A molecular model to identify patients who can safely forgo sentinel lymph node biopsy in primary cutaneous melanoma

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Background

- **Cutaneous melanoma accounts for less than 5% of skin cancer cases, but 75% of skin cancer deaths because of its ability to metastasize.[1,2]**
- **Staging of melanoma by AJCC 8th edition guidelines – the current classification system for describing the extent of disease and prognosis – relies on Breslow depth, ulceration, and whether disease has metastasized to sentinel lymph nodes (SLN) or beyond.[2,3,4]**
- **AJCC provides recommendations for patient selection for SLN biopsy (Fig. 1, right panel).**
- **More than 80% of patients who undergo SLN biopsy have no nodal metastasis, therefore they are unnecessarily exposed to procedure-induced morbidity such as lymphedema, seroma and infection.[5,6]**

Objective

Develop expression-based molecular markers to identify patients for whom the risk of nodal metastasis is so low that they can safely forego SLN biopsy, thus reducing procedure-associated morbidity and prioritizing care for high risk patients.

Method & Design

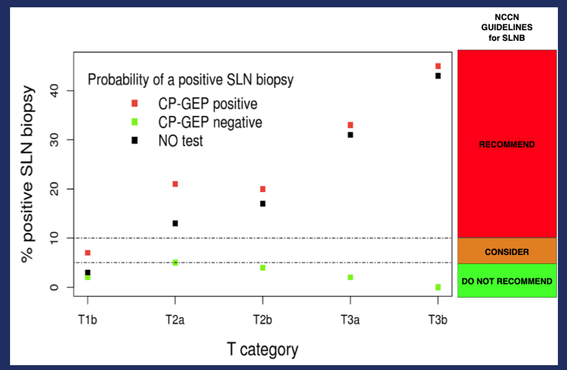
- **A targeted panel of gene expression assays was identified based on the analysis of RNA-Seq data, genomic databases, the cancer literature and case-control studies. 108 candidate biomarkers were selected and their expression was quantified in formalin-fixed paraffin-embedded diagnostic biopsy tissue across a cohort of 754 patients (Table 1).**
- **All patients underwent SLN biopsy at Mayo Clinic within 90 days of diagnosis between 2004 and 2018. Clinicopathologic variables were abstracted from the electronic medical record. Histopathology was reviewed by two or more board-certified Mayo Clinic dermatopathologists.**
- **We used a penalized maximum likelihood estimation algorithm[8] to train logistic regression models in a repeated cross-validation scheme.[7] Models were generated based on only clinicopathologic variables (CP), only gene expression profiling (GEP), or a combination of clinicopathologic and gene expression variables (CP-GEP).**

Table 1: Patient and tumor characteristics stratified by sentinel lymph node biopsy outcome.

Characteristic	SLNB negative (N=626)	SLNB positive (N=128)	P value †
Male gender, n (%)	393 (62.8%)	79 (61.7%)	0.82
Age at primary melanoma diagnosis (years), Mean (SD)	61.0 (15.87)	53.66 (17.11)	<0.001
Biopsy location, n (%)			0.13
Head and neck	122 (19.5%)	26 (20.3%)	
Trunk	202 (32.3%)	49 (38.3%)	
Upper extremity	160 (25.6%)	19 (14.8%)	
Lower extremity	91 (14.5%)	23 (18.0%)	
Acral	51 (8.1%)	11 (8.6%)	
Breslow depth (mm), n (%)			<0.001
0.5 – 1	186 (29.7%)	6 (4.7%)	
1.1 – 2	309 (49.4%)	50 (39.1%)	
2.1 – 4	131 (20.9%)	72 (56.2%)	
Clark level, n (%)			<0.001
II	6/620 (1.0%)	1/127 (0.8%)	
III	148/620 (23.9%)	7/127 (5.5%)	
IV	449/620 (72.4%)	118/127 (92.9%)	
V	17/620 (2.7%)	1/127 (0.8%)	
Mitotic rate type, n (%)			<0.001
Absent	92/623 (14.8%)	4/127 (3.1%)	
1 – 6	430/623 (69.0%)	82/127 (64.6%)	
> 6	101/623 (16.2%)	41/127 (32.3%)	
Ulceration, n (%)	114/622 (18.3%)	46 (35.9%)	<0.001
Angiolymphatic invasion, n (%)	19/526 (3.6%)	16/111 (14.4%)	<0.001
Regression, n (%)	47/518 (9.1%)	6/109 (5.5%)	0.22
Tumor invading lymphocytes, n (%)			0.04
Absent	140/550 (25.5%)	30/113 (26.5%)	
Non-brisk	302/550 (54.9%)	72/113 (63.7%)	
Brisk	108/550 (19.6%)	11/113 (9.7%)	
Microsatellitosis, n (%)	2/493 (0.4%)	2/107 (1.9%)	0.09
Histologic type, n (%)			--
Superficial spreading	336 (53.7%)	63 (49.2%)	
Nodular	112 (17.9%)	38 (29.7%)	
Desmoplastic	18 (2.9%)	0	
Lentigo maligna	32 (5.1%)	0	
Acral lentiginous	11 (1.8%)	4 (3.1%)	
Spindled	14 (2.2%)	1 (0.8%)	
Dermal	3 (0.5%)	0	
Spitzoid	5 (0.8%)	1 (0.8%)	
Nevoid	11 (1.8%)	0	
Unclassified	43 (6.9%)	13 (10.2%)	
Other	4 (0.6%)	0	
Mixed	6 (1.0%)	2 (1.6%)	
Not documented	31 (5.0%)	6 (4.7%)	

