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CASE SERIES

Clinical Utility of the 40-Gene Expression Profile (40-GEP) Test for Improved Patient Management Decisions and Disease-Related Outcomes when Combined with Current Clinicopathological Risk Factors for Cutaneous Squamous Cell Carcinoma (cSCC): Case Series

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ABSTRACT

Introduction: While improvements have been made to risk assessment of cutaneous squamous cell carcinoma (cSCC) patients, there is a critical need for a uniform and more precise stratification system of their care. To address this unmet clinical need, a prognostic 40-gene expression profile (40-GEP) test has recently been developed and independently validated to show improved stratification of metastatic risk in high-risk cSCC patients compared with current staging systems.

Methods: Two cSCC cases, both male with similar patient profiles and the same staging status across two different staging systems, yet with opposing outcomes, were chosen for retrospective review of their primary biopsy using the 40-GEP test.

Results: Case 1 declined further treatment, even when presented with evidence of a small focus of cSCC found in the last layer of non-marginal tissue obtained from Mohs micrographic surgery (MMS). Case 1 remained recurrence free, and retrospective analysis of the initial biopsy with the 40-GEP test provided a Class 1 result (low likelihood of metastasis). Case 2, even with subsequent clearing of the primary cSCC with MMS, noted another metastatic cSCC 3 months later. Case 2, after multimodal adjuvant treatments, died due to disease progression. Retrospective analysis of the initial biopsy with the 40-GEP test provided a Class 2B result (high likelihood of metastasis).

Conclusions: The cases discussed highlight the utility in 40-GEP to provide additional information to guide treatment decisions and improve outcomes. Integrating novel molecular prognostication with traditional clinicopathological risk factors can improve stratification of high-risk cSCC patients and may inform selection of risk-appropriate treatment and surveillance strategies.

Keywords: 40-GEP; Cutaneous squamous cell carcinoma; Gene expression profiling; Metastasis; Prognosis

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Key Summary Points

Managing cutaneous squamous cell carcinoma (cSCC) is a significant clinical issue, with an average of 1.8 million cases diagnosed per year, and a staggering increase in incidence over the past three decades.

We present two cases, in which both patients had similar clinical profiles and the same initial Brigham and Women's Hospital (BWH) and Cancer Staging Manual, 8th Edition (AJCC-8) staging, yet with distinctively different outcomes.

In a retrospective analysis of the initial biopsies of these patients, the 40-gene expression profile (40-GEP) test demonstrated its ability to distinguish between the biologically less aggressive and biologically more aggressive tumors.

These cases highlight the utility of the 40-GEP test as an adjunct to enhance cSCC risk stratification, with the potential to improve patient care and outcomes.

INTRODUCTION

While cutaneous squamous cell carcinoma (cSCC) has an overall favorable prognosis, a subset of patients will develop metastases and die from their disease. Even with a low fatality rate, the high (~1.8 million cases/year [1]) and increasing incidence of cSCC will perpetuate the occurrence of poor outcomes. Once nodal metastasis is detected, 5-year survival rates have been reported to be 50–70% [2, 3] even after appropriate treatments, and once distant metastasis has been identified, 5-year survival is rare [4]. Deaths from this disease are estimated to surpass those from melanoma, making the management of cSCC an increasingly significant clinical issue [5].

The National Comprehensive Cancer Network (NCCN) classifies cSCC patients as high risk for local recurrence or very high risk for metastasis or death by the presentation of selected risk factors and presents a range of downstream management approaches [6]. Tumor staging systems, such as the American Joint Committee on Cancer (AJCC) Cancer Staging Manual, 8th Edition (AJCC-8) [7] and Brigham and Women's Hospital (BWH) system [8], help determine recurrence and metastatic risk by translation of high-risk factors into tumor (T) stages. However, these systems can fail to fully assess patient risk, leading to low accuracy when identifying metastasis [9]. The subset of cSCC patients classified as high risk commonly require a more aggressive treatment regimen, but guidelines are vague and staging criteria has been noted as limited and lacking homogeneity [10–12], creating a burden for clinicians when establishing an appropriate treatment plan.

