Prognosis of Uveal Melanoma in 500 Cases Using Genetic Testing of Fine-Needle Aspiration Biopsy Specimens

Carol L. Shields, MD,[1] Arupa Ganguly, PhD,[2] Carlos G. Bianciotto, MD,[1] Kiran Turaka, MD,[1] Ali Tavallali, MD,[1] Jerry A. Shields, MD[1]

Purpose: To determine the relationship between monosomy 3 and incidence of metastasis after genetic testing of uveal melanoma using fine-needle aspiration biopsy (FNAB).

Design: Noncomparative retrospective case series.

Participants: Five hundred patients.

Methods: Fine-needle aspiration biopsy was performed intraoperatively immediately before plaque radiotherapy. The specimen underwent genetic analysis using DNA amplification and microsatellite assay. Systemic follow-up was obtained regarding melanoma-related metastasis.

Main Outcome Measures: Presence of chromosome 3 monosomy (loss of heterozygosity) and occurrence of melanoma metastasis.

Results: Disomy 3 was found in 241 melanomas (48%), partial monosomy 3 was found in 133 melanomas (27%), and complete monosomy 3 was found in 126 melanomas (25%). The cumulative probability for metastasis by 3 years was 2.6% for disomy 3, 5.3% for partial monosomy 3 (equivocal monosomy 3), and 24.0% for complete monosomy 3. At 3 years, for tumors with disomy 3, the cumulative probability of metastasis was 0% for small (0–3 mm thickness), 1.4% for medium (3.1–8 mm thickness), and 23.1% for large (>8 mm thickness) melanomas. At 3 years, for tumors with partial monosomy 3, the cumulative probability of metastasis was 4.5% for small, 6.9% for medium, and [insufficient numbers] for large melanomas. At 3 years, for tumors with complete monosomy 3, the cumulative probability of metastasis was 0% for small, 24.4% for medium, and 57.5% for large melanomas. The most important factors predictive of partial or complete monosomy 3 included increasing tumor thickness ($P = 0.001$) and increasing distance to optic disc ($P = 0.002$).

Conclusions: According to FNAB results, patients with uveal melanoma demonstrating complete monosomy 3 have substantially poorer prognosis at 3 years than those with partial monosomy 3 or disomy 3. Patients with partial monosomy 3 do not significantly differ in outcome from those with disomy 3.

Financial Disclosure(s): The author(s) have no proprietary or commercial interest in any materials discussed in this article. *Ophthalmology 2011;118:396–401 © 2011 by the American Academy of Ophthalmology.*

Several articles have been written on the relevance of genetic testing of uveal melanoma.[1,2] In 1996, Prescher et al[3] reported the landmark observation that uveal melanoma with chromosome 3 monosomy was an important predictor of worse patient prognosis. In that report, the authors retrospectively evaluated 54 enucleated eyes with uveal melanoma and correlated the copy number of chromosome 3 to the known patient outcome. Several publications on genetic testing of melanoma from enucleated eyes have confirmed their observations.[4–8]

In 2002, Naus and associates[9] reported that fine-needle aspiration biopsy (FNAB) could be reliably used to sample tumors for genetic testing of uveal melanoma and the results correlated to those obtained by open biopsy after enucleation. In 2006, Midena and associates[10] reported the first clinical series of 8 eyes with uveal melanoma sampled by FNAB for genetic testing using fluorescent in situ hybridization. Later, Young et al[11] used transscleral FNAB into the tumor base for genetic testing in 18 eyes and found a 50% yield. Shields et al[12] added their experience with 140 cases of FNAB for uveal melanoma and indicated that trans pars plana FNAB into the tumor apex provided adequate DNA for microsatellite assay in 97% of cases with little complication and no patient with tumor recurrence along the needle tract. This report provides an analysis of patient prognosis in 500 cases based on results of genetic testing from FNAB for uveal melanoma.

