

Metastasis-free survival prediction with the 40-gene expression profile test in patients with cutaneous squamous cell carcinoma risk stratified according to the National Comprehensive Cancer Network Guidelines®

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Introduction

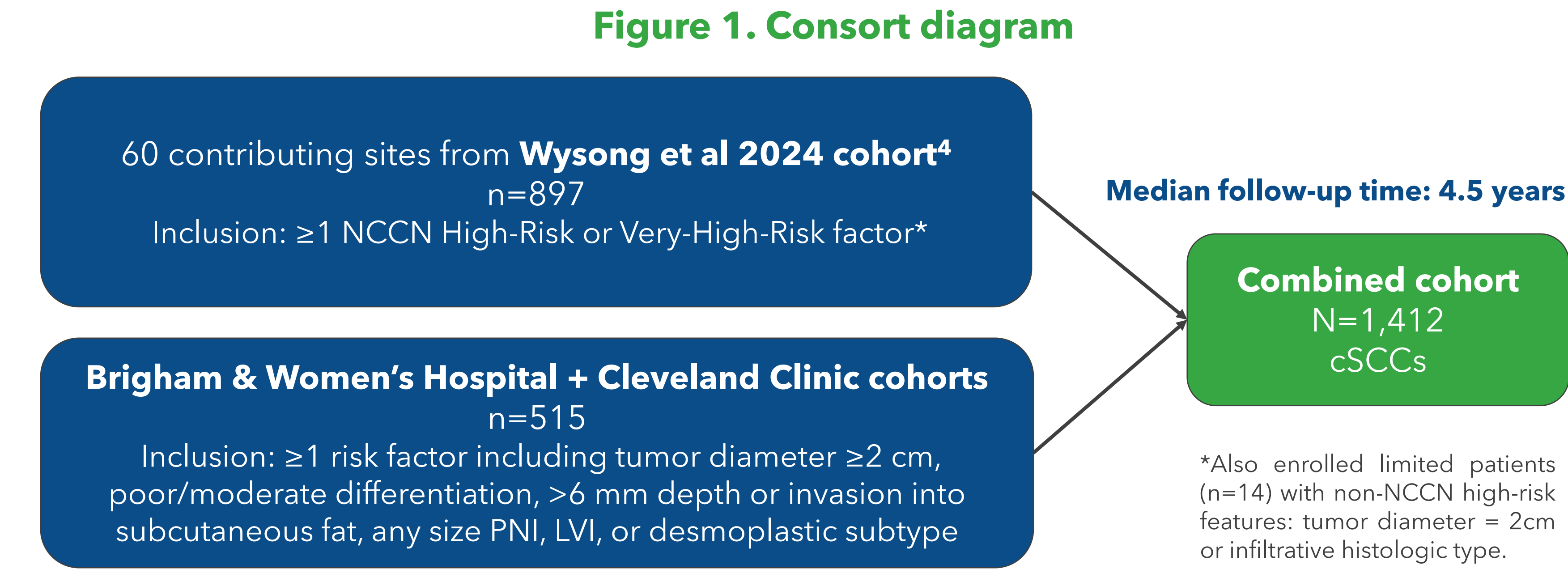
- Despite definitive surgical treatment, a subset of cutaneous squamous cell carcinomas (**cSCC**) has an increased risk of regional/distant metastasis making it challenging to identify patients most at risk.¹ The 40-gene expression profile (**40-GEP**) prognostic test improves risk stratification for patients with cSCC with known clinical or histologic risk factors, stratifying patients’ risk of metastasis as low (**Class 1**), higher (**Class 2A**), or highest (**Class 2B**) risk.²
- The 40-GEP can be used in conjunction with formalized risk assessment strategies^{3,4} such as the National Comprehensive Cancer Network (**NCCN**) Guidelines® and tumor staging criteria like Brigham & Women’s Hospital (**BWH**).^{5,6} However, it has not been shown how the 40-GEP adds prognostic value to BWH staging after first categorizing patients according to NCCN risk stratification into High-Risk or Very-High-Risk subgroups.

Objective

- To assess how the 40-GEP adds prognostic value for clinicians who may consider both BWH staging and NCCN risk stratification when evaluating treatment and follow-up plans for patients with cSCC.

