Real-world clinical usage data demonstrates appropriate utilization of the prognostic 40-gene expression profile test for cutaneous squamous cell carcinoma with one or more risk factors

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BACKGROUND

- The metastatic rate for cSCC is low, however the overall incidence is high (~1-2.5 million cases/year), and deaths from this disease are estimated to surpass those from melanoma.[1,2]
- The National Comprehensive Cancer Network (NCCN)[3] categorizes a patient as high or very high risk for recurrence and/or metastasis by the presence of risk factors, while current tumor staging systems, such as the American Joint Committee on Cancer (AJCC) Cancer Staging Manual, 8th Edition (AJCC8)[4] and Brigham and Women's Hospital (BWH) system,[5] help determine recurrence and metastatic risk by translation of high-risk factors into tumor (T) stages. However, these systems often fail to fully classify patient risk resulting in a broad range of downstream management guidelines.
- The clinically available 40-gene expression profile (40-GEP) test was developed and independently validated to accurately classify risk for regional or distant metastasis as low (Class 1), moderate (Class 2A), or high (Class 2B) in patients with primary cSCC and one or more high-risk factors.[6]

OBJECTIVE

To demonstrate independent prognostic value with existing risk assessment methods and report on the early clinical usage of the 40-GEP test.

cSCC with one or more risk factors

40-GEP

- Quantifies expression of 40 genes from primary tumor using RT-PCR
- Applies a validated neural network algorithm
- Accurately classifies patients based on risk for regional/distant metastasis

Class 1: Low Biological Risk
Less than half the study population risk

Class 2A: Moderate Biological Risk
Similar to strongest traditional factors

Class 2B: High Biological Risk
≥50% risk of metastasis

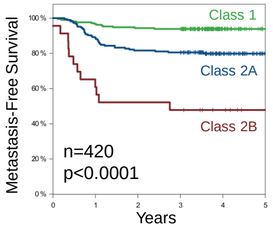
METHODS

- All primary FFPE cSCC samples underwent centralized pathology review and the 40-GEP test in a CAP-accredited, CLIA-certified laboratory.
- **Clinical Validation:** Archival cSCC tissue with verified clinicopathologic information and outcomes data were assayed under clinical testing conditions by the 40-GEP test (n=420). Kaplan-Meier for metastasis-free survival and Cox regression analysis were performed.
- **Clinical-usage Summary:** Summary metrics on the first 1000 samples received meeting clinical testing criteria, including 40% tumor content and sufficient RNA, were generated. The 40-GEP result and patient risk factors were captured by clinical requisition form review. Risk factors included lesion located on the H or M area,[3] ≥2cm diameter, poorly defined borders, patient immunosuppression, rapidly growing tumor, site of prior RT or chronic inflammation, History & Physical- other factor noted, high-risk subtype, Clark Level IV, >2mm invasion, poorly differentiated, LVI, PNI, invasion beyond the subcutaneous fat.

RESULTS

Clinical Validation (n=420)

Figure 1. The 40-GEP test classifies patients based on risk for metastasis. All cases were either high-risk by NCCN guidelines for localized cSCC or met Mohs Micrographic Surgery appropriate use criteria.[7]



40-GEP Risk Class	Overall Cohort 3-year MFS (95% CI)	Overall Event Rate
Class 1	93.9% (90.7-97.2%)	6.6%
Class 2A	80.5% (75.0-86.5%)	20.0%
Class 2B	47.8% (31.2-73.3%)	52.2%
Without 40-GEP	85.5% (82.2-88.9%)	15.0%

Table 1. The 40-GEP result demonstrates independent prognostic value in multivariate analysis. Cases were comprehensively staged based on medical records, pathology reports, and definitive surgical reports.[7]

