310 Uveal melanoma: From Clinical Trials to Molecular Mechanisms
Tuesday, May 09, 2017 8:30 AM–10:15 AM
Room 321 Paper Session
Program #/Board # Range: 2499–2504
Organizing Section: Anatomy and Pathology/Oncology

Program Number: 2499
Presentation Time: 8:30 AM–8:45 AM
Clinical course of radiation-induced choroidal tumor vasculopathy with progressive exudative retinal detachment following plaque radiotherapy for primary posterior uveal melanoma
James J. Augsburger, Cassandra C. Skinner, Zelia M. Correa.
Ophthalmology, University of Cincinnati, Cincinnati, OH.
Purpose: Occasional patients with primary posterior uveal melanoma treated by plaque radiotherapy develop radiation-induced "tumor vasculopathy" characterized by development of a progressive exudative retinal detachment surrounding the irradiated tumor residue. During the past several years, such eyes have frequently been treated by intravitreal anti-VEGF drugs, and favorable responses to such therapy have been reported in some of these patients. The purpose of this study was to determine the clinical course of such eyes that were encountered prior to the advent of intra-vitreal anti-VEGF drug therapy.
Methods: Retrospective chart review of all patients treated by plaque radiotherapy for a posterior uveal melanoma 1980 through 2000 with identification of those patients who developed radiation-induced chorioretinopathy following a post-treatment latent interval and exhibited a progressive exudative retinal detachment surrounding the residual irradiated tumor. The clinical course of the exudative retinal detachment, the visual status of the eye over time, and ultimate outcome of the eye in these patients were evaluated.
Results: The study group consisted of 34 patients (21 men, 13 women). The mean largest basal diameter (12.1 mm) and mean maximal tumor thickness (6.7 mm) were not substantially different than those dimensions in our total at-risk population of 1477 cases. The tumor was exclusively choroidal in 88.2% and involved the ciliary body in 38.2%. All 34 eyes became legally blind within a median post-treatment interval of 25 months, and 21 of the eyes (61.8%) ultimately became NLP. Most of these eyes developed neovascular glaucoma and recurrent intraocular bleeding from the surface of the tumor. Seven of these eyes were ultimately enucleated. The 6-yr cumulative actuarial probability of secondary enucleation was 0.2. Illustrative cases will be presented.
Conclusions: Eyes that experience radiation-induced "tumor vasculopathy" with progressive exudative retinal detachment after plaque radiotherapy for posterior uveal melanoma appear to have a very poor prognosis for retention of useful vision in the absence of intravitreal anti-VEGF drug injections.
Commercial Relationships: James J. Augsburger, None; **Cassandra C. Skinner**, None; **Zelia M. Correa**, Castle BioSciences (C)

Program Number: 2500
Presentation Time: 8:45 AM–9:00 AM
Adipophilin expression in primary and metastatic uveal melanoma: a pilot study
Sarah E. Coupland[1], Helen Kalirai[1], Periklis Katopodis[1], Heinrich Heimann[2, 1], Miltiadis Fiorentzis[2, 1]. [1]Molecular and Clinical Cancer Medicine, University of Liverpool, Liverpool, United Kingdom; [2]LOOC, Royal Liverpool University Hospital, Liverpool, United Kingdom.
Purpose: Adjustment of cancer cell metabolism to enable rapid cell growth and division is firmly established as one on the hallmarks of cancer. One of the changes associated with this metabolic phenotype is an over-activation of lipogenic pathways, characterized by high lipid droplet (LD) content in the cytoplasm of the cancer cells. The presence of large numbers of LDs has also been associated with chemoresistance. Adipophilin (ADP) is a protein that regulates the structure and formation of these LDs. Our observations of lipid droplets in UM cell lines led us to undertake this pilot study in which we evaluated the expression of ADP in uveal melanoma (UM).
Methods: Immunohistochemical analysis of ADP expression was performed in 34 consecutive enucleation specimens from 22 male and 12 female patients. These comprised 22 choroidal (64.7%) and 12 (35.3%) ciliochoroidal UM. The intensity of staining (IS) and the proportion of tumour cells stained (PS) for ADP were evaluated semiquantitatively on a scale 1-4. The total expression of ADP (tADP) was obtained by multiplying IS and PS scores together. Results were compared with clinical and histological parameters. In addition, 5 UM metastases to the liver were examined for ADP expression.
Results: The median age of the primary UM patients at diagnosis was 72.5 years (range 44 - 90). The tumours had a mean largest basal diameter (LBD) of 12.04mm and a mean thickness of 7.7mm. Seventeen UM (50%) contained epithelioid cells, and 21 cases (61.7%) were classified as monosomy 3 by Multiplex Ligation Dependant Probe Amplification (MLPA).
ADP was detected in all UM examined, although the proportion of UM cells containing ADP+ LD was variable across the tumour sections analysed. High ADP expression (tADP score >4) was documented in 17 specimens; however, this was not significantly correlated with any clinical, histological or genetic parameters. Notably, normal choroidal melanocytes in the enucleated eyes were ADP negative. Further, all examined hepatic UM metastases were ADP+, with most showing scores of >4 and with the surrounding liver parenchyma being ADP negative.
Conclusions: This is the first description of ADP protein expression in a small cohort of primary and metastatic UM. Understanding the biological significance of this trait of altered energy metabolism in UM may enable the development of therapies aimed at tumour cell metabolism.
Commercial Relationships: Sarah E. Coupland, None; **Helen Kalirai**, None; **Periklis Katopodis**, None; **Heinrich Heimann**, None; **Miltiadis Fiorentzis**, None

