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Primary cutaneous melanoma risk stratification using a clinicopathologic and gene expression model: a pilot study

Dear Editor,

We have recently reported on the CP-GEP model to identify patients with primary cutaneous melanoma who may forgo the sentinel lymph node (SLN) biopsy procedure because of their low risk of nodal metastasis.[1,2] The CP-GEP model combines clinicopathologic (CP) variables, Breslow thickness, patient age, and the expression of eight genes related to epithelial-to-mesenchymal transition[3,4] to categorize patients into two groups: low risk or high risk for nodal metastasis (Fig. 1). Here, we report on a feasibility study of cutaneous melanoma patients seen at Mayo Clinic (MC) between October and December of 2019 who had their diagnostic biopsy tissue tested by CP-GEP in a CAP/CLIA-certified laboratory in San Diego (operated by SkylineDx). The primary objective of this study was to evaluate the feasibility of standardized CP-GEP testing in a certified laboratory in the United States and to evaluate and optimize the requisitioning logistics. This pilot study was conducted under the framework of the Falcon Melanoma R&D program, which aims to investigate the relevance of gene expression-based testing in personalized healthcare. The CP-GEP model for predicting nodal metastasis, which we here refer to as the Merlin test, is the first diagnostic test developed under this program. The human investigations performed in this study were completed after approval by the Mayo Clinic Institutional Review Board and in accordance with the requirements of the Department of Health and Human Services, where appropriate.

Fifty consecutive patients were identified by daily reviews of pathology reports. Charts were checked for eligibility criteria (see below) and if met, we requested 50 micron tissue recuts either mounted on charged glass slides or as five times 10 micron tissue curls through the MC anatomic pathology or MC pathology research core laboratory. Eligibility was determined based on histopathology data derived from patient medical records and established by two or more board-certified MC dermatopathologists. Patients were eligible for this study if they met criteria for an SLN biopsy by National Comprehensive Cancer Network guidelines.[4] Specifically, a patient was eligible if their melanoma was (i) tumor (T) stage 1a (Breslow thickness of less than 0.8 mm) with at least one of the following risk

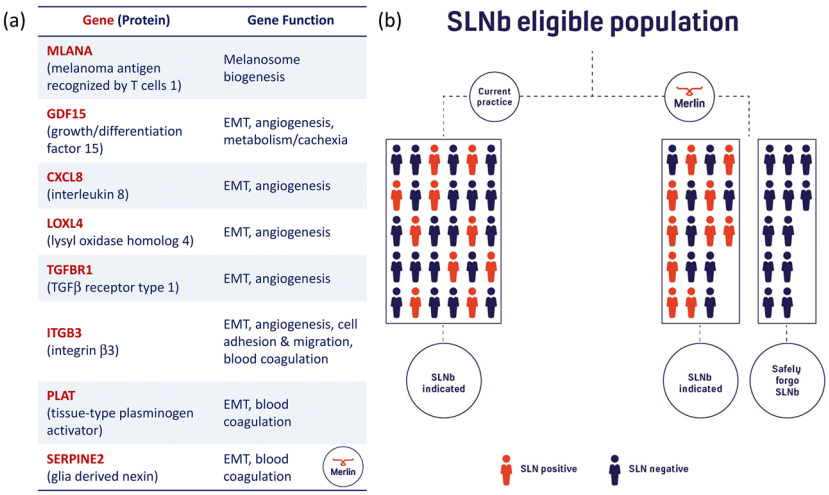

Figure 1 The CP-GEP model, which we refer to as the Merlin test, identifies patients with primary cutaneous melanoma who may forgo the sentinel lymph node biopsy (SLNb) procedure because of their low risk of nodal metastasis. (a) The CP-GEP model combines clinicopathologic variables, i.e. Breslow thickness and patient age, and the expression of eight genes. These genes serve biological functions in epithelial-to-mesenchymal transition (EMT) with specific roles in angiogenesis/hypoxia, coagulation, and melanosome biogenesis. (b) The Merlin test has the potential to reduce the sentinel lymph node biopsy rate by up to 80% for T1 disease and 42% for T2 disease.[1] In our pilot study, none of the negatively tested patients were found to have nodal metastasis.

factors: ulceration, mitoses present, and patient age <40 years; or (ii) T1b to T3 melanoma (Breslow thickness of 0.8–4 mm). All patients were at least 18 years of age and received care at MC. Formalin-fixed paraffin-embedded shave, punch, or excisional biopsy material was acceptable. If the pathology case consisted of more than one paraffin block, an MC dermatologist selected the block with the greatest tumor involvement for molecular testing. Exclusion criteria were: T4 melanoma (Breslow thickness greater than 4 mm); macroscopic nodal involvement, distant metastasis within 90 days of diagnosis, insufficient primary tumor tissue, and denial of access to medical records for research purposes (per Minnesota State law).

