Molecular Profiling & Precision Medicine

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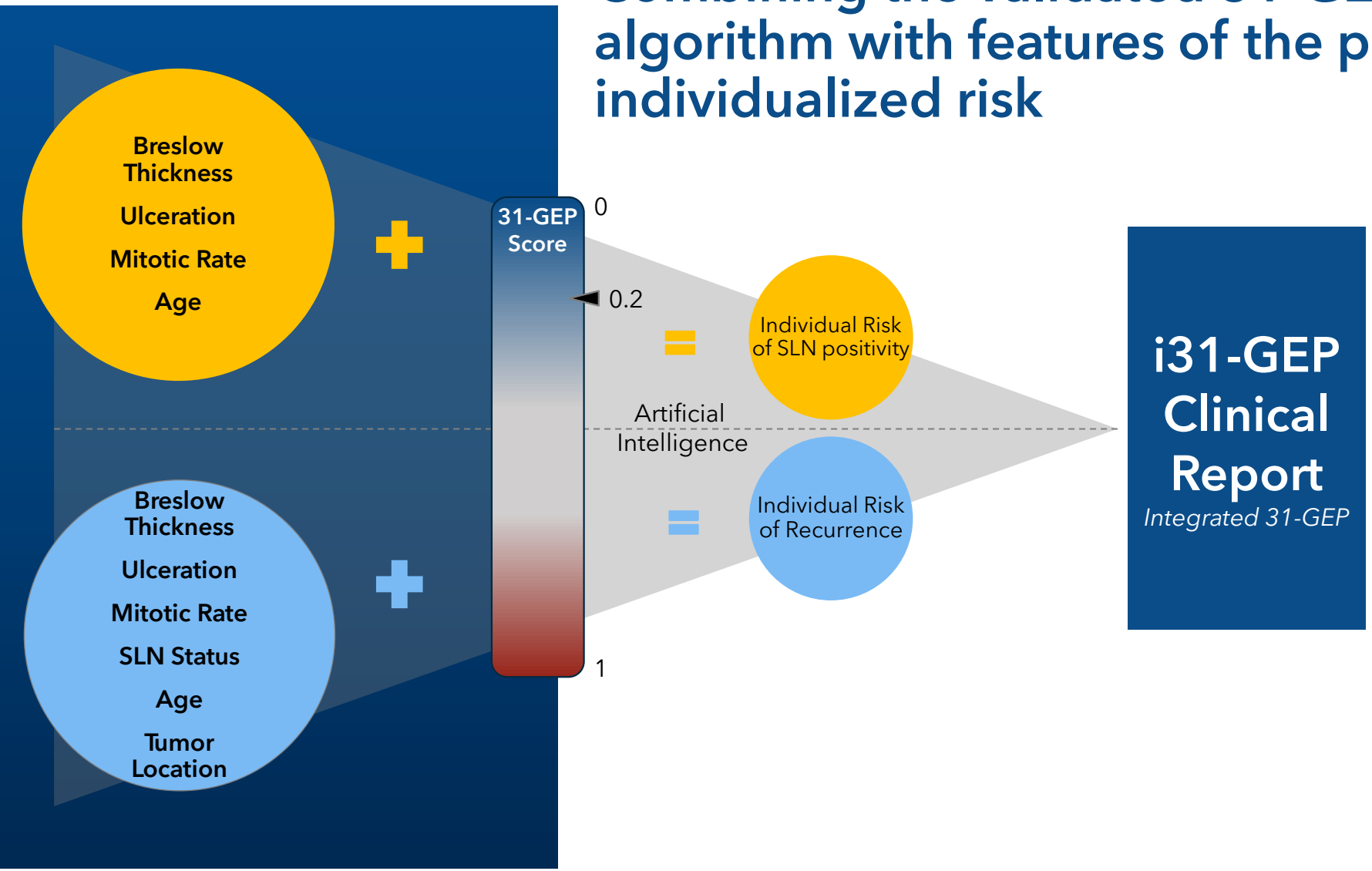
Conflicts of interest

- Investigator for Castle Biosciences

Objectives

- GEP for Melanoma Prognosis
- GEP for Cutaneous Squamous Cell Carcinoma Prognosis

Combining the validated 31-GEP molecular algorithm with features of the patient and tumor for individualized risk



31-GEP score was the most significant variable in predicting SLN positivity[1]

31-GEP score was an independent and significant variable in prognostication for precise RFS, DMFS, and MSS[2]

[1]Whitman et al. *JCO PO* 2021; [2]Jarell et al. *JAAD* in press

i31-GEP for SLNB provides a precise, personalized risk of SLN positivity

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original reports

PRECISION MEDICINE

Integrating 31-Gene Expression Profiling With Clinicopathologic Features to Optimize Cutaneous Melanoma Sentinel Lymph Node Metastasis Prediction

Eric D. Whitman, MD[1]; Vadim P. Koshenkov, MD[2]; Brian R. Gastman, MD[3]; Deri Lewis, MD[4]; Eddy C. Hsueh, MD[5]; Ho Pak, MD[6]; Thomas P. Trezona, MD[7]; Robert S. Davidson, MD[8]; Michael McPhee, MD[9]; J. Michael Guenther, MD[10]; Paul Toomey, MD[11]; Franz O. Smith, MD[12]; Peter D. Beitsch, MD[13]; James M. Lewis, MD[14]; Andrew Ward, NP[14]; Shawn E. Young, MD[15]; Parth K. Shah, MD[15]; Ann P. Quick, PhD[16]; Brian J. Martin, PhD[16]; Olga Zolochevska, PhD[16]; Kyle R. Covington, PhD[16]; Federico A. Monzon, MD[16]; Matthew S. Goldberg, MD[16]; Robert W. Cook, PhD[16]; Martin D. Fleming, MD[17]; David M. Hyams, MD[18]; and John T. Vetto, MD[19]

False-negative rate	1.9	2.6
Reduction rate	23.0[a]	32.1
Sensitivity	95.1	89.8
Pretest SLN positivity rate	10.9	8.0
PPV of ≥ 5% risk	14.4	10.6

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Brief Report

ONLINE FIRST

April 27, 2022

Utility of a Model for Predicting the Risk of Sentinel Lymph Node Metastasis in Patients With Cutaneous Melanoma

Michael A. Marchetti, MD[1]; Stephen W. Dusza, DrPH, MPH[1]; Edmund K. Bartlett, MD[2]

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JAMA Dermatol. Published online April 27, 2022. doi:10.1001/jamadermatol.2022.0970

JAMA Dermatology | Brief Report

Utility of a Model for Predicting the Risk of Sentinel Lymph Node Metastasis in Patients With Cutaneous Melanoma

Table 2. Net Benefit and Relative Utility of the i31-GEP-SLNB Prediction Model Using a 5% Risk Threshold

