Correlation of gene expression profile status and American Joint Commission on Cancer stage in uveal melanoma

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Abstract

Purpose: To study the relationship between gene expression profile (GEP) sub-class and AJCC stage in uveal melanoma (UM) patients.

Methods: A retrospective, multi-center study was undertaken with patients entered from 9 major ocular oncology centers from across the United States. 360 eligible patients had UM and underwent I-125 plaque brachytherapy with concurrent tumor biopsy with GEP testing between January 1, 2010 and October 28, 2014. Patient demographics and UM features were analyzed by both GEP and AJCC status.

Results: GEP class divided the cohort into three groups: Class 1a (n=186), Class 1b (n=77) and Class 2 (n=113). When classified using AJCC staging criteria, we found the following: Stage I in

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91 cases (25.3%), Stage IIA in 143 cases (39.7%), Stage IIB in 89 cases (24.7%), Stage IIIA in 36 cases (10%) and Stage IIIB in 1 case (0.3%). There were no stage IV cases as lymph node and metastatic data were not collected as a part of this study. Among Stage I tumors, both high tumor height and high largest basal diameter were associated with a higher frequency of Class 2 status ($p < 0.05$). As UMs progress to a larger AJCC tumor group (T1-T4), the odds ratio of having a worse prognosis based on GEP class was 1.75 (95% CI, 1.36–2.25; $p < 0.001$). Similarly, as UMs progress to a higher AJCC stage, the odds ratio of having a worse prognosis based on GEP class was 1.69 (95% CI, 1.36–2.10; $p < 0.001$).

Conclusion: This report details the differences in clinical features between GEP subclasses and how they are distributed among the AJCC stages. When the tumors were grouped by AJCC staging criteria, both larger AJCC tumor (T) group and worsening AJCC stage were associated with worsening predicted prognosis, based on GEP subclass.

Summary Statement: Gene expression profiling (GEP) and AJCC staging are two of the most commonly used methods of prognostication in uveal melanoma. This retrospective study examined their relationship and found that worsening AJCC stage was predictive of worsening prognosis based on GEP subclass.

Keywords

uveal melanoma; choroidal melanoma; gene expression profiling; AJCC

INTRODUCTION

Clinical and histopathologic features of uveal melanoma (UM) tumors have been the root of traditional metastatic risk assessment. Older patient age, high tumor thickness, high largest basal tumor diameter (LBD), ciliary body involvement, epithelioid tumor cell morphology, and extraocular tumor extension have all been identified as factors associated with increased risk of metastasis and disease-related mortality in UM.¹

The Collaborative Ocular Melanoma Study (COMS) was a landmark trial not just in terms of study design and execution but also because the designated size categories (small, medium and large) created a simple clinical framework and vernacular for the purposes of treatment stratification and prognostication.² The American Joint Committee on Cancer (AJCC) Cancer Staging Manual 8th edition uses tumor largest basal diameter, tumor height, ciliary body involvement, and extraocular growth at the time of presentation as the factors for prognostication in UM.³ The predictive model for metastatic disease and survival has been created with data from multiple centers and nearly 7000 patients.⁴ Although several commercial and research based genetic tests are used in clinical practice to predict UM metastatic risk⁵, the current 8th edition did not incorporate these into the stage group model.

In addition to anatomic features, chromosomal analysis has been used to identify individuals at risk for metastasis in UM. In particular, monosomy of chromosome 3⁶⁻⁸ and/or amplification of chromosome 8q⁸⁻¹⁰ have been strongly linked to the development of metastatic disease while gain of chromosome 6p¹¹ (in the absence of changes in chromosomes 3 and 8) and loss of chromosome 1p¹² have been associated with more

favorable outcomes. In a recent study, Dogrusöz and colleagues demonstrated that combining AJCC stage and chromosome 3 and 8q status allowed for more accurate prognostication in UM.¹³ This is in agreement with the findings of other groups who have shown that incorporating clinical, histologic and genomic information allows for individualized prognostication in UM.^{13–17}

Another modality in the realm of prognostication has been the development and validation of a gene expression profile (GEP) test which groups UM tumors into highly prognostic molecular subgroups using a 15-gene array.^{18, 19} This test identifies tumors as either Class 1, which have low metastatic risk; or Class 2, which have high metastatic risk within 3–5 years.^{18, 19} More recent work has shown that the relative expression of two genes on the array (CDH1 and RAB31) can be used to further divide Class 1 tumors into Class 1a (lowest metastatic risk) and Class 1b (moderate long-term metastatic risk).^{20, 21} For Class 1 tumors, the Ct values of RAB31 and CDH1 are added together to determine whether the sample is Class 1a (low summed expression) or Class 1b (high summed expression) based on a validated threshold.²²

