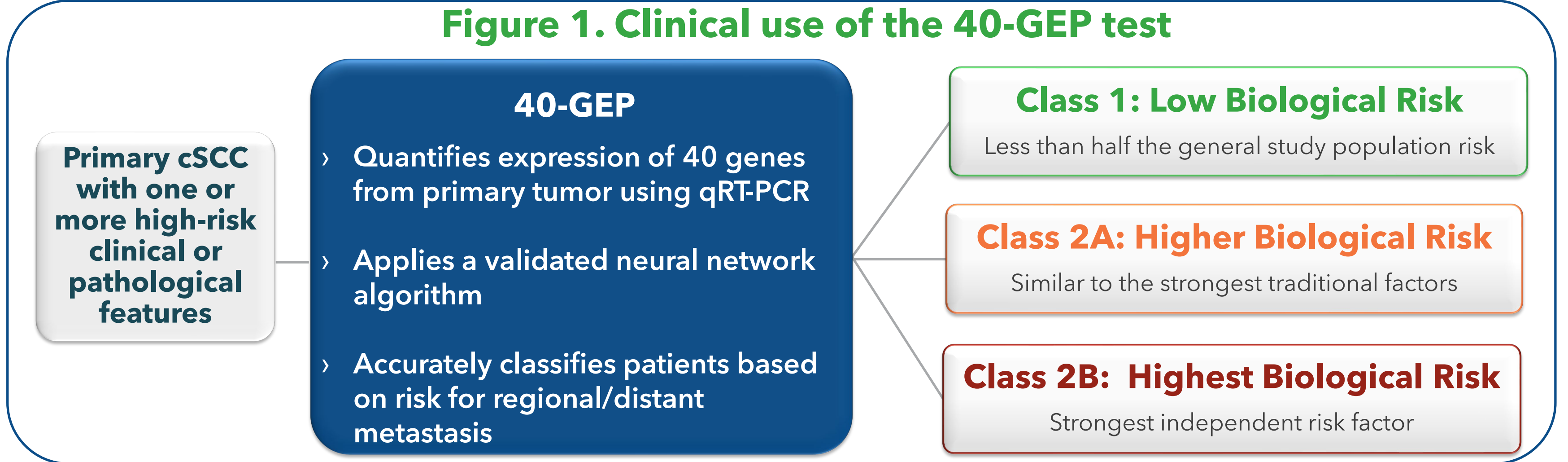# Addition of the 40-gene expression profile (40-GEP) test improves prognostic accuracy and risk stratification for high-risk cutaneous squamous cell carcinoma (HR-cSCC) of the head and neck treated with Mohs micrographic surgery (MMS)

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Introduction

› Mohs micrographic surgery (**MMS**) is the current standard-of-care for high-risk cutaneous squamous cell carcinoma (**HR-cSCC**), as it provides excellent cure rates. Despite meticulous margin control with MMS, 5-8% of patients treated with MMS will still develop metastasis.[1-3]

› Multiple guidelines, including the National Comprehensive Cancer Network (**NCCN**),[4] American Academy of Dermatology (**AAD**),[5] and the European Association of Dermato-Oncology (**EADO**)[6] recommend consideration of surveillance imaging as well as adjuvant radiation therapy (**ART**) for HR-cSCC with negative margins. However, available staging systems and prognostication tools are not particularly robust to guide such treatments.[7]

› The prognostic 40-gene expression profile (**40-GEP**) test stratifies patients with primary HR-cSCC and one or more clinicopathologic risk factors into three groups based on biological risk for regional and/or distant metastasis (Class 1, low risk; Class 2A, higher risk; Class 2B, highest risk). (**Figure 1**).[8-10] When the 40-GEP test is incorporated with staging, it offers a more accurate and personalized stratification of metastatic risk.[10,11] The 40-GEP also directs risk-aligned changes in clinical management,[12] including accurate identification of patients who are most likely to benefit from ART and those who may defer ART,[13,14] which has been shown could significantly reduce healthcare costs for Medicare-eligible patients.[15]

