

## BRIEF ARTICLE

## Biology Beyond the Margins: Impact of 40-Gene Expression Profiling on Risk Stratification in Cutaneous Squamous Cell Carcinoma

Heather Gates, PA-C, DFAAPA<sup>1</sup>, Cynthia Trickett, PA-C, Tiffani Botts Massey, PA-C, Francine Phillips, PA-C, Patrice Simon, PA-C, Angela Rosenberg, DO, Darrell Rigel, MD

<sup>1</sup> The Dermatology Institute & Skin Cancer Center, The Villages, FL, USA

<sup>2</sup> North Dallas Dermatology Associates, Dallas, TX, USA

<sup>3</sup> Boostology MedSpa LLC, St. Petersburg, FL, USA

<sup>4</sup> Florida Medical Clinic, Land O'Lakes, FL, USA

<sup>5</sup> Legacy Dermatology and Restoration Center, Frisco, TX, USA

<sup>6</sup> University of Texas Southwestern, Dallas, TX, USA

<sup>7</sup> Ronald O. Perelman Department of Dermatology, NYU Grossman School of Medicine, New York, NY, USA

### ABSTRACT

**Background** Cutaneous squamous cell carcinoma (cSCC) causes more annual mortality than metastatic melanoma. However, risk stratification protocols remain based solely on clinicopathologic features, excluding tumor biology.

**Objective** To illustrate how integration of 40-gene expression profile (GEP) testing influences prognostic assessment and post-treatment management in high-risk cSCC.

**Methods** We present a case report of a 70-year-old male whose incidental cSCC was reclassified as high-risk following 40-GEP testing despite histologic clearance, resulting in enhanced surveillance.

**Results** Tumor profiling yielded a Class 2A (higher risk) result, which altered post-operative follow-up strategies beyond conventional staging.

**Conclusion** Gene expression profiling provides valuable, individualized prognostic data, which may improve management of high-risk cSCC patients and should be considered for broader integration into clinical guidelines.

### INTRODUCTION

Cutaneous squamous cell carcinoma (cSCC), a type of nonmelanoma skin cancer (NMSC), is the second most common skin cancer in the United States, with increasing incidence among older, fair-skinned males. Although the metastatic potential is estimated at 3.7 - 5.2%, cSCC is responsible for over 15,000 deaths annually - surpassing that of melanoma.<sup>1</sup> The mortality rate of metastatic

SCC is approximately 1 - 2% with lymph nodes being the most common site of metastasis.<sup>2</sup> Current risk stratification guidelines (**Table 1**) - including those from the American Joint Committee on Cancer (AJCC)<sup>3</sup>, National Comprehensive Cancer Network (NCCN)<sup>4</sup>, and Brigham and Women's Hospital (BWH)<sup>5</sup> - rely on clinical and histologic parameters such as tumor

# SKIN

diameter, depth, grade, and perineural invasion. However, these metrics alone fail to

identify up to 30% of tumors that will ultimately metastasize.<sup>3</sup>

**Table 1.** High risk features of cSCC recognized by the American Joint Committee on Cancer (AJCC; 8th Edition), the National comprehensive Cancer Network (NCCN; Version 2.2025), and the Brigham and Women’s Hospital (BWH; 2013) Tumor Classification Staging Systems. \*=NCCN very high risk criteria only<sup>1-4,6</sup>

Risk Factors	AJCC	NCCN	BWH
History & Physical			
Location/Diameter	≥4cm	Trunk, Extremities: >2cm	>2cm
		Head, Neck, Hands, Feet, Pretibia, Anogenital Area: any size	
		>4cm any location*	
Clinical Borders		Poorly Defined	
Primary vs. Recurrent		Recurrent	
Immunosuppression		(+)	
Site of Prior Radiation or Chronic Inflammation		(+)	
Rapidly Growing Tumor		(+)	
Neurologic Symptoms		(+)	
Pathology			
Degree of Differentiation		Poorly Differentiated*	Poorly Differentiated
Histologic Subtype		(+)	
Invasion Beyond the Subcutaneous Fat	(+)	(+)*	(+)
Depth or Thickness	>6mm	2-6mm (>6mm*)	
Perineural Involvement	(+)	(+)	≥ 0.1mm in caliber
Bone Erosion	(+)		
Lymphatic or Vascular Involvement		(+)*	

