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

Carol L. Shields, MD; Arupa Ganguly, PhD; Miguel A. Materin, MD; Luiz Teixeira, MD; Arman Mashayekhi, MD; Lori Ann Swanson, BS; Brian P. Marr, MD; Jerry A. Shields, MD

Objective: To evaluate the feasibility of genetic testing of uveal melanoma using fine-needle aspiration biopsy (FNAB).

Methods: We reviewed the clinical records of all patients of the Ocular Oncology Service at Wills Eye Hospital with the diagnosis of uveal melanoma who underwent FNAB for genetic testing for chromosome 3 status between November 1, 2005, and March 1, 2006. The FNAB was performed immediately before plaque radiotherapy. The specimens underwent genetic analysis using DNA amplification and microsatellite assay to determine the presence of monosomy 3.

Results: A total of 140 eyes of 140 patients with uveal melanoma were sampled for chromosome 3 abnormalities using FNAB. Monosomy 3 was found in 44 cases (31%), disomy 3 was found in 76 cases (54%), and the genomic DNA yield was insufficient for genetic analysis in 20 cases (14%). Monosomy 3 was found in 16 of 61 small melanomas (26%), 24 of 67 medium melanomas (36%), and 4 of 12 large melanomas (33%). Adequate DNA was achieved in 97% of cases using a 27-gauge needle via the transvitreal tumor apex approach and in 75% of cases using a 30-gauge needle via the transscleral tumor base approach. Factors predictive of monosomy 3 included greater tumor basal dimension (P = .02) and greater distance from the optic disc (P = .02). Transient localized vitreous hemorrhage was found in 46% of eyes. No cases of diffuse vitreous hemorrhage, retinal detachment, or tumor recurrence along the biopsy tract were found.

Conclusion: We found that in most cases, FNAB provides adequate DNA for genetic analysis of uveal melanoma using microsatellite assay.

Arch Ophthalmol. 2007;125(8):1017-1024

For editorial comment see page 1122

Author Affiliations: Ocular Oncology Service, Wills Eye Hospital, Thomas Jefferson University (Drs C. L. Shields, Materin, Teixeira, Mashayekhi, Marr, and J. A. Shields), and Department of Genetics, University of Pennsylvania School of Medicine (Dr Ganguly and Ms Swanson), Philadelphia.

We reviewed the clinical records of all patients of the Ocular Oncology Service at Wills Eye Hospital with the diagnosis of uveal melanoma who underwent FNAB for genetic testing for chromosome 3 status between November 1, 2005, and March 1, 2006. The Wills Eye Hospital institutional review board issued approval for this retrospective study. Data were gathered regarding clinical and genetic features of the tumor. The clinical data at initial...
examination included age, race (African American, Hispanic, Asian, or white), sex (female or male), affected eye (right or left), visual acuity, and symptoms. The tumor data included location (iris, ciliary body, or choroid), quadrant location (inferior, temporal, superior, nasal, or macula), anterior-posterior location (macula, macula-equator, equator-ora, ciliary body, or iris), distance to the optic nerve (in millimeters), distance to the foveola (in millimeters), tumor basal dimension (in millimeters), tumor thickness (in millimeters by ultrasonography), subretinal fluid, orange pigment on the tumor surface, and previous documented tumor growth. The FNAB parameters included needle gauge, route (transscleral tumor base approach or transvitreal tumor apex approach), and fundus findings immediately after biopsy. Follow-up data included needle biopsy complications and recurrence at the site of biopsy.

