Performance of the prognostic 40-gene expression profile (40-GEP) test for high-risk cutaneous squamous cell carcinoma (cSCC) in a second independent cohort

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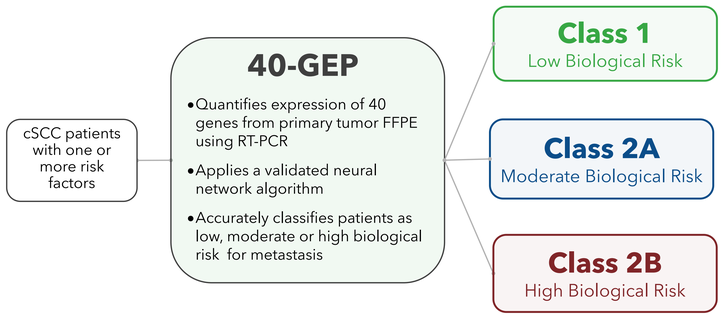
Synopsis

- cSCC is the second most common skin cancer with an estimated incidence of ~1.8 million cases per year. While cSCC has an overall favorable prognosis, mortality from this disease surpasses melanoma, due to this large incidence.[1,2]
- Management of cSCC is driven by patient's likelihood for poor outcomes, meaning decisions on nodal evaluation, adjuvant radiation, and follow-up are based upon an individual patient's estimated risk of recurrence and metastasis.[3]
- The 40-gene expression profile (40-GEP) 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).[4,5]
- When incorporated with current risk prediction methods, the 40-GEP test has been shown to improve risk assessment and provide more precise, individualized metastatic risk stratification.[4,5]
- Clinical utility studies have demonstrated the ability of 40-GEP test results to guide risk-aligned patient management decisions.[5-9]



Objective

- To confirm the performance of the 40-GEP test in a second 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=598). Clinicopathologic and outcomes data were collected from the 43 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 9.9%, 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, poor differentiation, perineural invasion (PNI), lymphovascular invasion (LVI), 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 4.55 and 10.71, respectively (p<0.001) (**Table 2**).
- High-risk clinicopathologic factors, including those used for either Brigham and Women's Hospital (BWH) or AJCCv8 T-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<0.05 for 2A and 2B, data not shown) or BWH T-stage (p<0.001 for 2A and p<0.002 for 2B) or AJCCv8 (p<0.001 for 2A and 2B) (**Table 2**).
- The addition of interaction terms to the multivariate analysis between the 40-GEP test and staging revealed no significant interactions (p>0.05), demonstrating that the 40-GEP test provides independent prognostic information from staging (**Table 2**).

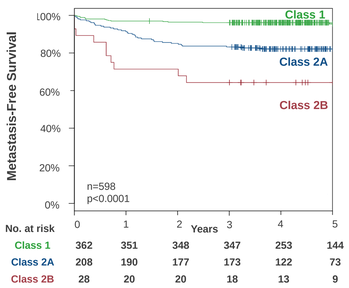
Table 1. Independent Cohort Demographics (n=598)

Risk Factor	All (n=598)	Non-Metastatic (n=536)	Metastatic (n=62)	p-value
Patient Characteristics				
Male sex, n (%)	428 (71.57%)	378 (70.52%)	50 (80.65%)	0.094
Immunosuppressed, n (%)	130 (21.74%)	109 (20.34%)	21 (33.87%)	0.014
Tumor Characteristics				
Tumor diameter*, cm, mean ± SD	1.93 (± 1.56)	1.74 (± 1.26)	3.68 (± 2.64)	<0.001
Tumor thickness*, mm, mean ± SD	7.50 (± 8.04)	5.82 (± 5.47)	12.37(± 11.70)	<0.001
Poorly differentiated, n (%)	93 (15.55%)	61 (11.38%)	32 (51.61%)	<0.001
Perineural invasion, n (%)	54 (9.03%)	30 (5.6%)	24 (38.71%)	<0.001
Lymphovascular invasion, n (%)	16 (2.68%)	7 (1.31%)	9 (14.52%)	<0.001
Invasion beyond subcutaneous fat, n (%)	67 (11.20%)	45 (8.40%)	22 (35.48%)	<0.001
40-GEP Risk Class				
Class 1	362 (60.53%)	347 (95.86%)	15 (4.14%)	
Class 2A	208 (34.78%)	171 (82.21%)	37 (17.79%)	<0.001
Class 2B	28 (4.68%)	18 (64.29%)	10 (35.71%)	

