Independent multi-center cohort study confirms the performance of the prognostic 40-gene expression profile (40-GEP) test to classify risk of metastasis for high-risk cutaneous squamous cell carcinoma (cSCC) patients

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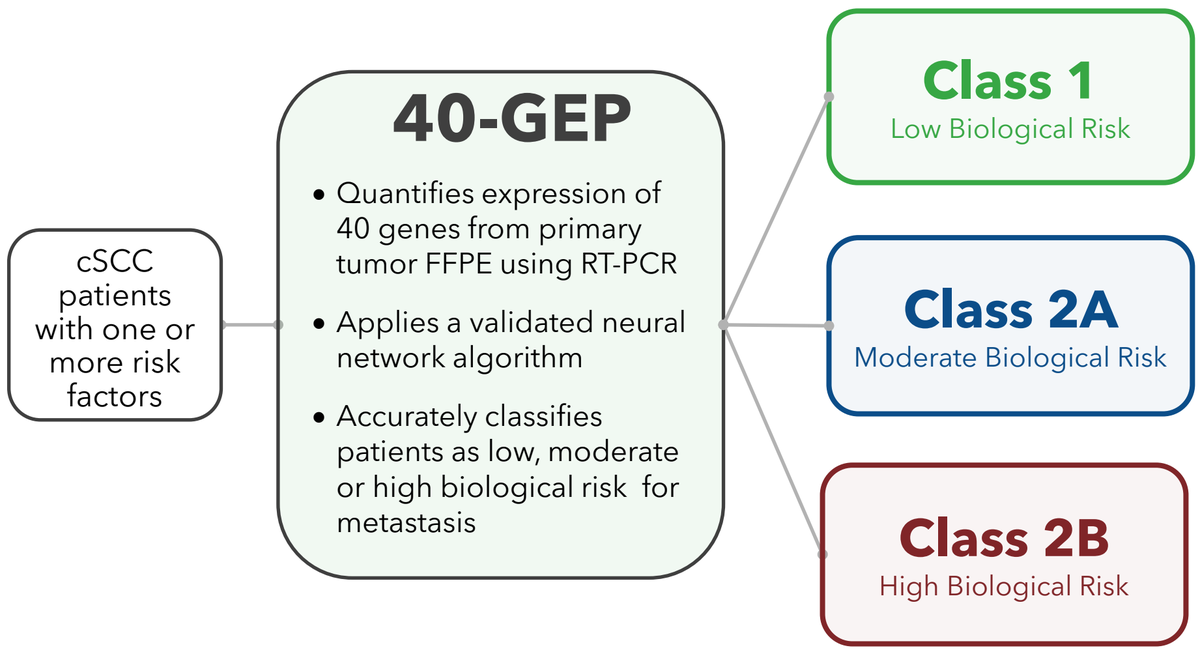
Background

- While the majority of cSCC tumors are curable by excision, approximately 20% of patients present with clinical or pathologic features that are associated with disease progression.[1]
- Since early detection of metastasis can lead to better outcomes through prompt and appropriate intervention, accurate identification of patients at high risk for metastasis is critical.
- The 40-GEP test has been validated to improve upon current metastasis risk prediction methods, providing for a more precise risk stratification for patients with high-risk cSCC.[2,3]
- Clinical utility studies have demonstrated the ability of 40-GEP test results to guide risk-aligned patient management decisions.[4-6]
- This test stratifies primary cSCC patients having one or more clinicopathologic risk factors into three biological risk groups based on risk for regional, nodal, or distant metastasis (Low = Class 1; Moderate = Class 2A; High = Class 2B).



Objective

- To confirm the performance of the 40-GEP test in an independent, multi-center cohort study of high-risk cSCC patients to augment prognostication of metastasis when compared to clinicopathologic information alone.

Methods

- Under an IRB-approved protocol, centralized pathology review and sample analysis were performed in a CAP-accredited, CLIA-certified laboratory using formalin-fixed paraffin-embedded archival primary cSCC tumor specimens (n=552). Clinicopathologic and outcomes data were collected from the 35 contributing centers.
- Clinicopathologic risk factors were comprehensively assessed, including review of original biopsy reports, definitive surgical reports, and independent review by a board-certified dermatopathologist.
- Kaplan-Meier analysis for metastasis-free survival (MFS) with log rank test and Cox regression analyses were performed.

Results

- The overall cohort had a metastatic rate of 10.9%, indicative of a high-risk cSCC cohort. Significant differences were observed between metastatic and non-metastatic groups among various parameters, including, tumor diameter and thickness, PNI, lymphovascular invasion, and invasion beyond the subcutaneous fat (**Table 1**).
- The 40-GEP demonstrated statistically significant risk stratification as demonstrated by Kaplan-Meier analysis (p<0.0001) (**Figure 1**). Univariate Cox regression analysis in this independent performance cohort was significant for Class 2A and Class 2B with a hazard ratio (HR) of 5.09 and 11.26, respectively (p<0.001) (**Table 2**).
- High-risk clinicopathologic factors (e.g. poor differentiation) and Brigham and Women's Hospital (BWH) staging were significantly associated with metastatic risk. Importantly, when combined into multivariate Cox models, the 40-GEP significantly contributed to risk stratification with the inclusion of either high-risk factors (p<0.01 for 2A and 2B) or BWH T-stage (p<0.001 for 2A and 2B) (**Table 2**).
- The addition of interaction terms to the multivariate analysis between the 40-GEP test and BWH revealed no significant interactions (p>0.05), demonstrating that the 40-GEP test provides independent prognostic information over BWH staging. Patients with Class 2B results had the highest risk of metastasis and a worse prognosis (**Table 2**).