Figure 1. Clinical use of the 40-GEP test

Primary cSCC with one or more high-risk clinical or pathological features

40-GEP
› Quantifies expression of 40 genes from primary tumor using qRT-PCR
› Applies a validated neural network algorithm
› Accurately classifies patients based on risk for regional/distant metastasis

Class 1: Low Biological Risk
Less than half the general study population risk

Class 2A: Higher Biological Risk
Similar to the strongest traditional factors

Class 2B: Highest Biological Risk
Strongest independent risk factor

Study Type, Setting, & Methods

› Under an IRB-approved, multi-institutional (n=46), retrospective study, primary HR-cSCC tumors with one or more risk factors were acquired. The current analysis included only patients with head and neck (**H&N**) tumors treated with MMS (obtaining clear margins) and having met both Mohs Appropriate Use Criteria and clinical usage requirements for the 40-GEP test, performed in a CAP-accredited, CLIA-certified laboratory. Patients with post-operative radiation therapy (**RT**) were excluded. Clinicopathologic risk factors were comprehensively assessed, including a review of original biopsy reports, definitive surgical reports, and independent review by a board-certified dermatopathologist. Other high-risk features identified included ≥2cm tumor diameter, poorly defined tumor borders, immunosuppression, rapidly growing tumor, site of prior RT, chronic inflammation, high-risk subtype, ≥Clark Level IV, >2mm invasion, poorly differentiated, lymphovascular invasion (**LVI**), perineural invasion (**PNI**), invasion beyond subcutaneous fat. Metastasis-free survival (**MFS**) was estimated by Kaplan-Meier analysis with log-rank test and Cox regression analysis. Cox regression models were used to determine whether adding 40-GEP results to staging (NCCN,[4] AJCC8,[16] or Brigham & Women's Hospital[17] [**BWH**]) enhanced risk prediction.

References

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Objectives

› Evaluate the 40-GEP test's ability to stratify metastatic risk for HR-cSCC on the H&N with clear margins after MMS.

› Assess the ability of the 40-GEP test to significantly improve metastatic risk prediction of NCCN, AJCC8, and BWH staging systems when included.

Results

Table 1. Cohort demographics: n=417 patients received definitive Mohs surgery for HR-cSCC on the H&N

Risk Factor	All patients n=417	No events n=366	Regional/distant metastasis n=51	p-value*
Patient Characteristics				
Age, years, median (range)	**72 (32-90+)**	72 (34-90+)	72 (32-90+)	ns
Male sex at birth, n (%)	**346 (83.0%)**	300 (82.0%)	46 (90.2%)	ns
Immunosuppression, n (%)	**112 (26.9%)**	90 (24.6%)	22 (43.1%)	<0.01
Follow-up**, years, median (range)	**4.2 (0.6-14.8)**	4.3 (3.0-14.8)	3.0 (0.58-9.5)	<0.001
Tumor Characteristics				
Tumor diameter*** >2 cm, n (%)	**117 (28.1%)**	95 (26.0%)	22 (43.1%)	<0.05
Poorly differentiated, n (%)	**63 (15.1%)**	48 (13.1%)	15 (29.4%)	<0.01
Staging, n (%)				
BWH[17] T1	**233 (55.9%)**	217 (59.3%)	16 (31.4%)	
T2a	**143 (34.3%)**	119 (32.5%)	24 (47.1%)	
T2b	**36 (8.6%)**	26 (7.1%)	10 (19.6%)	<0.001
T3	**5 (1.2%)**	4 (1.1%)	1 (2.0%)	
AJCC[16] T1	**270 (64.8%)**	246 (67.2%)	24 (47.1%)	
T2	**84 (20.1%)**	72 (19.7%)	12 (23.5%)	
T3	**59 (14.2%)**	45 (12.3%)	14 (27.5%)	<0.05
T4	**4 (1.0%)**	3 (0.9%)	1 (2.0%)	
NCCN[4] High risk	**276 (66.2%)**	253 (69.1%)	23 (45.1%)	<0.001
Very high risk	**141 (33.8%)**	113 (30.9%)	28 (54.9%)	
40-GEP Results, n (%)				
Class 1	**231 (55.4%)**	215 (58.7%)	16 (31.7%)	
Class 2A	**171 (41.1%)**	144 (39.3%)	27 (52.9%)	<0.001
Class 2B	**15 (3.6%)**	7 (1.9%)	8 (15.7%)	