High-risk GEP Class 2 status is significantly associated with increasing age,^{14, 18, 23–26} high LBD,^{14, 18, 24} high tumor height,^{14, 18, 23, 24, 26} and ciliary body involvement^{14, 18}. The Ocular Oncology Study Consortium has reported similar findings as well as the novel observation that as tumors progress to a larger COMS size class, the odds ratio of progressing to a worse prognosis GEP subclass was 2.73 (95% CI, 1.75 – 4.26; $p < 0.001$).²⁷ The purpose of this study was to determine the relationship between AJCC stage and GEP sub-classes (1a, 1b and 2) and specifically whether increasing metastatic risk based on AJCC stage agrees with the distribution of GEP sub-classes in UM patients undergoing plaque brachytherapy.

METHODS

Institutional Review Board (IRB) approval was obtained in each of the participating institutions in this study, but IRB rules did not require patient consent for retrospective noninterventive studies using deidentified patient data. The study adhered to the tenets of the Declaration of Helsinki and all work using patient information was performed in compliance with the Health Insurance Portability and Accountability Act.

A multi-institutional, retrospective study was performed of eligible patients meeting the following inclusion criteria: adult age (>18 years), pre-operative diagnosis of choroidal or ciliary body UM with clinical exam documenting size and location, concurrent choroidal tumor biopsy performed just prior to placement of I-125 brachytherapy plaque, and Decision Dx-UM (Castle Biosciences, Friendswood, Texas, USA) GEP testing performed between January 1, 2010 through October 28, 2014. Intraocular biopsy for cytologic examination was performed at the discretion of the surgeon and was not part of the inclusion criteria.²⁸ As the initial purpose of the Ocular Oncology Study Consortium (OOSC) was to study the regression of UM after brachytherapy, no patients treated with primary enucleation were included. The OOSC intends to report additional findings with regard to baseline features as they relate to GEP sub-class, tumor response to radiation treatment, and biopsy techniques in

this population, however study size will vary as inclusion criteria for each report may differ.
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Data was entered into a security encrypted Research Electronic Data Capture (REDCap) database, accessible by password, at each of the approved nine participating Ocular Oncology centers: Duke Eye Center, Durham, NC (*Coordinating center*), University of Miami/Bascom Palmer Eye Institute, Miami, FL, University of Southern California/USC Roski Eye Institute, Los Angeles, CA, Oregon Health Sciences/Casey Eye Institute, Portland, OR, Smilow Cancer Hospital at Yale New Haven, New Haven, CT, University of Michigan, Ann Arbor, MI, Colorado Retina Associates/Rocky Vista University, Denver, CO, Retina Specialists of Michigan and Michigan State University, and Retina Consultants of Houston/Blanton Eye Institute at Houston Methodist Hospital, Houston TX.

Relevant baseline information was collected consisting of: patient demographics (age, gender, eye involved); melanoma tumor status prior to therapy (location, size [clinical and ultrasound], presence of SRF, surface changes, color, presence of Bruch's membrane breakthrough, presence of exudative retinal detachment (RD)) and whether the tumor was previously followed as a nevus; and GEP class test result (Class 1 [1a/1b] or Class 2, and discriminant score value). It should be noted that, in this study, 'exudative retinal detachment' was defined as subretinal fluid inducing retinal detachment >3mm from the tumor borders based on clinical exam. Although all patients underwent systemic metastatic screening at presentation, specific data regarding the presence of metastatic disease and nodal involvement on presentation were not collected as a part of this study. Using baseline ultrasound measurements, tumors were classified using the AJCC staging system.³ A total of 21 patients were excluded: Cases that had an indeterminate GEP result (n = 3) or did not have enough information available for AJCC staging (n = 17) were excluded from this analysis. Only one Stage IIIB tumor was identified in our cohort. As such, this case was excluded from most of the statistical analyses.