Patients & Methods

- Under an IRB-approved, 60-institution retrospective study, patients with primary cSCC from a previously published cohort (n=897)⁴ having ≥1high-risk factor (closely modeled from NCCN risk stratification¹) were combined with a novel cohort from two academic centers (n=515) (**Figure 1**).
- Enrollment in the novel cohort required ≥1of the following risk factors: tumor diameter ≥2cm, poor or moderate differentiated histopathology, >6mm depth of invasion or invasion into/beyond subcutaneous fat, small/large caliber perineural invasion (PNI), lymphovascular invasion (LVI), or desmoplastic subtype.
- Patients were excluded if they had received adjuvant radiation therapy (**ART**). Kaplan-Meier survival analysis was used to calculate 3-year regional/distant metastasis-free survival (**MFS**). Cox regression of metastatic risk prediction modeled BWH ± 40-GEP within the context of NCCN risk groups⁷, High-Risk (**NCCN HR**) and Very-High-Risk (**NCCN VHR**), and compared using analysis of deviance. BWH staging⁶ was grouped into binary categories of low stage (T1+T2a) and high stage (T2b+T3).



References

1. Wysong A. *N Engl J Med.* 2023; 388(24):2262–73. **2.** Wysong A et al. *J Am Acad Dermatol.* 2021; 84(2):361–9. **3.** Ibrahim SF et al. *Future Oncol.* 2022; 18(7):833–47. **4.** Wysong A et al. *Dermatol Ther (Heidelb).* 2024; 14(3):593–612. **5.** NCCN Guidelines®, Squamous Cell Skin Cancer V.1.2025. **6.** Jambusaria-Pahlajani A et al. *JAMA Dermatol.* 2013; 149(4):402. **7.** NCCN Guidelines®, Squamous Cell Skin Cancer V.1.2024.

Results

Table 1. Patient demographics				
	All patients n=1412	Non-metastatic n=1256 (89.0%)	Metastatic n=156 (11.0%)	P-value*
Patient characteristics				
Age in years,** median (range)	73 (26-90+)	73 (26-90+)	73 (32-90+)	ns
Male, n (%)	999 (70.8%)	871 (69.4%)	128 (82.1%)	0.001
Immunosuppression, n (%)	372 (26.35%)	313 (24.9%)	59 (37.8%)	<0.001
Tumor characteristics & treatment				
Tumor diameter,*** median (range)	1.9 (0.1-22)	1.8 (0.1-22)	2.3 (0.35-18)	<0.001
Poorly differentiated, n (%)	210 (14.9%)	153 (12.2%)	57 (36.5%)	<0.001
Mohs as definitive surgery, n (%)	967 (68.5%)	880 (70.1%)	87 (55.8%)	<0.001
Risk stratification, n (%)				
NCCN ⁵ Low-Risk	64 (4.5%)	64 (5.1%)	0	<0.001
High-Risk	842 (59.6%)	798 (63.5%)	44 (28.2%)	
Very-High-Risk	506 (35.8%)	394 (31.4%)	112 (71.8%)	
40-GEP Class 1	815 (57.7%)	770 (61.3%)	45 (28.9%)	<0.001
Class 2A	538 (38.1%)	450 (35.8%)	88 (56.4%)	
Class 2B	59 (4.2%)	36 (2.9%)	23 (14.7%)	

Percentages shown were calculated as a fraction of the total n of each respective column header.
*P-values reported for Person Chi-squared or Wilcoxon F test, as appropriate
**Ages over 90 years reported as 90+ for privacy
***n=85 cases without tumor diameter available