*n= 542 for tumor diameter, n= 113 for tumor thickness

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

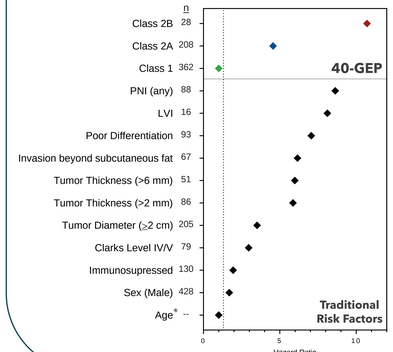
Figure 1. Performance of the 40-GEP to Stratify Patients by Risk of Regional or Distant Metastasis from cSCC



40-GEP Risk Class	Overall Cohort	
	3- year MFS (95% CI)	Overall Event Rate
Class 1	96.1% (94.2-98.1%)	3.9%
Class 2A	83.2% (78.2-88.4%)	16.8%
Class 2B	64.3% (48.8-84.7%)	35.7%
Without 40-GEP	90.1% (87.8-92.6%)	9.9%

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

Metastatic risk associated with 40-GEP Class and individual clinicopathologic factors in univariate analysis



*All variables, except age, were statistically significantly p<0.05

		Univariate		Multivariate†	
Risk Factor		HR	p-value	HR	p-value
40-GEP					
Class 1	(n=362)	1.00	---	1.00	---
Class 2A	(n=208)	4.55	<0.001	2.69	0.032
Class 2B	(n=28)	10.71	<0.001	6.85	0.014
AJCCv8 T-stage					
T1/T2	(n=458)	1.00	---	1.00	---
T3/T4	(n=140)	7.41	<0.001	4.78	0.003
Interaction:					
Class 2A: T3/T4		---	---	1.16	0.822
Class 2B: T3/T4		---	---	0.711	0.720
40-GEP					
Class 1	(n=362)	1.00	---	1.00	---
Class 2A	(n=208)	4.55	<0.001	3.40	0.003
Class 2B	(n=28)	10.71	<0.001	6.63	0.015
BWH T-stage					
T1/T2a	(n=503)	1.00	---	1.00	---
T2b/T3	(n=95)	8.53	<0.001	8.73	<0.001
Interaction:					
Class 2A: T2b/T3		---	---	0.57	0.378
Class 2B: T2b/T3		---	---	0.39	0.329

*n=598, 62 events; BWH = Brigham and Women's Hospital; AJCCv8 = American Joint Committee on Cancer version 8, HR = Hazard Ratio †interaction terms to the multivariate analysis between the 40-GEP test and BWH and AJCCv8 revealed no significant interactions (p>0.05)

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 bringing the total number of patients to 1,018.
- As in initial validation, the 40-GEP has demonstrated independent prognostic value for metastasis beyond either BWH and AJCCv8 staging or individual high-risk clinicopathologic factors, including, PNI, tumor size, poor differentiation, and invasion beyond the subcutaneous fat.
- This performance study complements the clinical utility studies showing incorporation of the 40-GEP test to guide treatment plan decisions.

References

1. Rogers et al JAMA Derm. 2015
2. skincancer.org, Skin Cancer Foundation
3. NCCN Guidelines®, 2021
4. Wysong, et al. J Am Acad Dermatol. 2021
5. Ibrahim, et al. Future Oncol. 2021
6. Teplitz, et al. J Drugs Dermatol. 2019
7. Litchman, et al. CMRO. 2020
8. Arron, et al. J Drugs Dermatol. 2022
9. Farberg, et al. presented WCD 2022

Disclosures

- JJS, SJK, JJS, ALF, AP, MSG are employees and shareholders of Castle Biosciences, Inc.
- AW is a member of the ACMS board of directors and Scientific Program Chair of the ACMS 2022 Annual Meeting

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