Early Exploratory Study Data for Potential Development of a Complementary Test to Accompany DecisionDx®-UM That Could Aid in the Early Detection of Uveal Melanomas

Except from presentation given by Amy Schefler, M.D., at the 2023 American Academy of Ophthalmology (AAO) Annual Meeting in San Francisco
Background: Value of a Minimally Invasive Test for Early Detection of Aggressive Uveal Melanomas

- Uveal melanoma (UM) affects approximately 2,000 people per year in the U.S.
- Up to 50% of patients will experience metastasis despite successful primary treatment, indicating that undetectable disease spread had occurred prior to primary treatment\(^1,2\).
- Earlier detection of UM would potentially enable clinicians to treat small tumors before they acquire the ability to spread.
- It can be clinically challenging to determine whether a small ocular lesion is a melanoma or an atypical benign nevus, resulting in the potential for misclassification, especially between larger nevi and smaller melanomas.
  - Number of “indeterminate” (difficult to diagnose) lesions is estimated to be between 4,000 - 12,000 cases/year\(^3\).
- Because biopsy of small ocular lesions can be challenging and is generally not repeated multiple times to detect malignant transformation, an alternative minimally invasive and repeatable test that can be done safely in the office is needed for early identification of lesions that are sufficiently likely to be malignant to warrant definitive tumor biopsy and treatment.
- A liquid biopsy approach to monitor suspicious lesions for malignant transformation would help inform more timely decision-making and earlier therapeutic interventions.

Liquid Biopsy Based Test for Earlier Detection of Aggressive UM Tumors

* The current standard is a “watch and wait” approach that consists of monitoring lesions for growth or appearance of high-risk features that would indicate transformation into a malignant melanoma.

** A liquid biomarker that is strongly associated with aggressive uveal melanoma (such as a Class 2 GEP signature) could be used as a more sensitive and objective biological marker of malignant transformation.
Study Design

- **Goal:** To develop an aqueous humor-based protein assay for early identification of aggressive UM tumors

160 uveal melanoma patients

Aqueous humor collection via paracentesis

Tumor biopsy for molecular testing
  - DecisionDx-UM
  - DecisionDx-PRAME
  - DecisionDx-UMSeq (optional)

Aqueous protein identification

Data analyzed and results compared with DecisionDx-UM and DecisionDx-PRAME results

Patients enrolled from 3 independent sites

High-throughput protein biomarker platform was used for discovery
Demographics

- Demographics are shown for the first half of the cohort (N=79 patients with clinically diagnosed uveal melanoma who underwent molecular prognostic biopsy)

- The cohort was representative of the clinically tested population in terms of the distribution of GEP Class and PRAME status

- A majority of tumors were small, consistent with the intended use population for this test in-development

### Demographics for first 79 UM samples
(1 sample excluded due to ineligibility)

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Class 1</th>
<th>Class 2</th>
<th>P-value</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>PRAME negative</td>
<td>PRAME positive</td>
<td>PRAME negative</td>
</tr>
<tr>
<td>No. Samples (%)</td>
<td>48 (60.8)</td>
<td>9 (11.4)</td>
<td>11 (13.9)</td>
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<tr>
<td>Sex, n (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>27 (56.2)</td>
<td>6 (66.7)</td>
<td>5 (45.5)</td>
</tr>
<tr>
<td>Female</td>
<td>21 (43.7)</td>
<td>3 (33.3)</td>
<td>6 (54.5)</td>
</tr>
<tr>
<td>Tumor Diameter, mean (mm)</td>
<td>9.76±3.59</td>
<td>10.68±5.58</td>
<td>11.69±4.0</td>
</tr>
<tr>
<td>Tumor Thickness, mean (mm)</td>
<td>3.72±2.37</td>
<td>3.81±2.20</td>
<td>4.76±3.42</td>
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Differential Expression of Aqueous Proteins Between Class 1 vs Class 2 patients

- >3000 proteins were assayed from 79 UM aqueous samples (Olink Explore 3072 panel).

- Several hundred proteins with statistically significant expression differences between high- and low-risk GEP classes (Class 1 vs 2) at the discovery level (p<0.0001) were identified and will be included in further analyses.

- Castle expects to share additional updates in 2024 if development of this complementary test is deemed possible.