

Integrating the 40-gene expression profile (40-GEP) test improves metastatic risk-stratification within clinically relevant subgroups of cutaneous squamous cell carcinoma (cSCC) patients

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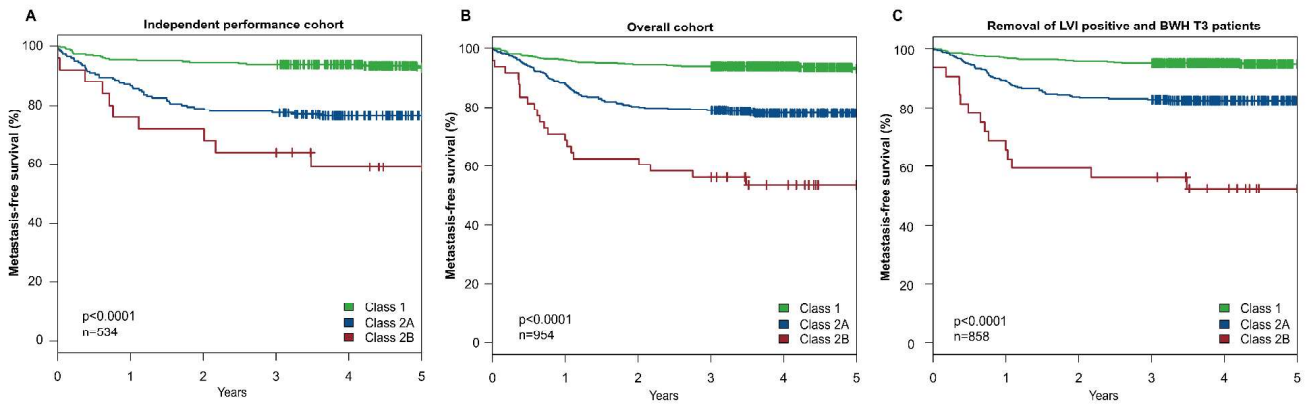
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Risk (n)

Class 1	309	295	292	290	223	134
Class 2A	200	173	158	155	115	69
Class 2B	25	19	18	16	12	9

	521	500	492	489	296	171
	305	337	309	304	190	100
	48	33	30	27	19	12

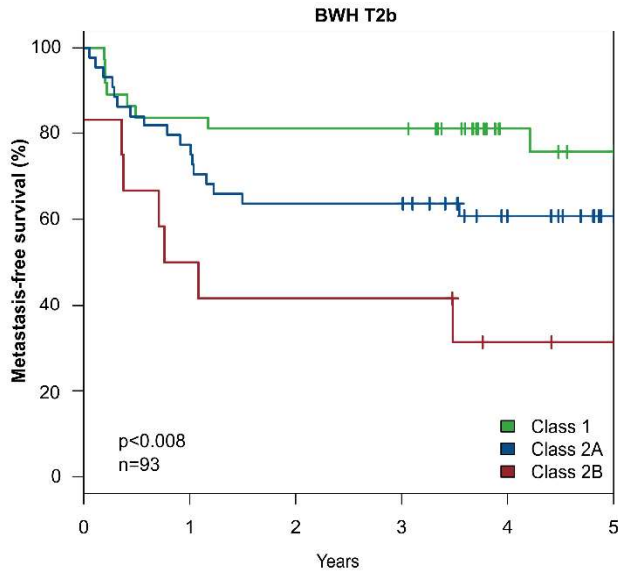
	496	481	475	472	282	163
	330	294	276	273	177	94
	32	21	19	18	12	6

Independent performance cohort		
40-GEP risk Class	3- year MFS (95% CI)	Event rate
Class 1	93.9% (91.2-96.6%)	6.8%
Class 2A	77.5% (71.9-83.5%)	23.5%
Class 2B	64.0% (47.7-85.9%)	40.0%
Overall	86.3% (83.5 -89.3%)	14.6%

Overall cohort		
40-GEP risk Class	3- year MFS (95% CI)	Event rate
Class 1	93.9% (91.8-95.9%)	6.7%
Class 2A	79.0% (75.0-83.1%)	21.8%
Class 2B	56.2% (43.8-72.2%)	45.8%
Overall	86.0% (83.8-88.2%)	14.8%

Removal of LVI positive and BWH T3 patients		
40-GEP risk Class	3- year MFS (95% CI)	Event rate
Class 1	95.2% (93.3-97.1%)	5.2%
Class 2A	82.7% (78.7-86.9%)	17.5%
Class 2B	56.2% (41.4-76.4%)	46.8%
Overall	88.9% (86.9-91.1%)	11.5%

SUPPLEMENTAL FIGURE 1. Kaplan-Meier survival analysis demonstrated statistically significant 3-year metastasis-free survival (MFS) between all 40-GEP classes for: A) the independent performance cohort (n=534); B) the overall cohort (n=954); C) removal of LVI positive and BWH T3 patients from the overall cohort (n=893); See Figure 1 for cohort specifics, and Figure 2 for table descriptions. P-values indicate log-rank test. LVI= lymphovascular invasion



Risk (n)						
Class 1	37	31	30	30	15	12
Class 2A	44	34	28	28	17	9
Class 2B	12	6	5	5	2	1

BWH T2b		
40-GEP risk Class	3- year MFS (95% CI)	Event rate
Class 1	81.1% (69.4-94.7%)	21.6%
Class 2A	63.6% (50.9-79.6%)	38.6%
Class 2B	41.7% (21.3-81.4%)	66.7%
Overall	67.7% (58.9-77.9%)	35.5%

SUPPLEMENTAL FIGURE 2. Kaplan-Meier survival analysis of Brigham and Women’s Hospital (BWH) higher risk stage, T2b, demonstrated statistically significant 3-year metastasis-free survival (MFS) between all 40-GEP classes. Tables as described in Figure 2; p-value indicates log-rank tests.