**FNAB Technique**

After obtaining informed patient consent for FNAB and genetic testing, tissue sampling was performed. A 3-ML blood sample in a purple-top EDTA tube at room temperature was obtained for isolation of constitutional DNA to be used as a control for comparison with microsatellite alleles detected in tumor DNA. The intraocular tumor sample was obtained at the time of plaque radiotherapy using retrobulbar anesthesia. After localization of the intraocular tumor and placement of intrascleral nylon sutures before securing the plaque, FNAB was performed (Figure 1). If the tumor was posterior to the equator of the eye, sampling was done via the trans pars plana transvitreal approach using a 27-gauge long needle on a connector tube and a 10-ML syringe. If the tumor was anterior to the equator of the eye, sampling was performed via the transscleral approach using a 30-gauge short needle on a connector tube and a 10-ML syringe. For the pars plana approach, the needle was entered along the meridian of the tumor 4 mm posterior to the limbus and directed into the extrafoveal apical portion of the tumor using indirect ophthalmoscopic guidance. For the transscleral approach, the tumor transillumination shadow was outlined on the sclera, and the needle was entered nearly perpendicular to the sclera at the tumor base, but with a slight bevel to make the wound self-sealing. The depth of penetration through the sclera depended on the measured tumor thickness, and the needle was aimed to sample the tumor base or midportion. In both approaches, the needle was held securely for the 10-ML syringe aspiration, and after needle withdrawal, pressure was applied to the globe puncture site with a cotton-tipped applicator. The microscopic cells were aspirated up the needle tip into the syringe using Hank solution and then flushed into a test tube and refrigerated until analysis by the genetic laboratory. The radioactive plaque was then applied to the eye into a test tube and refrigerated until analysis by the genetic laboratory. The microscopic cells were aspirated up the midportion. In both approaches, the needle was held securely and the needle was aimed to sample the tumor base or midportion. The depth of penetration through the sclera depended on the measured tumor thickness, and the needle was aimed to sample the tumor base or midportion. In both approaches, the needle was held securely for the 10-ML syringe aspiration, and after needle withdrawal, pressure was applied to the globe puncture site with a cotton-tipped applicator. The microscopic cells were aspirated up the needle tip into the syringe using Hank solution and then flushed into a test tube and refrigerated until analysis by the genetic laboratory. The radioactive plaque was then applied to the eye into a test tube and refrigerated until analysis by the genetic laboratory.

**Genetic Testing Technique**

DNA extractions from blood and the FNAB samples were performed using commercially available isolation kits (Qiagen, Valencia, California) following manufacturer-suggested protocols. Polymerase chain reaction–based diagnosis for monosomy 3 was performed by evaluating 10 polymorphic microsatellite markers on chromosome 3. These markers (ABI human genome mapping kit V2.5; Applied Biosystems, Foster City, California; http://www.appliedbiosystems.com/) were used according to the manufacturer’s instructions. The amplification products were analyzed on an ABI 3100 fragment analyzer, and the data analysis was performed with ABI Genemapper software V3.0 (Applied Biosystems).

**Statistical Analysis**

The clinical data were then analyzed with regard to the single outcome of presence of monosomy 3. The effect of each clinical variable on this outcome was analyzed using the Fisher exact test and logistic regression analysis. All variables were analyzed as discrete variables except for patient age, tumor thickness, tumor size, proximity to optic disc, and proximity to foveola, which were analyzed as continuous variables. The average age, tumor base, and tumor thickness in eyes with monosomy 3 vs disomy 3 were compared using an independent sample t test. The distributions of proximity to optic disc and proximity to foveola were compared using the Wilcoxon rank sum test. Statistical significance was assigned at P<.05.

A total of 140 eyes of 140 patients with uveal melanoma were sampled for chromosome 3 abnormalities using FNAB. The patient and tumor findings are listed in Table 1. The median patient age at FNAB was 59 years (range, 24-93 years). A total of 139 patients were white (99%), 1 was Hispanic (<1%), 75 were male (54%), and 65 were female (46%).