*p-values reported for Person Chi-squared or Wilcoxon F test, as appropriate; **Only patients without a non-local event were required to have a minimum follow-up of three years; ***n=385 cases with tumor diameter available.
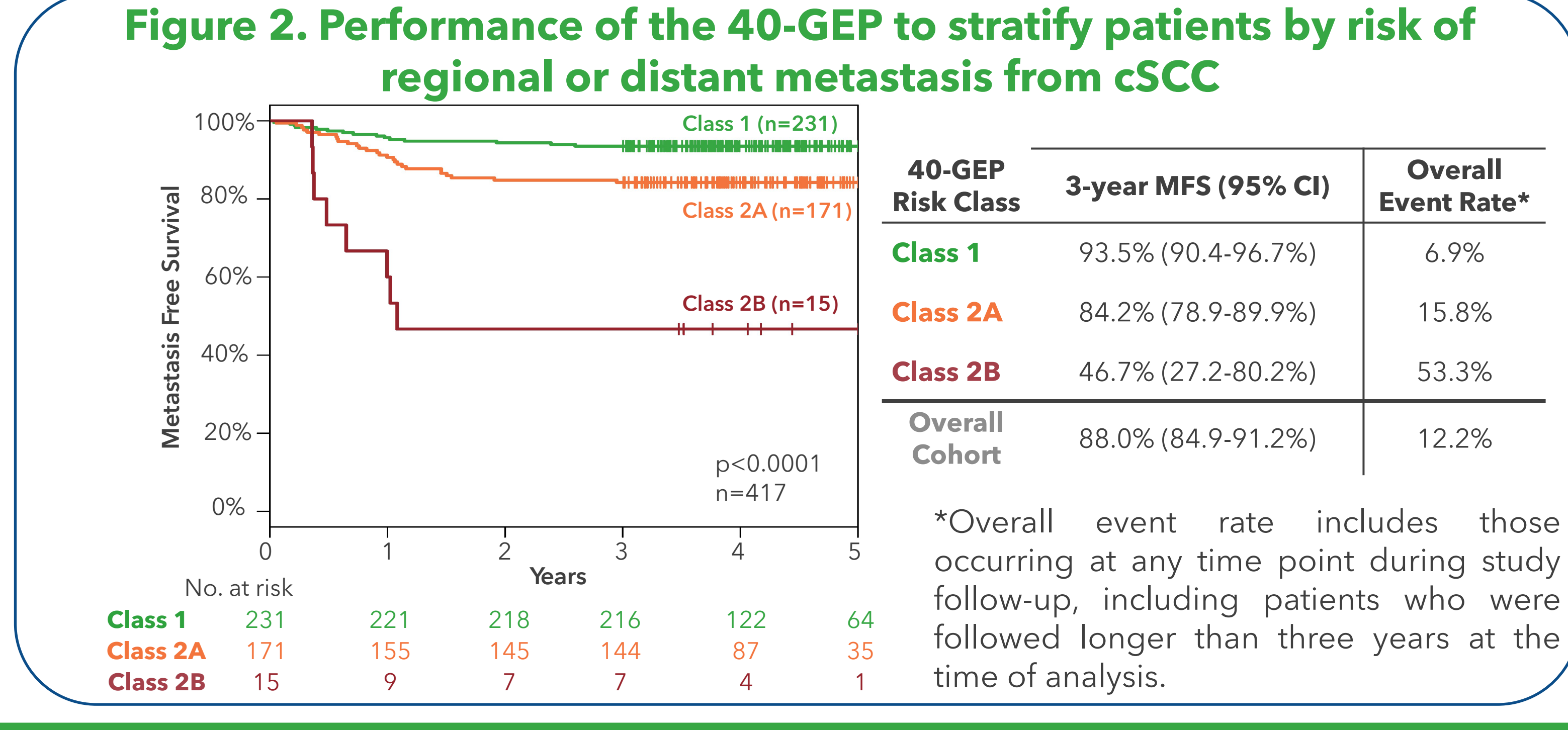
Figure 2. Performance of the 40-GEP to stratify patients by risk of regional or distant metastasis from cSCC