Molecular diagnostics, including the 40-gene expression profile (40-GEP), offer biologically-informed risk assessment. This test provides prediction of risk of regional and distant metastasis within 3 years, by stratifying tumors into Class 1 (low risk), Class 2A (higher risk), and Class 2B (highest risk) metastatic risk groups.<sup>2</sup> To be eligible for testing, patients must have one or more of the

risk factors listed in **Table 2**. Despite demonstrated clinical utility, integration into staging and treatment guidelines remains limited, even as emerging data shows the use of the 40-GEP can lead to improved clinical outcomes and more appropriate risk-aligned management strategies.<sup>7</sup>

**Table 2.** Testing criteria for 40-Gene Expression Profile (GEP) Testing<sup>7</sup>

Intended Use Population of 40-GEP	
History and Physical Examination	Surgical and Pathological Findings
Tumor size $\geq 2$ cm (anywhere on the body)	Perineural invasion (PNI)
Tumor location (areas H or M) <sup>#</sup> , head, neck, genitals, feet, or pretibial surfaces	Poorly differentiated tumor histology
Tumor at site of prior radiation therapy or chronic inflammation	Depth: i. Invasion beyond subcutaneous fat or into bone ii. Invasion $\geq 2$ mm iii. Clark level IV/V
Tumor with poorly defined borders	*Aggressive histological subtypes
Neurological symptoms around region of tumor	Lymphovascular invasion
Immunosuppression	Desmoplastic cSCC

#Area H: Mask areas of face including central face, eyelids (including inner/outer canthi), eyebrows, nose, lips, chin, ear and periauricular skin/sulci, temple), genitalia (including perineal and perianal), hands, feet, nail units, ankles, and nipples/areola.

#Area M: Cheeks, forehead, scalp, neck, jawline, pretibial surface.

\*Acantholytic (adenoid), adenosquamous, carcinosarcomatous (metaplastic) (Others will be considered on a case-by-case basis).

## CASE REPORT

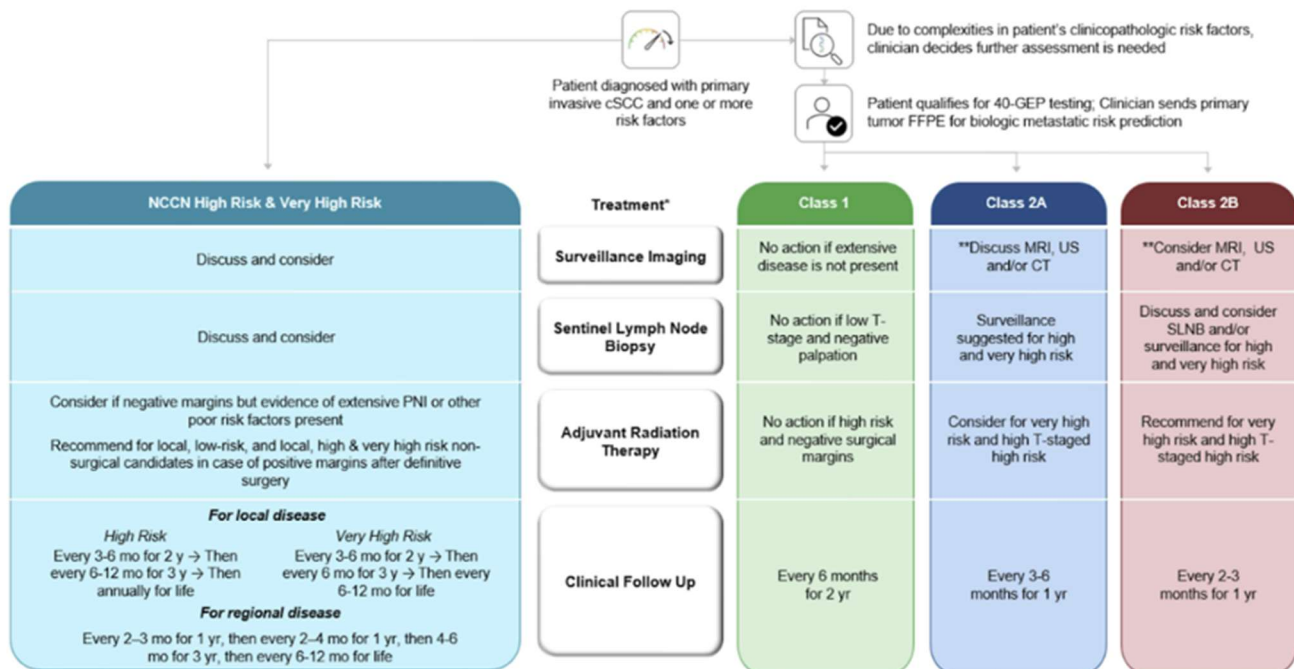
A 70-year-old Caucasian male with extensive sun exposure and history of NMSC presented for elective neurosurgical intervention for Parkinsonian tremors. During the electrode implantation procedure, a hyperkeratotic lesion on the frontal scalp was identified and biopsied. Histopathology revealed a moderately differentiated G2 (which signifies cSCC that is moderately

differentiated) unifocal cSCC with lymphovascular invasion and peripheral margin involvement.