Preoperative visual acuity was 20/20 to 20/50 in 106 eyes (76%), 20/60 to 20/100 in 15 (11%), and 20/200 or worse in 19 (14%). Patient symptoms included blurred vision in 54 cases (39%), flashes or floaters in 17 (12%), metamorphopsia in 4 (3%), visual field loss in 10 (7%), color vision loss in 1 (<1%), and pain in 1 (<1%). 53 patients were asymptomatic (38%). The tumor was located predominantly in the choroid in 129 eyes (92%), ciliary body in 9 (6%), and iris in 2 (1%). The median largest tumor basal dimension measured with ophthalmoscopy, transillumination, and ultrasonography was 11 mm (mean, 10.6 mm; range, 3-20 mm), and the median tumor thickness according to ultrasonography was 3.9 mm (mean, 4.6 mm; range, 1.6-11.6 mm). The FNAB approach was transscleral at the site of the tumor into the tumor base in 73 cases (52%) and trans pars plana through the vitreous into the tumor apex in 67 cases (48%). Localized vitreous or subretinal blood at the biopsy site occurred immediately at the time of FNAB in 64 cases (46%) and resolved in all cases. In no case was there extensive intraocular hemorrhage or retinal detachment. There have been no cases of tumor recurrence at the needle biopsy site during a median of 8 months of follow-up.

On the basis of tumor size, adequate yield was found in 49 of 61 small melanomas 3 mm thick or less (80%), in 62 of 67 medium melanomas between 3 and 8 mm thick (93%), and in 9 of 12 large melanomas 8 mm thick or more (75%). On the basis of the biopsy approach, adequate yield was found in 55 of 73 cases using the transscleral tumor base approach (75%) and 65 of 67 cases using the transvitreal tumor apex approach (97%). In all cases, the yield was microscopic and no cells were visible after aspiration with the exception of a large necrotic melanoma that yielded visible cellular debris, but because of extensive tumor necrosis, genetic studies were not possible.

According to tumor size, monosomy 3 was found in 16 of 61 small melanomas (26%), 24 of 67 medium melanomas (36%), and 4 of 12 large melanomas (33%).
(Figure 2 and Figure 3). On the basis of melanoma quadrant location, monosomy 3 was found in 7 of 13 tumors in the macular region (54%), 13 of 37 inferiorly (35%), 10 of 39 temporally (26%), 11 of 41 superiorly (27%), and 3 of 9 nasally (33%) (Table 1). According to the anteroposterior location of the melanoma, monosomy 3 was found in 7 of 13 tumors in the macular region (54%), 18 of 75 between the macular area and B. 

Figure 1. Technique of fine-needle aspiration biopsy (FNAB) for genetic analysis of uveal melanoma. A, A small choroidal melanoma with overlying orange pigment and subretinal fluid is found. Genetic testing confirmed monosomy 3. B, At the time of surgery, the tumor is localized and nylon sutures for securing the radioactive plaque are placed in the sclera. Immediately before placement of the radioactive plaque, FNAB is performed through the pars plana using a 27-gauge long needle. The tumor sample is obtained from an extramacular portion of the tumor, taking care to avoid the major retinal vessels. A small amount of localized vitreous hemorrhage at the site of tumor penetration is expected. C, The microscopic sample is aspirated into the syringe with pink Hank solution and then flushed into a test tube for analysis by the genetics laboratory. D, Microsatellite assay displaying 2 different samples. The top example shows blood results (normal peripheral blood lymphocyte DNA) with both copies of chromosome 3, but FNAB of the choroidal melanoma showed loss of 1 copy (monosomy 3). The bottom sample shows blood results with both copies of chromosome 3, and the FNAB of the choroidal melanoma showed retention of both copies (disomy 3).
equator (24%), and 19 of 52 anterior to the equator (37%). Melanomas with monosomy 3 were a median of 4.0 mm from the foveola and 4.8 mm from the optic disc compared with those with disomy 3, which were a median of 3.0 mm from the foveola and 2.0 mm from the optic disc. Melanomas with monosomy 3 had a median basal diameter of 12 mm and a median thickness of 4.1 mm relative to those with disomy 3, which had a median basal diameter of 9.5 mm and a median thickness of 3.8 mm.

Statistical analysis of the impact of each clinical variable on the single outcome of presence of monosomy 3 revealed a significant factor of greater basal dimension (odds ratio [OR], 1.17 per every 1-mm increase; \( P = .02 \)) (Table 2). A trend toward presence of monosomy 3 was found with melanoma location anterior to the equator (OR, 4.54; \( P = .05 \)) and within the macular region (OR, 3.18; \( P = .06 \)) (compared with location at macula-equator). The median distance to optic disc was also found to be greater among those with monosomy 3 (OR, 4.0; Wilcoxon rank sum test).