Program Number: 2501
Presentation Time: 9:00 AM–9:15 AM
Ocular Oncology Study Consortium Report 3: Baseline clinical features and relationship to GEP Class
Amy C. Schefler[1, 2], Duncan Berry[3], Michael Seider[3], Sandra Stinnett[3], Prithvi Mruthyunjaya[4]. [1]Ophthalmology, Retina Consultants of Houston, Houston, TX; [2]Blanton Eye Institute, Houston Methodist Hospital, Houston, TX; [3]Ophthalmology, Duke Eye Center, Durham, NC; [4]Stanford Department of Ophthalmology, Byers Eye Institute, Palo Alto, CA.
Purpose: To study the relationship between clinical features and Collaborative Ocular Melanoma Study (COMS)-based size categorization of gene expression profile (GEP) sub-classifications in a multi-center cohort of uveal melanoma (UM) patients.
Methods: This was a retrospective, multi-center study with patients entered from 9 major ocular oncology centers from across the United States. 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. Clinical and genomic

data were collected and statistical analysis was performed using Fisher's exact test, Wilcoxon rank sum test and Kruskal-Wallis test.
Results: Inclusion criteria were met for 379 patients. Class 2 tumors had a significantly larger baseline tumor height ($p<0.001$) and basal diameter ($p<0.001$) and were more likely to have ciliary body involvement ($p=0.007$) and exudative retinal detachment ($p<0.001$). There was no statistically significant difference between Class 1 and Class 2 tumors based on the presence of lipofuscin, drusen or subretinal fluid. Class 1a tumor patients, compared to Class 1b, were significantly older ($p=0.034$). However, Class 1b tumors had a non-significant trend towards larger baseline tumor height and basal tumor diameters compared to Class 1a. Class 2 tumors, when compared to Class 1b, were associated with increasing patient age ($p<0.001$), larger tumor height ($p=0.010$), ciliary body involvement ($p=0.001$), exudative RD ($p=0.024$), and anterior tumor location ($p<0.001$). When the tumors were grouped by COMS size categories, increasing tumor size category was significantly associated with Class 2 status: 19% of small tumors, 33% of medium tumors and 46% of large tumors were Class 2 ($p=0.009$).
Conclusions: Our findings confirm the previously reported associations between Class 2 status and increasing age, tumor height, largest basal diameter and ciliary body involvement. We are the first to report the differences in clinical features between the GEP sub-classes and our findings support the current molecular model of UM. When the tumors were grouped by size category, the distribution of the GEP sub-classes among the size groups was similar to reported time-to-metastasis data among the same size groupings.
Commercial Relationships: Amy C. Schefler, Allergan (R), Regeneron (F), Genentech (F); **Duncan Berry**, None; **Michael Seider**, None; **Sandra Stinnett**, None; **Prithvi Mruthyunjaya**, None