Materials and Methods

We reviewed the clinical records of all patients on the Ocular Oncology Service at Wills Eye Institute, Philadelphia, Pennsylvania, with the diagnosis of uveal melanoma managed with FNAB yielding DNA for genetic testing of chromosome 3 status at the

© 2011 by the American Academy of Ophthalmology
Published by Elsevier Inc.
ISSN 0161-6420/11/$–see front matter
doi:10.1016/j.ophtha.2010.05.023

time of plaque radiotherapy between November 1, 2005, and February 2, 2009. Institutional review board approval was obtained for this study. Data were gathered regarding clinical and genetic features of the tumor. The clinical data at initial examination included age, race, gender, affected eye, visual acuity, symptoms, and presence of ocular melanocytosis. The presence of cutaneous melanoma or systemic cancer was recorded. The tumor data included tumor anatomic location (iris, ciliary body, choroid), quadrant (superior, temporal, inferior, nasal, macula), anteroposterior location (macula, macula-equator, equator-ora, ciliary body, iris), distance to the optic disc (millimeters), distance to the foveola (millimeters), tumor basal dimension (millimeters), tumor thickness (millimeters by ultrasonography), and tumor pigmentation. Other features included presence of drusen, subretinal fluid, orange pigment on the tumor surface, Bruch's membrane rupture, retinal invasion, and extrascleral extension of the tumor. Ultrasonographic features were recorded on A and B scans for features of acoustic hollowness, internal reflectivity, and tumor configuration (plateau, dome, mushroom). Measurement of thickness was judged using both scan techniques.

The specific technique of FNAB of uveal melanoma for genetic testing has been described in a previous report.[12] The procedure was performed in the operating room under sterile condition at the time of plaque radiotherapy. The 10-ml syringe was attached to a 10-inch tube that was connected to the 27-gauge needle. The syringe and needle tip were dry, and after entering the tumor, aspiration to 10 ml was applied. Tumors posterior to the equator were sampled using the trans pars plana transvitreal approach with indirect ophthalmoscopic guidance of the needle through the pars plana and vitreous into the tumor. Tumors anterior to the equator were sampled by a transscleral approach using the needle through the sclera overlying the tumor into the tumor base or by a trans

Table 4. Chromosome 3 Status of Uveal Melanoma in 500 Patients: Tumor Characteristics

Features	Overall No. (%) n = 500	Disomy 3 No. (%) n = 241	Partial (Equivocal) Monosomy 3 No. (%) n = 133	Complete Monosomy 3 No. (%) n = 126
Location				
Iris	15	4 (27)	3 (20)	8 (53)
Ciliary body	75	17 (23)	23 (31)	35 (47)
Choroid	410	220 (54)	107 (26)	83 (20)
Tumor quadrant				
Superior	118	53 (45)	33 (28)	32 (27)
Temporal	80	36 (45)	19 (24)	25 (31)
Inferior	114	47 (41)	34 (30)	33 (29)
Nasal	66	30 (45)	17 (26)	19 (29)
Macula	122	75 (62)	30 (26)	17 (14)
Anteroposterior location of epicenter				
Iris	11	3 (27)	2 (18)	6 (54)
Ciliary body	58	14 (24)	16 (28)	28 (48)
Equator to ora	53	15 (28)	22 (42)	16 (30)
Macula to equator	256	134 (52)	62 (24)	60 (23)
Macula	122	75 (61)	31 (25)	16 (13)
Distance to the optic nerve (mm)				
Median (mean, range)	3 (4.1, 0–18)	3 (3.4, 0–18)	4 (4.9, 0–16)	4 (5.0, 0–18)
Distance to the foveola (mm)				
Median (mean, range)	3 (3.8, 0–18)	2 (3.2, 0–15)	3 (4.3, 0–18)	4.0 (4.8, 0–18)
Largest base (mm)				
Median (mean, range)	10 (10.6, 3–20)	10 (9.9, 3–18)	11 (10.8, 4–18)	12 (11.9, 4–20)
Thickness (mm)				
Median (mean, range)	3.8 (4.7, 1.4–12.3)	3.4 (4.1, 1.4–11.6)	4.2 (4.9, 1.6–12)	4.7 (5.5, 1.7–12.3)
Pigment				
Melanotic	346	155 (45)	89 (26)	102 (29)
Amelanotic	86	48 (56)	25 (29)	13 (15)
Combination	68	38 (56)	19 (28)	11 (16)
Related features				
Drusen	58	28 (48)	16 (28)	14 (24)
Orange pigment	240	132 (55)	63 (26)	45 (19)
Subretinal fluid	397	200 (50)	104 (26)	93 (23)
Bruch's membrane rupture	89	40 (45)	29 (33)	20 (22)
Retinal invasion	28	14 (50)	7 (25)	7 (25)
Extrascleral extension	9	2 (22)	2 (22)	5 (56)
Ultrasound features				
Configuration				
Plateau	37	23 (62)	6 (16)	8 (22)
Dome	399	190 (48)	105 (26)	104 (26)
Mushroom	64	28 (44)	22 (34)	14 (22)
Acoustic hollowness	437	215 (49)	115 (26)	107 (24)
Internal reflectivity low	461	224 (49)	125 (27)	112 (24)
Choroidal excavation	462	221 (48)	127 (27)	114 (25)