The only complication of needle biopsy was localized transient vitreous hemorrhage at the tumor site in 64 eyes (46%). No cases of diffuse vitreous hemorrhage, retinal detachment, or tumor recurrence along the biopsy tract were found.

### Table 1. Monosomy 3 Analysis of Uveal Melanoma in 140 Cases Using FNAB

<table>
<thead>
<tr>
<th>Feature</th>
<th>All Patients (N = 140 [100%])</th>
<th>Monosomy 3 (n = 44 [31%])</th>
<th>Disomy 3 (n = 76 [54%])</th>
<th>Quantity Not Sufficient for Genetic Testing (n = 20 [14%])</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patient age, median (mean) [range], y</td>
<td>59 (59) [24-93]</td>
<td>60 (60) [24-99]</td>
<td>57 (58) [26-85]</td>
<td>61 (61) [42-93]</td>
</tr>
<tr>
<td>Tumor quadrant</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Macula</td>
<td>13 (9)</td>
<td>7 (16)</td>
<td>6 (8)</td>
<td>0</td>
</tr>
<tr>
<td>Inferior</td>
<td>37 (26)</td>
<td>13 (29)</td>
<td>15 (20)</td>
<td>9 (45)</td>
</tr>
<tr>
<td>Temporal</td>
<td>39 (28)</td>
<td>10 (23)</td>
<td>22 (29)</td>
<td>7 (35)</td>
</tr>
<tr>
<td>Superior</td>
<td>41 (29)</td>
<td>11 (25)</td>
<td>27 (35)</td>
<td>3 (15)</td>
</tr>
<tr>
<td>Nasal</td>
<td>9 (6)</td>
<td>3 (7)</td>
<td>5 (6)</td>
<td>1 (5)</td>
</tr>
<tr>
<td>Diffuse</td>
<td>1 (1)</td>
<td>0 (0)</td>
<td>1 (2)</td>
<td>0</td>
</tr>
<tr>
<td>Tumor epicenter</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Macula</td>
<td>13 (9)</td>
<td>7 (16)</td>
<td>6 (8)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Macula-equator</td>
<td>75 (54)</td>
<td>18 (41)</td>
<td>49 (64)</td>
<td>8 (40)</td>
</tr>
<tr>
<td>Anterior to equator</td>
<td>52 (37)</td>
<td>19 (43)</td>
<td>21 (28)</td>
<td>12 (60)</td>
</tr>
<tr>
<td>Tumor proximity, median (mean) [range], mm</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>To foveola</td>
<td>3.5 (5.4) [0-25]</td>
<td>4.0 (5.6) [0-25]</td>
<td>3.0 (4.1) [0-25]</td>
<td>7.0 (8.7) [0-25]</td>
</tr>
<tr>
<td>To optic disc</td>
<td>4.0 (5.8) [0-28]</td>
<td>4.8 (6.0) [0-25]</td>
<td>2.0 (4.2) [0-28]</td>
<td>7.2 (10.0) [2-25]</td>
</tr>
<tr>
<td>Tumor size, median (mean) [range], mm</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Basal dimension</td>
<td>11.0 (10.6) [2-20]</td>
<td>12.0 (11.4) [5-18]</td>
<td>9.5 (10.0) [5-18]</td>
<td>11.5 (11.3) [3-20]</td>
</tr>
<tr>
<td>Thickness</td>
<td>3.9 (4.8) [2-12]</td>
<td>4.1 (4.9) [2-10]</td>
<td>3.8 (4.6) [2-12]</td>
<td>3.1 (4.0) [2-9]</td>
</tr>
<tr>
<td>Tumor thickness, mm</td>
<td>0.0-3.0</td>
<td>61 (44)</td>
<td>16 (36)</td>
<td>33 (44)</td>
</tr>
<tr>
<td>3.1-8.0</td>
<td>67 (48)</td>
<td>24 (55)</td>
<td>38 (50)</td>
<td>5 (25)</td>
</tr>
<tr>
<td>&gt;8.0</td>
<td>12 (8)</td>
<td>4 (9)</td>
<td>5 (6)</td>
<td>3 (15)</td>
</tr>
<tr>
<td>Tumor features</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Subretinal fluid</td>
<td>109 (78)</td>
<td>35 (80)</td>
<td>64 (84)</td>
<td>10 (50)</td>
</tr>
<tr>
<td>Orange pigment</td>
<td>69 (49)</td>
<td>23 (52)</td>
<td>40 (53)</td>
<td>6 (30)</td>
</tr>
<tr>
<td>FNAB approach</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Transvitreall approach into tumor apex</td>
<td>67 (48)</td>
<td>16 (36)</td>
<td>49 (64)</td>
<td>2 (10)</td>
</tr>
<tr>
<td>Transscleral approach into tumor base</td>
<td>73 (52)</td>
<td>28 (64)</td>
<td>27 (36)</td>
<td>18 (90)</td>
</tr>
<tr>
<td>FNAB needle size</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>27 Gauge</td>
<td>67 (48)</td>
<td>16 (36)</td>
<td>49 (64)</td>
<td>2 (10)</td>
</tr>
<tr>
<td>30 Gauge</td>
<td>73 (52)</td>
<td>28 (64)</td>
<td>27 (36)</td>
<td>18 (90)</td>
</tr>
<tr>
<td>FNAB complications</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vitreous blood localized</td>
<td>64 (46)</td>
<td>15 (34)</td>
<td>48 (63)</td>
<td>1 (5)</td>
</tr>
<tr>
<td>Vitreous blood diffuse</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Retinal detachment</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Tumor recurrence along tract</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