Statistical methods

Secure data was downloaded from the REDCap database as a SAS file and analyzed using SAS 9.3 (SAS Institute Inc., Cary, NC, USA). Descriptive statistics were computed. The significance of the difference between 2 subgroups was assessed using the Wilcoxon rank sum test of difference among medians and Fisher's exact test was used to test differences for categorical variables. Non-parametric tests were used because few of the variables tested are normally distributed. A proportional odds logistic regression model with a three-level outcome (GEP class 1A, 1B and 2) was created. The proportional odds assumption means that for each term included in the model (tumor category or AJCC Stage), the estimate between each pair of outcomes (across two response levels) are assumed to be the same regardless of which partition we consider. This means that the estimate of the odds ratios between outcomes of class 2 versus classes 1B and 1A combined or classes 2 and 1B combined versus class 1A are the same. A score test was performed which was nonsignificant and confirmed that the calculated odds ratios could be interpreted as constant across all possible outcomes. This common odds ratio with 95% confidence intervals was obtained from the model.

P-values less than 0.05 were considered significant. Although we conducted multiple statistical tests, we did not apply the Bonferroni correction because it increases the risk of type II error.

RESULTS

Baseline Characteristics of Patients and Tumors: Entire Cohort

The primary cohort consisted of 360 UM patients meeting entry criteria with 179 (50%) women and median age 61 years (range 19.0 – 94.4 years). Baseline median Snellen visual acuity was 20/32 (logMAR 0.2; range –0.1 – 1.6) and median intraocular pressure was 15 mmHg (range 6.0 – 34.0). The tumor LBD ranged from 2.4 mm to 20.5 mm (median LBD = 12.0 mm) and the maximal tumor height ranged from 0.7 mm to 13.0 mm (median height = 3.9 mm). The GEP results included Class 1a in 176 cases (49%), Class 1b in 73 cases (20%), Class 2 in 111 cases (31%).

AJCC Staging and GEP: Entire Cohort

When classified using AJCC staging criteria, we found the following: Stage I in 91 cases (25%), Stage IIA in 143 cases (40%), Stage IIB in 89 cases (25%), Stage IIIA in 36 cases (10%), Stage IIIB in 1 case (0.3%) and Stage IIIC in 0 cases. There were no stage IV cases as lymph node and metastatic data was not collected. The distribution among the AJCC tumor (T) groups was as follows: T1 in 105 cases (29%), T2 in 148 cases (41%), T3 in 99 cases (28%), and T4 in 8 cases (2%). Ciliary body involvement was reported in 59 patients (16%). Extraocular extension was reported in 4 cases (1%) – all of which were classified as Stage IIIA tumors. The distribution of clinical features by AJCC stage is shown in Table 1.

The distribution of GEP subclasses among the AJCC stages was also examined and is shown in Figure 1. A proportional odds analysis found that as UMs progress to larger AJCC T group (T1-T4), the odds ratio of having a worse prognosis GEP class was 1.75 (95% CI, 1.36–2.25; $p < 0.001$). Similarly, as UMs progress to worse AJCC stage, the odds ratio of having a worse prognosis GEP class was 1.69 (95% CI, 1.36–2.10; $p < 0.001$).

Clinical Features: AJCC Stage I and GEP

There were 91 tumors classified as AJCC Stage I with a median tumor height of 2.6 mm (range 1.4 – 4.4 mm) and median LBD of 9.1 mm (range 4.0 – 12.0 mm). The GEP results for this group included Class 1a in 55 cases (60%), Class 1b in 19 cases (21%) and Class 2 in 17 cases (19%). Among Stage I tumors only, Class 1 tumors, compared to Class 2, had significantly smaller median LBD (9.0 mm vs 10.0 mm; $p = 0.030$) and tumor height (2.5 mm vs 2.8 mm; $p = 0.006$) (Table 2). There was no significant difference with regard to tumor height or LBD when Stage I Class 1a tumors were compared to Stage I Class 1b tumors. The only statistically significant differences in clinical features between Stage I Class 1a tumors and Stage I Class 1b tumors was that younger age was significantly associated with Class 1b status ($p = 0.022$). When Stage 1 Class 1b tumors were compared to Stage I Class 2 tumors, Class 2 tumors were significantly thicker ($p = 0.014$) and had significantly larger LBD ($p = 0.018$). No surface clinical features were different between the groups (Supplemental Table 1).

