# The 40-gene expression profile (40-GEP) test allows for an improved prognostication of the likelihood of metastasis in patients with T1 cutaneous squamous cell carcinoma (cSCC) with high-risk factors

Ally-Khan Somani, MD, PhD[1]; Javier Cañueto MD, PhD[2]; Vincent Smith, DNP, ANP-C[3]; Andrew Berg, NP-C[4]; Alison L. Fitzgerald, PhD[5]; Jennifer J. Siegel, PhD[5]; Anesh Prasai, PhD[5]; Sherrif F, Ibrahim, MD, PhD[6]; Sarah T. Arron, MD, PhD[7]

[1] Department of Dermatology, Indiana University School of Medicine, Indianapolis, Indiana, USA; [2] Department of Dermatology, Universitario de Salamanca, Salamanca, Spain; [3] Pinnacle Dermatology, Little Rock, Arkansas, USA; [4] Dermatology Specialists of Kansas City, Kansas City, MO, USA; [5] Research and Development, Castle Biosciences, Inc., Friendswood, Texas, USA; [6] Rochester Dermatologic Surgery, Victor, NY, USA, [7] Peninsula Dermatology, Burlingame, California, USA

## Synopsis

- The metastatic rate for cSCC is low, however, the overall incidence is high (~1-2.5 million cases/year), and deaths from this disease now surpass those from melanoma. [1,2]
- Guidelines recommend that patient management decisions should be determined by the individual patient's probability of disease progression. However existing prognostic tools are variable and have demonstrated low accuracy, thus a significant number of risky patients are missed. [3-5] **(Figure 1)**
- The 40-gene expression profile (40-GEP) test stratifies primary cSCC patients having one or more clinicopathologic risk factors into three biological risk groups based on risk for regional, nodal, or distant metastasis 3-years post diagnosis (low = Class 1; moderate = Class 2A; high = Class 2B).[6] **(Figure 2)**
- Studies have demonstrated that when the 40-GEP test is incorporated with staging, it enhances risk assessment and offers a more accurate and personalized stratification of metastatic risk. [6-8]

### Figure 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 | ✓ | ✓ | Blue shaded area connotates T1 Stage Tumors | |
| 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## | ✓ | ✓ | | |

*NCCN- National Comprehensive Cancer Network guidelines (high or very-high risk factors); AJCC8- American Joint Committee on Cancer Eighth Edition; BWH- Brigham and Women's Hospital **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; ##Acanthotylic, adenosquamous, or metaplastic subtypes (40-GEP- others will be considered on a case-by-case basis). †40-GEP is not validated for local recurrence

### Figure 2. Clinical use of the 40-GEP test

**Primary cSCC with one or more risk factors**

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

**Class 1: Low Biological Risk**
~7% risk of metastasis

**Class 2A: Moderate Biological Risk**
20% risk of metastasis

**Class 2B: High Biological Risk**
≥50% risk of metastasis

## Methods

- Retrospective study consisted of cSCC patients with confirmed clinicopathologic information, which met requirements for 40-GEP clinical testing and follow up of at least 3 years (n=954). 446 cases were staged as BWH T1 (see **Figure 1**) and 501 cases were staged as AJCC8 T1 (**Figure 1**).
- Clinicopathologic risk factors were comprehensively assessed by performing the following on the T1 subsets: review of original biopsy and surgical reports, independent review by a board-certified dermatopathologist, and Kaplan-Meier analysis for metastasis-free survival (MFS) with log rank test and Cox regression analyses.
- For real world clinical usage data, summary metrics on T1 (n=2,924) samples were identified from all n=7317 patients who received test reports during the first two years of clinical ordering (August 31, 2020–August 31, 2022).

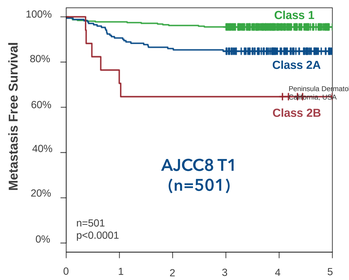
## Clinical Issue and Objective

Staging systems for cSCC have shown limited accuracy in predicting the prognosis of the disease to metastasize.[3,4] Approximately 30% of BWH or AJCC8 T1 cSCCs (deemed at a low risk of metastasis) will go on to metastasize, potentially due to risk factors not captured in clinicopathologic risk assessment, but within the intended use of 40-GEP.[9,10] The lack of accurate risk assessment methods complicates aligning treatment plans with risk of progression.

