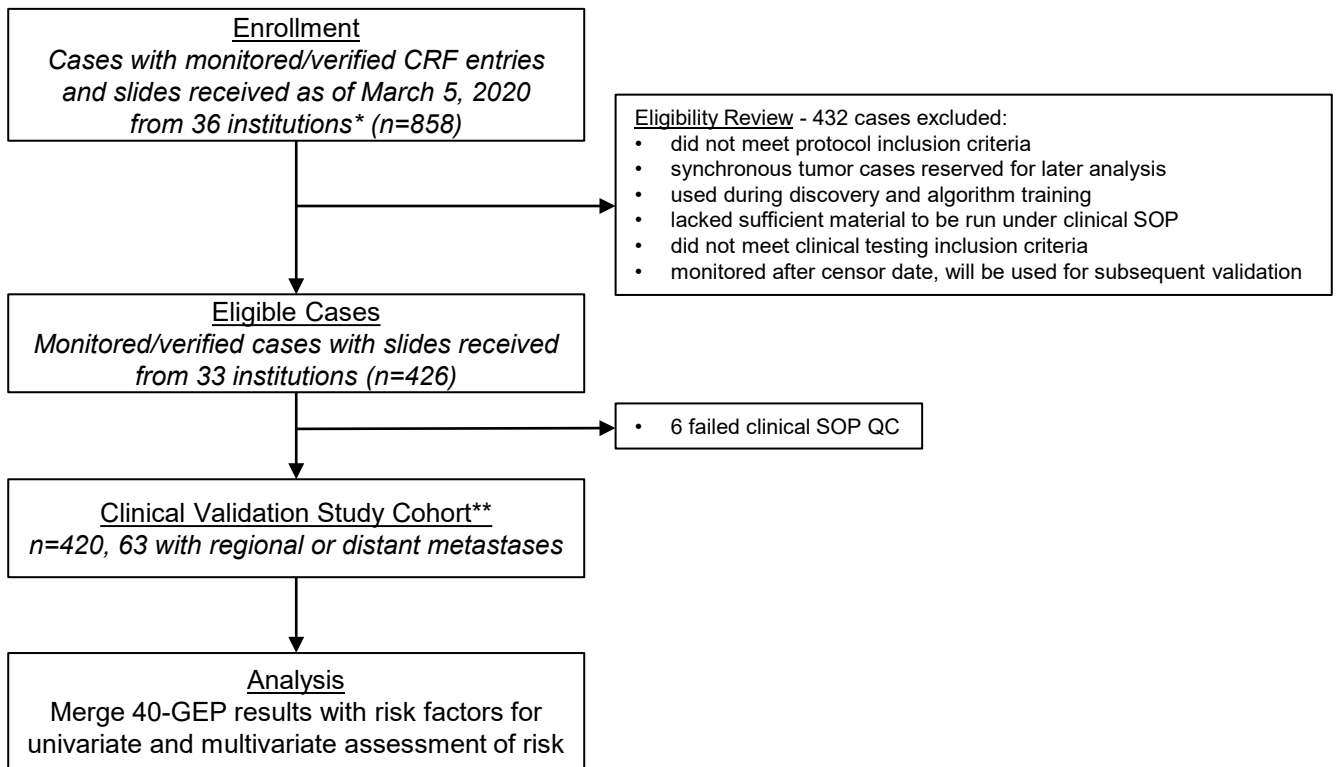


**Enhanced Metastatic Risk Assessment in Cutaneous Squamous Cell Carcinoma with
the 40-Gene Expression Profile Test**

SUPPLEMENTAL FIGURES AND TABLES

(Suppl Fig: 2, Suppl Tables: 3)

