Assessing Genetic Expression Profiles in Melanoma Prognosis

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KEYWORDS

• Skin cancer • Melanoma • Prognosis • DecisionDx-melanoma • Genetic expression profile

KEY POINTS

- A 31-genetic expression profile (31-GEP) test to predict metastatic risk of melanoma has been previously validated and classifies patients as either class 1 (low risk) or class 2 (high risk).
- The 31-GEP in combination with other prognostic characteristics or tools (American Joint Committee on Cancer online tool and sentinel lymph node biopsy) provides superior prognostic capability.
- Clinical utilization studies reveal the 31-GEP test had a significant and appropriate impact on management while remaining within the context of established guidelines.
- Limited follow-up data required to correlate the 31-GEP with outcomes are available. The 31-GEP has not been included in any official guideline recommendations, either as standard of care or as part of clinical trials.

INTRODUCTION

The incidence of cutaneous malignant melanoma (CMM) has continued to increase, and although it accounts for less than 5% of all skin cancers, it causes the greatest number of skin cancer–related deaths worldwide.[1] Following a diagnosis of CMM, patients are classified by the American Joint Committee on Cancer (AJCC) system that defines CMM staging.[2] A patient's staging status in conjunction with national guidelines can then be used for subsequent evidenced-based management by their dermatologist.

Despite advances in management and treatment, the factor that most impacts prognosis remains early detection of the malignancy that is responsible for the detection of thinner CMM lesions at diagnosis. Although it is well demonstrated that Breslow thickness predicts disease-free survival and overall survival, other potential characteristics have been evaluated for the prognosis of patients with CMM.[3]

Currently, the following clinical and pathologic prognostic markers of CMM are incorporated for clinical use: Breslow thickness, presence of ulceration, presence of microsatellites, and regional lymph node involvement.[2] Mitotic rate is included only for melanomas $\leq$1 mm in thickness. Unfortunately, even after decades of research on various prognostic markers, the guideline recommendations are often similar across several tumor stages in part because of their inability to stratify different

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risk groups that may have markedly different outcomes.[4,5]

The difficulties in discretely stratifying CMM staging are apparent. Although sentinel lymph node biopsy (SLNB) has been shown to be the most accurate independent prognostic parameter in CMM, positive SLNB status only identifies one-third of patients with CMM who develop metastatic disease and ultimately die.[6,7] The SLNB negative patients are generally managed with lower intensity strategies that include less frequent physician-patient interaction, yet 2 out of 3 patients who die from melanoma are initially diagnosed with stage I and II disease, and most recurrences (up to 70%) are detected by the patient.[6,8] Furthermore, prognosis for clinical stage II and III cases by TNM is highly variable, as evidenced by a 5-year survival rate of 53% to 82% for stage II patients and a 5-year survival rate of 22% to 68% for stage III patients.[2,7] Although the use of prognostic factors in conjunction with staging is a strong predictor of metastatic spread, the clinical use of each factor has limitations.

Several new molecular tests for melanoma have been developed that are based on gene expression patterns from RNA obtained from formalin-fixed paraffin-embedded sections from the biopsy specimens of lesions. These molecular techniques provide information that cannot be gleaned from clinical or histologic examination and may provide significant prognostic capability.

A 31-genetic expression profile (31-GEP) test (DecisionDx-Melanoma, Castle Biosciences Inc, Friendswood, TX, USA) was developed as a diagnostic test to assist physicians in the management of CMM.[9] Based on a patient's primary tumor expression levels of a panel of genes, a lesion is classified as either ''low risk'' (class 1) or ''high risk'' (class 2) for metastasis. The 31-GEP has significant potential to affect clinical practice in the management of CMM.

CONTENT

31-Genetic Expression Profile Test

The quantitative reverse transcription polymerase chain reaction–based 31-GEP test is obtained from samples that are collected from formalin-fixed paraffin-embedded CMM tissue and arranged in 5-μm sections on microscope slides.[9] RNA isolation is performed followed by an assessment of its quality and quantity. The RNA is then converted to complementary DNA and undergoes amplification before being loaded to microfluidics gene cards containing primers specific for the 31 gene targets. The gene expression assay is performed in triplicate. Radial basis machine predictive modeling is performed, which is a nonlinear classification based on the normalized values for each gene. The modeling transforms the gene measurements using a kernel function to find an optimal hyperplane in multivariate dimension, thus providing a predicted classification of high and low risk tumor biology.

Initial Development and Validation

For the development of the 31-GEP, Gerami and colleagues[9] used published genomic analysis of CMM tumors to determine a unique prognostic genetic signature for metastatic risk. Genes were selected on the basis of significant genetic expression variation in metastatic and nonmetastatic CMM across several published studies. Of 54 identified genes, the investigators selected 20 based on chromosomal location. Genes from a similar uveal melanoma panel were added in addition to specific BAP1 gene probes. A signature comprising 28 prognostic genetic targets and 3 control genes was developed from the expression data. The 31-GEP was applied to 268 primary CMM cases (collected from 7 independent centers) with clinical follow-up of at least 5 years unless there was a well-documented metastatic event, including positive SLNB.

The study initially reported the use of the test to predict metastasis in patients diagnosed with stage I or II CMM using an independent validation set consisting of 104 cases.[9] Of these cases, 35 had developed metastatic disease, and there was median follow-up of 7.3 years for the cases that did not. The 5-year disease-free survival was 97% among the 61 cases with a class 1 "low-risk" signature and 31% for the 43 cases with a class 2 "high-risk" signature. Negative predictive value and positive predictive value were 93% and 72%, respectively. The receiver operating characteristic curve was 0.91 for the validation set and 0.93 for the original training set, which is consistent with a clinically relevant predictive model.

For stage I and II cases in the validation set that had either a metastatic event or more than 5 years of follow-up without metastasis, class 1 disease-free survival was 98% compared with class 2 with a rate of 37%.[9] Median follow-up for cases in this cohort was 7.6 years. When combined, the validation and training cohorts consisted of 220 stage I and II CMM cases. Overall, the 31-GEP accurately identified 120 of 134 (90%) stage I/IIA cases without documented evidence of metastasis as class 1 (low risk) and 24 of 30 (80%) stage I/IIA cases with documented metastasis as class 2 (high risk)