Abbreviation: FNAB, fine-needle aspiration biopsy.

a Data are presented as number (percentage) of cases unless otherwise indicated.

b The vitreous blood spontaneously resolved in all cases.

In 1990, Sisley et al. in England published cytogenetic findings in 6 eyes with posterior uveal melanoma that showed monosomy 3 and 8q abnormalities (n = 3, 50%), chromosome 1 abnormality (n = 2, 33%), and chromosome 6 abnormality (n = 4, 67%). Two years later, Sisley et al. analyzed 10 cases of uveal melanoma, and abnormalities of chromosomes 3, 6, and 8 were found in 5 cases...
(50%), chromosome 11 in 3 cases (30%), and chromosome 13 in 2 cases (20%). One tumor showed normal chromosome complement. In 1992, Horsthemke et al from Germany found loss of chromosome 3 alleles and multiplication of chromosome 8 alleles in uveal melanoma. Later, Prescher et al, from the same laboratory in Germany, published the prognostic implications of monosomy 3. They evaluated 54 patients who under-

Figure 2. Examples of choroidal melanomas sampled using fine-needle aspiration biopsy, which revealed disomy 3 (A, B, and C).

Figure 3. Examples of choroidal melanomas sampled using fine-needle aspiration biopsy, which revealed monosomy 3 (A, B, and C).
went enucleation for uveal melanoma and found monosomy 3 in 30 tumors (56%) and disomy 3 in 24 tumors (44%). By 3 years, 50% of the patients with monosomy 3 showed metastasis, whereas those with disomy 3 showed no metastatic disease. They concluded that monosomy 3 was a significant predictor of poor life prognosis. Similar findings were published in 1997 by Sisley et al,7 who discovered that monosomy 3 and additional copies of 8q statistically correlated with reduced patient survival. Three years later, they recognized that the amount of chromosomal abnormalities increased with increasing tumor size.8

More refined global gene expression patterns of uveal melanoma have been recently studied by Onken et al9 from the United States with fresh tumor samples obtained at the time of enucleation. They performed gene expression microarray analysis of 3075 significant genes in 25 enucleated eyes and found a distinctive separation of uveal melanoma, which they classified into 2 groups: class 1 (low-grade tumor) in 14 cases (56%) and class 2 (high-grade tumor) in 11 cases (44%). They found that class 2 tumors displayed down-regulated gene clusters on chromosome 3 and up-regulated clusters on chromosome 8q. These findings paralleled those of Sisley et al.7 Onken and colleagues further evaluated prognostic implications of the 2 classes and found that 95% of class 1 patients and 31% of class 2 patients were still alive at 8 years.