SUPPLEMENTAL TABLE 1. Variability in risk factor inclusion and classifications for cSCC

Clinicopathologic risk factor	40-GEP testing criteria	Risk classifications systems		
		NCCN (v1.2024)	AJCC8	BWH
Tumor size ≥ 2 cm	✓	✓	✓	✓
Invasion beyond subcutaneous fat or $>6\text{mm}^{\ddagger}$	✓	✓	✓	✓
Perineural invasion [#]	✓	✓	✓	✓
Poorly differentiated	✓	✓	-	✓
Recurrent [†]	-	✓	-	-
Immunosuppression	✓	✓	-	-
Site of prior RT or chronic inflammation	✓	✓	-	-
Located on head, neck, genital, hands, and feet or pretibial surface	✓	✓	-	-
Borders poorly defined	✓	✓	-	-
Rapidly growing tumor	✓	✓	-	-
Neurological symptoms	✓	✓	-	-
Lymphatic or vascular involvement	✓	✓	-	-
Desmoplastic SCC	✓	✓	-	-
Specific high-risk subtypes ^{##}	✓	✓	-	-

NCCN= National Comprehensive Cancer Network v1.2024 (high- or very high-risk factors); AJCC8= American Joint Committee on Cancer version 8; BWH= Brigham and Women's Hospital ‡AJCC8 and NCCN- $>6\text{mm}$ and bone erosion/invasion included, BWH- bone invasion automatically upstages to highest risk stage- T3; #AJCC8 and NCCN- $\geq 0.1\text{mm}$ nerve or deeper than dermis, BWH- $\geq 0.1\text{mm}$ 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)

SUPPLEMENTAL TABLE 2. Demographics and clinical characteristics of the combined cohort (n=897) based on BWH staging

Risk Factor	Combined (n=897)	T1 (n=444)	T2a (n=335)	T2b (n=93)	T3 (n=25)
Metastatic events	118 (13.15%)	29 (24.6%)	45 (38.1%)	33 (28.0%)	11 (9.3%)
40-GEP Distribution					
Class 1	510 (56.9%)	291 (57.1%)	175 (34.3%)	37 (7.3%)	7 (1.4%)
Class 2A	350 (39.0%)	141 (40.3%)	149 (42.6%)	44 (12.6%)	16 (4.6%)
Class 2B	37 (4.1%)	12 (32.4%)	11 (29.7%)	12 (32.4%)	2 (5.4%)
Patient Characteristics					
Age, y, median (range)	72 (26-90+)	71 (26-90+)	73 (44-90+)	71 (38-90)	74 (43-90)
Male sex, n (%)	653 (72.8%)	322 (49.3%)	244 (37.4%)	67 (10.3%)	20 (3.1%)
Immunosuppression, n (%)	230 (25.6%)	146 (63.5%)	63 (27.4%)	17 (7.4%)	4 (1.7%)
Tumor Characteristics					
Head and Neck, n (%)	577 (64.3%)	293 (50.8%)	192 (33.3%)	73 (12.7%)	19 (3.3%)
Tumor diameter, cm, mean ± SD	1.907 (±1.63)	0.989 (±0.45)	2.445 (±1.39)	3.293 (±2.50)	4.722 (±2.82)
Tumor thickness, mm, mean ± SD	5.259 (±6.63)	1.899 (±1.66)	7.373 (±6.81)	10.044 (±7.22)	10.68 (±14.23)
Poorly differentiated, n (%)	130 (14.5%)	0 (0%)	58 (44.6%)	58 (44.6%)	14 (10.8%)
Perineural invasion [§] , n (%)	46 (5.1%)	8 (17.4%)	13 (28.3%)	18 (39.1%)	7 (15.2%)
Lymphovascular invasion, n (%)	14 (1.6%)	3 (21.4%)	1 (7.1%)	7 (50.0%)	3 (21.4%)
Invasion beyond subcutaneous fat, n (%)	81 (9.03%)	0 (0%)	34 (42.0%)	38 (46.9%)	9 (11.1%)
Surgery Type					
Mohs [‡]	601 (67.0%)	312 (51.9%)	236 (39.3%)	48 (8.0%)	5 (0.8%)

p-value reported for Person Chi-squared or Wilcoxon F test, as appropriate; SD = standard deviation; § presence of PNI; ‡ other surgery types: biopsy, electrocauterization, and wide local excision. BWH= Brigham and Women's Hospital