	SLN biopsy		
T Category	Strategy 1: none[a]	Strategy 2: all[b]	Strategy 3: using i31-GEP SLNB model
Net benefit (95% CI)			
T1a-HR	0	−0.021 (−0.044 to 0.001)	−0.003 (−0.018 to 0.119)
T1b	0	0.005 (−0.019 to 0.030)	0.017 (−0.006 to 0.040)
T2a	0	0.069 (0.037 to 0.100)	0.070 (0.039 to 0.101)
T2b	0	0.081 (0.026 to 0.136)	0.083 (0.025 to 0.142)
Relative utility, % (95% CI)[c]			
T1a-HR	0	NA	NA
T1b	0	9 (0-64)	31 (0-69)
T2a	0	60 (46-73)	61 (48-74)
T2b	0	64 (38-89)	66 (46-85)

Abbreviations: SLN, sentinel lymph node; T1a-HR, T1a high-risk patients (mitotic index ≥2 mm^2; lymphovascular invasion, absence of tumor infiltrating lymphocytes, age <40 years, microsatellites, regression, or transected base).

[a] The SLN biopsy for none is equivalent to a strategy with 0% sensitivity and 100% specificity.

[b] The SLN biopsy for all is equivalent to a strategy with 100% sensitivity and 0% specificity.

[c] Relative utility is calculated by dividing the net benefit by the maximum achievable utility (prevalence) and ranges from 0% to 100%. In other words, relative utility is the maximum fraction of expected utility achieved by risk prediction compared with perfect prediction. Relative utility allows an assessment of the potential for improved performance with better prediction models.

Collaboration with the National Cancer Institute (NCI)

Linking 31-GEP testing data with patients captured in the NCI-SEER Registry

Collaboration with the National Cancer Institute (NCI) to link 31-GEP testing data with data from the Surveillance, Epidemiology and End Results (SEER) program's registries on cutaneous melanoma (CM) cases

Phase 1 Collaboration Objectives:

- **Validate:** Confirm the performance of DecisionDx-Melanoma
  - Unselected and prospectively tested cohort of patients with CM
  - Provide unbiased real-world data, showing clinical benefit of 31-GEP testing
  - Patients diagnosed from 2013 – 2018
- **Compare:** Does the addition of DecisionDx-Melanoma test results improve outcomes?
  - Survival outcomes in patients receiving 31-GEP testing vs. untested patients
  - A total of 5,226 patients who received 31-GEP testing met the initial selection criteria,
  - Of these 3,621 had all necessary information to be included in the matching process

Matching 31-GEP tested patients to untested patients to isolate the potential effect of 31-GEP testing on outcomes

Patient selection

- All incident cases of cutaneous melanoma diagnosed between 2013-2018 registered in SEER
- Cases that were tested with 31-GEP
- Analysis included all patients within the SEER Database
- **Diagnosed in 2016-2018 to account for potential access to adjuvant therapy**

Matching

- Patients tested with 31-GEP were matched to untested patients (1:3 ratio)
- **No significant differences between 31-GEP tested and non-tested patients**

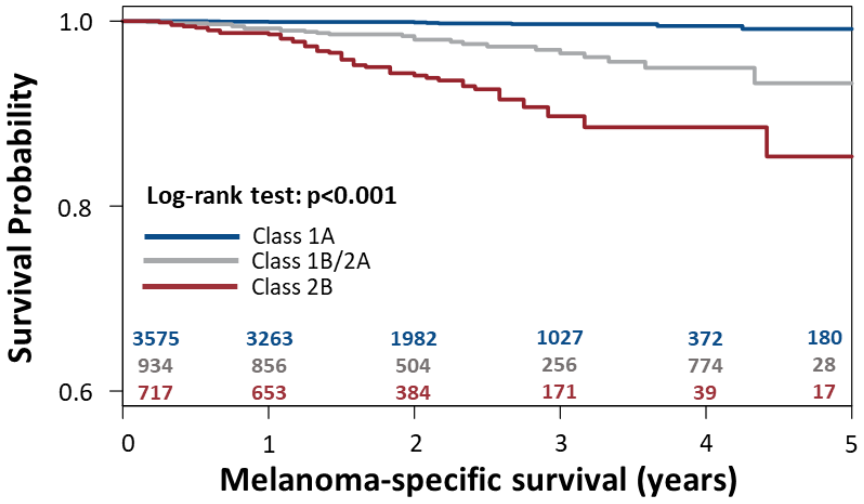
Successful matching of a cohort of non-31-GEP tested patients to the 31-GEP tested population

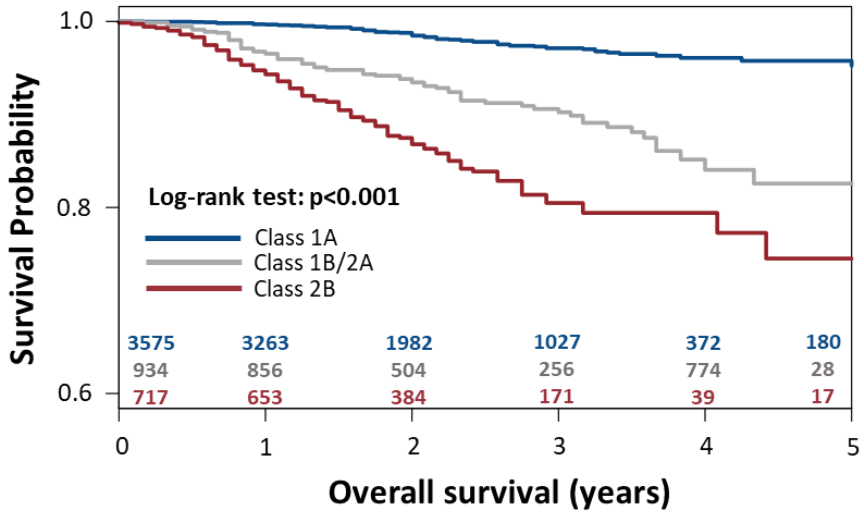
Covariates	31-GEP Tested (n=3,621) vs. Non-31-GEP Tested (n=10,863)
Age *(median)*	p=0.607
Follow-up time *(median)*	p=0.474
T-stage	p>0.999
Year of diagnosis (2016-2018)	p=0.327
Sex	p=0.199
Yost index (quintile)	p=0.888
SLN assessment	p=0.813
SLN positivity	p=0.757
Mitotic rate *(median)*	p=0.524
Primary tumor location	p=0.956
Race	p=0.506

Kurley et al. European Association of Dermato Oncology (EADO) conference in Seville, Spain; April 21-23, 2022

NCI/SEER cohort of unselected prospectively tested patients confirms previously reported risk stratification for patients with Stage I-III cutaneous melanoma (n=5226)

