Title: Detection of Extrascleral Extension in Uveal Melanoma with Histopathological Correlation

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Running Title: Extrascleral Extension in Uveal Melanoma

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Abstract:

**Purpose:** Uveal melanoma is the most common primary intraocular malignancy. Extrascleral extension (ESE) by this tumor is rare, but associated with an increased rate of orbital recurrence and an overall poor prognosis. Clinical studies show low rates when compared with histological studies. Due to the prognostic importance of ESE, we seek to compare our clinical, intraoperative, and histological detection rates.

**Design:** A retrospective cross-sectional case series.

**Methods:** A list of eyes enucleated for uveal melanoma was compiled from the admissions records of the London Ocular Oncology Service during the 28-month period between January 2010 and April 2012. The surgical and clinical notes of patients with histopathology reports positive for ESE were reviewed to determine when it was first diagnosed or suspected. The subsequent management of these cases is discussed.

**Results:** 16 of 174 (9%) eyes had histologically proven ESE. 8 of 16 cases were detected preoperatively at clinical examination including the use of ocular ultrasound, 3 of 16 were discovered intra-operatively, and 5 of 16, deemed microscopic ESE, were first detected on histological examination. 7 out of 7 (100%) of cases with anterior ESE were detected clinically by slit lamp biomicroscopy, while only 1 out of 9 (11%) of cases with posterior ESE were detected preoperatively with ultrasound.

**Conclusions:** Slit lamp biomicroscopy is sensitive for detecting anterior ESE. Most cases of posterior ESE are microscopic, but B-scan ocular ultrasound may also miss macroscopic posterior ESE. Orbital surgeons should be aware of the possibility of clinically undetected posterior ESE, and consider adjuvant orbital radiotherapy in cases with macroscopic extrascleral extension to minimize the risk of orbital recurrence.
INTRODUCTION

Uveal melanoma is the most common primary intraocular malignancy [1]. Mortality is typically due to liver failure secondary to metastatic disease. [2] Extrascleral extension (ESE) is rare, but has been associated with an increased rate of orbital recurrence, as well as an overall poor prognosis. [3,4] Other features correlated with early metastatic death include: advanced patient age, large tumor size, ciliary body involvement, epithelioid cellular morphology, periodic acid-Schiff (PAS) positive vascular mimicry patterns, increased nucleolar size, and loss of a copy of chromosome 3 (monosomy 3). [5-10] Several large studies show low rates of clinical versus histological detection. Due to its prognostic importance, we seek to compare our clinical, intraoperative, and histological detection rates of ESE in cases with histologically proven uveal melanoma.

METHODS

Clinical cohort analysis was performed on all enucleations (primary and secondary) undertaken in the London Ocular Oncology Service following a diagnosis of uveal melanoma between January 2010 and April 2012. Histopathology reports documenting ESE were correlated with the histology. The surgical and clinical notes were obtained for these cases to determine if and when the ESE was detected preoperatively or intraoperatively.

All patients underwent complete clinical examination with slit lamp biomicroscopy, indirect ophthalmoscopy and transpupillary transillumination. B-scan ocular ultrasound (Acuson Sequoia 512 by Siemens) using the 14MHz probe was performed on all patients. MRI scans of the orbit were not requested unless the clinician was suspicious of ESE following ocular ultrasound. Tumor locations, sizes and pigmentation were recorded. Any treatments prior to enucleation and adjuvant therapies were also recorded. Histopathologic assessment was done with standard hematoxylin and eosin stain. Melanoma histopathologic cell type, mitotic figures and routes of ESE were recorded. In most of the cases cytogenetic analysis of chromosomes 3 and 8 was performed with Interphase fluorescence in situ hybridization (FISH) analysis following a punch biopsy of
the intraocular tumor (Abbott Molecular (Des Plaines, IL, U.S.A.) IGH/MYC, CEP8 tricolor dual fusion probe, CEP3 (D3Z1)SpectrumOrange Probe, and CEP8 (D8Z2) SpectrumGreen Probes).