The vast majority of tissue samples tested, i.e. 46 of 50, was from shave biopsies. Of the remaining four samples, two were from punch and two from excisional biopsies. Twenty-four samples were received according to specifications defined in the tissue request form of which 16 arrived in presupplied containers and eight on glass slides. All samples and forms were received within 48 hours after sending. Turnaround time from sample receipt to test reporting was five working days during the peak phase of the study. On average more than two micrograms of total RNA could be extracted from samples. However, for three of the 50 samples, RNA yield did not meet prespecified quality control criteria, and the Merlin test was not performed. Of the 47 remaining patients, 34 underwent successful SLN biopsy within 90 days of diagnosis whereas 13 were without known SLN status because (i) SLN biopsy was not requested for T1a disease with risk factors (n = 9); (ii) SLN biopsy was attempted but failed (n = 2); (iii) patient no-showed for the procedure (n = 1). A summary of patient and tumor characteristics of all patients (n = 50) and patients with Merlin test results and known SLN status (n = 34) is shown in Table 1. One of 13 (7.7%) T1 patients, four of 13 (30.1%) T2 patients, and one of eight (12.5%) T3 patients were SLN positive. All SLN positive patients were correctively identified as high risk by the Merlin test. Metastatic tumor volume ranged from individual tumor cells to cell clusters 26 mm in diameter.

This study was a feasibility study and not intended for test validation. Merlin test development and initial validation data has been published.[1,5] Additional validation studies are ongoing as part of the Merlin Study Initiative under the Falcon R&D Program. We conclude from our pilot study that Merlin testing is feasible as a send-out test using primary melanoma diagnostic biopsy tissue which is routinely obtained in patient care.

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

Characteristic	Total samples (N = 50)	Merlin tested (N = 34)
Male gender, n (%)	30/50 (60%)	19/34 (55.9%)
Age at SLN (years), mean (SD)	56.59 (18.48)	56.76 (17.11)
Biopsy location, n (%)		
Head and neck	8/50 (16%)	5/34 (14.7%)
Trunk	22/50 (44%)	13/34 (38.2%)
Upper extremity	13/40 (26%)	11/34 (32.4%)
Lower extremity	5/50 (10%)	3/34 (8.8%)
Acral	2/626 (4%)	2/34 (5.9%)
Breslow thickness (mm), n (%)		
0.5–1 (T1)	25/50 (50%)	13/34 (38.2%)
1.1–2 (T2)	14/50 (28%)	13/34 (38.2%)
2.1–4 (T3)	11/50 (22%)	8/34 (23.5%)
Mitotic rate type, n (%)		
Absent	13/50 (26%)	8/34 (23.5%)
1–6	32/50 (64%)	23/34 (67.7%)
>6	5/50 (10%)	3/34 (8.8%)
Ulceration, n (%)	12/50 (12%)	8/34 (8.8%)
Histologic type, n (%)		
Superficial spreading	31/50 (62%)	18/34 (52.9%)
Nodular	10/50 (20%)	9/34 (26.5%)
Desmoplastic	2/50 (4%)	1/34 (2.9%)
Acral lentiginous	1/50 (2%)	1/34 (2.9%)
Spitzoid	2/50 (4%)	2/34 (5.9%)
Nevoid	1/50 (2%)	1/34 (2.9%)
Unclassifiable	3/50 (6.0%)	2/34 (5.9%)

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Evaluation of the sale of unregulated dermal fillers on e-commerce websites

Dear Editor,

Dermal fillers are cosmetic injectables that enhance facial contours by volumizing the skin to diminish signs of aging. In the United States, the Food and Drug Administration (FDA) oversees drug quality, efficacy, and safety to protect patients from substandard or dangerous medications.[1] Despite their high-risk (class III) classification, third party injectables from unlicensed suppliers remain available to the public.[2-4] In this cross-sectional study, we sought to characterize dermal fillers and manufacturer claims on two leading e-commerce websites.