Clinical Features: AJCC Stage IIA and GEP

For tumors classified as Stage IIA, there were 143 patients included with a median tumor height of 3.9 mm (range 1.4 – 9.0 mm) and a median LBD of 12.0 mm (range 2.4 – 17.0 mm). The GEP results for this group included Class 1a in 76 cases (53%), Class 1b in 29 cases (20%), and Class 2 in 38 cases (27%). Among Stage IIA tumors only, Class 2 tumors, when compared to Class 1, were significantly associated with older patient age ($p = 0.018$) and anterior tumor location ($p = 0.007$) (Table 3). Stage IIA Class 1 tumors were significantly more likely to have been previously followed as a nevus when compared to their Class 2 counterparts ($p = 0.026$). There were no significant differences in tumor height, LBD or tumor surface features when Stage IIA Class 2 tumors were compared to Stage IIA Class 1 tumors. When Stage IIA tumors were divided by GEP subclass, patients with Class 1b tumors were found to have both significantly lower IOP ($p = 0.009$), significantly thicker tumors than patients with Class 1a tumors ($p = 0.037$), and no difference in surface clinical features. (Supplemental Table 2).

Clinical Features: AJCC Stage IIB and GEP

There were 89 Stage IIB tumors with a median tumor height of 6.5 mm (range 2.6 – 11.7 mm) and a median LBD of 15.1 mm (range 9.2 – 18.0 mm). The GEP results for this group included Class 1a in 38 cases (43%), Class 1b in 17 cases (19%) and Class 2 in 34 cases (38%). When Stage IIB Class 1 tumors were compared to Stage IIB Class 2 tumors, the only significant difference was that subretinal fluid was more likely to be associated with Stage IIB Class 2 tumors ($p = 0.029$) (Table 4). The significance of subretinal fluid was also noted when Stage IIB Class 1a tumors were compared to Stage IIB Class 2 tumors ($p=0.041$) (Supplemental Table 3). When Stage IIB Class 1a tumors were compared to Stage IIB Class 1b tumors, there were no significant differences in size or clinical feature.

Clinical Features: AJCC Stage IIIA and GEP

Only 36 tumors were classified as Stage IIIA and had a median tumor height of 8.9 mm (range 2.2 – 12.3 mm) and a median LBD of 16.0 mm (range 8.9 – 20.5 mm). The GEP results for this group included Class 1a in 7 cases (19%), Class 1b in 8 cases (22%) and Class 2 in 21 cases (58%). There were no significant differences between Stage IIIA Class 1 and Stage IIIA Class 2 tumors (Table 5). When Stage IIIA Class 1a tumors were compared to Stage IIIA Class 1b tumors, it was found that Stage IIIA Class 1b tumors were significantly more likely to have associated subretinal fluid ($p = 0.015$) (Supplemental Table 4). When Stage IIIA Class 1b tumors were compared to Stage IIIA Class 2 tumors, Class 2 tumors were significantly more likely to feature ciliary body involvement (60% vs 86%; $p=0.043$).

DISCUSSION

UM staging by means of anatomic size and extent is perhaps the oldest method used for survival prognostication, whereas GEP status predicts metastatic risk based purely on tumor genetics. This retrospective study is the first large-scale, multi-center study to examine the relationship between AJCC stage and GEP sub-class status. The current study confirms the findings of an exploratory study by Nguyen and colleagues which showed that AJCC stage

and LBD were the only clinical features statistically associated with GEP class.²⁹ Moreover, it has been demonstrated by other groups that incorporating clinical, histologic, and genomic information from GEP and other validated commercial tests can enhance prognostication in a more individualized manner.^{13–17, 30} As GEP and AJCC are among the most commonly employed prognostic tools, further exploration of their particular relationship is a necessary step towards providing the most accurate prognostic information to our patients.

When we examined this relationship using a proportional odds model, we found that as UMs progress to a worse AJCC stage, the odds ratio of having a worse prognosis GEP class was 1.69 (CI 1.36–2.10). This agreement confirms the accuracy of these important prognostic tools. Interestingly, a similar model using only tumor size group (T1–T4) based on AJCC criteria was slightly more predictive of worse GEP class (OR 1.75; CI 1.36–2.25) than was the model using AJCC stage. This finding is likely due to the fact that our cohort only included patients undergoing brachytherapy and not primary enucleation which shifted the study population away from the largest tumor size categories.