		Univariate Cox Regression		Multivariate Cox Regression	
Risk Factor	n	Hazard Ratio (95% CI)	p value	Hazard Ratio (95% CI)	p value
40-GEP Result					
Class 1	212	1.00 (---)	---	1.00 (---)	---
Class 2A	185	3.22 (1.74-5.95)	<0.001	**2.33** (1.20-4.53)	0.013
Class 2B	23	11.61 (5.36-25.15)	<0.001	**6.86** (2.73-17.22)	<0.001
Clinicopathologic Risk Factors					
Poor Differentiation	58	3.93 (2.34-6.60)	<0.001	2.29 (1.21-4.33)	0.011
Perineural Invasion	53	3.28 (1.41-14.36)	<0.001	1.22 (0.58-2.59)	ns
Deep Invasion	72	3.11 (1.86-5.20)	<0.001	2.05 (1.04-4.04)	0.039
Tumor Diameter	N/A	1.15 (1.08-1.22)	<0.001	1.07 (0.97-1.17)	ns
Immunosuppression	103	1.46 (0.86-2.49)	ns	---	---
40-GEP Result					
Class 1	212	1.00 (---)	---	1.00 (---)	---
Class 2A	185	3.22 (1.74-5.95)	<0.001	**2.98** (1.61-5.53)	<0.001
Class 2B	23	11.61 (5.36-25.15)	<0.001	**9.42** (4.28-20.7)	<0.001
BWH T Stage					
T1/T2a	364	1.00 (---)	---	1.00 (---)	---
T2b/T3	56	2.55 (1.26-5.17)	0.009	**2.38** (1.38-4.13)	0.002

Clinical-usage Summary (n=1000)

Primary cSCC with qualifying high-risk factor reported → First n=1000 40-GEP tests

- **69.0%** Class 1 (n=690)
- **26.0%** Class 2A (n=260)
- **1.3%** Class 2B (n=13)

Figure 2. The 40-GEP test has high technical reliability. Data reporting on the first 1000 orders since availability in September 2020

Figure 3. Most tested patients have ≥3 risk factors. Histogram of risk factor count per patient.

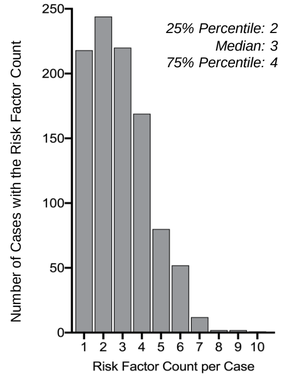


Table 2. Patient Characteristics. NCCN risk group[3] and BWH T-stage[5] for each patient were approximated using physician-reported risk factors

Clinicopathologic Risk*		% of Patients
NCCN:	High Risk	56.8%
	Very High Risk	43.2%
BWH T-stage:	T1	33.4%
	T2a	42.7%
	T2b	23.4%
	T3	0.5%

*Estimated based on factors reported, all reported PNI was considered an upstaging factor

Table 3. Frequency of reported risk factors in the clinical cohort.

Risk Factor	% of Patients
Located on H or M	77.3%
Size ≥2 cm	44.5%
Rapidly growing tumor	41.1%
Borders poorly defined	27.6%
Poorly differentiated	20.6%
Invasion beyond subcutaneous fat	19.3%
Perineural invasion	13.5%
Specific high-risk subtype	13.8%
Clarks Level IV or >2mm	12.7%
Patient Immunosuppression	9.4%
History and Physical- Other Factor	4.9%
Lymphovascular Invasion	1.9%
Prior RT/Chronic Inflammation	1.6%

CONCLUSIONS

- The 40-GEP test is validated to classify risk for metastasis in cSCC patients with one or more risk factors and provides prognostic information independent from known high risk factors or established staging systems.
- These findings demonstrate the utility of the 40-GEP test as an adjunct to enhance cSCC risk stratification and the intended use population aligns with the cases submitted for clinical testing.
- Incorporating 40-GEP test results in clinical assessments with traditional clinicopathological risk factors can improve stratification of high-risk cSCC patients and contribute to risk-appropriate surveillance and treatment decisions.

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