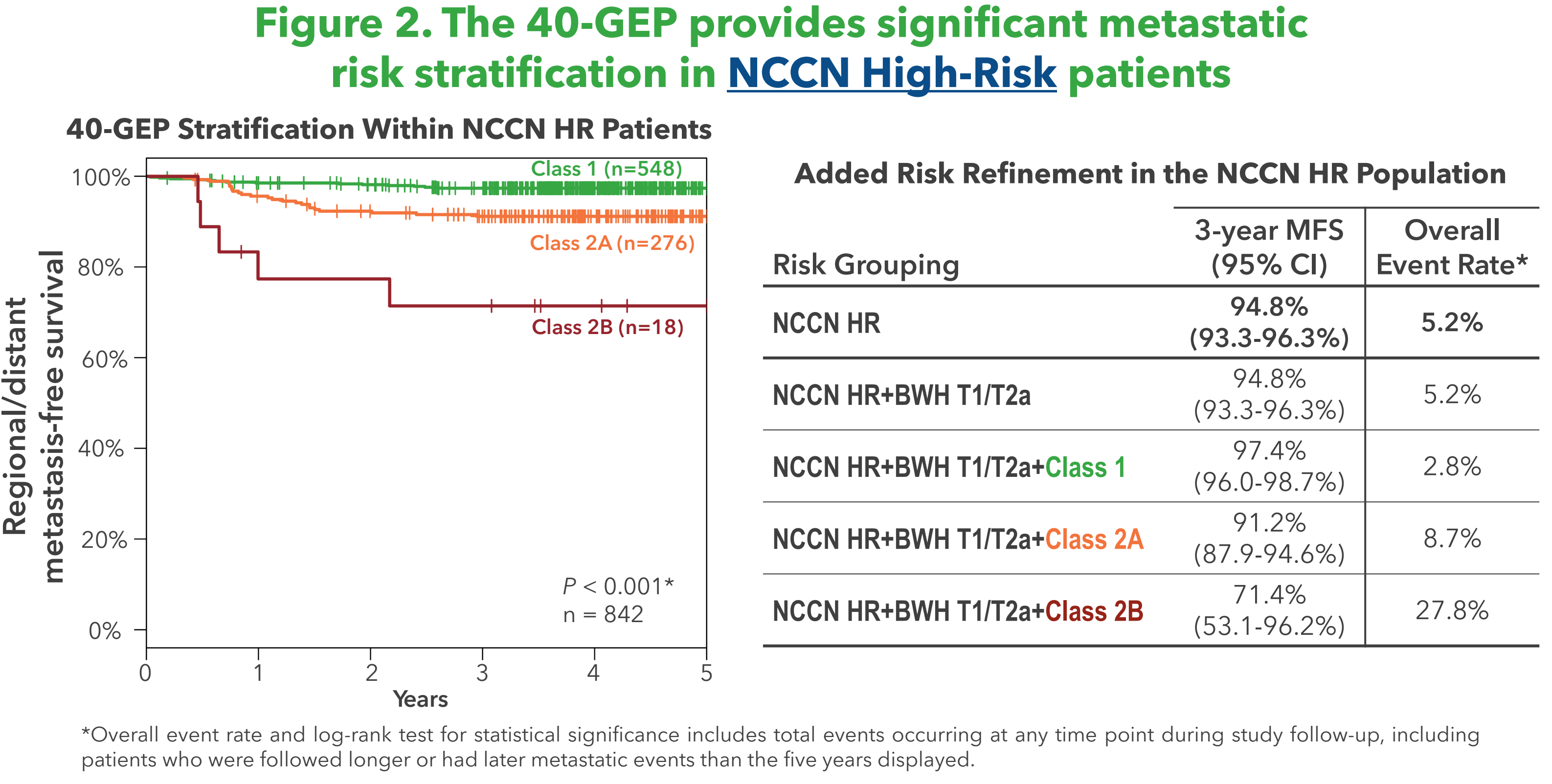


Table 2. Metastatic risk prediction of the BWH staging system is significantly improved when 40-GEP is included for NCCN High-Risk patients

Risk Stratification*	Model Likelihood Ratio	Model ANOVA P-value
BWH Staging	0.21	<0.001
BWH Staging + 40-GEP	23.35	

*Models employed binary staging of BWH T1/T2a vs BWH T2b/T3, and three groups for the 40-GEP: Class 1, Class 2A, and Class 2B. (n=842)

- In the **NCCN High-Risk** population, model performance comparison of BWH staging ± 40-GEP showed significantly improved predictive accuracy of metastatic events by the addition of the 40-GEP.
- Inclusion of interaction terms revealed no significant interactions (P>0.05), indicating that the 40-GEP adds prognostic information that is independent of BWH staging.