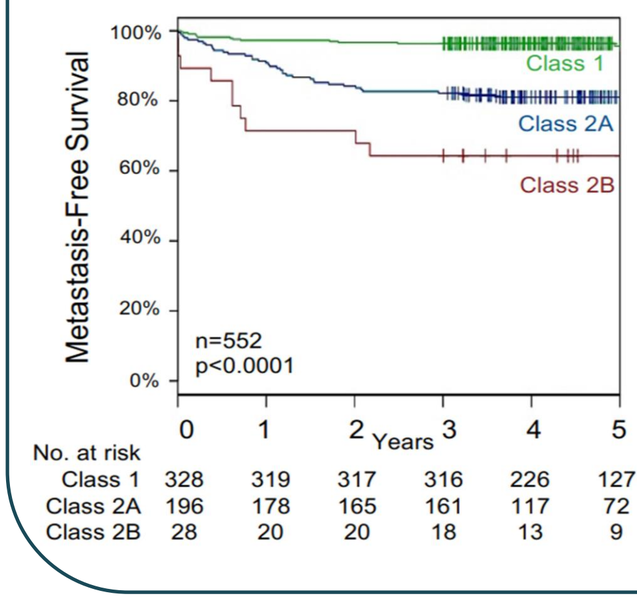
Results

Table 1. Independent Cohort Demographics (n=552)

Risk Factor	All (n=552)	Non-Metastatic (n=492)	Metastatic (n=60)	p-value
Patient Characteristics				
Male sex, n (%)	388 (70.3%)	340 (69.1%)	48 (80%)	0.081
Immunosuppressed, n (%)	121 (21.9%)	102 (20.7%)	19 (31.7%)	0.053
Tumor Characteristics				
Tumor diameter, cm, mean ± SD	1.92 (± 1.59)	1.73 (± 1.30)	3.60 (± 2.60)	<0.001
Tumor thickness, mm, mean ± SD	8.04 (± 9.09)	6.01 (± 5.60)	13.6 (± 13.6)	<0.001
Poorly differentiated, n (%)	45 (8.15%)	28 (5.69%)	17 (28.33%)	<0.001
Perineural invasion, n (%)	51 (9.2%)	28 (5.69%)	23 (38.3%)	<0.001
Lymphovascular invasion, n (%)	16 (2.9%)	7 (1.4%)	9 (15%)	<0.001
Invasion beyond subcutaneous fat, n (%)	60 (10.9%)	38 (7.7%)	22 (36.7%)	<0.001

p-value reported for Person Chi-squared or Wilcoxon F test, as appropriate; SD = standard deviation

Figure 1. Independent Performance of the 40-GEP to Stratify Patients by Risk of Regional or Distant Metastasis from cSCC



40-GEP Risk Class	Overall Cohort 3-year MFS (95% CI)	Overall Event Rate
Class 1	96.3% (94.3-98.4%)	4.0%
Class 2A	82.1% (77.0-87.7%)	18.9%
Class 2B	64.3% (48.8-84.7%)	35.7%
Without 40-GEP	89.7% (87.2-92.2%)	10.9%

Table 2. Independent Performance of the 40-GEP to Identify cSCC Patients with Metastasis

	Univariate		Multivariate*	
Risk Factor	Hazard Ratio	p-value	Hazard Ratio	p-value
40-GEP				
Class 1	1.00	---	1.00	---
Class 2A	5.09	<0.001	3.84	<0.001
Class 2B	11.26	<0.001	5.99	<0.001
BWH T-stage				
T1/T2a	1.00	---	1.00	---
T2b/T3	5.41	<0.001	3.55	<0.001

*n=552, 60 events; BWH = Brigham and Women's Hospital

	Univariate		Multivariate*	
Risk Factor	Hazard Ratio	p-value	Hazard Ratio	p-value
40-GEP				
Class 1	1.00	---	1.00	---
Class 2A	5.09	<0.001	3.61	<0.001
Class 2B	11.26	<0.001	4.23	0.006
Clinicopathologic Factor				
Immunosuppressed	1.73	0.049	1.86	0.044
Diameter ≥2 cm	4.78	<0.001	2.53	0.005
Invasion	6.40	<0.001	2.83	0.002
PNI	4.00	<0.001	0.95	0.906
Poor Differentiation	5.56	<0.001	1.60	0.186

*n=499, 53 events; excluding cases without tumor diameter reported; PNI = perineural invasion; Invasion = beyond the subcutaneous fat or >6mm

Conclusions

- This second independent, multi-center cohort study confirms the performance of the 40-GEP test to classify risk for metastasis in cSCC patients with one or more risk factors.
- The independent contribution of the 40-GEP in risk stratification when compared to high-risk factors or clinical staging systems suggests that incorporating the 40-GEP test results into treatment decisions will guide patient care in a more risk-aligned manner and improve patient outcomes.

References

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Disclosures

- MSG, SJK, JJS, AP, ALF are employees and shareholders of Castle Biosciences, Inc.

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