These studies have been performed on fresh or paraffin-embedded tissue from eyes with melanoma after enucleation. Most of these studies have concluded that loss (monosomy) or down-regulation of chromosome 3 is the

| Table 2. Monosomy 3 Analysis of Uveal Melanoma in 140 Cases Using FNABa |
|------------------------|-----------------|-----------------|
| Variable               | Monosomy 3 (n = 44) | Disomy 3 (n = 76) |
| Age, mean (SD), y      | 60.2 (12.8)      | 58.6 (13.9)     |
| Race, white vs Hispanic | 44 (100)         | 75 (99)         |
| Sex, male vs female    | 28 (64)          | 40 (53)         |
| Eye, left vs right     | 22 (50)          | 32 (42)         |
| Visual acuity, 20/20 vs <20/20 | 12 (27) | 20 (26) |

Symptoms

- Floaters vs loss of visual field
  - Monosomy 3: 1 (2)
  - Disomy 3: 4 (5)

- Asymptomatic vs loss of visual field
  - Monosomy 3: 15 (34)
  - Disomy 3: 28 (37)

- Blurred vision vs loss of visual field
  - Monosomy 3: 19 (43)
  - Disomy 3: 29 (38)

- Photopsia vs loss of visual field
  - Monosomy 3: 4 (9)
  - Disomy 3: 5 (7)

- Metamorphopsia vs loss of visual field
  - Monosomy 3: 2 (4)
  - Disomy 3: 2 (3)

Location

- Ciliary body vs choroid
  - Monosomy 3: 2 (4)
  - Disomy 3: 2 (3)

- Ciliochoroidal vs choroid
  - Monosomy 3: 6 (14)
  - Disomy 3: 4 (5)

Tumor quadrant

- Macula vs superior
  - Monosomy 3: 7 (16)
  - Disomy 3: 6 (8)

- Inferior vs superior
  - Monosomy 3: 13 (30)
  - Disomy 3: 15 (20)

- Temporal vs superior
  - Monosomy 3: 10 (23)
  - Disomy 3: 22 (29)

- Nasal vs superior
  - Monosomy 3: 3 (7)
  - Disomy 3: 5 (7)

Anteroposterior location

- Macula vs macula-equator
  - Monosomy 3: 7 (16)
  - Disomy 3: 6 (8)

- Equator to ora vs macula-equator
  - Monosomy 3: 9 (20)
  - Disomy 3: 14 (18)

- Ciliary body and equator to ora vs macula-equator
  - Monosomy 3: 5 (11)
  - Disomy 3: 3 (4)

- Ciliary body vs macula-equator
  - Monosomy 3: 2 (4)
  - Disomy 3: 2 (3)

- Ciliary body and ora vs macula-equator
  - Monosomy 3: 1 (2)
  - Disomy 3: 1 (1)

- Distance to optic disc, mean (SD) [median], mm
  - Monosomy 3: 6.0 (5.8) [4.8]
  - Disomy 3: 4.2 (5.8) [2.0]

- Distance to foveola, mean (SD) [median], mm
  - Monosomy 3: 5.6 (6.3) [4.0]
  - Disomy 3: 4.1 (5.4) [3.0]

- Largest base, mean (SD), mm
  - Monosomy 3: 11.4 (3.4)
  - Disomy 3: 9.9 (2.9)

- Thickness, mean (SD), mm
  - Monosomy 3: 4.9 (2.4)
  - Disomy 3: 4.6 (2.3)