A 40-gene expression profile (40-GEP) test was recently developed to assess the biology of primary archival formalin-fixed paraffin-embedded (FFPE) cSCC tissue, and has been validated to significantly improve metastasis risk prediction when compared with the current staging systems listed above [9]. The 40-GEP test classifies patients into three groups based on risk for regional and/or distant metastasis (Class 1: low risk; Class 2A: moderate risk; Class 2B: high risk). As national guidelines for high-risk cSCC patients are unclear on which patients warrant additional follow-up and management, treatment of high-risk cSCC often relies on risk assessment based on individual risk factors weighted by physician judgement, leading to management intensity heterogeneity and highlighting the critical need for an unbiased method of risk assessment. The purpose of developing the 40-GEP test was to identify high-risk cSCC early in the disease state, such that its result could complement current risk assessment methods for development of more personalized management plans to reduce the risk of poor outcomes for cSCC patients. The cases discussed herein highlight the utility of 40-GEP to provide additional information to guide treatment decisions and improve outcomes.

METHODS

Sample Acquisition and Analysis

Formalin-fixed paraffin-embedded (FFPE) samples from primary cSCC tissue and associated de-identified clinical data were obtained from Department of Dermatology, Indiana University School of Medicine. All reported clinicopathological and outcomes patient data were monitored onsite, including review of pathology reports and medical records. Staging was performed by a board-certified dermatopathologist and included all available data in the medical record and centralized pathology review. Briefly, the generation of a 40-GEP test result requires FFPE tumor tissue macrodissection and processing by real-time PCR, with samples run in triplicate, as previously described [9].

Compliance with Ethics Guidelines

This study received institutional approval for institutional review board (IRB) exempt status from Indiana University (Protocol #1708728214). This study was performed in accordance with the Helsinki Declaration of 1964. A waiver of informed consent was granted by Indiana University Human Research Protection Program policy on informed consent.

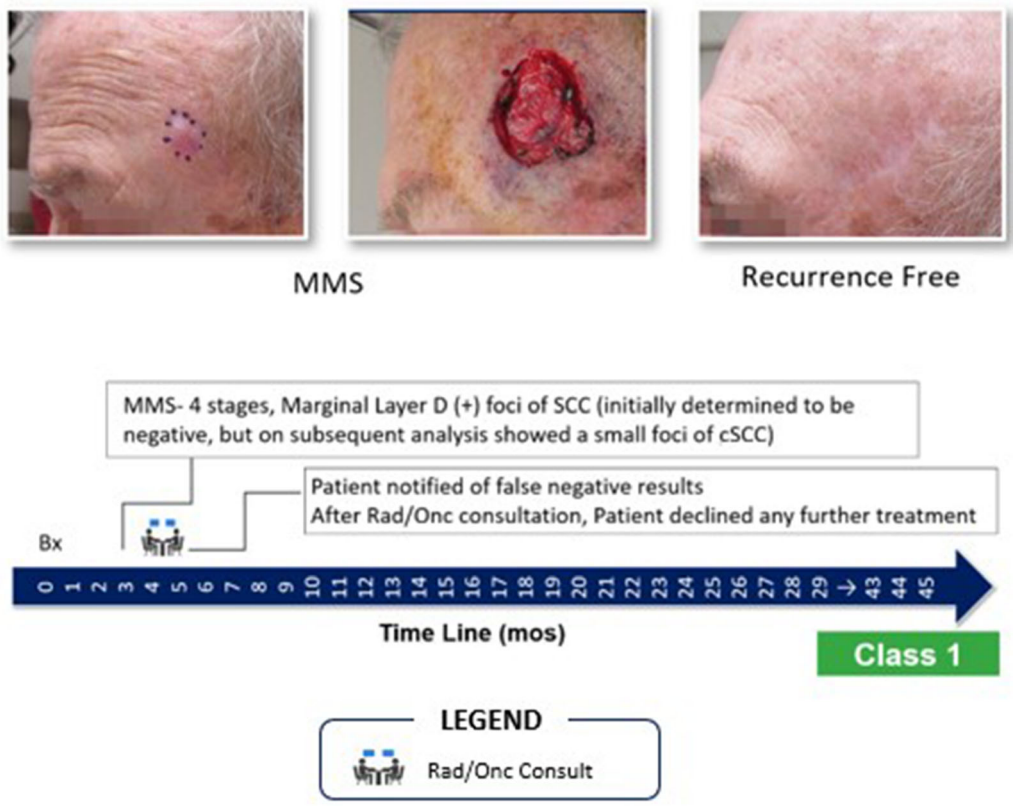

Fig. 1 Case 1 receiving a retrospective Class 1 result using the 40-GEP test. Small foci of cSCC present on subsequent analysis following Mohs micrographic surgery (MMS). Recurrence free for 4 years after declining further treatment (death by myocardial infarction)