Table 5. Kaplan–Meier Estimates of Probability of Metastasis of Uveal Melanoma Based on Tumor Thickness in 500 Patients

Tumor Thickness (mm)	Uveal Melanoma		Cumulative Probability of Metastasis (95% CI) (No. Failed/No. Left)		
	No. of Patients	*No. of Patients with Metastasis (%)*	*1 yr*	*2 yrs*	*3 yrs*
Small (0–3 mm)	173	1 (0.6)	0% (0–2.5) (0/118)	1.2% (0–3.5) (1/66)	1.2% (0–3.5) (1/15)
Medium (3.1–8 mm)	264	10 (3.8)	1.1% (0–2.5) (2/179)	5.4% (1.7–9.2) (8/85)	9.2% (2.7–15.7) (10/17)
Large (>8 mm)	63	7 (11.1)	2.1% (0–6.3) (1/44)	8.0% (0–17.0) (3/19)	39.4% (7.1–71.7) (6/3)
Overall	500	18 (3.6)	0.85% (0–1.8) (3/341)	4.3% (1.9–6.7) (12/170)	9.3% (4.2–14.3) (17/35)

CI = confidence interval.

pars plana transvitreal approach. The cells were stored in refrigerated Hank's solution and submitted for genetic studies. During the same operation, a radioactive plaque was applied immediately after the FNAB.

Genetic testing was performed on DNA extractions from blood (control) and conjunctiva (control) and FNAB samples using commercially available isolation kits (Qiagen Inc., Valencia, CA). Polymerase chain reaction-based diagnosis for monosomy of chromosome 3 was performed by evaluating 10 polymorphic microsatellite markers on chromosome 3. These markers are listed in Table 1 (available at http://aaojournal.org) and were purchased from Applied Biosystems (ABI, Carlsbad, CA) (http://www.appliedbiosystems.com/; accessed December 20, 2005) human genome mapping kit V2.5. The amplification products were analyzed on ABI 3730 fragment analyzer. Data analysis was performed using ABI GeneMapper software V3.0. Tumors that retained only 1 allele of all 10 markers missing were classified as loss of heterozygosity and inferred complete monosomy 3, tumors that retained only 1 allele of 1 to 9 markers missing were classified as partial (equivocal) monosomy 3, and tumors that retained both alleles for all 10 markers were classified as disomy 3. The follow-up data were gathered regarding the presence or absence of metastasis and the time interval to date of metastasis.