The separation of Class 1A, Class 1B/2A, and Class 2B MSS and OS risk in 31-GEP tested patients within the SEER registry mirrors the risk separation in previously reported studies[1,2]





Kurley et al. Presented at EADO, April 21-23, 2022, [1]Gastman et al. *JAAD* 2019, [2]Hsueh et al. *JCO Precis Oncol* 2021

NCI/SEER cohort of unselected prospectively tested patients confirms previously reported risk stratification for patients with Stage I-III cutaneous melanoma (n=5226)

Melanoma-specific survival	
Feature	*Multivariate HR (95% CI)*
31-GEP class 1A	Reference
31-GEP class 1B/2A	4.86 (1.97-12.03)
31 GEP class 2B	7.0 (2.7-18.00)
Age (continuous)	1.05 (1.03-1.07)
Breslow Thickness (continuous)	1.16 (1.05-1.27)
Ulceration Absent	Reference
Ulceration Unknown	1.31 (0.18-9.78)
Ulceration Present	1.59 (0.86-2.94)
LN Negative	Reference
LN Status Unknown	0.84 (0.40-1.77)
LNB Positive	2.64 (1.45-4.79)

Overall survival	
Feature	*Multivariate HR (95% CI)*
31-GEP class 1A	Reference
31-GEP class 1B/2A	2.22 (1.51-3.25)
31 GEP class 2B	2.39 (1.54-3.70)
Age (continuous)	1.08 (1.07-1.10)
Breslow Thickness (continuous)	1.14 (1.07-1.21)
Ulceration Absent	Reference
Ulceration Unknown	0.85 (0.21-3.45)
Ulceration Present	1.45 (1.02-2.06)
LN Negative	Reference
LN Status Unknown	1.45 (1.06-2.00)
LNB Positive	1.45 (0.93-2.25)

Bailey et al. *JCO PO*, 2023. Multivariable analysis for melanoma-specific and overall survival for patients linked to SEER data registry diagnosed from 2016-2018. LN: lymph node. HR: Hazard ratio: CI: Confidence interval. Unit increase for each continuous variable: Breslow thickness: 1.0 mm; age: 1 year. N=4,226 after removing 459 observations with missing data for one or more variables.

Collaboration with the National Cancer Institute (NCI) to link 31-GEP testing data with data from the Surveillance, Epidemiology and End Results (SEER) Program's registries on cutaneous melanoma (CM) cases

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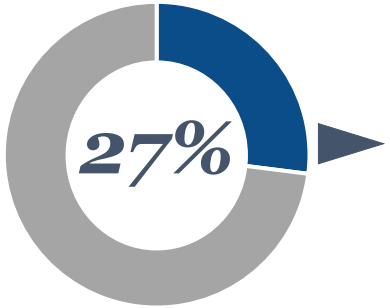
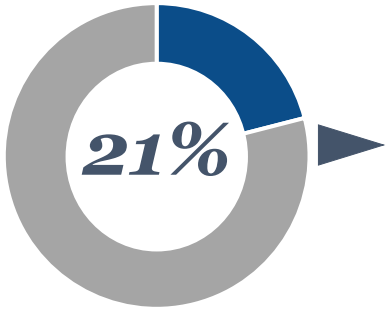
Patients receiving 31-GEP test results had improved melanoma-specific survival and overall survival compared to those not tested, (n=3,621)



Benefit in 3-year MSS in patient that were tested over those that were not tested

	3-year MSS (95% CI)	Deaths, % (n/N)
31-GEP Tested	97.7% (97.0-98.4%)	1.6% (58/3621)
Matched Untested	96.6% (96.2-97.1%)	2.2% (238/10863)
Hazard ratio‡	**0.73 (0.54-0.97)**	**P=0.03**



Benefit in 3-year OS in patients that were tested over those that were not tested

	3-year OS (95% CI)	Deaths, % (n/N)
31-GEP Tested	93.1% (92.0-94.2%)	4.8% (174/3621)
Matched Untested	91.2% (90.4-91.9%)	6.1% (658/10863)
Hazard ratio‡	**0.79 (0.67-0.93)**	**P=0.006**

Study data provide direct evidence that patients tested with 31-GEP have better survival rates relative to untested patients

Study data suggest that 31-GEP testing can aid in risk-aligned treatment plans for improved patient outcomes and survival rates

‡Hazard ratio (HR) was computed using the untested patients as reference for 31-GEP tested cohort. An HR less than 1.0 demonstrates improved survival in 31-GEP tested patients. Diagnosis date 2016 and onward.

31-GEP shows similar or better performance when compared to other standard of care prognostic tests

MSLT-1 Study[1]:

What is the impact of a traditional risk-stratification test (i.e.SLN biopsy)?

- MSLT-1 found that SLN biopsy had no impact on 10-year MSS
- 31-GEP had a statistically significant absolute MSS benefit at 3 years over those not tested ($p<0.05$)

Tumor size	P-value	10-Year MSS
Thin (<1.2mm)	Not reported	Not impacted
Intermediate (1.2-3.5mm)	not significant (p=.18)	Not impacted
Thick (>3.5)	not significant (p=.56)	Not impacted

Other NCI/SEER collaborative studies[2]

How significant is the absolute benefit?

- NCI/SEER collaborated with Oncotype DX Breast (ODX) on a similar analysis for use in guiding management decisions in breast cancer.
- Patients who were tested with Oncotype DX Breast had improved breast cancer specific-survival (BCSS) compared to untested patients ($p<0.05$)

	3-Year MSS
31-GEP Tested	97.7%
Matched Untested	96.6%
Absolute Mortality Difference	**1.1% (p<0.05)**

31-GEP showed absolute MSS mortality difference of 1.1% at 3 years over those not tested

	3-Year BCSS
ODX Tested	97.6%
Matched Untested	99.1%
Absolute Mortality Difference	**0.50% (p<0.05)**

ODX showed absolute BCSS mortality difference of 0.5% at 3 years over those not tested

1. Morton et al *N Engl J Med* 2014;370:599-609 2. Zhang et al *Breast Cancer Res Treat*. 2020 April; 180(2): 491-501.

Real-world utility incorporating the 31-GEP: GEP-guided surveillance imaging

Routine imaging guided by a 31-gene expression profile assay results in earlier detection of melanoma with decreased metastatic tumor burden compared to patients without surveillance imaging studies