RESULTS

Case Presentations

Case 1 (Fig. 1) was a 65-year-old male patient with a history of renal and liver transplantation and cSCC, who presented with a papule on his left temple previously treated with cryotherapy. The 1.3 cm tumor was diagnosed to be a poorly differentiated cSCC and staged as a BWH T2a and AJCC-8 stage T1. Mohs micrographic surgery (MMS) was completed in four stages and was initially determined to be margin negative. However, subsequent analysis of the last layer of nonmarginal tissue was positive for cSCC. This prompted a review of the marginal frozen

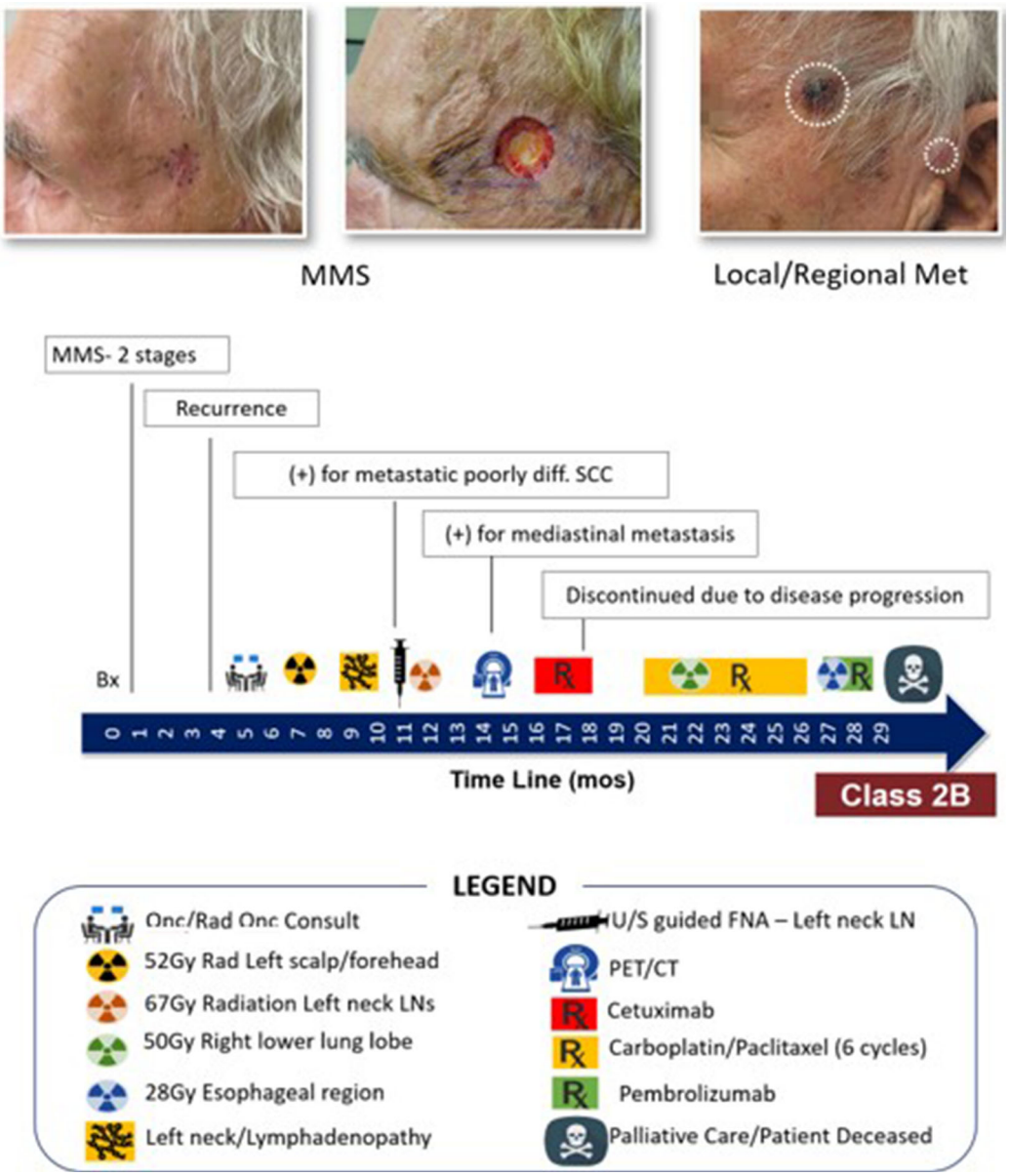

Fig. 2 Case 2 receiving a retrospective Class 2B result using the 40-GEP test. Metastatic cSCC presenting 3 months following Mohs micrographic surgery (MMS) with subsequent metastasis to mediastinum. Patient died due to disease progression

sections, which showed a small focus of cSCC. While the residual cSCC may have been removed with the standing cone, there was no histologic confirmation. The patient was informed but declined any further treatments. The patient was recurrence-free for 4 years, but subsequently died due to unrelated causes (myocardial infarction). Retrospective analysis of the initial biopsy with the 40-GEP test provided a Class 1 result.