Statistical Methods

The clinical data were then analyzed with regard to the outcome of disomy 3, partial monosomy 3, or complete monosomy 3. The effect of each individual clinical variable on this outcome was analyzed by logistic regression analysis. All variables were analyzed as discrete variables except for patient age, tumor base, tumor thickness, proximity to optic disc, and proximity to foveola, which were analyzed as continuous variables. Statistical significance was assigned at $P<0.05$. Odds ratios accompanied by 95% confidence intervals were provided for each clinical risk factor for partial or complete monosomy 3. Cumulative survival probability of metastasis using Kaplan–Meier estimates were provided at 1, 2, and 3 years after treatment for small (0–3 mm thickness), medium (3.1–8 mm thickness), and large (>8 mm thickness) melanomas; for disomy 3, partial monosomy 3, and complete monosomy 3 melanomas; and for the various combinations of tumor size (small, medium, and large) with genetic results (disomy 3, partial monosomy 3, and complete monosomy 3).

Results

There were 500 eyes with uveal melanoma sampled for chromosome 3 status using FNAB. The patient age, race, gender, and other cancers are listed in Table 2 (available at http://aaojournal.org). The overall median patient age at diagnosis was 58 years: 55 years for those with disomy 3 and 61 years for complete monosomy 3. There was a history of cutaneous melanoma in 2% of cases. The ocular features are presented in Table 3 (available at http://aaojournal.org). Overall, 31% were asymptomatic: 33% for disomy 3 and 25% for complete monosomy 3. Ocular melanocytosis was found in 4% of affected eyes: 4% of those with disomy 3 and 6% with monosomy 3.

The tumor features are listed in Table 4. Overall, the tumor location was iris in 15 cases (3%), ciliary body in 75 cases (15%), and choroid in 410 cases (82%). Complete monosomy 3 was found in 6 cases (54%) of iris melanoma, 28 cases (48%) of ciliary body melanoma, and 92 cases (21%) of choroidal melanoma. The median distance to the foveola was 3 mm overall, 3 mm for disomy 3 tumors, and 4 mm for partial or complete monosomy 3. The median tumor thickness was 3.8 mm overall, 3.4 mm for disomy 3 tumors, and 4.7 mm for complete monosomy 3.

After needle biopsy through the retina to obtain a sample of underlying tumor, there was no sign of rhegmatogenous retinal detachment; persistent vitreous, subretinal, or intratumoral hemorrhage; infection; or tumor dissemination in any case. In many cases, after withdrawal of the needle, there was 1 drop of preretinal blood at the site of retinal perforation, and further hemorrhage was prevented by gentle cotton swab applicator to the scleral entry site for 1 to 2 minutes under indirect ophthalmoscopic guidance. The drop of blood completely resolved by the 4-month examination.

The cumulative probability for metastasis at 1, 2, and 3 years was 0%, 1.2%, and 1.2% for small melanomas; 1.1%, 5.4%, and 9.2% for medium melanomas; and 2.1%, 8.0%, and 39.4% for large melanomas, respectively (Table 5). Combination of tumor size with chromosome 3 results (Table 6) revealed cumulative probability for metastasis at 1, 2, and 3 years for small melanomas with disomy 3 at 0%, 0%, and 0%, respectively. The greatest probability for metastasis at 1, 2, and 3 years was found for large melanomas with complete monosomy 3 at 5.6%, 15.0%, and 57.5%, respectively. At 3 years, the probability of metastasis was 2.6% for patients with disomy 3, 5.3% for patients with partial monosomy 3, and 24.0% for patients with complete monosomy 3 ($P < 0.001$, odds ratio [OR] = 5.8, compared with disomy 3).

By multivariate analysis, the factors predictive of partial or complete monosomy 3 melanoma included greater tumor thickness ($P = 0.001$, OR 1.16 per millimeter increase), greater distance to the optic disc ($P = 0.002$, OR 1.09 per millimeter increase), and presence of cutaneous melanoma ($P = 0.023$, OR 11.15) (Table 7) (Fig 1, available at http://aaojournal.org).

