

FINAL REPORT

Patient:	Type of Specimen: Fresh Frozen FNAB
Sex:	Specimen ID:
DOB:	Collected:
MRN:	Received:
Client:	Reported:
Clinician:	

DecisionDx-UM Result

Class
1A

Discriminant Value: 0.52

Class 1 molecular signature is associated with a low risk of near term (within 5 years) clinical metastasis. Subanalysis 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 report for discussion on discriminant value confidence.

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.

CLINICAL EXPERIENCE FOR CLASS 1A, 1B AND 2¹⁻⁸

The **DecisionDx-UM** assay has been evaluated in over 1300 patients with uveal melanoma to date, including those from a prospective, multi-center study to validate the predictive accuracy of this gene expression-based molecular assay. Outcomes were collected and the ability of the molecular signature to predict metastasis was evaluated. The most recent censor date (June 9, 2011) of the prospective study included 514 patients with follow-up data available for analysis. The 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, Inc. | Sherri Borman, PhD, HCLD, Lab Director



This test was developed and its performance characteristics determined by Castle Biosciences Inc. It has not been cleared or approved by the FDA. The laboratory is regulated under CLIA as qualified to perform high-complexity testing. This test is used for clinical purposes. It should not be regarded as investigational or for research. Patent Pending.

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

The **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 Δ Ct values for each of the twelve genes of interest. The Δ Ct 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 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 Δ Ct values for CDH1 and RAB31 are summed.

ADDITIONAL BACKGROUND INFORMATION^{2,9-11}

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. 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. 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 ($p = 0.6$).

REFERENCE LIST

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