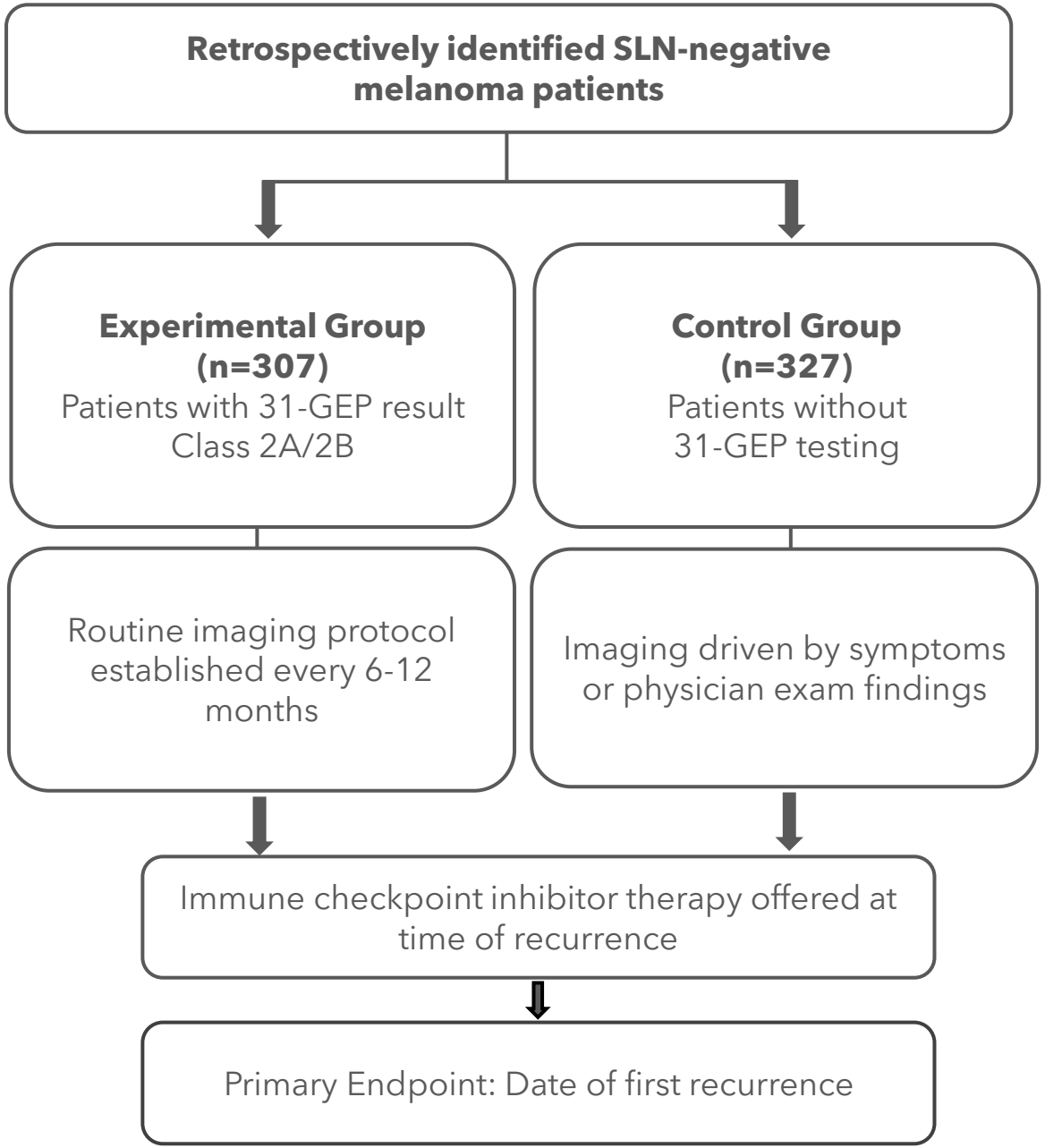
Soneet Dhillon[1] · Daniela Duarte-Bateman[2] · Graham Fowler[3] · Michael Norman Eun Hagstrom[1] · Nathaniel Lampley[1] · Shantel Olivares[1] · Mónica Stella Fumero-Velázquez[1] · Kathryn Vu[3] · Jeffrey D. Wayne[4] · Brian R. Gastman[2] · John Vetto[3] · Pedram Gerami[1,5]

Received: 13 March 2023 / Revised: 13 March 2023 / Accepted: 22 March 2023

- **Primary endpoint:** The date of detection of the first evidence of recurrence
- **Secondary endpoint:** Date of the last chart review
- Routine imaging protocol typically consisted of chest CT without contrast, abdominal pelvic CT with contrast, and brain MRIs with and without contrast at an average of 6-month intervals.

Dhillon et al. *Archives of Derm Research.* 2023.

Real-world utility incoporating the 31-GEP: GEP-guided surveillance imaging



Dhillon et al. *Archives of Derm Research.* 2023.

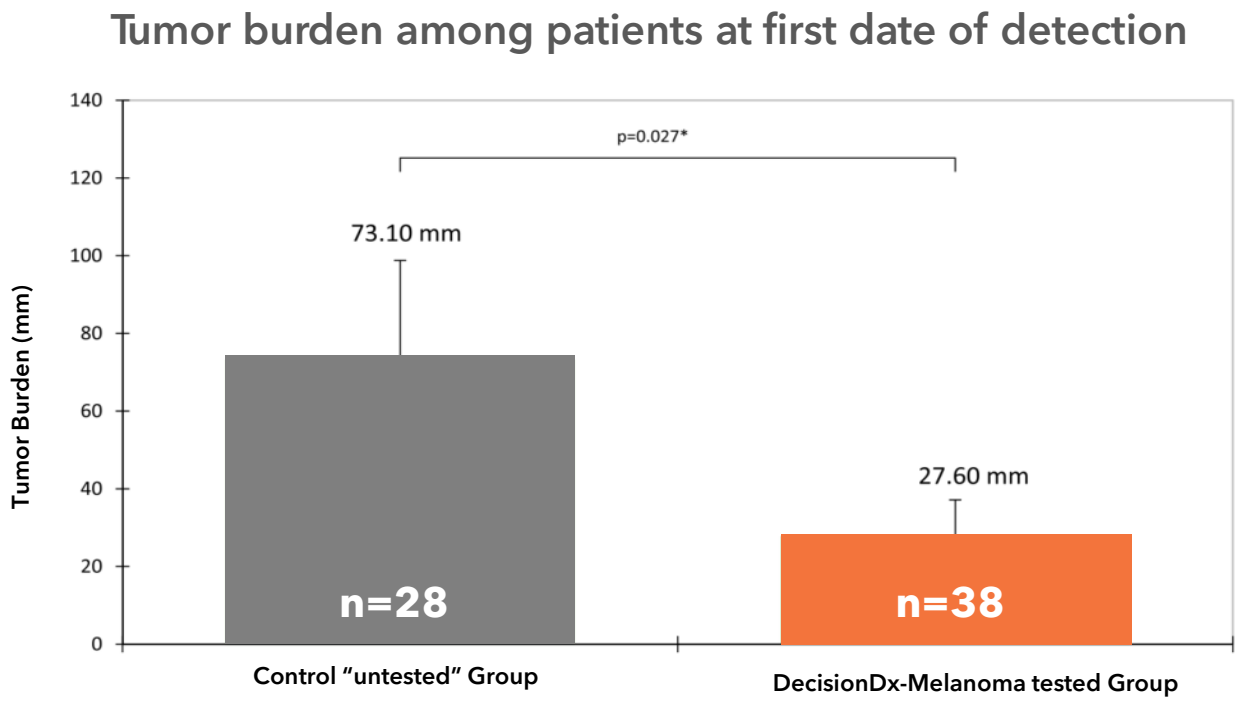
Real-world utility incorporating the 31-GEP: GEP-guided surveillance imaging