Discussion

Several cytogenetic abnormalities have been identified in uveal melanoma involving chromosomes 1, 3, 6, 8, 11, 13, and others. Kilic and associates[13] evaluated 74 eyes enucle-

Table 6. Kaplan–Meier Estimates of Probability of Metastasis of Uveal Melanoma Based on Tumor Thickness and Genetic Results in 500 Patients

Tumor Thickness (mm)	Uveal Melanoma		Cumulative Probability of Metastasis (95% CI) (No. Failed/No. Left)		
	No. of Patients	*No. of Patients with Metastasis (%)*	*1 yr*	*2 yrs*	*3 yrs*
Disomy (N = 241)					
Small (0–3 mm)	101	0	0 (0/62)	0 (0/37)	0 (0/8)
Medium (3.1–8 mm)	123	1 (0.8)	0% (0–3.3) (0/91)	1.4% (0–4.1) (1/47)	1.4% (0–4.1) (1/7)
Large (>8 mm)	17	2 (11.8)	0% (0–18.7) (0/13)	7.7% (0–22.2) (1/6)	23.1% (0–53.1) (2/1)
Overall	241	3 (1.2)	0% (0–1.8) (0/165)	1.4% (0–3.4) (2/90)	2.6% (0–5.6) (3/16)
Partial monosomy (N = 133)					
Small (0–3 mm)	42	1 (2.4)	0% (0–32) (0/32)	4.5% (0–13.2) (1/16)	4.5% (0–13.2) (1/3)
Medium (3.1–8 mm)	71	3 (4.2)	2% (0–5.9) (1/45)	6.9% (0–14.4) (3/17)	6.9% (0–14.4) (3/3)
Large (>8 mm)	20	0	0 (0/15)	0 (0/6)	0 (0/1)
Overall	133	4 (3.0)	1.0% (0–3.0) (1/92)	5.3% (0.1–10.5) (4/39)	5.3% (0.1–10.5) (4/7)
Complete monosomy (N = 126)					
Small (0–3 mm)	30	0	0 (0/24)	0 (0/13)	0 (0/4)
Medium (3.1–8 mm)	70	6 (8.6)	2.2% (0–6.5) (1/43)	11.7% (0.6–22.7) (4/21)	24.4% (5.2–43.7) (6/7)
Large (>8 mm)	26	5 (19.2)	5.6% (0–16.1) (1/16)	15.0% (0–35.0) (2/7)	57.5% (14.7–100) (4/1)
Overall	126	11 (8.7)	2.3% (0–5.5) (2/83)	8.9% (1.9–16.0) (6/41)	24.0% (8.9–39.0) (10/12)

CI = confidence interval.
Partial refers to equivocal monosomy 3.

ated for uveal melanomas for chromosomal losses or gains and found the most frequent abnormality involved chromosome 8q gain (53%), 8p gain (18%), 8p loss (24%), chromosome 3p loss (41%), 3q loss (42%), chromosome 1p partial loss (24%), chromosome 6p gain (18%), 6q loss (28%), and chromosome 16q loss (16%). By statistical analysis of clinical, histopathologic, and cytogenetic results, the most important factors predictive of patient survival

Table 7. Univariate and Multivariate Analyses of Factors Predictive of Partial or Complete Monosomy 3 in 500 Patients Based on Clinical Features at Presentation

