The 40-gene expression profile (40-GEP) test identifies cutaneous squamous cell carcinoma (cSCC) patients at high risk of metastasis within lower-staged tumors to better guide treatment decisions

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Background

- Available tumor staging systems for cSCC include the American Joint Committee on Cancer, 8th Edition (AJCC8) and Brigham and Women's Hospital (BWH) staging. Each system utilizes different risk factors to determine the T-stage[3,4] and have limited and variable accuracy for determining metastasis risk.[4,5]
- The 40-GEP test is validated to independently stratify for regional and/or distant metastasis for cSCC patients with one or more risk factors into three risk categories: Class 1 (low), Class 2A (higher) and Class 2B (highest).[5,6]

Table 1. Variability in risk factor assessment for cSCC impacts staging and therefore treatment decisions

Clinicopathologic risk factor	40-GEP testing criteria	Factors used for risk assessment		
		NCCN (v1.2023)	AJCC8	BWH
Tumor size ≥2 cm	✓	✓	✓	✓
Invasion beyond subcutaneous fat or >6mm‡	✓	✓	✓	✓
Perineural invasion#	✓	✓	✓	✓
Poorly differentiated	✓	✓		✓
Recurrent†		✓		
Immunosuppression	✓	✓		
Site of prior RT or chronic inflammation	✓	✓		
Located on head, neck, anogenital, hands, and feet, any size	✓	✓		
Borders poorly defined	✓	✓		
Rapidly growing tumor	✓	✓		
Neurological symptoms	✓	✓		
Lymphatic or vascular involvement	✓	✓		
Desmoplastic SCC	✓	✓		
Specific high-risk subtypes##	✓	✓		

Blue shaded area connotates T1 Stage Tumors

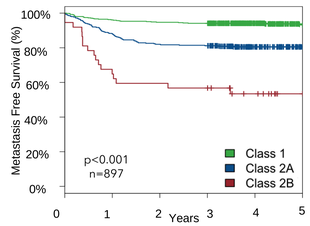
NCCN- National Comprehensive Cancer Network guidelines (high or very-high risk factors); ‡AJCC8 and NCCN- >6mm and bone erosion/invasion included, BWH- bone invasion automatically upstages to highest risk stage- T3; #AJCC8 and NCCN- ≥0.1mm nerve or deeper than dermis, BWH- ≥0.1mm nerve required; †40-GEP is not validated for local recurrence; ##Acantholytic, adenosquamous, or metaplastic subtypes (40-GEP- others will be considered on a case-by-case basis)

Methods

- In an IRB-approved, retrospective, multi-center study, primary tumor tissue and associated clinical data from patients with cSCC and one or more clinicopathologic risk factors (n=897) were collected. Within this overall cohort, lower BWH and AJCC8 T-staged samples were evaluated by Kaplan-Meier survival analysis to determine metastasis-free survival according to 40-GEP risk class.

Results

Figure 1. 40-GEP accurately stratifies metastatic risk (n=897)



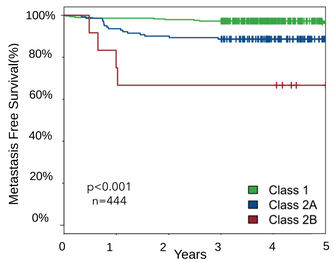
40-GEP Risk Class	Overall Cohort: 3- year MFS (95% CI)	Overall Event Rate
Class 1	94.1% (92.1-96.2%)	6.5%
Class 2A	81.1% (77.1-85.3%)	19.4%
Class 2B	56.8% (42.8-72.2%)	45.9%
Overall	87.5% (85.4-89.7%)	13.2%

› Kaplan-Meier survival analysis of the overall cohort (n=897) demonstrated statistically significant 3-year metastasis-free survival between all classes.

Clinical Issue and Objective

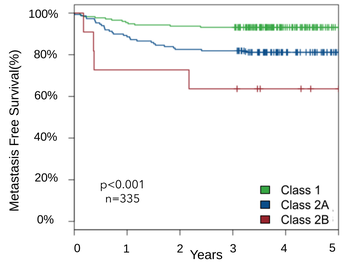
Lower-stage tumors are those that lack risk factors that are considered at higher risk for disease progression but can be characterized by other high-risk factors that are clinically concerning **(Table 1)**. Improved risk assessment in these lower staged subsets is important because up to one-third of all metastatic events have been reported for patients originally staged as T1.[1,2] The current study investigated whether the 40-GEP test could independently identify lower-staged patients at increased risk of metastasis.

Figure 2. 40-GEP stratifies metastatic risk in lower T-staged BWH and AJCC8 cohorts



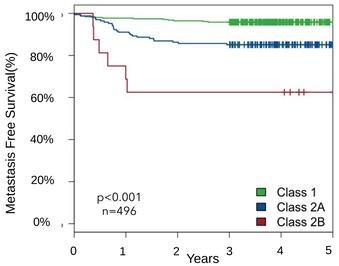
BWH T1

40-GEP risk Class	3- year MFS (95% CI)	Event rate
Class 1	97.3% (95.4-99.1%)	3.1%
Class 2A	88.7% (83.6-94.0%)	11.3%
Class 2B	66.7% (44.7-99.5%)	**33.3%**
Overall	93.7% (91.5-96.0%)	6.5%