Program Number: 2502
Presentation Time: 9:15 AM–9:30 AM
Molecular classification of uveal melanoma subtypes using integrative mutational and whole-genome copy number analysis
Serdar Yavuzyigitoglu[1, 2], Wojtek Drabarek[1, 2], Kyra N. Smit[1, 2], Askar Obulkasim[1], Natasha van Poppelen[1, 2], Anna Koopmans[1], Jolanda Vaarwater[1, 2], Tom Brands[2], Bert Eussen[2], Hendrikus J. Dubbink[3], Robert Verdijk[3], Nicole Naus[1], Dion Paridaens[4], Emine Kilic[1], Annelies de Klein[2]. [1]Ophthalmology, Erasmus University Medical Center, Rotterdam, Netherlands; [2]Clinical Genetics, Erasmus University Medical Center, Rotterdam, Netherlands; [3]Pathology, Erasmus University Medical Center, Rotterdam, Netherlands; [4]The Rotterdam Eye Hospital, Rotterdam, Netherlands.
Purpose: Classification of structural and numerical chromosomal changes to elucidate the observed metastatic risk difference in uveal melanoma (UM) patients with mutually exclusive *BAP1*, *SF3B1* and *EIF1AX* mutations.
Methods: Unsupervised hierarchical clustering of genome-wide single nucleotide polymorphism (SNP) array data was used to identify molecular subclasses with distinct chromosomal patterns. These subclasses were then investigated for mutations in *BAP1*, *SF3B1* and *EIF1AX* by next-generation sequencing, Sanger sequencing and immunohistochemistry (IHC).
Results: Unsupervised clustering identified five clusters with distinct copy number aberrations patterns, based on copy number variations (CNVs) of chromosome 3, chromosome 6 and chromosome 8. Chromosome 3 divided the cases in two major clusters, in which monosomy 3 UMs were enriched for BAP1 IHC negative UMs. BAP1 IHC positive UMs harboring *BAP1* mutations also clusters along BAP1 negative UMs. Within the disomy 3 UMs, the three subclusters were based on chromosome 6 CNVs. UMs with disomy 6 and gain of entire chromosome 6 UMs were enriched for *EIF1AX* mutations, whereas partial chromosome 6p accompanied by partial chromosome 8q gain were *SF3B1*-mutated UMs.
Conclusions: UMs are characterized by recurrent CNVs and recurrent mutated genes. Unsupervised clustering revealed that UMs can be classified in distinct subclasses which are characterized by the same CNVs and recurrent mutated genes. UMs harboring mutations in *BAP1*, *SF3B1* or *EIF1AX* have distinct chromosomal aberration patterns that mainly differ by the affected chromosomes, the absolute number of CNVs and the type of CNVs. Mutations in these genes are strongly associated with distinct molecular subclasses. This highlights and reflects the biological difference between UMs on a genetic level.
Commercial Relationships: Serdar Yavuzyigitoglu, None; **Wojtek Drabarek**, None; **Kyra N. Smit**, None; **Askar Obulkasim**, None; **Natasha van Poppelen**, None; **Anna Koopmans**, None; **Jolanda Vaarwater**, None; **Tom Brands**, None; **Bert Eussen**, None; **Hendrikus J. Dubbink**, None; **Robert Verdijk**, None; **Nicole Naus**, None; **Dion Paridaens**, None; **Emine Kilic**, None; **Annelies de Klein**, None

Program Number: 2503
Presentation Time: 9:30 AM–9:45 AM
Correlation of BAP1 immunoreactivity with metastasis in uveal melanoma
Eszter Szalai[1], Jill R. Wells[1], Hans E. Grossniklaus[1, 2]. [1]Department of Ophthalmology, Emory University School of Medicine, Atlanta, GA; [2]Department of Pathology, Emory University School of Medicine, Atlanta, GA.
Purpose: To examine the BRCA1-associated protein-1 (BAP1) expression of primary uveal melanomas without and with metastasis, and to analyze the correlation between the BAP1 immunoreactivity and clinico-pathologic features of the tumor.
Methods: Medical records and histology slides of patients with primary uveal melanoma treated by enucleation were reviewed. BAP1 expression was evaluated immunohistochemically on formalin-fixed paraffin-embedded sections, and immunoreactivity in the nucleus and cytoplasm was graded by estimating the percentage of tumor cells showing a positive stain per high power field (grade 0 to 4).
Results: Forty uveal melanoma patients (mean age: 57.98±14.75 years) were included in this analysis, of which twenty patients had no metastatic disease and 20 patients had metastasis. The median nuclear BAP1 grade was 2, the median cytoplasmic BAP1 grade was 3. Significantly lower nuclear ($P=0.006$) and cytoplasmic ($P=0.009$) BAP1 immunoreactivity was observed in the metastatic melanoma group. Patients with grade 3-4 nuclear and grade 4 cytoplasmic BAP1 stain had significantly longer metastasis-free survival than patients with low nuclear ($P<0.0001$) and cytoplasmic ($P=0.019$) BAP1 expression (Figure 1). Greater tumor thickness, basal diameter, more advanced TNM stage and higher GEP class were associated with increased odds of developing metastasis ($P<0.05$). Additionally, higher grade nuclear ($P=0.004$) and cytoplasmic ($P=0.006$) BAP1 expression were associated with decreased odds of developing metastatic disease.
Conclusions: We conclude that there is a difference in time to metastasis in uveal melanoma patients with different grades of nuclear and cytoplasmic BAP1 immunoreactivity. Patients with low nuclear and cytoplasmic BAP1 staining showed an increased risk of metastasis. Our data support the assumption that both high nuclear and cytoplasmic BAP1 expressions are associated with decreased metastasis in patients with uveal melanoma.