40-GEP Risk Class	3-year MFS (95% CI)	Overall Event Rate*
Class 1	93.5% (90.4-96.7%)	6.9%
Class 2A	84.2% (78.9-89.9%)	15.8%
Class 2B	46.7% (27.2-80.2%)	53.3%
Overall Cohort	88.0% (84.9-91.2%)	12.2%

*Overall event rate includes those occurring at any time point during study follow-up, including patients who were followed longer than three years at the time of analysis.

p<0.0001 n=417

No. at risk	0	1	2	3	4	5
Class 1	231	221	218	216	122	64
Class 2A	171	155	145	144	87	35
Class 2B	15	9	7	7	4	1

Table 2. Multivariable analysis combining the 40-GEP with BWH, AJCC, or NCCN staging systems

Group	HR (95% CI)	P-value
Class 1	Reference	--
Class 2A	2.28 (1.23-4.25)	0.009
Class 2B	9.02 (3.74-21.77)	<0.001
BWH Low Risk	Reference	--
BWH High Risk	2.10 (1.05-4.20)	0.034
Class 1	Reference	--
Class 2A	2.33 (1.26-4.33)	0.007
Class 2B	13.67 (5.73-32.64)	<0.002
AJCC Low Risk	Reference	--
AJCC High Risk	3.08 (1.66-5.72)	<0.001
Class 1	Reference	--
Class 2A	2.15 (1.15-4.01)	0.017
Class 2B	8.74 (3.66-20.88)	<0.001
NCCN High Risk	Reference	--
NCCN Very High Risk	2.06 (1.17-3.62)	0.012

Binary categorizations of staging were employed: AJCC8 T3/T4 (high risk), BWH T2b/T3 (high risk), NCCN very high risk, and the 40-GEP Class 2A/2B. Addition of interaction terms to the multivariate analysis revealed no significant interactions (p>0.05).

Table 3. Metastatic risk prediction of NCCN, AJCC8, and BWH staging systems are significantly improved when 40-GEP is included

› When the performance of staging-alone models was compared to multivariate models that included the 40-GEP, a significant improvement in predictive accuracy of metastatic events was observed. Inclusion of interaction terms revealed no significant interactions (p>0.05), verifying the 40-GEP as contributing independent prognostic value to the prediction of metastatic risk relative to staging alone.

Model*	Likelihood ratio	ANOVA (p-value)
40-GEP	24.29	
NCCN very high	11.25	p<0.0001
NCCN very high + 40-GEP	30.59	
AJCC8 Staging	8.42	p<0.0001
AJCC8 Staging + 40-GEP	35.05	
BWH Staging	7.97	p<0.0001
BWH Staging + 40-GEP	28.19	

*The models employed binary staging of AJCC8 T3/T4 (high risk), BWH T2b/T3 (high risk), NCCN very high risk, and 40-GEP Class 2A/B.

Conclusions

› Some patients with HR-cSCC on the H&N will experience metastasis despite MMS with clear margins; the 40-GEP can help identify patients at high risk.

› The 40-GEP significantly increases the accuracy of metastatic event prediction, alone and when combined with NCCN, AJCC8, or BWH staging systems to better guide risk-aligned care decisions for metastatic surveillance or ART.

Disclosures