- Thickness >2 mm (positive vs negative)
  - Monosomy 3: 40 (91)
  - Disomy 3: 67 (88)

- Fluid (negative vs positive)
  - Monosomy 3: 5 (11)
  - Disomy 3: 9 (12)

- Symptoms (positive vs negative)
  - Monosomy 3: 26 (59)
  - Disomy 3: 47 (62)

- Orange pigment (positive vs negative)
  - Monosomy 3: 23 (52)
  - Disomy 3: 40 (53)

- Documented growth (positive vs negative)
  - Monosomy 3: 14 (32)
  - Disomy 3: 20 (26)

Abbreviations: CI, confidence interval; FNAB, fine-needle aspiration biopsy; OR, odds ratio.

a Data are presented as number (percentage) of cases unless otherwise indicated.

b Fisher exact test.
c Logistic regression analysis.
d Independent sample t test.
e Per 10-year increase.
f Reference variable.
g Wilcoxon rank sum test.
h Per 1-mm increase.
most important genetic factor related to prognosis of patients with uveal melanoma. In our analysis, we specifically focused on our ability to detect abnormalities of chromosome 3 with only an FNAB sample of the tumor and without a solid tissue sample. We were able to harvest adequate cells for analysis in 86% of cases, despite the fact that 61 of 140 tumors (44%) were 3 mm thick or less. Of the 20 cases in which genetic testing was not feasible, most tumors (12) were 3 mm thick or less. Of the 61 tumors that were 3 mm thick or less, genetic testing was feasible in 49 cases (80%). We suspect that failure to obtain adequate samples in 20 of our cases occurred because of 1 or more reasons, including small tumor size, small needle bore, tightly cohesive spindle cells not yielding to aspiration, necrotic cells without intact DNA, or loss of cells during transfer to the test tube. Of the 79 melanomas that measured more than 3 mm in thickness, adequate aspirate was obtained in 71 (90%).

Naus et al validated that FISH analysis for uveal melanoma obtained by needle aspirate was reliable. They evaluated 40 eyes with uveal melanoma managed with enucleation. The mean tumor thickness was not evaluated, but the mean tumor diameter was 12.9 mm. After eye removal, a 23-gauge needle attached to a 10-mL syringe was inserted through the sclera into the tumor (transscleral approach), and cells were aspirated. In 39 of 40 eyes (98%), the FISH results of both chromosomes 3 and 8 could be analyzed. In 11 of 249 hybridizations (4%), discrepancies between the results of FNAB and the solid tumor were detected, but the overall weighted \( \kappa \) was 0.95, indicating good agreement between the FNAB and solid tumor results. Such discrepancies included mostly differences in the extent of chromosome or subclone abnormality on FNAB vs solid tumor analysis. These authors concluded that FNAB of uveal melanoma provided sufficient samples and was reliable for FISH analysis for chromosome 3 or 8 abnormalities. Sisley et al found similar accurate correlation of cytogenetic analysis in FNAB compared with solid tumor samples after enucleation in 10 cases.

In our series of 140 consecutive cases, we used FNAB at the time of plaque radiotherapy rather than enucleation. In 67 cases (48%) the route was transvitreal using indirect ophthalmoscopy guidance with a 27-gauge needle, whereas in 73 cases (52%) the route was transscleral, directly through the sclera into the tumor base after transillumination using a 30-gauge needle. We preferred the small needle bore for the transscleral route to minimize possible tumor seeding through the site of scleral perforation. Results were obtained in 65 of 67 melanomas with the transvitreal route (97%) and 55 of 73 melanomas with the transscleral route (75%). Most uveal melanomas are currently treated with radiotherapy rather than enucleation, so genetic analysis of FNAB specimens provides a method of obtaining important genetic information on all patients with uveal melanoma. For eyes that undergo enucleation, fresh tissue can be harvested for the same genetic studies.

In this analysis, greater basal dimension and greater distance from the optic disc were factors associated with monosomy 3. Monosomy 3 was noted in 26% of small melanomas, 36% of medium melanomas, and 33% of large melanomas. These findings could indicate that monosomy 3 mutation develops during tumor enlargement, but further analysis is warranted because this could reflect the difficulty in obtaining sufficient DNA in the smaller tumors.