RESULTS

During the 28-month study period, a total of 174 patients with uveal melanoma underwent enucleation. Histopathology reported the presence of ESE in 16 of 174 (9%) of patients. Patients with ESE had a median age of 79.5 years (mean 74.5 ± 6.4, range 49-88), of which 8 were male and 8 were female patients. There was no significant difference in age between the patients with anterior versus posterior ESE (p-value 0.227, Kruskal-Wallis). Patient demographics and tumor features are presented in Tables 1 and 2 for anterior and posterior ESE respectively.

Eight cases were diagnosed at preoperative clinical examination, 3 were discovered intraoperatively, and 5 were first detected on histopathologic examination. The cell types of the tumors were: 10 spindle, 4 mixed, and 2 epithelioid. More than half (9/16) of the tumors were medium sized according to the Collaborative Ocular Melanoma Study (COMS). Of the 14 cases that had cytogenetic testing, 12 (86%) were found to have chromosomal abnormalities in chromosomes 3 and/or 8.

All patients with clinically or intraoperatively detected ESE received modified enucleation surgery with care to avoid manipulation of the tissues overlying the extraocular portion of the tumor. 10 patients had well-encapsulated or microscopic ESE. These patients did not receive any adjuvant treatment but were monitored in the Ocular Oncology Service. Adjuvant external beam radiotherapy (EBRT), 50 Gy in 20 fractions, was administered to the orbit of 6 patients who had non-encapsulated macroscopic ESE.

Anterior ESE

Seven patients had anterior ESE. These patients had a median age of 84 years (mean 74.6, range 49-88) and nearly all were female (6/7). Six out of 7 patients had not received prior radiation treatment. The melanomas were predominantly located
in the ciliochoroidal area (5/7), were medium-sized (5/7), dome-shaped (4/7) and melanotic (6/7). Regardless of the size of the ESE, all anterior cases were clinically detected by slit lamp biomicroscopy. Clinical presentation of the extension ranged from transscleral discoloration to a frank sizeable subconjunctival nodule (Table 1A – Notes). Histopathologically, 4/7 were spindle cell type with 2 cases of mixed type and only one case with epithelioid morphology. Cytogenetic analysis indicated the presence of monosomy of chromosome 3 in 4/7 cases and abnormalities in chromosome 8 in 6/7 cases. Interestingly, two patients developed anterior ESE following previous transcleral manipulation at the site of the extension (fine needle aspiration biopsy site, and trabeculectomy scleral flap). The other route of the ESE for these ciliochoroidal melanomas was also determined to be the aqueous drainage channels or adjacent to ciliary arteries or nerves. One patient had liver metastasis at the time of initial diagnosis.

**Posterior ESE**

Nine patients had posterior ESE. These patients had a median age of 79 years (mean 74.4, range 56-86) and most of the patients (7/9) were male. Seven out of 9 patients had not received prior treatment. The intraocular tumors were predominantly isolated to the posterior pole (5/9), were large (5/9) or medium-sized (4/9), collar-stud shaped (6/9), and were all melanotic. B-scan ultrasound only detected the ESE in the case with the largest posterior extension (a 6 mm nodule, see Table 1B – Notes). The other cases of macroscopic and microscopic posterior ESE were first discovered intraoperatively (3 during enucleation), or following microscopic examination (5). Histopathologically, 6/9 were spindle type with 2 cases of mixed type and only one case with epithelioid morphology. Cytogenetic analysis in this group was available for 6 cases. Chromosome 3 abnormalities were detected in 5/6 cases and chromosome 8 abnormalities in 5/6 cases.

The routes of the extension were posterior ciliary artery or nerve emissary canals in 4/9 cases, the vortex veins in 3/9 cases, the optic nerve in 1/9 case, and transcleral extension with ciliary artery and nerve involvement in 1/9.
DISCUSSION

In this study, the incidence and detection of ESE in eyes enucleated for uveal melanoma were assessed retrospectively in a large series from a single tertiary referral Ocular Oncology center. The overall incidence of histopathologically confirmed extrascleral extension in our series was 9%. This is in accordance with previous studies noting extrascleral extension in 13% of choroidal melanomas [4,11] though older series have reported percentages as high as 29% [4]. The ages of the patients with anterior vs. posterior ESE were comparable.