Feature	Partial or Complete Monosomy 3 n = 259 Patients	Disomy 3 n = 241 Patients	*P* Value	Odds Ratio	Confidence Interval
Univariate analysis					
Age (mean)	58.17	55.17	0.014	1.18†	1.03–1.34
Skin MM (present vs. absent*)	10 (3.9)	1 (0.4)	0.031	9.64	1.22–75.87
Tumor location					
(Ciliary body vs. choroid*)	24 (9.3)	4 (1.7)	<0.001	6.98	2.38–20.49
(Ciliochoroid vs. choroid*)	34 (13.2)	13 (5.4)	0.001	3.04	1.56–5.94
Tumor quadrant					
(Inferior vs. macula*)	67 (25.9)	47 (19.5)	0.002	2.28	1.35–3.83
(Temporal vs. macula*)	44 (17.0)	36 (14.9)	0.022	1.95	1.10–3.46
(Superior vs. macula*)	65 (25.1)	53 (22.0)	0.011	1.96	1.17–3.27
(Nasal vs. macula*)	36 (13.9)	30 (12.4)	0.036	1.92	1.04–3.51
Anteroposterior tumor epicenter					
(Equator to ora vs. macula*)	38 (14.7)	15 (6.2)	<0.001	4.04	2.01–8.14
(Ciliary body vs. macula*)	44 (17.0)	14 (5.8)	<0.001	5.02	2.48–10.13
(Iris vs. macula*)	8 (3.1)	3 (1.2)	0.039	4.26	1.08–16.85
Distance to optic nerve (mean)	4.97	3.37	<0.001	1.12‡	1.07–1.18
Distance to foveola (mean)	4.53	3.21	<0.001	1.10‡	1.04–1.15
Largest base (mean)	11.31	9.93	<0.001	1.12‡	1.07–1.18
Thickness (mean)	5.19	4.14	<0.001	1.19‡	1.11–1.29
Multivariate analysis					
Skin MM (present vs. absent*)	—	—	0.025	11.15	1.35–92.4
Distance to optic disc (mean)	—	—	0.002	1.09‡	1.04–1.16
Thickness (mean)	—	—	0.001	1.16‡	1.07–1.27

Logistic regression analysis. Partial refers to equivocal monosomy 3.
*Reference variable.
†Per 10-yr increase.
‡Per 1-mm increase.

were presence of chromosome 3 monosomy and largest tumor diameter.[13] Damato and associates[14] and Damato and Coupland[15] found that uveal melanoma prognosis was associated with chromosome 3 monosomy, largest tumor diameter, and the presence of epithelioid cell type. Previous analyses of the prognostic effect of chromosome 3 monosomy have focused mostly on enucleated eyes and patients with retrospectively known outcomes. In our analysis, we chose to focus on the outcome of each patient after FNAB-performed cytogenetic analysis. Each patient was treated with plaque radiotherapy at the time of FNAB, and there were no enucleations for cytogenetics in this series because all biopsy specimens were obtained by needle sampling.[12] In addition, our analysis is clinically important in that we evaluated complete monosomy 3, partial monosomy 3, and disomy 3 results specifically for small, medium, and large melanomas. In this way, a more specified prognostication was made on the basis of tumor size and cytogenetics.

Previous reports by Kilic and associates[13] and Damato and associates[14] emphasized that the important factors for uveal melanoma prognosis are chromosome 3 monosomy and tumor size. In their extensive 7-year experience with uveal melanoma cytogenetic testing after enucleation or resection in 356 cases, Damato and associates commented in the opening sentence of the discussion that their study "confirms that monosomy 3 is associated with a high rate of metastatic disease in the first 5 years after treatment" and "prognosis [estimation] is improved [with] cytogenetic results together with largest basal tumor diameter and tumor cell type."[14] In our study, we chose to evaluate cytogenetic results and tumor size because this was the common factor in the above 2 large reports.[13,14] We investigated both factors independently and jointly in 500 consecutive cases, but our study is different because we obtained tissue by FNAB without enucleation and not open biopsy after enucleation, as was performed for both previous landmark studies.[13,14] We chose to use ultrasound measurement of tumor thickness to represent size because this quantified number was interpreted to be more reliable and less arbitrary than basal dimension estimation with the indirect ophthalmoscope or with ultrasonography, in which flat tumor extension might not be imaged.