All patients	Control group (N= 327)	Experimental group (N=307)	Total (N=634)	P-value
Melanoma recurrence	14.1% (46/327)	20.5% (62/307)	17.1% (109/634)	0.031[a]
Recurrent melanoma patients	Control group patients (N=28)	Experimental Group patients (N=38)	Total (N=66)	P-value
Average Breslow	3.31 mm	3.72 mm	3.55 mm	0.171[b]
Tumor staging at primary diagnosis				0.060[a]
Clinical Stage I				
T1a	1	0	1	
T1b	2	0	2	
T2a	5	4	9	
Total Stage I	28.6% (8/28)	10.5% (4/38)	18.2% (12/66)	
Clinical Stage ≥II				
T2b	2	4	6	
T3a	7	6	13	
T3b	6	10	16	
T4a	1	3	4	
T4b	4	11	15	
Total Stage ≥II	71.4% (20/28)	89.5% (34/38)	81.80%	
Sex				
Male	17	19	36	0.388[a]
Female	11	19	30	
Age at primary diagnosis				
Both sexes (mean)	59.2	65.75	63	0.181[b]
Both sexes (range)	27-85	41-89	27-89	
Immunotherapy[c]				
Number of patients	71.4% (20/28)	81.6% (31/38)	77.3% (51/66)	0.331[a]
Patient status				
Alive patients	50.0% (14/28)	76.3% (29/38)	65.2% (43/66)	0.027[a]
Deceased patients	50.0% (14/28)	23.7% (9/38)	34.8% (26/66)	

Dhillon et al. *Archives of Derm Research.* 2023.

a. Chi-Square p-value
b. Kruskal-Wallis p-value
c. Patients who did not start immunotherapy when offered were excluded

Real-world utility incorporating the 31-GEP: GEP-guided surveillance imaging



Bar diagram representing the average tumor burden (measured in mm) between the control group and experimental group. If multiple foci were identified, measurements were added together to determine a total tumor burden.

Control "untested" group:
- 46 recurrences
- Average tumor burden at first detection was 73.10mm

31-GEP "tested" group:
- 63 recurrences
- Average tumor burden at first detection was 27.60mm

Dhillon et al. *Archives of Derm Research.* 2023.

Real-world utility incorporating the 31-GEP: GEP-guided surveillance imaging

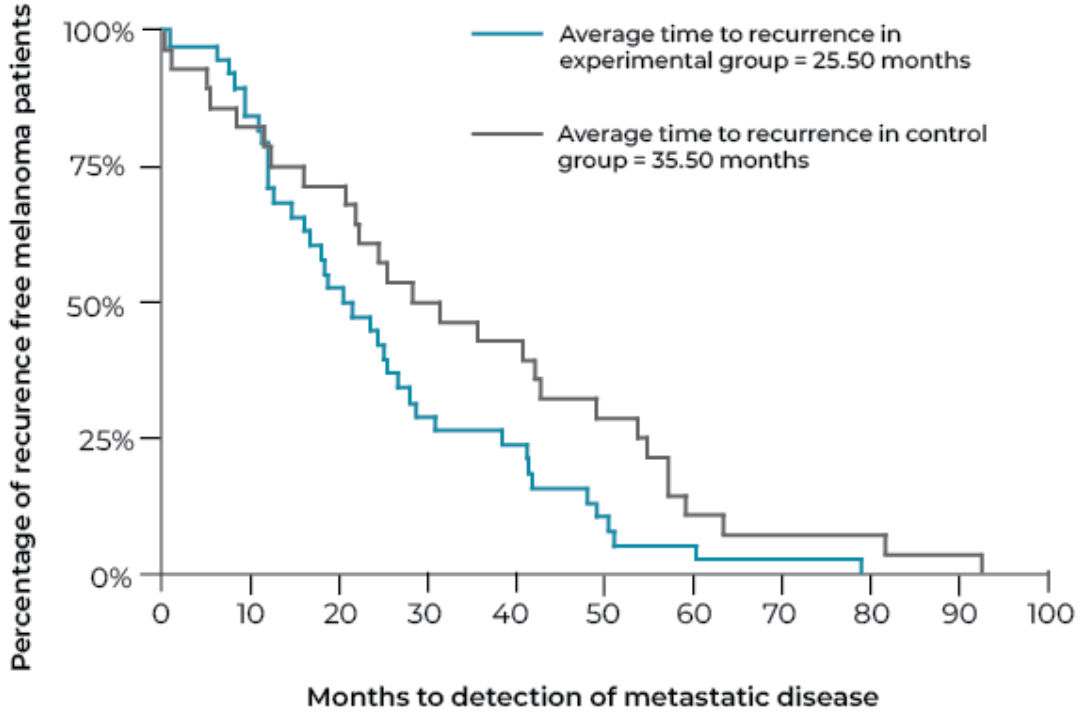


Figure 2: Kaplan–Meier curve illustrating percentage of recurrence free patients over time. P-value is significant at p=0.049.

Dhillon et al. *Archives of Derm Research.* 2023.

Summary: Real-world utility incorporating the 31-GEP: GEP-guided surveillance imaging

- Routine surveillance imaging in SLN-, high-risk patients detected melanoma recurrence ~10 months earlier than those without routine imaging.
- Tumor burden at detection was significantly lower in patients tested compared to those not tested (27.6mm vs 73.1mm)
- At study end, patients tested had better overall survival than those not tested (76% vs 50%, p-value= 0.027).

Dhillon et al. *Archives of Derm Research.* 2023.