PRIOR TREATMENTS

Enucleation was the primary treatment modality for 13 patients, but secondary in 3 patients after failed ruthenium plaque brachytherapy. It is unlikely that the brachytherapy facilitated ESE, as the sclera has been shown to be able to withstand more than 1000 Gy before induction of scleral atrophy/thinning. [14] All of the patients who received prior brachytherapy had a dose of less than 1000 Gy to the sclera. The two patients with full thickness direct transcleral extension had previous transcleral procedures overlying their intraocular tumors (Patient #6 required two trans-scleral fine needle aspiration (FNA) biopsies to confirm the diagnosis, Patient #7 had a previous trabeculectomy). Tumor seeding along the FNA biopsy needle track is a known and well-described risk of the procedure. [15] Direct transcleral tumor extension after glaucoma filtration surgery has also been described. [16]

MELANOMA SIZE, HISTOPATHOLOGY TYPE AND CYTOGENETICS

Although previously published studies [4,11] found ESE to be associated with increased tumor size and epithelioid morphology, we did not have an overabundance of tumors meeting the COMS criteria for large tumors [2] and most of our cases were spindle cell tumors (10/16).
Importantly, more than 80% of melanomas showing ESE harbored high-risk cytogenetic features with abnormalities in chromosomes 3 and or 8 known to be associated with a poor survival prognosis. Researchers in Holland have also reported monosomy of chromosome 3 and gains of chromosome 8q in patients with ESE [12]. In our study the intraocular component of the tumor was tested for cytogenetic analysis, since biopsy from extraocular tumor has not been shown to be representative of the underlying cytogenetic changes with respect to chromosome 1p, 3, 6, and 8q abnormalities [13]

**ROUTES OF EXTRASCLERAL EXTENSION**

Most of the ESE occurred via aqueous outflow vessels, or along emissary canals carrying posterior ciliary nerves, arteries, and vortex veins. Complete direct extension through the sclera was uncommon in our series as in other studies [11]. Similarly, despite the fact that many uveal melanomas are juxtapapillary, direct invasion of the optic nerve to the lamina cribrosa is rare, and we had only one case in our series. [17,18]

**ESE DETECTION**

Our clinical detection rate of ESE among cases of proven uveal melanoma was 5% versus the 9% histological detection rate. This compares favorably with recent large studies, which have found clinical detection rates of 3% versus 10-15% histologically. [7,8,14,19-20] The clinical detection of ESE was largely related to an anterior or posterior location. Clinical detection of anterior ESE was excellent regardless of the extent, with all 7 of these cases noted on slit lamp biomicroscopy either as a fixed subconjunctival nodule or flat subconjunctival pigment overlying the intraocular melanoma (Figure Top left, Top right). Murray et al published on the value of ocular ultrasound in detecting ESE, with ultrasound being superior to MRI scanning (22). In the current study we found B-scan ocular ultrasound detected 1 out of 4 cases with posterior macroscopic ESE. The case detected using ultrasound was a 6mm nodular extension (Figure Middle left, Middle right). The 3 cases missed using B-scan ocular ultrasound
were all small nodular extensions of less than 2mm in thickness. The route of ESE for all of these 3 cases was via the emissary channel containing the posterior ciliary artery or nerve. Extrascleral spread of melanoma into the orbital fat is detected on the B-Scan image as a region of lower echogenicity than the surrounding strongly scattering orbital fat. Tumor appears as grey spots of varying brightness surrounded by and outlined by the brighter white spots of the orbital fat. Small extrascleral extensions at muscle insertions can be difficult to detect. Retrobulbar tissues that can obscure B-scan ultrasound images may also impede direct intraoperative visualization of episcleral pigment and macroscopic nodules (Figure Bottom left, Bottom right) from orbital surgeons. Post surgical cystic spaces at plaque sites may lead to false positives. Conversely, if there is tumor spread into the optic nerve then tumor echoes are detected within the normally echoluent nerve complex and the dark area of the nerve complex outlines the grey of the tumor. In the latter situation, blood flow imaging using Ultrasound Color Flow Mapping (CFM) has a particularly useful role. Blood vessels detected within the nerve complex, other than normal retinal artery and vein, represent abnormal flow and suggests tumor spread.