In our 500 cases, the cumulative probability for metastasis was 1.2% for small, 9.2% for medium, and 39.4% for large melanomas (Table 5). Independently, the probability for metastasis was 2.6% for tumors with disomy 3, 5.3% for tumors with partial monosomy 3, and 24.0% for tumors with complete monosomy 3. By combining these 2 factors, it was apparent that the prognosis varied greatly on the basis of tumor size and cytogenetics (Table 6). Complete monosomy 3 was present in 17% (30/173) of small melanomas, 27% (70/264) of medium melanomas, and 41% (26/63) of large melanomas. At 3 years follow-up, the cumulative probability for metastasis for tumors with complete monosomy 3 was 0% for small, 24.4% for medium, and 57.5% for large melanomas. By comparison, the probability for tumors with disomy 3 was 0% for small, 1.4% for medium, and 23.1% for large melanomas. In confirmation of previous retrospective investigations in enucleated specimens,[13,14] we have shown using FNAB in non-enucleated eyes that monosomy 3 and tumor size are defining in melanoma-related metastasis.

The correlation of uveal melanoma cytogenetics in FNAB specimens with open biopsy specimens has been established.[9,16] Even small choroidal melanoma or those in the macula can be biopsied to provide sufficient DNA for cytogenetic evaluation.[17–20] However, it should be realized that needle biopsy of tumor tissue could lead to sampling error based on the possibly heterogeneous distribution of monosomy 3 abnormality within uveal melanoma.[21,22] The higher rate of metastasis in large melanoma with disomy 3 could represent sampling error or indicate other factors involved in prognostication.

In conclusion, this preliminary evaluation showed that the combination of increasing tumor size and the presence of complete monosomy 3 in uveal melanoma portends a worse prognosis. Partial monosomy 3 and disomy 3 portend a better prognosis. Longer follow-up will be necessary to determine the validity of these observations.

References

1. Harbour JW. Eye cancer: unique insights into oncogenesis: The Cogan Lecture. Invest Ophthalmol Vis Sci 2006;47:1736–45.
2. Shields CL. The hunt for the secrets of uveal melanoma. Perspective. Editorial. J Clin Exp Ophthalmol 2008;36:277–80.
3. Prescher G, Bornfeld N, Hirche H, et al. Prognostic implications of monosomy 3 in uveal melanoma. Lancet 1996;347:1222–5.
4. Sisley K, Rennie IG, Cottam DW, et al. Cytogenetic findings in six posterior uveal melanomas: involvement of chromosomes 3, 6, and 8. Genes Chromosomes Cancer 1990;2:205–9.
5. Horsthemke B, Prescher G, Bornfeld N, et al. Loss of chromosome 3 alleles and multiplication of chromosome 8 alleles in uveal melanoma. Genes Chromosomes Cancer 1992;4:217–21.
6. Sisley K, Rennie IG, Parsons MA, et al. Abnormalities of chromosomes 3 and 8 in posterior uveal melanoma correlate with prognosis. Genes Chromosomes Cancer 1997;19:22–8.
7. Onken MD, Worley LA, Ehlers JP, Harbour JW. Gene expression profiling in uveal melanoma reveals two molecular classes and predicts metastatic death. Cancer Res 2004;64:7205–9.
8. Hughes S, Damato BE, Giddings I, et al. Microarray comparative genomic hybridisation analysis of intraocular uveal melanomas identifies distinctive imbalances associated with loss of chromosome 3. Br J Cancer 2005;93:1191–6.
9. Naus NC, Verhoeven AC, van Drunen E, et al. Detection of genetic prognostic markers in uveal melanoma biopsies using fluorescence in situ hybridization. Clin Cancer Res 2002;8:534–9.
10. Midena E, Bonaldi L, Parrozzani R, et al. In vivo detection of monosomy 3 in eyes with medium-sized uveal melanoma using transscleral fine needle aspiration biopsy. Eur J Ophthalmol 2006;16:422–5.
11. Young T, Rao NP, Glasgow BJ, et al. Fluorescent in situ hybridization of monosomy 3 via 30-gauge fine-needles aspiration biopsy of choroidal melanoma in vivo. Ophthalmology 2007;114:142–6.
12. Shields CL, Ganguly A, Materin MA, et al. Chromosome 3 analysis of uveal melanoma using fine needle aspiration biopsy at