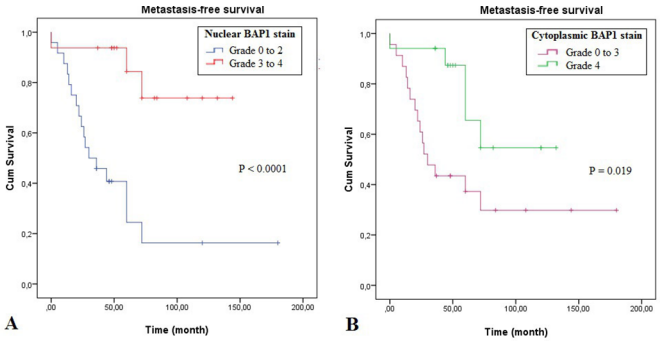

Figure 1. Kaplan-Meier metastasis-free survival curves for uveal melanoma patients stratified by the median nuclear (A) and cytoplasmic (B) BAP1 score.

Commercial Relationships: Eszter Szalai; **Jill R. Wells**, None; **Hans E. Grossniklaus**, None

Support: This study was supported by NIH P30EY06360 and an unrestricted departmental grant from Research to Prevent Blindness, Inc.

Program Number: 2504
Presentation Time: 9:45 AM–10:00 AM

Monosomy 3 uveal melanoma cells have a unique metabolic phenotype distinct from disomy 3

Chandrani Chattopadhyay, Jason Roszik, Elizabeth Grimm, Scott E. Woodman. Melanoma Medical Oncology, UT MD Anderson Cancer Center, Houston, TX.

Purpose: Uveal melanoma (UM) is the most frequent primary intraocular cancer in adults and about 50% of primary uveal melanoma metastasize, preferentially in liver with very poor prognosis. The most important genetic alterations associated with poor prognosis in UM are BAP1 gene alterations and loss of an entire copy of Chromosome 3 (monosomy3), which are most often concurrent, BAP1 is encoded on chromosome 3. It is unknown whether monosomy 3 and Bap1 loss are independent or interdependent mechanisms.

Methods: Cell survival analysis with monosomy and disomy 3 UM cells in presence and absence of Oxidative phosphorylation (Ox Phos) inhibitor, IACS-10759, developed at UT MD Anderson Cancer Center were performed. Cells were cultured under ambient oxygen or 1% oxygen. Mitotracker Red dye staining was used to assay the extent of active mitochondria in monosomy 3 and disomy 3 cells. Other analyses to differentiate monosomy and disomy 3 cells included seahorse analysis for Ox Phos and metabolic profile analysis with mass spectrometric methods followed by Ingenuity Pathway Analysis.

Results: Three UM cell lines with monosomy3 were more resistant to IACS-10759 when compared to three lines with normal chromosome 3 copy number. This resistance diminishes under hypoxic conditions or in galactose media, when Ox Phos is not the predominant form of sugar metabolism. Further studies show more active mitochondria and a larger mitochondrial reserve capacity in monosomy3 cells. SDHA, a key enzyme connecting the TCA cycle to mitochondrial ETC, is up regulated in monosomy 3 cells and may be responsible for the resistant phenotype.

Conclusions: We conclude that UM cells with monosomy3 are more resistant to mitochondrial Ox Phos inhibitor due to larger mitochondrial reserve capacity. We are now in the process of elucidating the underlying molecular basis that can account for this difference in monosomy3 containing UM cells. Genes/proteins responsible for this resistance can be developed as a target for combination therapy with the Ox Phos inhibitor, such as, IACS-10759

Commercial Relationships: Chandrani Chattopadhyay, None; **Jason Roszik**; **Elizabeth Grimm**, None; **Scott E. Woodman**, None