Cutaneous Squamous Cell Carcinoma

Clinicopathologic risk assessment methods are in flux

Implication: Broad treatment plan options, need for improved risk stratification tools

Previous NCCN Guidelines (v1.2020):

Presence of a single clinicopathologic risk factor deems a patient "High Risk"

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NCCN Guidelines Version 1.2020
Squamous Cell Skin Cancer

RISK FACTORS FOR LOCAL RECURRENCE OR METASTASES

	Low Risk	High Risk
H&P		
Location/size[1]	Area L <20 mm Area M <10 mm[4]	Area L ≥20 mm Area M ≥10 mm Area H[5]
Borders	Well-defined	Poorly defined
Primary vs. recurrent	Primary	Recurrent
Immunosuppression	(-)	(+)
Site of prior RT or chronic inflammatory process	(-)	(+)
Rapidly growing tumor	(-)	(+)
Neurologic symptoms	(-)	(+)
Pathology (See SCC-A)		
Degree of differentiation	Well or moderately differentiated	Poorly differentiated
Acantholytic (adenoid), adenosquamous (showing mucin production), desmoplastic, or metaplastic (carcinosarcomatous) subtypes	(-)	(+)
Depth[2,3]: Thickness or level of invasion	≤6 mm and no invasion beyond subcutaneous fat	>6 mm or invasion beyond subcutaneous fat
Perineural, lymphatic, or vascular involvement	(-)	(+)

Area H = "mask areas" of face (central face, eyelids, eyebrows, periorbital, nose, lips [cutaneous and vermilion], chin, mandible, preauricular and postauricular skin/sulci, temple, and ear), genitalia, hands, and feet
Area M = cheeks, forehead, scalp, neck, and pretibia
Area L = trunk and extremities (excluding hands, nail units, pretibia, ankles, and feet)

Current NCCN Guidelines (v1.2024):

Presence of a risk factor deems a patient "High Risk" or "Very High Risk," depending on the factor

National Comprehensive Cancer Network®

NCCN Guidelines Version 1.2024
Squamous Cell Skin Cancer

NCCN Guidelines Index
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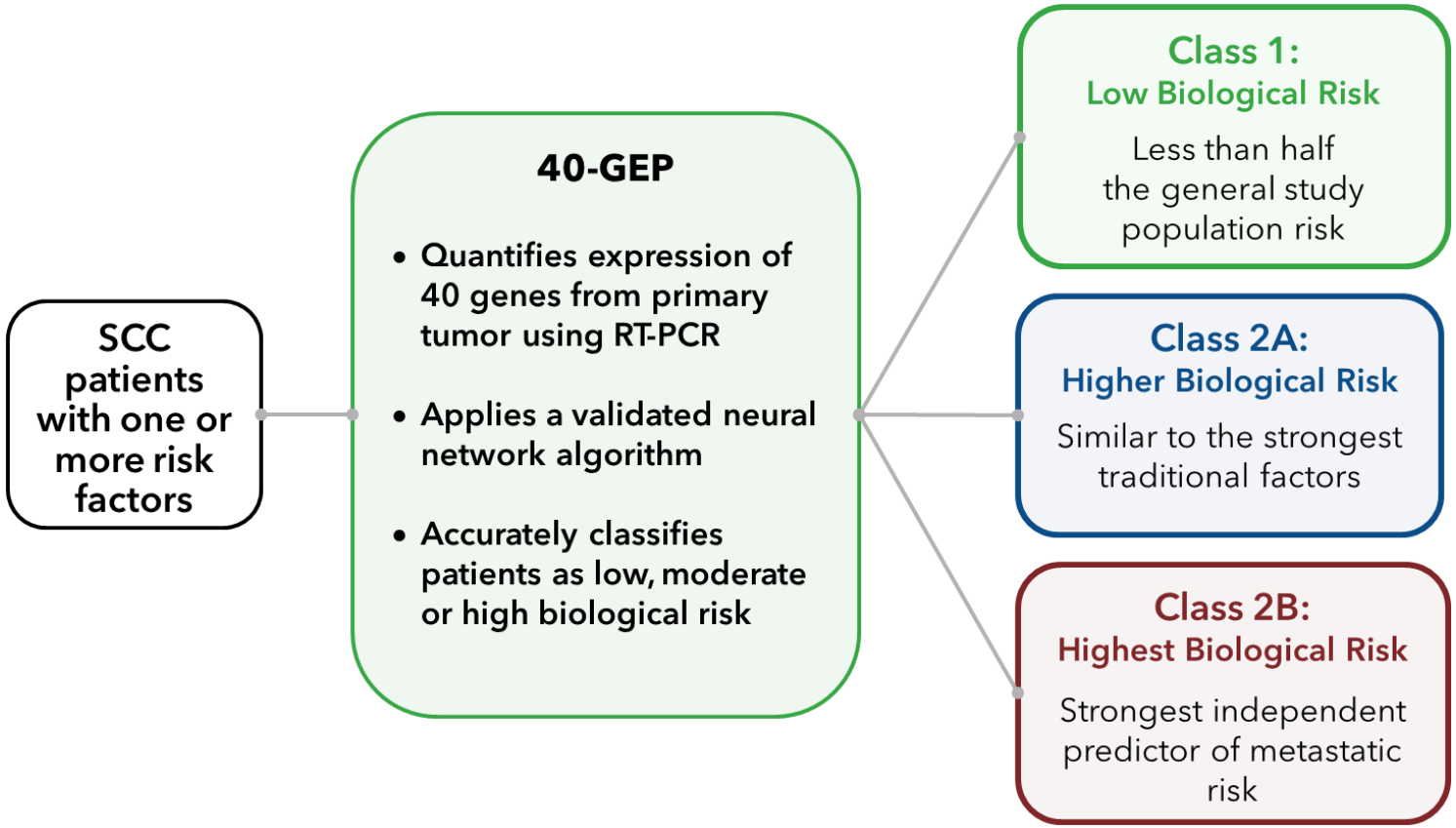
Discussion

STRATIFICATION TO DETERMINE TREATMENT OPTIONS AND FOLLOW-UP FOR LOCAL CSCC BASED ON RISK FACTORS FOR LOCAL RECURRENCE, METASTASES, OR DEATH FROM DISEASE

Risk Group[a]	Low Risk	High Risk	Very High Risk
Treatment options	SCC-3	SCC-4	SCC-4 and SCC-5
H&P			
Location/size[b]	Trunk, extremities ≤2 cm	Trunk, extremities >2 cm – ≤4 cm	>4 cm (any location)
		Head, neck, hands, feet, pretibia, and anogenital (any size)[e]	
Clinical extent	Well-defined	Poorly defined	
Primary vs. recurrent	Primary	Recurrent	
Immunosuppression	(-)	(+)	
Site of prior RT or chronic inflammatory process	(-)	(+)	
Rapidly growing tumor	(-)	(+)	
Neurologic symptoms	(-)	(+)	
Pathology (SCC-A)			
Degree of differentiation	Well or moderately differentiated		Poor differentiation
Histologic features: Acantholytic (adenoid), adenosquamous (showing mucin production), or metaplastic (carcinosarcomatous) subtypes	(-)	(+)	Desmoplastic SCC
Depth[c,d]: Thickness or level of invasion	<2 mm thick and no invasion beyond subcutaneous fat	2–6 mm depth	>6 mm or invasion beyond subcutaneous fat
Perineural involvement	(-)	(+)	Tumor cells within the nerve sheath of a nerve lying deeper than the dermis or measuring ≥0.1 mm
Lymphatic or vascular involvement	(-)	(-)	(+)