ESE TREATMENT

If ESE is suspected enucleation approaches should be modified with care to avoid handling the tissues overlying the intraocular tumor. The presence of microscopic ESE did not change our overall management; however non-encapsulated macroscopic ESE was treated with EBRT. This has been the longstanding management of ESE in uveal melanoma in London since Hykin et al. reported a low orbital recurrence rate of only 6% in these high-risk cases. As of March 2017, none of the patients who received EBRT and none of the patients monitored with microscopic ESE have had an orbital recurrence. Though there is currently no long-term data to support improved mortality, we believe offering adjuvant orbital radiotherapy in cases of non-encapsulated macroscopic ESE is a prudent choice to prevent orbital recurrences. [3] Paul Finger et al. have also reported success in preventing orbital recurrence with high-dose-rate (HDR) orbital brachytherapy. [21]
In conclusion, while detailed examination using slit lamp biomicroscopy is very sensitive for detection of anterior ESE, small posterior ESE may be missed with B-scan ultrasound. Most microscopic ESE not seen intraoperatively does not require adjuvant radiotherapy to the socket, and thus does not change overall patient management. The surgical technique should be modified when anterior or posterior ESE is suspected. As in all cases of uveal melanoma, a cautious surgical approach with good visualization is recommended.

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B. No financial disclosures
C. No other acknowledgements
REFERENCES


11. Coupland SE, Campbell I, Damato B. Routes of extraocular extension of


**Figure Caption:** Presentation of anterior ESE on slit lamp exam varied between fixed subconjunctival nodules with dilated and tortuous sentinel vessels (Upper left, case #3) and flat subconjunctival pigment (Upper right, case #4). Middle left shows B-scan ultrasound and, Middle right demonstrates corresponding Hematoxylin and Eosin stained slide showing a 6mm posterior extrascleral nodule (case #11, ‘T’ denotes tumor, ‘S’ the sclera, and ‘ON’ the optic nerve). Bottom left shows tumor cells exiting the eye via the vortex vein (‘VV’), noted as episcleral pigment intraoperatively (case #12), Bottom right shows 2mm extrascleral nodule hidden intraoperatively by the inferior oblique (‘IO’) muscle stump (case #10).
**Table 1 Anterior ESE**

<table>
<thead>
<tr>
<th>Case #</th>
<th>Age</th>
<th>Patient sex</th>
<th>Histology type</th>
<th>Tumor size (COMS classification)</th>
<th>AJCC Staging</th>
<th>Cytogenetics</th>
<th>Pigment</th>
<th>Tumor location</th>
<th>Tumor shape</th>
<th>ESE detection</th>
<th>Route</th>
<th>Previous therapy</th>
<th>Notes</th>
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<tr>
<td>1</td>
<td>85</td>
<td>F</td>
<td>Spindle cell</td>
<td>Large</td>
<td>Stage III B (T3dNxM0)</td>
<td>Monosomy 3/ Gain of 8/8q</td>
<td>Melanotic</td>
<td>Ciliochoroidal</td>
<td>Dome shaped</td>
<td>Clinical</td>
<td>Aqueous Drainage channels</td>
<td>No</td>
<td>3mm focus</td>
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<tr>
<td>2</td>
<td>88</td>
<td>F</td>
<td>Spindle cell</td>
<td>Large</td>
<td>Stage III B (T3dNxM0)</td>
<td>Monosomy 3/ Gain of 8</td>
<td>Melanotic</td>
<td>Ciliochoroidal</td>
<td>Bilobed</td>
<td>Clinical</td>
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<td>Medium</td>
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<td>Melanotic</td>
<td>Ciliochoroidal</td>
<td>Dome shaped</td>
<td>Clinical</td>
<td>Aqueous Drainage channels</td>
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<td>14x10mm subconjunctival nodule, liver metastasis at initial diagnosis</td>
</tr>
<tr>
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<td>Epithelioid</td>
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<td>Stage III A (T2cNxM0)</td>
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<td>Melanotic</td>
<td>Choroidal nasal</td>
<td>Dome shaped</td>
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<td>Stage III A (T2dNxM0)</td>
<td>Dismomy 3/ Dismomy 8</td>
<td>Amelanotic</td>
<td>Ciliochoroidal</td>
<td>Collar stud</td>
<td>Clinical</td>
<td>Transcleral</td>
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<td></td>
</tr>
<tr>
<td>6</td>
<td>49</td>
<td>F</td>
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<td>Stage III A (T2dNxM0)</td>
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<td>Melanotic</td>
<td>Anterior</td>
<td>Bilobed</td>
<td>Clinical</td>
<td>Transcleral</td>
<td>Brachytherapy + (FNA x2)</td>
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<tr>
<td>7</td>
<td>68</td>
<td>F</td>
<td>Spindle cell</td>
<td>Medium</td>
<td>Stage III A (T2dNxM0)</td>
<td>Monosomy 3/ Gain of 8/8q</td>
<td>Melanotic</td>
<td>Ciliochoroidal</td>
<td>Dome shaped</td>
<td>Clinical</td>
<td>Trabeculectomy flap</td>
<td>No</td>
<td>4.5mm extraclear focus</td>
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## Table 2 Posterior ESE