Case 2 (Fig. 2) is a 69-year-old male patient with a history of liver transplantation and cSCC, who presented with a 2-month history of an exophytic growth on his left temple. The 1.5 cm tumor was diagnosed to be a poorly differentiated cSCC (BWH T2a, AJCC-8 stage T1) with subsequent clearing with MMS in two stages 1 month later. The patient then noted another growth immediately inferior to the linear scar line, as well as one on the ipsilateral helical root 3 months later. The biopsy results were consistent with metastatic cSCC. Despite aggressive therapies, the patient died due to disease progression. Retrospective analysis of the initial biopsy with the 40-GEP test provided a Class 2B result.

Table 1 Clinicopathologic characteristics, 40-GEP class designation, and outcome of Case 1 and Case 2

Case 1	Case 2
65-year-old male	69-year-old male
Liver/kidney transplant	Liver transplant
1.3 cm diameter	1.5 cm diameter
Poorly differentiated	Poorly differentiated
AJCC-8 Stage T1	AJCC-8 Stage T1
BWH Stage T2a	BWH Stage T2a
40-GEP Class 1	**40-GEP Class 2B**
Recurrence free	**Regional/distant met**

The bolded cells in the two columns highlights the differences between the two cases

DISCUSSION AND CONCLUSION

We present two cases that highlight the utility of the 40-GEP test as an adjunct to enhance cSCC risk stratification. Each case was similar in patient background and tumor characteristics and had the same initial BWH and AJCC-8 staging, yet there were distinctively different outcomes between them (Table 1). Case 1 highlighted a biologically less aggressive tumor (with a retrospective 40-GEP Class 1 result) that did not recur despite incomplete surgical clearance. Case 2 highlighted a biologically aggressive tumor (with a retrospective 40-GEP Class 2B result) that developed regional metastasis despite clear surgical margins obtained through MMS. Adjuvant treatment might have been appropriate for this patient earlier in the disease course and thus altered his prognosis.

Although the majority of cSCC tumors are associated with a favorable outcome, patients presenting with clinicopathologic high-risk factors show a higher risk of local recurrence, as well as regional and distant metastasis, which are associated with high levels of cSCC mortality [12]. Unfortunately, an unclear agreement on what factors are most influential in determining a high-risk cSCC tumor that may most benefit from a particular adjuvant therapy, as seen in the ambiguity concerning treatment recommendations (as governed by the NCCN) and the diversity of risk factors considered high risk by staging criteria (i.e., AJCC-8 and BWH), has led to a wide range of and variation in patient management decisions [13, 14]. These inconsistencies have a profound effect on the accuracy of applying staging to risk assessment [9] and emphasize the need for an objective tool to complement these systems.

Effective molecular prognostic assays provide reproducible and reliable risk assessment (analytical and clinical validity) to alter decision making (clinical utility). The advancement of gene expression profiling signatures for use in clinical management make them a powerful prognostic tool, when complementing staging, for many other tumor types [15–19]. A recent publication from Ibrahim et al. [20] demonstrated how combining both clinicopathologic

features with molecular testing for cSCC can augment stratification of metastatic risk. This may reduce expanding health care costs by focusing more intense treatments (i.e., therapeutics, surgeries, and/or imaging) toward patients who will see the utmost benefit, while reducing unnecessary procedures for those who would be appropriately deemed low risk for metastatic disease. With this study, we demonstrate that the integration of novel molecular prognostication for cSCC in combination with traditional clinicopathologic risk factors, has the potential to improve stratification of high-risk cSCC patients and posits selection of risk-appropriate treatment and surveillance strategies aligned with a patient's biological risk for poor outcomes.

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Disclosures. Jeremiah H. Au and Perry B. Hooper declare that they have no conflict of interest. Alison L. Fitzgerald is an employee and option holder of Castle Biosciences, Inc. Ally-Khan Somani is an investigator and receives honoraria as a member of the Castle Bioscience's Speakers Bureau.

Compliance with Ethics Guidelines. This study received institutional approval for IRB exempt status from Indiana University (Protocol #1708728214). This study was performed in accordance with the Helsinki Declaration of 1964. A waiver of informed consent was granted by Indiana University Human Research Protection Program policy on informed consent.

Data Availability. The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request. The authors would like to thank the participants of the study for their contribution.

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