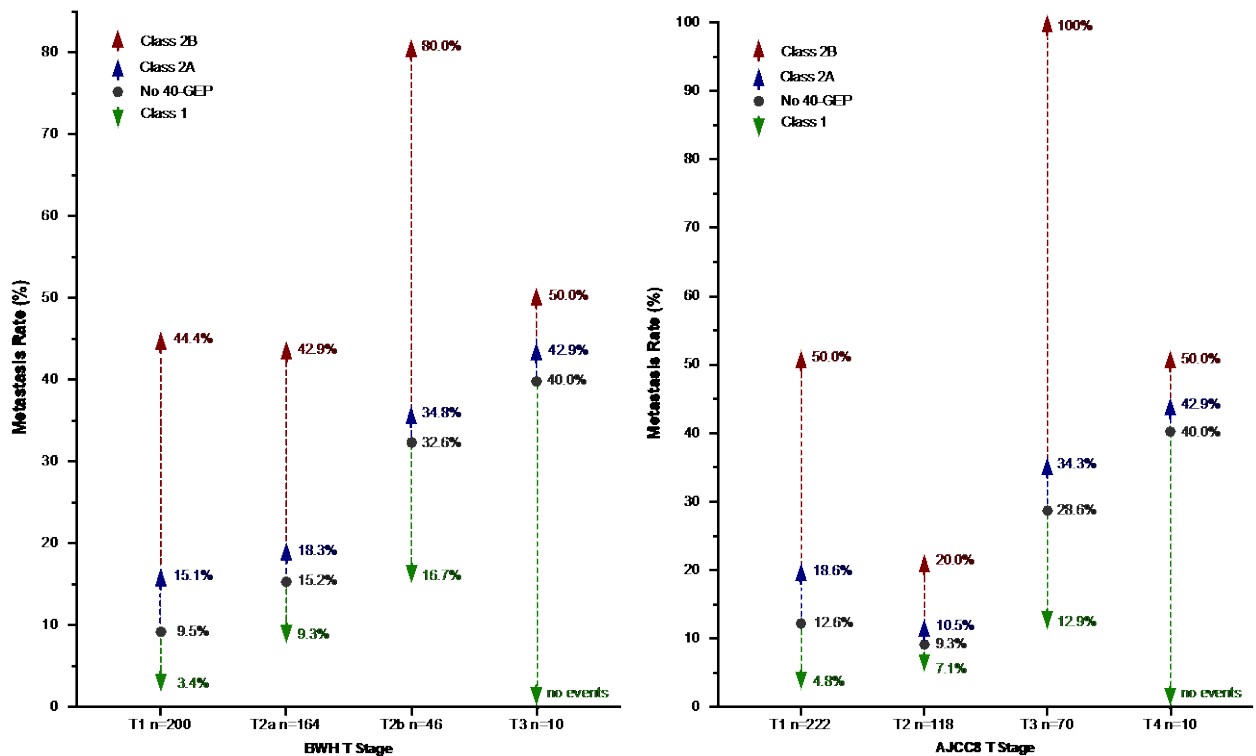


Supplemental Figure 1. Study design for the clinical validation cohort (n=420). *Archival sample collection ongoing. **Comprised of 307 previously-assessed (but not under clinical SOP, Wysong *et al.*, *JAAD* 2021)²¹ and 113 novel samples. CRF: case report form; SOP: standard operating procedure; QC: quality control; 40-GEP: 40-gene expression profile; cSCC: cutaneous squamous cell carcinoma.

Supplemental Table 1. Risk factors captured and used for factor count by case.

<i>Risk Factors</i>	<i>Factor Count</i>
Tumor size and location* <ul style="list-style-type: none"> Any size on the head, neck, genitalia, hands, feet or pretibial surface (Areas H or M), or ≥2 cm size on any other area of the body (Area L) 	1
Immunosuppression**	1
Perineural invasion: Large (≥0.1 mm), named nerve involvement, <0.1 mm in caliber, or unknown caliber	1
Depth (any one or combination of) <ul style="list-style-type: none"> Invasion beyond subcutaneous fat Depth ≥2 mm Clark level ≥IV 	1
Poorly differentiated tumor histology	1
Aggressive histologic subtypes***	1
Lymphovascular invasion	1
TOTAL POSSIBLE RISK FACTOR COUNT#	7

*Size and location criteria as defined in NCCN guidelines and more inclusive Mohs appropriate use criteria: Area H, 'mask areas' of face (central face, eyelids, eyebrows, periorbital, nose, lips [cutaneous and vermillion], chin, mandible, preauricular and postauricular skin/sulci, temple, and ear), genitalia, hands, and feet; Area M, cheeks, forehead, scalp, neck, and pretibia; and Area L, trunk and extremities (excluding hands, nail units, pretibial, ankles, and feet). **Types of immunosuppression included per protocol were from organ transplant, leukemia, lymphoma, HIV. ***Any of: acantholytic, adenosquamous, pagetoid spread, desmoplastic, sclerosing, basosquamous, small cell, spindle cell/sarcomatoid, infiltrating, clear cell, lymphoepithelial, single cell spread, or metaplastic/carcinosarcomatous. #Note, tumors with poorly defined borders, that were rapidly growing, with neurologic symptoms in tumor region, and/or at a site of prior radiation therapy or chronic inflammatory process were not captured by this current study; however, they will be allowed for clinical testing as defined as high risk per NCCN.



Supplemental Figure 2. Further stratification of metastatic risk by the 40-GEP test per BWH or AJCC8 T stage for the cSCC Validation Cohort (n=420). 40-GEP, 40-gene expression profile; BWH, Brigham and Women's Hospital; AJCC8, American Joint Committee on Cancer, Cancer Staging Manual, Eighth Edition; cSCC, cutaneous squamous cell carcinoma.

Supplemental Table 2. Metastasis rates by 40-GEP and BWH T stage or AJCC8 T stage.