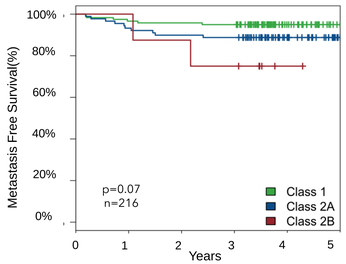
BWH T2a

40-GEP risk Class	3- year MFS (95% CI)	Event rate
Class 1	93.1% (89.5-97.0%)	7.4%
Class 2A	81.9% (75.9-88.3%)	18.8%
Class 2B	63.6% (40.7-99.5%)	**36.4%**
Overall	87.2% (83.7-90.8%)	13.4%



AJCC8 T1

40-GEP Risk Class	3- year MFS (95% CI)	Event Rate
Class 1	95.8% (93.6-98.1%)	4.2%
Class 2A	85.1% (79.9-90.7%)	14.9%
Class 2B	62.5% (42.8-91.4%)	**37.5%**
Overall	91.1% (88.7-93.7%)	8.9%



AJCC8 T2

40-GEP risk Class	3- year MFS (95% CI)	Event rate
Class 1	95.0% (91.1-99.0%)	5.0%
Class 2A	88.8% (82.4-95.6%)	11.2%
Class 2B	75.0% (50.3-100%)	**25.0%**
Overall	91.7% (88.1-95.4%)	8.3%

› Kaplan-Meier survival analysis of the BWH T1, T2a and AJCC8 T1 cohorts demonstrated statistically significant 3-year metastasis-free survival between all classes. AJCC8 T2 cohort was underpowered for statistical significance but shows a similar trend to the other subsets.

Table 2. Demographics and clinical characteristics of the overall cohort (n=897) based on lower T-staged cohorts

Risk Factor	Combined (n=897)	BWH		AJCC8	
		T1 (n=444)	T2a (n=335)	T1 (n=496)	T2 (n=216)
Metastatic events	118 (13.15%)	29 (6.53%)	45 (13.43%)	44 (8.9%)	18 (8.3%)
40-GEP Distribution					
Class 1	510 (56.9%)	291 (65.5%)	175 (52.2%)	312 (62.9%)	119 (55.1%)
Class 2A	350 (39.0%)	141 (31.8%)	149 (44.5%)	168 (33.9%)	89 (41.2%)
Class 2B	37 (4.1%)	12 (2.7%)	11 (3.3%)	16 (3.2%)	8 (3.7%)
Patient Characteristics					
Age, y, median (range)	72 (26-95)	71 (26-95)	73 (44-95)	70 (26-95)	76 (44-95)
Male sex, n (%)	653 (72.8%)	322 (72.5%)	244 (72.8%)	364 (73.4%)	154 (71.3%)
Immunosuppression, n (%)	230 (25.6%)	146 (32.9%)	63 (18.81%)	164 (33.1%)	30 (13.9%)
Tumor Characteristics					
Head and Neck, n (%)	577 (64.3%)	293 (66.0%)	192 (57.3%)	336 (67.7%)	105 (48.6%)
Tumor diameter*, cm, mean ± SD	1.91 (±1.63)	0.99 (±0.45)	2.45 (±1.39)	1.01 (± 0.45)	2.48 (± 0.50)
Tumor thickness**, mm, mean ± SD	5.26 (±6.63)	1.90 (±1.66)	7.37 (±6.81)	1.78 (± 1.39)	3.23 (± 2.06)
Poorly differentiated, n (%)	130 (14.5%)	0 (0%)	58 (17.3%)	56 (11.3%)	25 (11.6%)
Perineural invasion§, n (%)	46 (5.1%)	8 (1.8%)	13 (3.9%)	11 (2.2%)	7 (3.2%)
Lymphovascular invasion, n (%)	14 (1.6%)	3 (0.68%)	1 (0.3%)	2 (0.4%)	2 (0.9%)
Invasion beyond subcutaneous fat, n (%)	81 (9.03%)	0 (0%)	34 (10.15%)	0 (0.0%)	0 (0.0%)
Surgery Type					
Mohs	601 (67.0%)	312 (70.3%)	236 (70.5%)	352 (71.0%)	166 (76.9%)

› SD=standard deviation; *=(n=820); **=(n=204); §=presence of perineural invasion

Conclusions

- Within a cohort of cSCC patients considered lower risk by current staging alone, the 40-GEP identified those who experienced a substantial increase in metastatic risk.
- These results represent a clinically significant improvement in risk assessment for cSCC patients with observed rates of metastasis over 10% and 20% which are clinically actionable for nodal staging or post-operative adjuvant radiation.
- Combining clinicopathologic risk assessment with individual biologic risk, as provided by the 40-GEP test, improves the accuracy of risk assessment used clinically as the basis of treatment decisions.

References

1. Eggermont, JEADV 2023 2. Farberg, Derm Ther 2022 3. Amin, Cancer J Clin. 2017 4. Karia, JCO 2014 5. Wysong, JAAD 2021 6. Ibrahim, Future Onc 2021

Disclosures

This study was sponsored by Castle Biosciences, Inc. (CBI), which provided funding to the contributing centers for tissue and clinical data retrieval. AKS is a speaker for CBI. JS, AP, AF and MG are employees and options holders of CBI. ASF is a consultant for CBI. SA is a paid consultant for CBI.