The relationship between high risk clinical features, particularly tumor size, and GEP status within the AJCC stages was also examined and produced several noteworthy findings. Among Stage I tumors only, for example, GEP Class 2 status was significantly associated with both high tumor height ($p = 0.006$) and high tumor LBD ($p = 0.030$). The correlation between larger tumor size and GEP class has been reported by others^{14, 18, 23–26} and recent studies have further identified LBD > 12 mm as an additional risk factor for metastatic disease independent of GEP status.^{14, 15} Using this same cohort, our group recently reported that as tumors progress to a larger COMS size category, the odds ratio of progressing to a worse prognosis GEP subclass was 2.73 (CI 1.75 – 4.26).²⁷ As COMS tumor size categories are defined by tumor height and diameter, this finding is not surprising.³¹ These findings are similar to those of Shields and colleagues who recently reported a strong correlation between increasing tumor size (both thickness and LBD) and the presence of cytogenetic abnormalities.^{32, 33}

Similarly, AJCC staging is also driven largely by tumor height and diameter given that, at the time of diagnosis of UM, lymph node metastases (N) are found in fewer than 1% of patients and systemic metastases (M) in only 2–3% of patients.^{34, 35} Two reports, each with over 7000 patients, found that high tumor LBD and high tumor height were among the clinical features that were most predictive of both worsening AJCC tumor size category and anatomic stage.^{36, 37} Thus, the concordance we are seeing with high tumor height and diameter with Class 2 status suggests that tumor size-based classifications, such as the COMS classification, still provide some degree of predictive information. Finally, in terms of risk stratification, these findings may help identify those patients in generally low-risk AJCC stages who are at the highest risk of developing metastases and may need closer surveillance.

While the relationship between tumor size and GEP class may have important prognostic implications when examined at baseline, this relationship has also been the subject of much study with regard to tumor response to plaque brachytherapy. Our group has previously published that Class 1 tumors had a significantly greater percentage reduction in tumor

height than did Class 2 tumors at 3 and 6 months.²⁸ This finding supported the work of Rao and colleagues²⁴ although several other groups have found no relationship between GEP class and tumor regression.^{23, 25} Ours was an unexpected finding and may be due to a combination of mechanisms including but not limited to differences between Class 1 and Class 2 tumors in terms of immunologic recognition and/or radiation-induced inflammation. Nevertheless, this finding, taken together with other clinical and genetic data, is an important adjunct to providing the best prognostic information to our patients.

Class 1 tumors have a lower risk of metastasis than Class 2 tumors and Class 1b tumors have an increased predicted risk of metastatic disease compared to Class 1a. Class 1 tumors that are classified as Stage I AJCC should have the lowest risk of metastasis; yet there is still a small percentage that do develop metastatic spread. Among only AJCC Stage I GEP Class 1 tumors, we found that Class 1b status was significantly associated with younger patient age ($p = 0.022$). Several independent groups have identified a subset of low-risk patients who are younger and lack ciliary body involvement but still go on to develop late onset metastatic disease^{20, 38, 39}. Field et al²⁰ found this to be true among Class 1 UMs that metastasized and both Decatur et al³⁸ and Yavuziyigitoglu et al³⁹ have reported an association between late metastases and mutations in the SF3B1 (splicing factor 3 subunit B1) gene.

Nearly half the tumors in this study were classified as AJCC Stage IIA ($n = 143$), which is to be expected as these patients all underwent plaque brachytherapy. Among all AJCC Stage IIA tumors, anterior location was significantly associated with GEP Class 2 status; a finding which is not likely explained by ciliary body involvement alone as there was no significant difference in terms of ciliary body involvement between AJCC Stage IIA GEP Class 1 and Class 2 tumors. As AJCC Stage IIA is limited to tumor size groups T1 and T2, the more frequent anterior location of Class 2 tumors may be due to the challenge in detecting smaller UM in the peripheral choroid or ciliary body, allowing these tumors to grow longer and undergo further genomic changes toward Class 2 status.⁴⁰ In fact, the most common presentation in patients with UM is identification on a routine examination, as lesions that don't cause visual loss often go undetected.^{41–43} When AJCC Stage IIA tumors were compared by GEP subclass, we found that Class 1b tumors were significantly thicker than their Class 1a counterparts. Since there were no Stage IIA GEP Class 1b tumors that had either ciliary body involvement or extrascleral extension, all were classified as Stage IIA based on a T2a tumor grouping and therefore were larger in height and LBD which would potentially explain this difference in tumor height.

When we examined the AJCC Stage IIB and AJCC Stage IIIA tumors, we found almost no significant clinical differences among the GEP subclasses within these groups. Our findings are consistent with those of Dogrusöz and colleagues in which they examined the relationship between chromosome 3 and 8 status and AJCC stage and found that AJCC stage II and stage III tumors appeared to be less dependent on chromosome status.¹³ Of note, the current study only had one Stage IIIB tumor and thus was not included in the analysis.