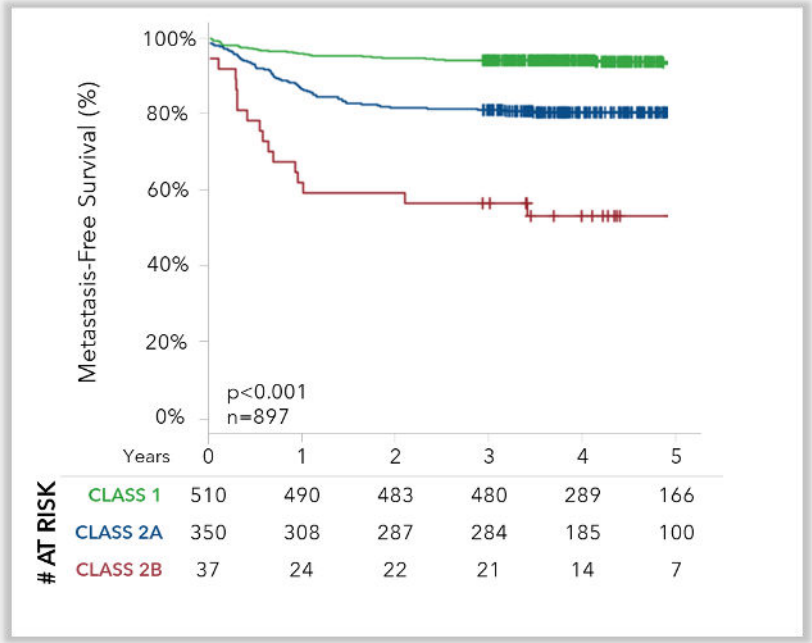
While the specific risk factors in NCCN and staging systems will likely change, clinicians use the presence or absence of clinicopathologic risk factors to identify patients at high risk for metastasis who will benefit from improved risk stratification to guide risk-aligned management decisions within guidelines

40-GEP predicts metastatic risk for SCC patients with one or more risk factors



Wysong et al. *JAAD* 2021; Ibrahim et al. *Future Oncology* 2021; Wysong et al. Oral Late-Breaking Abstract Presented at AAD 2023; Castle Biosciences data on file

40-GEP stratifies risk for metastasis in a merged cohort



40-GEP RISK CLASS	3-YEAR MFS (95% CI)	EVENT RATE
Class 1	94.1% (92.1-96.2%)	6.5%
Class 2A	81.1% (77.1-85.3%)	19.4%
Class 2B	56.8% (42.8-72.2%)	45.9%
OVERALL	87.5% (85.4-89.7%)	13.2%

40-GEP Merged Cohort

40-GEP performance is now confirmed across two independent studies.

Wysong et al. Oral Late-Breaking Abstract Presented at AAD 2023; Ibrahim et al. *Future Oncology* 2021; Castle Biosciences data on file; Manuscript submitted and under review with merged validation cohort data

40 –GEP Identifies Patients Likely to Respond, and not Respond, to Adjuvant Radiation Therapy (ART)

Adjuvant radiation therapy (ART) is a recommended treatment plan option for 'high-risk' SCC patients by all relevant guideline groups

ART recommended for cSCC with:

- Concern for perineural invasion (PNI) or
- High risk for regional or distant metastasis

ART recommended for primary cSCC if:

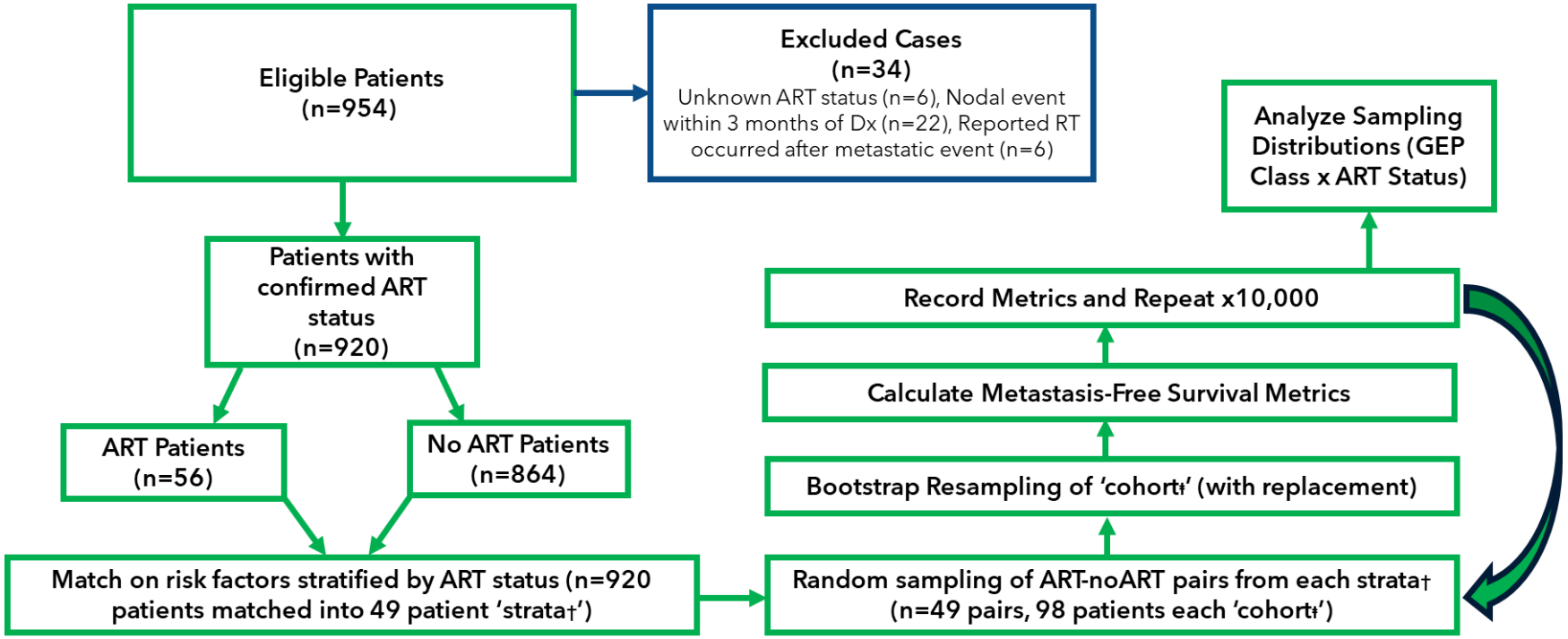
- Extensive perineural invasion (PNI)
- Large caliber nerve invasion (LCNI)
- Positive margins following surgery

