Huntersville, NC Uveal Melanoma Scope of Work

Background: There have been 12 cases of uveal melanoma diagnosed in patients within the past 10 years who have resided or worked in Huntersville, NC. Of these 12, 9 affected women with 6 of these women diagnosed under the age of 30. Two of these women and 2 men have died from disease. In addition to the incidence of cases, the number cases affecting young female patients are unusual. Epidemiologic and clinical genetic (BAP1) analyses are ongoing. Deep molecular analyses of biospecimens available from these cases may contribute to a further understanding of this group of patients.

Plan: After obtaining regulatory and IRB approval, we will obtain all available tumor and germline biospecimens. The tumor specimens that have been identified thus far and are available are outlined in the table below and include:

1. Five enucleation specimens (primary tumor samples)
2. Four liver biopsy specimens (metastatic tumor samples; three liver biopsy sample available from patient who also underwent enucleation and one from a patient who also underwent FNA of the primary lesion)
3. Three fine needle aspirate sample of primary (primary tumor samples; one FNA available from a patient who underwent liver biopsy)

Of these, it may be possible to obtain matched normal tissue from 4 patients for purposes of whole exome sequencing analysis.

Case	Status	Enucleation Specimen	FNA of Primary	Liver Biopsy	Matched Normal
1 (KC)	Deceased	X		X	
2 (ML)	Deceased		X	X	
3 (SH)	Alive		X		X
4 (CP)	Alive	X			X
5 (GG)	Alive		X		X
6 (BM)	Alive	X			X
7 (BP)	Deceased	X		X	
8 (JK)	Deceased	X		X	

We propose the following testing:

1. We will perform next generation sequencing and transcriptome analysis for cases with sufficient material through the Columbia University Medical Center (CUMC) Genomics Technologies Core of the Herbert Irving Comprehensive Cancer Center (http://www.hiccc.columbia.edu/research/sharedresources/genomics). For cases where matched normal-frozen tumor specimens are available with sufficient quality and quantity, we will perform whole exome/transcriptome

sequencing. We will perform more targeted sequencing as need based upon biospecimen quantity and quality. We will compare results obtained from these Huntersville, NC cases from publically available results obtained from uveal melanoma samples collected as part of The Cancer Genome Atlas project (https://cancergenome.nih.gov/cancersselected/UvealMelanoma) and other available datasets in an effort to identify genetic similarities and differences.

2. Similar epigenetic analysis will be performed on all cases with sufficient DNA material. We will perform genome-wide analysis of CpG methylation using the Illumina "800k" MethylationEPIC BeadChip Assay through the CUMC Epigenetics Shared Resource of the Herbert Irving Comprehensive Cancer Center (http://www.hiccc.columbia.edu/research/sharedresources/molecularcyto-epigenetics).

3. We will assess the cellular immune infiltrate within the tumor microenvironment of the available cases using available archived tumor specimens. We determine cell phenotypes and localization as well as perform nearest neighbor analysis using the MANTRA system from Perkin Elmer and Inform software in the laboratory of Dr. Yvonne Saenger at CUMC (http://www.cumc.columbia.edu/hematology-oncology/about_us/yvonne_saenger).

DNA and RNA extraction from either fresh frozen material or paraffin embedded material will be performed by the Herbert Irving Comprehensive Cancer Center Molecular Pathology Core (http://www.hiccc.columbia.edu/research/sharedresources/molecular/using).

Budget: The estimated cost per sample for each of the proposed assays, including bioinformatics analysis costs, is below.

Assay	Estimated Cost/Sample	Testing Location
DNA and RNA Extraction	$50	Molecular Pathology Core
Whole Exome/Transcriptome	$5000	Genomics Technologies Core
CpG Methylation Analysis	$1000	Epigenetics Shared Resource
Multiplex Immunohistochemistry	$500	Saenger Laboratory

Assuming full analysis of 9 cases, including the five enucleation samples and four liver biopsy samples (with limited if any analysis possible on three FNA samples), we anticipate a maximum total cost of $58,950 (9 x $6550). The total cost may be lower

depending on sample availability, specimen quality, and assays ultimately felt to be feasible.

Timeline: We have initiated the process to obtain local IRB approval of this project and will obtain verbal consent of patients and/or family members to obtain available biospecimens once approval has been obtained. We anticipate this process being complete by May 2017. We will then request all available biospecimens to be shipped to our center and hope to have all specimens on site by July 2017. Once all specimens are obtained, we anticipate completion of all testing and analysis within 2 months. A final report will be generated by December 2017 or sooner.