There are several limitations to this study. First, this was a retrospective and multi-center study which we felt allowed for the best chance to survey a large patient cohort and offer a 'real-world snapshot' of UM clinical and GEP data across 9 geographically distinct ocular

oncology centers in the United States. All patients received standard-of-care plaque radiation therapy, though there were no specific protocols for B-scan ultrasound measurements or biopsy technique. The retrospective nature of this study led to incomplete clinical data in a very small number of cases. It should also be noted that this study did not include eyes that were enucleated or small melanomas that were observed and thus likely resulted in a study population that was biased away from traditionally very small or very large tumors both in apical height and basal dimension and extraocular extension. Moreover, given the known relationship between high risk GEP Class 2 status and larger tumor size, we suspect this may have also led to a relative underrepresentation of Class 2 tumors in this cohort. Finally, no conclusions regarding patient survival can be made based on this study, as development of metastases and vital status were not obtained due to the short study period. Long-term follow up is planned to enable us to complete these analyses.

In summary, our study demonstrates an association between GEP class and AJCC staging in UM. Our findings detail the clinical differences between the GEP subgroups in relation to AJCC stage and support the association of certain features such as height, largest basal dimension, tumor location, and patient age with both advancing AJCC stage and GEP class. These features can be used to modify predicted prognosis in patients treated in the past who do not have access to GEP or other chromosomal data.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1.
Distribution of GEP subclasses among AJCC stages

Table 1:

Baseline features based on AJCC Stage

Variable	Statistic	Stage I N=91	Stage IIA N=143	Stage IIB N=89	Stage IIIA N=36
Age	Min, Median, Max	22.9, 56.9, 87.4	35.2, 65.4, 94.4	19.3, 59.0, 83.4	19.0, 58.1, 93.0
Sex: Male	N (%)	42 (46)	71 (50)	43 (48)	18 (50)
Affected Eye: Right	N (%)	40 (44)	73 (51)	46 (52)	18 (50)
Visual Acuity (LogMAR)	Mean (SD)	0.21 (0.32)	0.24 (0.30)	0.35 (0.36)	0.37 (0.33)
	Min, Median, Max	-0.1, 0.1, 1.4	0.0, 0.2, 1.6	0.0, 0.2, 1.5	0.0, 0.3, 1.5
Visual Acuity (Snellen)	Mean	20/32	20/34	20/45	20/47
	Min, Median,	20/15, 20/25,	20/20, 20/32,	20/20, 20/32,	20/20, 20/40,
	Max	20/500	20/800	20/630	20/630
IOP	Mean (SD)	15.60 (3.01)	15.05 (3.92)	14.48 (3.49)	13.48 (3.14)
	Min, Median, Max	8.0, 15.0, 23.0	6.0, 15.0, 34.0	6.0, 14.0, 24.0	8.0, 13.0, 21.0
Subretinal Fluid	N (%)	65 (75)	101 (73)	63 (72)	25 (71)
Exudative retinal detachment	N (%)	10 (16)	33 (33)	35 (56)	14 (56)
Drusen	N (%)	19 (23)	28 (20)	8 (10)	1 (3)
Lipofuscin	N (%)	28 (31)	47 (35)	21 (24)	2 (6)
Ciliary body involvement	N (%)	0	14 (10)	17 (19)	27 (75)
Location					
Posterior	N (%)	74 (84)	94 (68)	48 (59)	5 (15)
Anterior	N (%)	14 (16)	45 (32)	34 (41)	29 (85)
Tumor height	Mean (SD)	2.53 (0.51)	3.96 (1.32)	6.51 (2.05)	8.35 (2.54)
	Min, Median, Max	1.4, 2.5, 4.4	1.4, 3.9, 9.0	2.6, 6.5, 11.7	2.2, 8.8, 12.3
Largest basal diameter	Mean (SD)	9.03 (1.88)	11.83 (2.35)	14.73 (2.07)	16.03 (2.45)
	Min, Median, Max	4.0, 9.0, 12.0	2.4, 12.0, 17.0	9.2, 15.5, 18.0	8.9, 16.0, 20.5
Followed as suspicious nevus	N (%)	32 (36)	34 (25)	14 (16)	6 (19)

Table 2:

