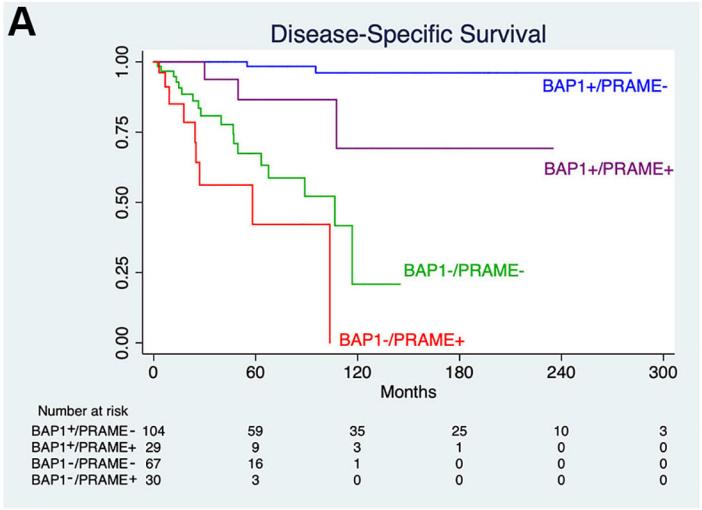
| | BAP1+ / PRAME- | BAP1+ / PRAME+ | BAP1- / PRAME- | BAP1- / PRAME+ |
|---|---|---|---|---|
| 5-year MFS (95%CI) | 93.2% (84.3 - 97.1) | 73% (46.8 - 87.8) | 57.1% (40.9 - 40.4) | 28.2% (10.9 - 48.5) |
| 10-year MFS (95% CI) | 88.3% (76.2 - 94.4) | 56.8% (21.5- 81.2) | 35.7% (15.9 - 56.2) | 28.2% (10.9 - 48.5) |

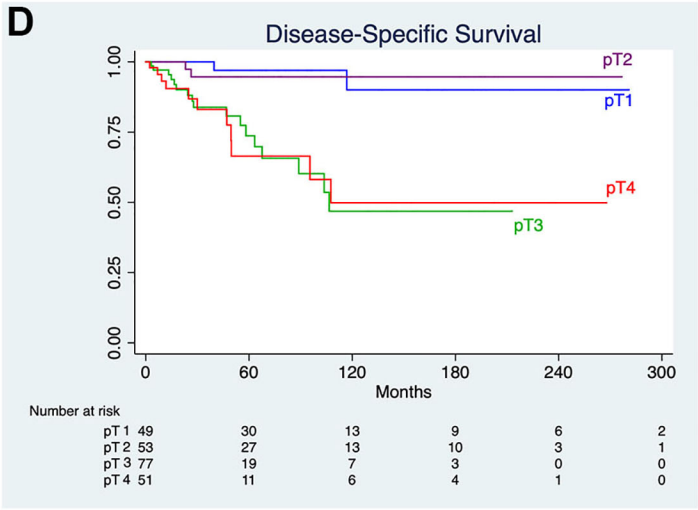
**F**

| | pT1 | pT2 | pT3 | pT4 |
|---|---|---|---|---|
| 5-year MFS (95%CI) | 97.3% (82.3 - 99.6) | 86.8% (71.2 - 94.3) | 58.3% (44.5 - 69.7) | 50.8% (32.7 - 66.3) |
| 10-year MFS (95% CI) | 87.6% (65.1 - 95.9) | 81.7% (62.4 - 91.8) | 43.1% (25.5 - 59.5) | 43.5% (23.8 - 61.8) |

**Figure 2: Metastasis-free survival (MFS) results for uveal melanomas stratified by study risk groups and pTNM stage.**

**A)** Kaplan-Meier curves demonstrating the MFS stratified by study risk groups based on BAP1 and PRAME immunohistochemistry (IHC). **B)** Cox Regression analysis with pairwise comparison of MFS among study risk groups based on BAP1 and PRAME IHC. HR: Hazard ratio. **C)** 5-year and 10-year MFS rates among study risk groups based on BAP1 and PRAME IHC. 95%CI: 95% confidence interval). **D)** Kaplan-Meier curves demonstrating the MFS stratified by pTNM stage (American Joint Committee on Cancer (AJCC), 8th edition). **E)** Cox Regression analysis with pairwise comparison of MFS among pTNM stages (AJCC, 8th edition). HR: Hazard ratio. **F)** 5-year and 10-year MFS rates among pTNM stages (AJCC, 8th edition). 95%CI: 95% confidence interval).




**B**

Pairwise comparison of study groups based on BAP1 and PRAME results

| | BAP1+ / PRAME- | BAP1+ / PRAME+ | BAP1- / PRAME- |
|---|---|---|---|
| BAP1+ / PRAME+ | HR: 8.3 p=0.021 | | |
| BAP1- / PRAME- | HR: 33.9 p<0.0001 | HR: 4.1 p=0.025 | |
| BAP1- / PRAME+ | HR: 73.9 p<0.0001 | HR: 8.9 p=0.001 | HR: 2.2 p=0.061 |

**E**

Pairwise comparison of TNM stages

| | pT1 | pT2 | pT3 |
|---|---|---|---|
| pT2 | HR: 0.6 p=0.612 | | |
| pT3 | HR: 6.9 p=0.002 | HR: 10.9 p=0.001 | |
| pT4 | HR: 7.3 p=0.002 | HR: 11.5 p=0.001 | HR: 1.1 p=0.881 |

**C**

| | BAP1+ / PRAME- | BAP1+ / PRAME+ | BAP1- / PRAME- | BAP1- / PRAME+ |
|---|---|---|---|---|
| 5-year DSS (95%CI) | 98.8% (91.7 - 99.8) | 89.2% (63.3 - 97.2) | 70.5% (54.6 - 81.7) | 55.6% (30.5 - 74.8) |
| 10-year DSS (95% CI) | 96.7% (86.7 - 99.2) | 73% (29.7 - 92.1) | 38.4% (17.6 - 59.1) | 27.8% (1.9 - 66.1) |

**F**

| | pT1 | pT2 | pT3 | pT4 |
|---|---|---|---|---|
| 5-year DSS (95%CI) | 97.7% (84.5 - 99.7) | 95.2% (82.3 - 98.8) | 77.4% (64.3 - 86.2) | 70.6% (52.2 - 83) |
| 10-year DSS (95% CI) | 93.5% (75.2 - 98.4) | 95.2% (82.3 - 98.8) | 53.9% (33.8 - 70.4) | 54% (28.9 - 73.6) |

**Figure 3: Disease-specific survival (DSS) results for uveal melanomas stratified by study risk groups and pTNM stage.**

**A)** Kaplan-Meier curves demonstrating the DSS stratified by study risk groups based on BAP1 and PRAME immunohistochemistry (IHC). **B)** Cox Regression analysis with pairwise comparison of DSS among study risk groups based on BAP1 and PRAME IHC. HR: Hazard ratio. **C)** 5-year and 10-year DSS rates among study risk groups based on BAP1 and PRAME IHC. 95%CI: 95% confidence interval). **D)** Kaplan-Meier curves demonstrating the DSS stratified by pTNM stage (American Joint Committee on Cancer (AJCC), 8th edition). **E)** Cox Regression analysis with pairwise comparison of DSS among pTNM stages (AJCC, 8th edition). HR: Hazard ratio. **F)** 5-year and 10-year DSS rates among pTNM stages (AJCC, 8th edition). 95%CI: 95% confidence interval).