40-GEP Result	T1/T2a BWH T stage		T2b/T3 BWH T stage	
	No. Cases	No. Mets (rate)	No. Cases	No. Mets (rate)
Class 1	193	11 (5.7%)	19	3 (15.8%)
	<i>T1: 118</i>	<i>4 (3.4%)</i>	<i>T2b: 18</i>	<i>3 (16.7%)</i>
	<i>T2a: 75</i>	<i>7 (9.3%)</i>	<i>T3: 1</i>	<i>0 (0%)</i>
Class 2A	155	26 (16.8%)	30	11 (36.7%)
	<i>T1: 73</i>	<i>11 (15.1%)</i>	<i>T2b: 23</i>	<i>8 (34.8%)</i>
	<i>T2a: 82</i>	<i>15 (18.3%)</i>	<i>T3: 7</i>	<i>3 (42.9%)</i>
Class 2B	16	7 (43.8%)	7	5 (71.4%)
	<i>T1: 9</i>	<i>4 (44.4%)</i>	<i>T2b: 5</i>	<i>4 (80.0%)</i>
	<i>T2a: 7</i>	<i>3 (42.9%)</i>	<i>T3: 2</i>	<i>1 (50.0%)</i>
Pre-Test	364	44 (12.1%)	56	19 (33.9%)
	<i>T1: 200</i>	<i>19 (9.5%)</i>	<i>T2b: 46</i>	<i>15 (32.6%)</i>
	<i>T2a: 164</i>	<i>25 (15.2%)</i>	<i>T3: 10</i>	<i>4 (40.0%)</i>
40-GEP Result	T1/T2 AJCC8 T stage		T3/T4 AJCC8 T stage	
	No. Cases	No. Mets (rate)	No. Cases	No. Mets (rate)
Class 1	180	10 (5.6%)	32	4 (12.5%)
	<i>T1: 124</i>	<i>6 (4.8%)</i>	<i>T3: 31</i>	<i>4 (12.9%)</i>
	<i>T2: 56</i>	<i>4 (7.1%)</i>	<i>T4: 1</i>	<i>0 (0%)</i>
Class 2A	143	22 (15.4%)	42	15 (35.7%)
	<i>T1: 86</i>	<i>16 (18.6%)</i>	<i>T3: 35</i>	<i>12 (34.3%)</i>
	<i>T2: 57</i>	<i>6 (10.5%)</i>	<i>T4: 7</i>	<i>3 (42.9%)</i>
Class 2B	17	7 (41.2%)	6	5 (83.3%)
	<i>T1: 12</i>	<i>6 (50.0%)</i>	<i>T3: 4</i>	<i>4 (100.0%)</i>
	<i>T2: 5</i>	<i>1 (20.0%)</i>	<i>T4: 2</i>	<i>1 (50.0%)</i>
Pre-Test	340	39 (11.5%)	80	24 (30.0%)
	<i>T1: 222</i>	<i>28 (12.6%)</i>	<i>T3: 70</i>	<i>20 (28.6%)</i>
	<i>T2: 118</i>	<i>11 (9.3%)</i>	<i>T4: 10</i>	<i>4 (40.0%)</i>

Abbreviations: GEP, gene expression profile; BWH, Brigham and Women's Hospital; AJCC8, American Joint Committee on Cancer (AJCC) Cancer Staging Manual, 8th Edition.

Supplemental Table 3. Univariate and multivariate Cox regression analyses of risk for metastasis per 40-GEP Class and individual BWH and AJCC8 T stages in cSCC validation cases.

Risk Factor	n	Univariate Cox Regression		Multivariate Cox Regression*	
		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	2.87 (1.54-5.35)	<0.001
Class 2B	23	11.61 (5.36-25.15)	<0.001	9.86 (4.44-21.9)	<0.001
BWH T Stage					
T1	200	1.00 (---)	---	1.00 (---)	---
T2a	164	1.64 (0.90-2.97)	0.106	1.48 (0.81-2.71)	0.199
T2b	46	3.97 (2.02-7.82)	<0.001	3.21 (1.62-6.35)	<0.001
T3	10	4.68 (1.59-13.77)	0.005	2.14 (0.70-6.50)	0.181
40-GEP Result					
Class 1	212	1.00 (---)	---	1.00 (---)	---
Class 2A	185	3.22 (1.74-5.95)	<0.001	3.10 (1.67-5.75)	<0.001
Class 2B	23	11.61 (5.36-25.15)	<0.001	13.27 (5.90-29.84)	<0.001
AJCC8 T Stage					
T1	222	1.00 (---)	---	1.00 (---)	---
T2	118	0.72 (0.36-1.44)	0.347	0.65 (0.32-1.30)	0.222
T3	70	2.47 (1.39-4.39)	0.002	2.63 (1.46-4.74)	0.001
T4	10	3.46 (1.21-9.88)	0.020	1.48 (0.50-4.38)	0.474

Abbreviations: 40-GEP, 40-gene expression profile; BWH, Brigham and Women's Hospital; AJCC8, American Joint Committee on Cancer (AJCC) Cancer Staging Manual, 8th Edition; cSCC, cutaneous squamous cell carcinoma. *n=393, 54 events, excluding cases without tumor diameter reported. **Perineural invasion was considered positive regardless of nerve caliber. #Deep invasion: beyond the subcutaneous fat, depth >6mm or Clark level V. ##Tumor diameter: continuous variable per cm.