Figure 3. The 40-GEP stratifies metastatic risk in NCCN Very-High-Risk patients, who have a >4-fold overall increase in metastatic event rate vs High-Risk patients

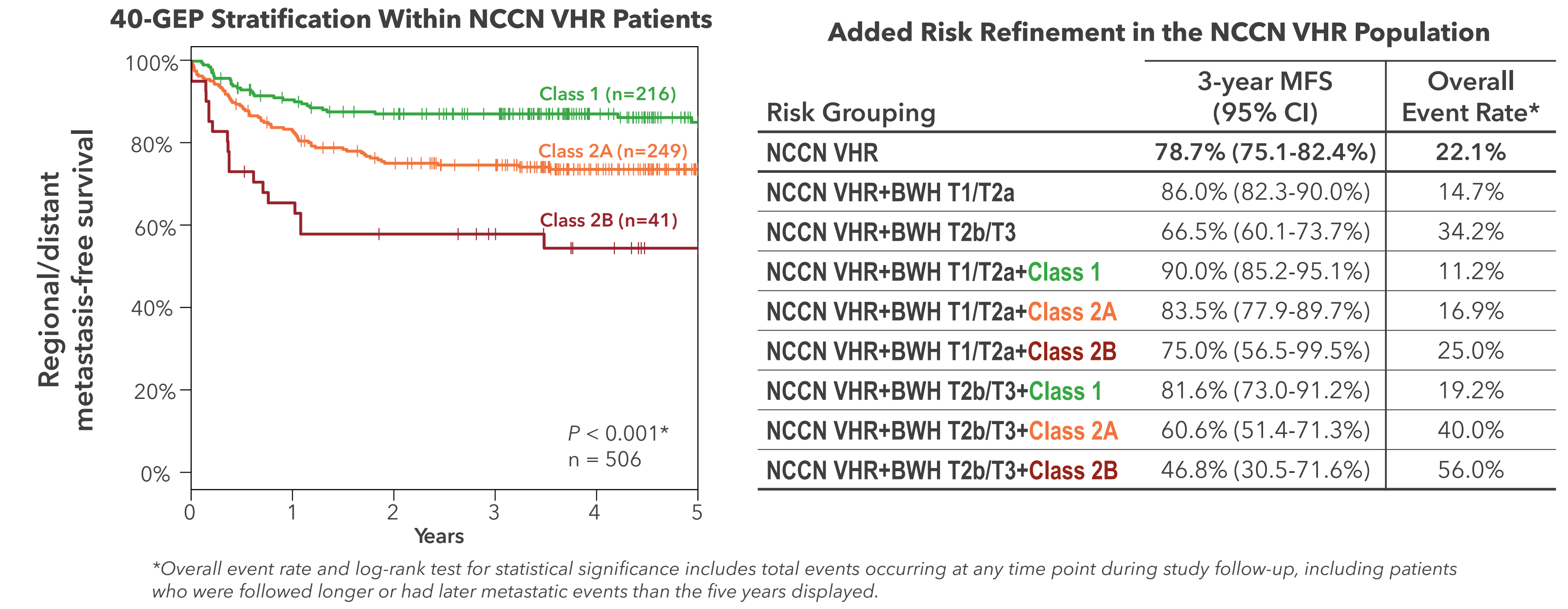


Table 3. Metastatic risk prediction of the BWH staging system is also significantly improved when 40-GEP is included for NCCN Very-High-Risk patients

Risk Stratification*	Model Likelihood Ratio	Model ANOVA P-value
BWH Staging	28.45	<0.001
BWH Staging + 40-GEP	45.38	

- In the **NCCN Very-High-Risk** population (n=506), model performance comparison of BWH staging ± 40-GEP showed significantly improved predictive accuracy of metastatic events by the addition of the 40-GEP.
- Inclusion of interaction terms revealed no significant interactions (P>0.05), indicating that the 40-GEP adds prognostic information that is independent of BWH staging in this patient subset.

Conclusions

- The 40-GEP provided significant metastatic risk stratification in both NCCN High-Risk and Very-High-Risk patients with cSCC.
- Incorporation of the 40-GEP with BWH T-staging significantly improved the accuracy of metastatic risk stratification in both NCCN High- and Very-High-Risk subgroups of patients.
- Integrating the 40-GEP with BWH staging, even within NCCN High or Very-High-Risk profiles, refines risk stratification and supports more accurate clinical decisions, improving personalized care.

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