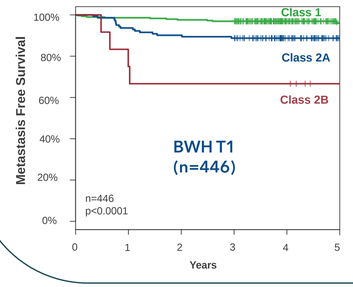
The objective of the study was to evaluate the performance of the 40-GEP test in identifying BWH or AJCC T1 staged tumors.

## Results

### Figure 3. The 40-GEP accurately classifies patients by metastatic risk within AJCC8 and BWH T1 staged subset




| 40-GEP Risk Class | Overall Cohort 3- year MFS (95% CI) | Overall Event Rate |
|---|---|---|
| Class 1 | 95.5% (93.3-97.8%) | 4.5% |
| Class 2A | 84.8% (79.6-90.4%) | 15.2% |
| Class 2B | 64.7% (45.5-91.9%) | 35.3% |
| Overall | 90.8% (88.3-93.4%) | 9.2% |




| 40-GEP Risk Class | Overall Cohort 3- year MFS (95% CI) | Overall Event Rate |
|---|---|---|
| Class 1 | 96.9% (95.0-98.9%) | 3.4% |
| Class 2A | 88.7% (83.7-94.1%) | 11.3% |
| Class 2B | 66.7% (44.7-99.5%) | 33.3% |
| Overall | 93.5% (91.2-95.8%) | 6.7% |

## References

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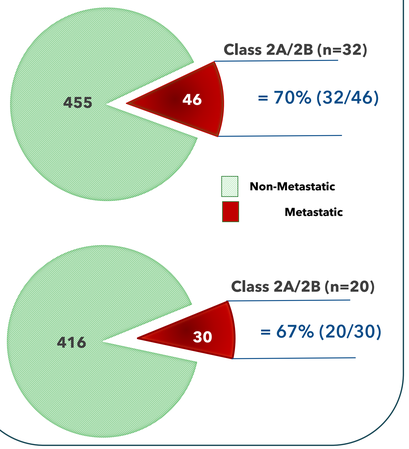
## Disclosures

- JJS, ALF, and AP are employees and shareholders of Castle Biosciences, Inc.

## Results

### Figure 4. 40-GEP identified ~70% of the T1 tumors that metastasized as biologically high risk




### Table 1. Distribution of NCCN high risk factors missed by AJCC8/BWH T1 staging that are captured by 40-GEP in real world data

**A)**

| Risk Factor | % of Patients |
|---|---|
| Located on Areas H or M* | 86.7 |
| Rapidly growing tumor | 28.3 |
| Borders poorly defined | 17.1 |
| Immuno-suppressed | 6.0 |
| Neurological symptoms in tumor region | 2.0 |
| Other† | 1.7 |
| Tumor at site of prior radiation therapy or chronic inflammatory process | 1.2 |

**B)**

| Test Result | % of Tests | # of Patients |
|---|---|---|
| Class 1 | 80.0% | 2,338 |
| Class 2A | 18.6% | 545 |
| Class 2B | 1.4% | 41 |

Clinicopathologic risk factors of patients tested with the 40-GEP that are BWH and AJCC8 T1 (n=2,.924) samples. *mask areas of face, genitalia, hands, feet, cheeks, forehead, scalp, neck and pretibial. † high risk subtypes and infiltrating tumors that are deemed eligible for testing

## Conclusions

- This study shows that ~70% of the metastasis events that are missed by AJCC8 and BWH T1 staging are captured by 40-GEP Class 2 test results.
- Real world clinical testing data shows that 20% of patients were staged as T1 and received Class 2 results, identifying potentially 584 patients that could metastasize if only staging criteria were applied and used to determine treatment decisions.
- These findings demonstrate the ability of the 40-GEP to identify biologically high-risk tumors in patients deemed low risk by traditional staging methods.
- Incorporating 40-GEP test results in clinical assessments with traditional clinicopathological risk factors can improve the stratification of high-risk cSCC T1 patients and contribute to risk-appropriate surveillance and treatment decisions.

For more information: aprasai@castlebiosciences.com