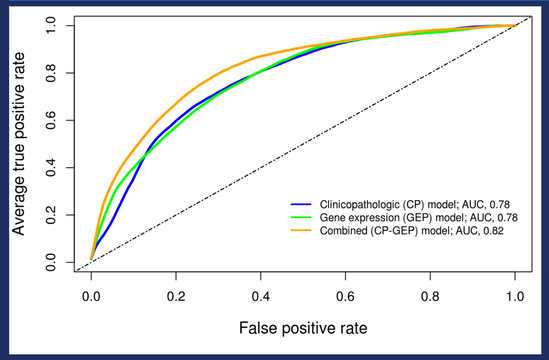
†Comparisons of the SLN biopsy negative and positive patients were performed using the chi-square test for categorical variables, the two-sample t-test for patient age, and the Wilcoxon rank sum test for all other variables.

Figure 1: Risk of SLN positivity by T categories



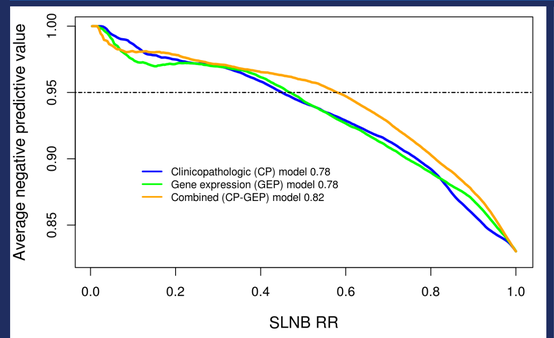
Risk of SLN biopsy positivity, starified by T categories. For each T group, we report the pre-test probability of a positive SLN (black square); the risk upon a positive CP-GEP test result (red square); and the risk upon a negative CP-GEP test result (green square). In the right panel, we report, with a traffic-light color scheme, the reccomendation for SLN biopsy, according to NCCN guidelines.

Figure 2: ROC curves



ROC curves. Blue line, model based on clinicopathologic variables (CP); green line, model based on gene expression (GEP); orange line, model based on gene expression and clinicopathologic variables combined (CP-GEP). Curves are averages over repeated cross validation runs.

Figure 3: SLNB RR vs NPV



Sentinel lymph node biopsy reduction rates (SLNB RR) versus negative predictive value (NPV). Blue line, model based on clinicopathologic variables (CP); green line, model based on gene expression (GEP); orange line, model based on gene expression and clinicopathologic variables combined (CP-GEP). Curves are averages over repeated cross validation runs.

Results

- **The combined model (CP-GEP) actionably changes the pre-test probability of SLN positivity for each T group (Fig. 1). In fact, across all T categories, the risk of having a positive SLN is not higher than 5%, upon a negative CP-GEP test result. This allows, in accordance with NCCN guidelines, to safely forgo the SLN biopsy for CP-GEP negative patients.**
- **The combined model (CP-GEP) can discriminate SLN+ from SLN- patients better than the models based solely on clinicopathologic variables or GEP, as quantified by the area under the curve (Fig. 2).**
- **The combined model (CP-GEP) can be used to reduce SLN biopsies, while maintaining a high negative predictive value (Fig. 3). More in detail, the CP-GEP model achieves a SLN biopsy reduction rate of about 60%, while keeping the false omission rate (i.e. 100%-NPV) below 5%.**

Conclusions

The CP-GEP model based on age, Breslow depth and stromal gene expression outperforms all other models in:

- **Identifying patients with a low risk (≤ 5%) of nodal metastasis who can safely forego a SLN biopsy across all T categories.**
- **Reducing SLN biopsy rates by up to 60%, while keeping the incorrect omission rate below 5%.**

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Disclosures

M.H.v.V., J.D., and D.B. are employees and option holders of SkylineDx BV; A.M. and Mayo Clinic have financial interest related to this research.