In September and October 2019, the retailing websites Alibaba.com and Made-in-China.com were queried using the search terms "dermal filler," "hyaluronic acid," "collagen injectable," and "silicone injection." Product and seller data for the first 100 products for each search term, excluding injection devices without injectable material, topical cosmetic products (i.e., serums), and non-dermatologic products, were collected for each item. To compare price data, the average cost per syringe, per 1 ml and per 20 mg, was calculated for products in syringes, in vials, and as powders, respectively.

On Alibaba.com, a total of 342,405 products resulted for search terms "dermal filler" (n = 65,712), "hyaluronic acid" (n = 215,708), "collagen injectable" (n = 2,489), and "silicone injection" (n = 58,496). On Made-in-China.com, 57,802 products resulted for search terms "dermal filler" (n = 7,067), "hyaluronic acid" (n = 13,833), "collagen injectable" (n = 541), and "silicone injection" (n = 36,361).

After exclusions, 265 and 286 unique products were identified on Alibaba.com and Made-in-China.com, respectively. Most products were composed of hyaluronic acid (Alibaba.com 95.09%, Made-in-China.com 97.19%) and sold as prefilled syringes with needle attachments provided (Alibaba.com 87.55%, Made-in-China.com 73.78%) (Table 1). Some products were sold as vials of injectable liquid without an injection device (Alibaba.com 6.79%, Made-in-China.com 1.05%) or in powder form (Alibaba.com 5.66%, Made-in-China.com 25.17%). Per injection, prefilled syringes were the most expensive (Alibaba.com \$60.55 ± 51.41 per syringe, Made-in-China.com \$63.96 ± 51.52 per syringe), and powder was least expensive (Alibaba.com

Table 1 Characteristics of products

	Alibaba	Made-in-China
Injection material	Percentage (n)	Percentage (n)
Hyaluronate/ hyaluronic acid	95.09% (252)	97.19% (277)
Poly-L-lactic acid	1.13% (3)	0% (0)
Herbal/ homeopathic	0.75% (2)	1.05% (3)
Other	1.13% (3)	1.75% (5)
Not provided	1.89% (5)	0% (0)
Product type	Percentage (n)	Percentage (n)
Prefilled syringe with needles	87.55% (232)	73.78% (211)
Vial with liquid injectable	6.79% (18)	1.05% (3)
Powder	5.66% (15)	25.17% (72)
Price	Mean ± SD (range, n)	Mean ± SD (range, n)
Syringe (per 1 syringe)	\$60.55 ± 51.41 (15–300, 231)	\$63.96 ± 51.52 (1–160, 107)
Vial (per 1 ml)	\$3.33 ± 3.02 (0.30–8.00, 8)	\$2.37 ± 1.09 (1.60–3.14, 2)
Powder (per 20 mg)	\$1.73 ± 4.26 (0.0025–13.86, 13)	\$0.64 ± 4.03 (0.0002–32.00, 63)
Minimum quantity per purchase	Mean ± SD (range, n)	Mean ± SD (range, n)
Syringe (syringe)	4.55 ± 15.46 (1–100, 232)	4.28 ± 11.98 (1–100, 211)
Vial (ml)	39.09 ± 14.80 (5–50, 11)	212.50 ± 53.03 (175–250, 2)
Powder (g)	802.07 ± 649.11 (1–2000, 15)	19,409.50 ± 112,896.39 (0.01–907185.00, 66)
Certifications	Percentage (n)	Percentage (n)
CE/ISO	86.79% (230)	87.41% (250)
FDA	16.98% (45)	12.59% (36)
GMP	26.79% (71)	29.02% (83)
SGS	11.32% (30)	36.36% (104)
	Mean ± SD (range, n)	
Product rating	4.75 ± 0.64 (1–5, 57)	[a]
	Mean ± SD (range, n)	
Number of reviews	3.02 ± 2.96 (1–11, 57)	[a]

SD, Standard Deviation; CE, Conformité Européene; ISO, International Organization for Standardization; FDA, Food and Drug Administration; GMP, Good Manufacturing Practices; SGS, Société Générale de Surveillance.
[a]Product ratings were unavailable on Made-in-China.com.