We used a microsatellite assay rather than FISH analysis for our FNAB specimens. The microsatellite assay is more refined and provides more information on chromosomal segments than does FISH analysis. The microsatellite-based assay is more robust from the inherent amplification of signals owing to polymerase chain reaction. The main advantage of this assay is ready adaptability of this test in any molecular biology laboratory. In contrast, FISH requires access to specialized microcopy and operator skills to correctly identify and quantify the signals that infer monosomy vs disomy. For microsatellite-based assays, the analysis software aids in making automated calls for loss of heterozygosity and infers monosomy vs disomy. A drawback of the latter assay might be the inability to distinguish between loss of heterozygosity and copy neutral amplification (loss of heterozygosity followed by reduplication of the lost allele). There has been only 1 report, to our knowledge, of an alternate form of chromosome 3 abnormality for uveal melanoma; thus, this concern is minimal.

Percutaneous biopsy for prognostication via genetic testing has been found feasible and is used in other fields of medicine. Teixeira et al found that genomic analysis of prostate carcinoma obtained using ultrasonography-guided needle biopsy was possible in 34 of 35 cases with chromosome banding analysis and comparative genomic hybridization. They found aberrations in 69% of samples and noted that specific imbalances, such as 16q and 8q abnormalities, imparted a worse prognosis. Hoffer et al found that percutaneous needle biopsy in 21 children with neuroblastoma provided genetic prognostic information in 95%, DNA index (ploidy) in 90%, and N-myc gene expression in 70%. They concluded that percutaneous biopsy of advanced neuroblastoma was a feasible alternative to open biopsy. Our study has shown that FNAB of uveal melanoma for genetic information is possible, and this finding, combined with previous knowledge that needle biopsy specimens correlate with open biopsy specimens, suggests that this technique may be useful in assessing ultimate patient prognosis.

Submitted for Publication: October 5, 2006; final revision received December 12, 2006; accepted January 8, 2007.

Correspondence: Carol L. Shields, MD, Ocular Oncology Service, Suite 1440, Wills Eye Hospital, 840 Walnut St, Philadelphia, PA 19107 (carol.shields@shieldsoncology.com).

Author Contributions: Dr C. L. Shields has full access to all of the data in this study and takes responsibility for the integrity of the data and the accuracy of the data analysis. Financial Disclosure: None reported.

Funding/Support: Support was provided by the Retina Research Foundation Award of the Retina Society (Dr C. L. Shields), the Paul Kayser International Award of Merit in Retina Research (Dr J. A. Shields), a donation from Michael, Bruce, and Ellen Ratner (Drs J. A. Shields and C. L. Shields), Mellon Charitable Giving from the Martha W. Rogers Charitable Trust (Dr C. L. Shields), the LuEsther Mertz Retina Research Foundation (Dr C. L. Shields), and the Eye Tu-
mor Research Foundation (Drs C. L. Shields and J. A. Shields).

Previous Presentations: Presented in part at the Retina Research Foundation Award/Charles L. Schepens Lecture at the Combined Retina Society/Gonin Society Meeting; October 17, 2006; Capetown, South Africa (Dr C. L. Shields); and at the Deborah Iversion, MD Lecture-ship; November 1, 2006; Detroit, Michigan (Dr C. L. Shields).

Additional Contributions: Statistical analysis was provided by Rishita Nutheti, MSc, International Centre for Advancement of Rural Eye Care, L.V. Prasad Eye Institute, Hyderabad, India.

REFERENCES


From the Archives of the Archives

Forty years ago it was difficult to find a prominent American ophthalmologist, or even a professor of ophthalmology, who had any real knowledge of such basic subjects as physiologic optics or ophthalmic pathology. On the other hand, on the European Continent it was difficult to find any ophthalmologist who did not have such knowledge. . . . And almost equally as widespread ignorance of physiologic optics also still prevails.