CASTLE
BIOSCIENCES INCORPORATED
3737 N. 7th Street, Suite 160
Phoenix, AZ 85014
T: (866) 788-9007 F: (866) 712-5207

UM DECISIONDx
Uveal Melanoma

ACCN#: _____

PATIENT REPORT

Patient:
Sex:
DOB:
Medical Record/Patient #:
Date of Surgery:
Tissue Received:
Date Reported:

Type of Specimen:
Ordering Clinician:
Client:

ASSAY DESCRIPTION

DecisionDx-UM® gene expression assay for uveal melanoma is a proprietary assay that uses RT-PCR to determine the expression of a panel of 15 genes (3 control) in the supplied tumor tissue. The **DecisionDx-UM** classification is calculated from the gene expression results and comparing these results to a training set of patients with known outcomes.

RESULTS

DecisionDX-UM Class = 1A

Discriminant Value = 1.00

Class 1 molecular signature is associated with a low risk of near term (within 5 years) clinical metastasis. Sub-analysis indicates a Class 1A tumor which carries the lowest metastatic risk. A discriminant value ≥ 0.100 is reported with normal confidence.

Test Results should be interpreted using the Clinical Experience information contained in this report which is derived from clinical studies involving patient populations with specific clinical features as noted in section titled Clinical Experience. These results have not been validated in patients with clinical features different from those described. The discriminant value relates to Class 1 vs 2. See page 2 of initial report for discussion on discriminant value confidence.

CLINICAL EXPERIENCE FOR CLASS 1A, 1B AND 2

The **DecisionDx-UM** assay has been evaluated in over 700 patients with uveal melanoma to date. The majority of these patients participated in a prospective, multi-center study to validate the predictive accuracy of this gene expression-based molecular assay. Outcomes are collected and the ability of the molecular signature to predict metastasis is being evaluated at regular intervals. The most recent censor date (June 9, 2011) of the prospective study included 514 patients with follow-up data available for analysis. The censor date for this addendum is June 9, 2011. The actuarial outcomes for metastasis of the predicted low-risk (Class 1A), intermediate-risk (Class 1B), and the high-risk (Class 2) molecular signatures are shown below.

Molecular Signature Class	Percent Metastasis Free at 3 Years	Percent Metastasis Free at 5 Years
Class 1A	98%	98%
Class 1B	93%	79%
Class 2	50%	28%
n=514; Log-rank (Mantel-Cox) test; p<0.0001		

CASTLE BIOSCIENCES INCORPORATED
3737 N. 7th Street, Suite 160
Phoenix, AZ 85014
T: (866) 788-9007 F: (866) 712-5207

UM DECISIONDx
Uveal Melanoma

RAW DATA AND CALCULATION OF DECISIONDX-UM CLASS 1A, 1B AND 2

DecisionDx-UM® uveal melanoma assay Uses RT-PCR to determine the expression of a panel of 15 genes (3 control) in the supplied tumor tissue. The twelve genes of interest are: CDH1, ECM1, EIF1B, FXR1, HTR2B, ID2, LMCD1, LTA4H, MTUS1, RAB31, ROBO1, and SATB1. The three control genes are: MRPS21, RBM23, and SAP130. The optimized molecular model determines ΔC_t values for each of the twelve genes of interest. The ΔC_t values are imported into a support vector machine learning algorithm (SVM), which analyzes their combined expression profile. SVM calculates a predicted classification and a discriminant value. As the absolute value of the discriminant approaches zero, the probability that the prediction is incorrect increases. Discriminant values from the concordance study conducted on both FNAB and FFPE specimens were analyzed at a 97.5% and 95% confidence levels. The 97.5% confidence level equated to a discriminant value of 0.069; the 95% confidence level equated to a discriminate value of 0.060. Based on these findings, a conservative discriminant value cut-point of 0.100 has been set to differentiate between normal and reduced confidence.

For the identification of Class 1 tumor subgroups 1A and 1B, the ΔCt values for CDH1 and RAB31 are summed.

ADDITIONAL BACKGROUND INFORMATION

Comparison of gene expression profile (molecular signature) to other clinicopathologic factors
A study of subjects was undertaken to compare the gene expression profile (molecular signature) to the chromosomal marker - monosomy 3 (Worley, 2007). The same study compared the assay to clinical and pathologic factors for predicting metastasis in uveal melanoma. This study of 67 patients treated by enucleation was the largest study using metastatic outcome to compare prognostic factors to date in uveal melanoma. Monosomy 3 was assessed through fluorescence in situ hybridization (FISH) and array comparative genomic hybridization (aCGH). The clinicopathologic factors of age, sclera invasion, histopathologic cell type, tumor thickness, gender, largest tumor diameter, and anterior tumor location were also assessed. By Cox univariate proportional hazards, only Class 2 gene expression profile ($p = 0.0001$), advanced patient age ($p = 0.01$), and scleral invasion ($p = 0.007$) were significantly associated with metastasis (Table 2). Kaplan-Meier analysis rendered similar results. When all three significant variables were entered into a Cox multivariate model, only the Class 2 molecular signature exhibited significant association with metastasis.
Comparison of the DecisionDx-UM assay to clinical and pathologic factors for predicting metastasis was also undertaken in the blinded prospective study noted above. This study is the largest prospective study using metastatic outcome to compare prognostic factors to date in uveal melanoma. Monosomy 3 was assessed by SNP_LOH. Using multivariate Cox modeling, DecisionDx-UM Class ($p<0.0001$) and tumor diameter ($p=0.02$) were the only two variables that contributed independent prognostic information. Chromosome 3 status did not contribute additional prognostic information that was independent of DecisionDx-UM ($p=0.6$).

_____
John F. Stone, PhD, FACMG, Laboratory Director

REFERENCE LIST

Onken, et al. Gene expression profiling in uveal melanoma reveals two molecular classes and predicts metastatic death. Cancer Res 2004;64: 7205-9.
Onken, et al. Prognostic testing in uveal melanoma by transcriptomic profiling of fine needle biopsy specimens. Jrnl Mol Diagn; 2006;8: 567-73.
Worley, et al. Transcriptomic versus Chromosomal PrognosticMarkers and Clinical Outcome in Uveal Melanoma Clin Cancer Res 2007;13(5): 1466-71.
Ehlers, et al. Integrative genomic analysis of aneuploidy in uveal melanoma. Clin Cancer Res 2008;14:115-122.
AJCC Cancer Staging Manual, Version 7. Malignant Melanoma of the Uvea. 2010.
Onken, et al. An accurate, clinically feasible multi-gene expression assay for predicting metastasis in uveal melanoma. J Mol Diagn; 2010;12:461-8.
Harbour. Molecular research in uveal melanoma: ushering in a new standard of care. Retina Times 2011;29: 36-7.
Onken, et al. Collaborative Ocular Oncology Group Report No. 1: Prospective Validation of a Multi-Gene Prognostic Assay in Uveal Melanoma. Ophthalmology 2012;119:1596–1603.