According to current AJCC, NCCN, and BWH guidelines, the only universally required intervention for this lesion is complete surgical excision with histologically clear margins. Additional measures such as imaging, multidisciplinary tumor board review, and consideration of adjuvant radiation therapy are not mandated, but are only recommended or considered based on the presence of multiple high-risk features. The lesion was excised via Mohs micrographic surgery, achieving negative margins in three stages. No other treatment or diagnostics were completed initially.

However, the specimen was later sent for 40-GEP testing and returned a Class 2A result (Figure 1). Given the elevated molecular risk signified by a Class 2A result, the case was sent for review at a multidisciplinary tumor board, which recommended alternative management than originally intended. Updated management included enhanced

surveillance with lymph node palpation and skin examinations every three months. No imaging or adjuvant radiation was pursued due to prolonged timing of secondary diagnostics and guideline constraints. To date, no recurrence or nodal disease has been noted.



**Figure 1.** A treatment algorithm for incorporation of 40-Gene Expression Profile (GEP) test results into treatment decisions for a high-risk cSCC compared to current NCCN guideline recommendations. \*Multidisciplinary board should be considered; \*\*Choice of imaging technique dependent on number and type of high-risk factors

Image from: Singh G, Tolkachjov SN, Farberg AS. Incorporation of the 40-Gene Expression Profile (40-GEP) Test to Improve Treatment Decisions in High-Risk Cutaneous Squamous Cell Carcinoma (cSCC) Patients: Case Series and Algorithm. *Clin Cosmet Investig Dermatol*. 2023 Apr 5;16:925-935. doi: 10.2147/CCID.S403330. PMID: 37051586; PMCID: PMC10083143.

## DISCUSSION

This case highlights a critical deficiency in current cSCC staging: omission of tumor-intrinsic biology. Although traditional metrics did not indicate high risk, gene expression analysis reclassified this tumor as moderate-

to-high risk. In response, management was adapted to include a more aggressive surveillance plan.

The 40-GEP assay has been validated as an independent predictor of metastasis in high-risk cSCC offering enhanced risk stratification beyond clinical pathology staging.<sup>7</sup> However, limited awareness and

lack of incorporation into national guidelines have slowed widespread clinical use.

In a multicenter cohort study, 40-GEP identified 60% of tumors that ultimately metastasized, many of which were classified as low-risk by AJCC or BWH criteria.<sup>7</sup> Approximately 25% of Class 2A tumors were initially staged as low risk, underscoring the limitations of conventional metrics.<sup>7</sup> Patients with Class 2A results demonstrated a 3-year metastasis-free survival rate of 87%, compared to 95% in Class 1, thus warranting closer post-treatment monitoring.<sup>7</sup> These findings support using 40-GEP results for cSCC to guide escalation of care, including earlier referral, imaging, and multidisciplinary review.<sup>7,8</sup> In a survey of practicing dermatologists, over 70% reported altering their clinical management based on 40-GEP results, citing improved confidence in surveillance and treatment decisions.<sup>8</sup> In this case, the Class 2A result prompted intensified follow-up every three months along with lymph node evaluation, a change not supported by traditional staging alone. Broader integration of gene expression profiling into risk assessment algorithms could improve outcomes by aligning surveillance intensity with biological behavior, as demonstrated here.

## CONCLUSION

Tumor biology, as captured by gene expression profiling, provides critical insight into metastatic risk not evident from histologic features alone. This case supports broader implementation of 40-GEP testing to augment current risk models, personalize care, and potentially improve clinical outcomes in cSCC.

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**Corresponding Author:**

Heather Gates, PA-C, DFAAPA  
The Dermatology Institute, 2783 Brownwood Blvd.,  
The Villages, FL 32163  
Phone: 309-370-6355  
Email: heather@thegatewaypro.com

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