the time of plaque radiotherapy in 140 consecutive cases. The Deborah Iverson MD Lectureship. Arch Ophthalmol 2007;125: 1017–24.
13. Kilic E, van Gils W, Lodder E, et al. Clinical and cytogenetic analyses in uveal melanoma. Invest Ophthalmol Vis Sci 2006; 47:3703–7.
14. Damato B, Duke C, Coupland SE, et al. Cytogenetics of uveal melanoma: a 7 year clinical experience. Ophthalmology 2007; 114:1925–31.
15. Damato B, Coupland SE. Translating uveal melanoma cytogenetics into clinical care. Arch Ophthalmol 2009;127: 423–9.
16. Sisley K, Nichols C, Parsons MA, et al. Clinical applications of chromosome analysis, from fine needle aspiration biopsies, of posterior uveal melanomas. Eye 1998;12:203–7.
17. Shields CL, Materin MA, Teixiera L, et al. Small choroidal melanoma with chromosome 3 monosomy on fine needle aspiration biopsy. Ophthalmology 2007;114:1919–24.
18. Young TA, Burgess BL, Rao NP, et al. Transscleral fine needle aspiration biopsy of macular choroidal melanoma. Am J Ophthalmol 2008;145:297–302.
19. Young TA, Burgess BL, Rao NP, et al. High density genoma array is superior to fluorescence in-situ hybridization analysis of monosomy 3 in choroidal melanoma fine needle aspiration biopsy. Mol Vis 2007;13:2328–33.
20. Bonaldi L, Midena E, Filippi B, et al. FISH analysis of chromosomes 3 and 6 on fine needle aspiration biopsy samples identifies distinct subgroups of uveal melanomas. J Cancer Res Clin Oncol 2008;134:1123–7.
21. Maat W, Jordanova ES, van Zelderen-Bhola SL, et al. The heterogeneous distribution of monosomy 3 in uveal melanomas: implications for prognostication based on fine needle aspiration biopsies. Arch Pathol Lab Med 2007;131:91–6.
22. Schoenfield L, Pettay J, Tubbs RR, Singh AD. Variation of monosomy 3 status within uveal melanoma. Arch Pathol Lab Med 2009;133:1219–22.

Footnotes and Financial Disclosures

Originally received: October 29, 2009.
Final revision: May 25, 2010.
Accepted: May 26, 2010.
Available online: September 23, 2010. Manuscript no. 2009-1506.

[1] Ocular Oncology Service, Wills Eye Institute, Thomas Jefferson University, Philadelphia, Pennsylvania.

[2] Department of Genetics, University of Pennsylvania School of Medicine, Philadelphia, Pennsylvania.

This article contains online-only material. The following should appear online-only: Tables 1 to 3 and Figure 1.

Presented in part at: the Robert M. Ellsworth Lecture, November 19, 2009, New York, New York (CLS). Presented as a poster at: the American Academy of Ophthalmology, October 24–26, 2009, San Francisco, California (CGB).

Financial Disclosure(s):
The author(s) have no proprietary or commercial interest in any materials discussed in this article.

Support provided by the Retina Research Foundation of the Retina Society in Cape Town, South Africa (CLS); the Paul Kayser International Award of Merit in Retina Research, Houston, Texas (JAS); a donation from Michael, Bruce, and Ellen Ratner, New York, New York (JAS, CLS); Mellon Charitable Giving from the Martha W. Rogers Charitable Trust, Philadelphia, Pennsylvania (CLS); the LuEsther Mertz Retina Research Foundation, New York, New York (CLS); and the Eye Tumor Research Foundation, Philadelphia, Pennsylvania (CLS). Statistical analysis provided by Rishita Nutheti, PhD, Hyderabad, India.

The funders had no role in the design and conduct of the study; in the collection, analysis, and interpretation of the data; or in the preparation, review, or approval of the manuscript. Carol L. Shields, MD, had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

Correspondence:
Carol L. Shields, MD, Ocular Oncology Service, Suite 1440, Wills Eye Institute, 840 Walnut Street, Philadelphia, PA 19107. E-mail: carol.shields@shieldsoncology.com.