Baseline tumor characteristics based on GEP class – AJCC Stage I

Variable	Statistic	Class 1 (n = 74)	Class 2 (n = 17)	Class 1 vs Class 2 (P value)
Age	Mean (SD)	56.57 (15.43)	61.86 (12.37)	0.184
	Min, Median, Max	22.9, 56.3, 87.4	37.0, 64.8, 77.5	
Sex: Male	N (%)	39 (53)	10 (59)	0.789
Affected Eye: Right	N (%)	34 (46)	6 (35)	0.589
Visual Acuity (LogMAR)	Mean (SD)	0.21 (0.33)	0.18 (0.28)	0.996
	Min, Median, Max	-0.1, 0.1, 1.4	-0.1, 0.1, 1.0	
Visual Acuity (Snellen)	Mean	20/32	20/30	
	Min, Median, Max	20/15, 20/20, 20/500	20/15, 20/20, 20/200	
IOP	Mean (SD)	15.79 (3.01)	14.81 (2.99)	0.232
	Min, Median, Max	8.0, 16.0, 23.0	10.0, 15.0, 20.0	
Subretinal Fluid	N (%)	53 (72)	12 (75)	1.000
Exudative retinal detachment	N (%)	7 (13)	3 (25)	0.381
Drusen	N (%)	17 (25)	2 (13)	0.502
Lipofuscin	N (%)	22 (30)	6 (37)	0.565
Ciliary body involvement	N (%)	0	0	---
Tumor location				
Posterior	N (%)	60 (85)	14 (82)	1.000
Anterior	N (%)	11 (15)	3 (18)	
Tumor height	Mean (SD)	2.47 (0.53)	2.76 (0.32)	0.006
	Min, Median, Max	1.4, 2.4, 4.4	1.9, 2.8, 3.3	
Largest basal diameter	Mean (SD)	8.83 (1.88)	9.89 (1.64)	0.030
	Min, Median, Max	4.0, 9.0, 12.0	6.0, 10.0, 12.0	
Followed as a suspicious nevus	N (%)	25 (35)	7 (44)	0.570

P-values for categorical variables are based on Fisher's exact test of difference between proportions.

*P-values for continuous variables are based on Mann-Whitney test of difference between medians.

Table 3:

Baseline tumor characteristics based on GEP class – AJCC Stage IIA

Variable	Statistic	Class 1 (n = 105)	Class 2 (n = 38)	Class 1 vs Class 2 (P value)
Age	Mean (SD)	61.07 (12.89)	67.16 (9.89)	
	Min, Median, Max	35.2, 64.5, 94.4	41.2, 68.4, 85.0	0.018
Sex: Male	N (%)	56 (53)	15 (39)	0.185
Affected Eye: Right	N (%)	51 (49)	22 (58)	0.349
Visual Acuity (LogMAR)	Mean (SD)	0.25 (0.31)	0.23 (0.25)	
	Min, Median, Max	0.0, 0.1, 1.6	0.0, 0.2, 1.0	0.724
Visual Acuity (Snellen)	Mean	20/35	20/34	
	Min, Median, Max	20/20, 20/25, 20/800	20/20, 20/30, 20/200	
IOP	Mean (SD)	15.01 (3.33)	15.17 (5.25)	
	Min, Median, Max	8.0, 15.0, 23.0	6.0, 14.0, 34.0	0.436
Subretinal Fluid	N (%)	73 (73)	28 (74)	1.000
Exudative retinal detachment	N (%)	20 (28)	13 (46)	0.098
Drusen	N (%)	22 (22)	6 (16)	0.634
Lipofuscin	N (%)	32 (33)	15 (42)	0.415
Ciliary body involvement	N (%)	10 (10)	4 (11)	1.000
Tumor location				
Posterior	N (%)	76 (75)	18 (49)	
Anterior	N (%)	26 (25)	19 (51)	0.007
Tumor height	Mean (SD)	4.00 (1.41)	3.87 (1.06)	
	Min, Median, Max	1.4, 3.9, 9.0	1.9, 3.8, 5.8	0.826
Largest basal diameter	Mean (SD)	11.91 (2.31)	11.61 (2.49)	
	Min, Median, Max	3.5, 12.0, 17.0	2.4, 12.0, 15.5	0.389
Followed as a suspicious nevus	N (%)	30 (30)	4 (11)	0.026

P-values for categorical variables are based on Fisher's exact test of difference between proportions.

*P-values for continuous variables are based on Mann-Whitney test of difference between medians.

