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.

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
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, LMCDD1, 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 discriminant 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**

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**