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<tr>
<th>Case #</th>
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<th>Patient sex</th>
<th>Histology type</th>
<th>Tumor size (COMS classification)</th>
<th>AJCC Staging</th>
<th>Cytogenetics</th>
<th>Pigment</th>
<th>Tumor location</th>
<th>Tumor shape</th>
<th>ESE detection</th>
<th>Route</th>
<th>Previous therapy</th>
<th>Notes</th>
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<td>1</td>
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<td>M</td>
<td>Spindle cell</td>
<td>Large</td>
<td>Stage IIIA</td>
<td>Monosomy 3/ Gain of 8/8q</td>
<td>Melanotic</td>
<td>Superior</td>
<td>Collar stud</td>
<td>Histopathologic</td>
<td>Not noted</td>
<td>No</td>
<td>2mm extrascleral deposit</td>
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<tr>
<td>2</td>
<td>81</td>
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<td>Mixed</td>
<td>Large</td>
<td>Stage IIIIB</td>
<td>Not done</td>
<td>Melanotic</td>
<td>Ciliochoroidal to posterior</td>
<td>Collar stud</td>
<td>Histopathologic</td>
<td>Posterior ciliary nerve, artery</td>
<td>Brachytherapy &lt; 1mm encapsulated nodule</td>
<td></td>
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<td>Not done</td>
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<td>Posterior</td>
<td>Dome shaped</td>
<td>Histopathologic</td>
<td>Posterior ciliary artery</td>
<td>Brachytherapy x 2 2mm encapsulated nodule</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>84</td>
<td>F</td>
<td>Spindle cell</td>
<td>Large</td>
<td>Stage IIIC</td>
<td>Monosomy 3/ Gain of 8/8q</td>
<td>Melanotic</td>
<td>Superior</td>
<td>Multilobed</td>
<td>Clinical</td>
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<td>No</td>
<td>6mm nodule</td>
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<td>Medium</td>
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<td>Disomy 3/ Disomy B</td>
<td>Melanotic</td>
<td>Ciliochoroidal</td>
<td>Collar stud</td>
<td>Intraoperative</td>
<td>Vortex vein</td>
<td>No</td>
<td>Cells coating 3mm of external sclera</td>
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<tr>
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<tr>
<td>7</td>
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<td>M</td>
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<td>Medium</td>
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<td>Monosomy 3/ Gain of 8/8q</td>
<td>Melanotic</td>
<td>Posterior</td>
<td>Dome shaped</td>
<td>Intraoperative</td>
<td>Vortex vein</td>
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<td></td>
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<tr>
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<td>86</td>
<td>M</td>
<td>Spindle cell</td>
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<td>2/mm^2</td>
<td>Monosomy 3/ Gain of 8/8q</td>
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<td>Posterior</td>
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<td>Posterior ciliary nerve</td>
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<td>2mm nodule</td>
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<tr>
<td>9</td>
<td>80</td>
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<td>Epithelioid</td>
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<td>1/10 high powered fields</td>
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<td>Posterior</td>
<td>Collar stud - filling most of the globe</td>
<td>Histopathologic</td>
<td>Optic nerve</td>
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<td>Focal infiltration of lamina cribrosa</td>
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