Table 4:

Baseline tumor characteristics based on GEP class – AJCC Stage IIB

Variable	Statistic	Class 1 (n = 55)	Class 2 (n = 34)	Class 1 vs Class 2 (P value)
Age	Mean (SD)	55.80 (16.61)	60.42 (14.81)	
	Min, Median, Max	25.3, 55.2, 83.3	19.3, 60.7, 83.4	0.191
Sex: Male	N (%)	23 (42)	20 (59)	0.132
Affected Eye: Right	N (%)	26 (47)	20 (59)	0.383
Visual Acuity (LogMAR)	Mean (SD)	0.35 (0.39)	0.33 (0.32)	
	Min, Median, Max	0.0, 0.2, 1.5	0.0, 0.3, 1.2	0.984
Visual Acuity (Snellen)	Mean	20/45	20/43	
	Min, Median, Max	20/20, 20/30, 20/600	20/20, 20/40, 20/300	
IOP	Mean (SD)	14.54 (3.87)	14.39 (2.87)	
	Min, Median, Max	6.0, 14.0, 24.0	10.0, 14.0, 22.0	0.877
Subretinal Fluid	N (%)	34 (63)	29 (85)	0.029
Exudative retinal detachment	N (%)	16 (47)	19 (66)	0.204
Drusen	N (%)	5 (9)	3 (10)	1.000
Lipofuscin	N (%)	11 (21)	10 (30)	0.439
Ciliary body involvement	N (%)	11 (20)	6 (18)	1.000
Tumor location				
Posterior	N (%)	30 (57)	18 (62)	
Anterior	N (%)	23 (43)	11 (38)	0.649
Tumor height	Mean (SD)	6.48 (2.17)	6.57 (1.85)	
	Min, Median, Max	2.6, 6.6, 11.7	3.6, 6.4, 10.5	0.774
Largest basal diameter	Mean (SD)	14.62 (2.12)	14.90 (2.01)	
	Min, Median, Max	9.2, 15.5, 18.0	9.8, 15.5, 18.0	0.687
Followed as a suspicious nevus	N (%)	11 (20)	3 (9)	0.235

P-values for categorical variables are based on Fisher's exact test of difference between proportions.

*P-values for continuous variables are based on Mann-Whitney test of difference between medians.

Table 5:

Baseline tumor characteristics based on GEP class – AJCC Stage IIIA

Variable	Statistic	Class 1 (1A and 1B) (n =15)	Class 2 (n = 21)	Class 1 vs Class 2 (P value)
Age	Mean (SD)	52.70 (21.60)	65.06 (16.52)	0.183
	Min, Median, Max	19.0, 56.0, 85.9	38.1, 60.0, 93.0	
Sex: Male	N (%)	8 (53)	10 (48)	1.000
Affected Eye: Right	N (%)	5 (33)	13 (62)	0.176
Visual Acuity (LogMAR)	Mean (SD)	0.36 (0.30)	0.38 (0.35)	0.970
	Min, Median, Max	0.0, 0.4, 1.0	0.0, 0.3, 1.5	
Visual Acuity (Snellen)	Mean	20/60	20/48	
	Min, Median, Max	20/20, 20/50, 20/200	20/20, 20/40, 20/600	
IOP	Mean (SD)	12.77 (2.49)	14.00 (3.51)	0.372
	Min, Median, Max	8.0, 12.0, 18.0	9.0, 13.5, 21.0	
Subretinal Fluid	N (%)	10 (71)	15 (71)	1.000
Exudative retinal detachment	N (%)	6 (60)	8 (53)	1.000
Drusen	N (%)	0	1 (5)	1.000
Lipofuscin	N (%)	2 (15)	0	0.139
Ciliary body involvement	N (%)	9 (60)	18 (86)	0.122
Tumor location				
Posterior	N (%)	4 (29)	1 (5)	0.135
Anterior	N (%)	10 (71)	19 (95)	
Tumor height	Mean (SD)	7.58 (3.28)	8.90 (1.73)	0.183
	Min, Median, Max	2.2, 8.4, 12.3	4.8, 9.2, 12.3	
Largest basal diameter	Mean (SD)	15.84 (3.10)	16.16 (1.93)	0.685
	Min, Median, Max	8.9, 16.0, 20.5	12.0, 16.0, 20.0	
Followed as a suspicious nevus	N (%)	2 (15)	4 (21)	1.000

P-values for categorical variables are based on Fisher's exact test of difference between proportions.

*

P-values for continuous variables are based on Mann-Whitney test of difference between medians.