ART recommended for cSCC if:

- Gross clinical or radiologic perineural invasion (PNI)
- Close surgical margins where further surgery cannot be performed
- Recurrent tumors
- AJCC8 T3 and T4 tumors
- Desmoplastic or infiltrative tumors in chronically immunosuppressed patients

Newman et al. *Head & Neck* 2021; Ruiz et al. *JAAD* 2022

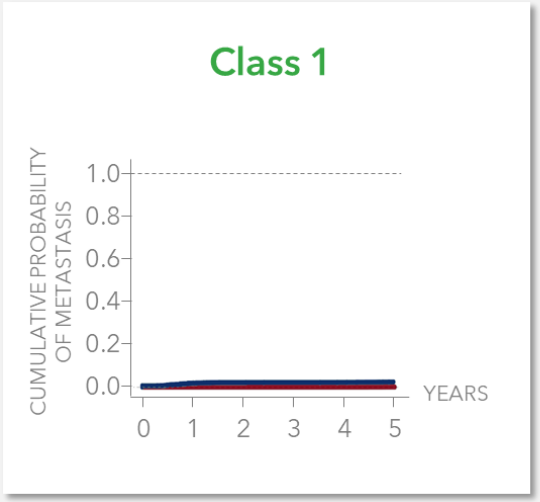
Validation approach to confirm 40-GEP's impact on adjuvant radiation therapy (ART) treatment benefit



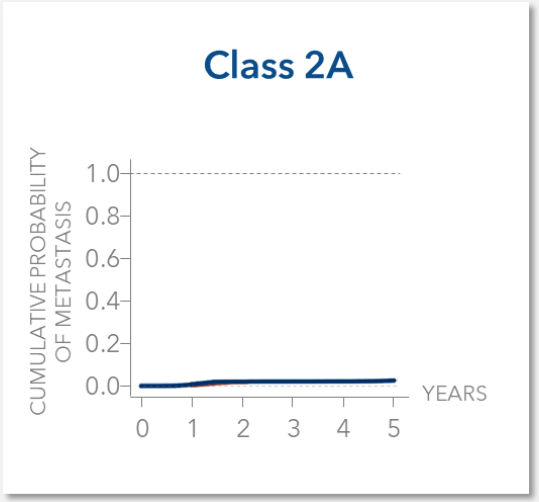
Wysong et al. Oral Late-Breaking Abstract Presented at AAD 2023; Ibrahim et al. *Future Oncology* 2021; Castle Biosciences data on file; Arron et al. Presented at Fall Clinical 2023; Manuscript submitted and under review with merged validation cohort data; † defined in notes; ‡ defined in notes

Adjuvant radiation therapy (ART)-treated class 2B patients see a significant reduction in the cumulative probability of metastasis over time

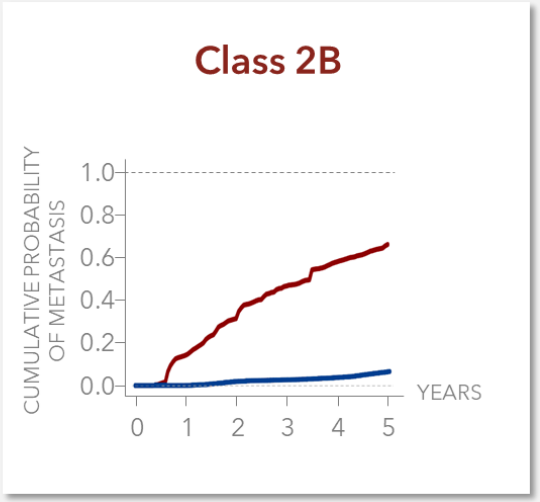
LEGEND:
- No ART
- ART



No significant impact of ART in cohort as a whole or within **Class 1**



No significant impact of ART in cohort as a whole or within **Class 2A**

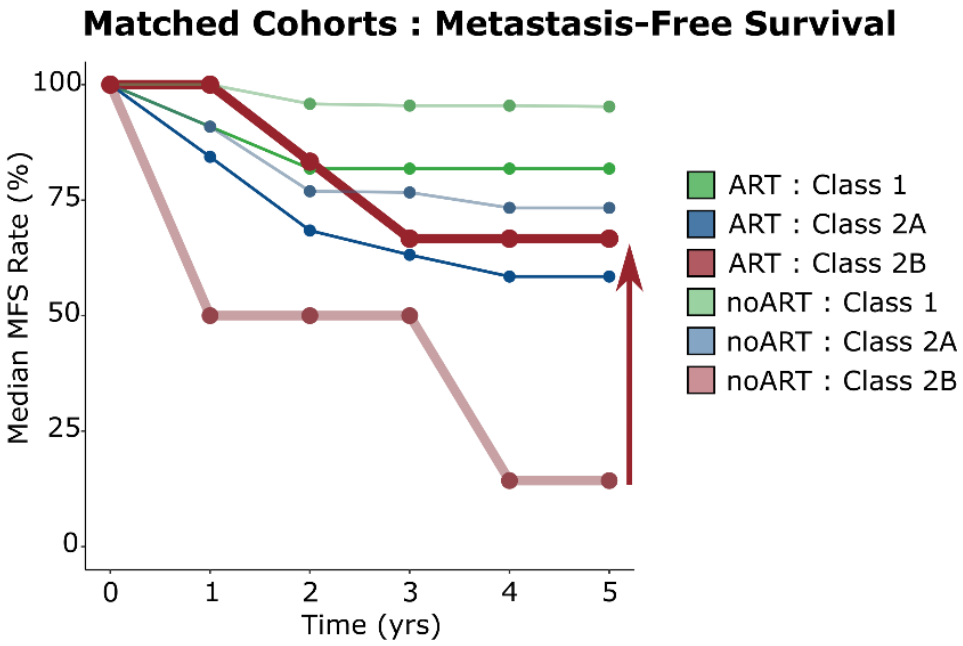


ART treated **Class 2B** patients see significant reduction in metastasis*

No ART merged cohort n=864, ART-treated merged cohort n=56

Arron et al. *The Journal of Skin* 2024; *Class 2B p<0.001

Matched control analysis supports use of 40-GEP to inform adjuvant radiation therapy (ART) treatment decisions



- This study is the single largest study evaluating benefit of ART.
- Patients were matched for risk factors and resampled into matched cohorts for ART treated and untreated patients.
- ART treated Class 2B* patient cohorts experienced a >50% reduction in metastasis on average compared to untreated patients.
- No significant ART benefit noted for Class 1 patients in this analysis.

*Class 2B $p<0.001$, n=920
Arron et al *The Journal of Skin* 2024

Thank You

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