Next Generation Sequencing of Uveal Melanoma

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OBJECTIVE This was a pilot study to clinically apply a next-generation sequencing (NGS) assay in uveal melanoma (UM).

PURPOSE To investigate next-generation genetic sequencing clinically in uveal melanoma (UM) and to compare results with gene expression profiling (GEP), the current standard of care for prognostic genetic testing of uveal melanoma in the USA.

METHODS This was a prospective, non-randomized, case controlled study. 51 UM samples were obtained via trans-scleral or trans-retinal fine-needle aspiration or vitrector-assisted biopsy, or after enucleation. Tumor tissue was sent for cytopathology testing in the UCSF Department of Pathology and for gene expression profiling (Castle Biosciences, Friendswood, Texas) as well as for next generation sequencing at UCSF. Following extraction, NGS library preparation was performed. The target regions spanned ~1.8 Mb of the genome and included exonic, intronic, and untranslated regions of 538 cancer genes including genes with known relevance to uveal melanoma.

RESULTS Of the 51 patients, 28 were male and 23 were female. Tumors were choroidal (39), ciliochoroidal (6), ciliary body (4), and iris (2). 24 were GEP Class 1A, 11 were Class 1B and 16 were Class 2. There was high concordance between Class 2 GEP and Chromosome 3 loss and BAP1 mutation on NGS, which was statistically significant (t test, $p<0.05$). There was also a high concordance between Class 1A GEP and reassuring features on NGS (i.e., lack of chromosome 3 loss, no BAP1 mutation), which was

statistically significant (t-test, $p<0.05$). There were 3 (13%) Class 1A tumors on GEP that showed chromosome 3 loss and BAP 1 mutation. Of the Class 1B GEP tumors, 3 (27%) showed chromosome 3 loss, indicating high risk of metastasis. All Class 2 tumors showed Chromosome 3 loss, which was statistically significant (t-test, $p<0.05$). GNAQ/11 mutations were seen in 46 (90%) patients, consistent with the incidence of these mutations the literature.

CONCLUSION NGS identified chromosome 3 loss in some GEP Class 1A and Class 1B UMs, and may assist in determining which of these tumors carry increased metastatic risk. All GEP Class 2 tumors showed chromosome 3 loss with NGS, indicating high concordance between GEP and NGS for identifying tumors with highest metastatic risk. NGS also identified GNAQ/11 mutations, confirming presence of UM cells in samples.

TAKE HOME MESSAGE Genetic testing of uveal melanomas is helpful in prognostication; gene expression profiling is the current gold-standard in the US. Next-generatio sequencing methods may offer additional information.

HUMAN RESEARCH This study involves human research.
IRB Approval Status: Approved by institutional review board

Randomized, Prospective Study of Aflibercept for Visually Significant Radiation Maculopathy - Interim Analysis

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OBJECTIVE This is the first randomized, prospective clinical trial of aflibercept in the management of visually significant radiation maculopathy in patients with treated uveal melanoma.

PURPOSE To report a randomized, prospective clinical trial of aflibercept in the treatment of radiation maculopathy comparing: Fixed interval dosing versus Treat and Adjust

METHODS Randomized clinical trial of Iodine-125 brachytherapy treated patients with uveal melanoma presenting with visually significant radiation maculopathy. All patients were evaluated with widefield photography, wide field FA/ICG, standardized echography, and ophthalmological examination at baseline. IRB approved informed consent was obtained and patients were randomized to fixed interval every 6 week injections OR treat and extend with initial 6 week injection of aflibercept. All patients were followed at each visit with ETDRS VA, sdOCT, widefield photography and q 6month FA/ICG angiography. Grading of sdOCT images was performed in a masked fashion. Patients were scheduled for 12 month aflibercept therapy prior to study termination.

RESULTS 50 patients were scheduled for randomized participation. Mean age at study entry was 64 years (42-86 years). Presenting mean VA was 20/80. Mean sdOCT maculopathy grading was 3.2. Mean follow-up was 4 months (3-6 months) and mean VA improved to 20/30. Mean sdOCT grading improved to 1.4. No cases of significant vitreous hemorrhage, choroidal detachment or endophthalmitis were seen. No cases of tumor progression or metastases were seen during the study.

CONCLUSION Aflibercept is effective in treatment of visually compromising radiation maculopathy. Treatment can be extended beyond every 6 weeks utilizing a treat-and-adjust injection schedule. This randomized, prospective clinical study establishes a framework for consideration of aflibercept as a primary agent in the treatment of radiation maculopathy.

TAKE HOME MESSAGE Aflibercept is effective in treating visually significant radiation maculopathy and may allow for extended intervals between treatment.

HUMAN RESEARCH This study involves human research.
IRB Approval Status: Approved by institutional review board

Ocular Oncology Study Consortium (OOSC) Report No 2: Effect of Clinical and Pathologic Variables on Biopsy Complication Rates

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OBJECTIVE The objective of this study was to examine the effect of various surgical intraocular biopsy techniques in uveal melanoma on the incidence of complications, tissue yield, and genomic results.

PURPOSE The OOSC study is a collaborative retrospective study designed to examine the details of intraocular biopsy procedures for uveal melanoma patients undergoing surgery at multiple centers across the U.S. Multiple clinical variables related to technique, complications, and surgical outcome were examined.

METHODS An IRB-approved, multi-institutional retrospective study including consecutive adult patients with uveal melanoma undergoing intraocular tumor biopsy to obtain tissue for Gene Expression Profile (GEP) testing just prior to treatment with I-125 plaque brachytherapy from 1/1/2010 to 6/30/2014. The data were entered into a secure REDCap database that allowed online inputting of de-identified patient data. Nine ocular oncologists contributed data. Data were collected regarding

demographics and surgical technique including approach (transvitreal versus transscleral), needle/cutter gauge, number of passes, tissue yield, and complications.

RESULTS 354 patients underwent biopsies with GEP testing. 163 cases were found to be GEP Class 1A (47%), 78 were BEP Class 1B (22%), 105 were GEP Class 2 (30%), and there were 3 technical failures (1%). Mean GEP discriminant factor, a measure of specimen quality and quantity, was 0.84 (range 0.01 – 1.64). Complications included: mild vitreous hemorrhage (4 cases, 1%) and rhegmatogenous retinal detachment (1 case, 0.2%). Statistical association between the occurrence of a surgical complication and various clinical variables including GEP result, surgical approach, needle/cutter gauge, use/non-use of a microscope/viewing system, number of passes, age of patient, and thickness of tumor, was examined. All vitreous hemorrhages occurred in patients who had undergone a trans-vitreal biopsy approach, 75% of whom underwent a second pass for cytology ($p<0.1$). No statistically significant association was found between occurrence of a surgical complication and all other clinical variables.

CONCLUSION Complication rates were low after both transscleral and transvitreal biopsies despite varying techniques at different centers. There were no cases of biopsy-induced extraocular extension, and nearly all specimens produced tumor material suitable for genomic analysis. The proportion of patients with a GEP Class 2 result (high metastatic risk) was similar to previously published series.

TAKE HOME MESSAGE Transscleral and transvitreal biopsies are safe for uveal melanoma patients. Complication rates are low with rare occurrences of vitreous hemorrhage and rhegmatogenous retinal detachment.

HUMAN RESEARCH This study involves human research.
IRB Approval Status: Approved by institutional review board