Using a 31-gene expression profile test to stratify patients with sentinel lymph node negative stage I-II cutaneous melanoma according to recurrence risk: Longer-term follow-up from a prospective, multicentre study

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BACKGROUND

- Patients diagnosed with stage I-II cutaneous melanoma (CM) according to the American Joint Committee on Cancer staging guidelines have ≥90% 5-year melanoma-specific survival.[1] However, many patients do experience a recurrence.
- The 31-gene expression profile (31-GEP) prognostic test for cutaneous melanoma uses gene expression measurements from primary tumors to classify a patient's recurrence risk as low (Class 1A) intermediate (Class 1B/2A), or high (Class 2B) (**Figure 1**) and performance has been validated in multiple prospective and retrospective studies.[2-15]
- We previously reported early outcome data from a prospective, multicentre study with a median follow-up of 2.2 years, and we hypothesized that longer follow-up would confirm the previously reported 31-GEP risk of recurrence stratification for disease-free survival (DFS) in this sentinel lymph node negative stage I-II CM population.

Results

Table 1**. Patient Demographics.**

Feature	Class 1A (n=40)	Class 1B/2A (n=25)	Class 2B (n=21)	*P*-value
Age, median years, (range)	58 (26-79)	66(23-82)	65 (32-86)	.165*
Male sex, % (n/N)	40% (16/40)	44% (11/25)	62% (13/21)	.380†
Breslow, mm median (range)	1.3 (0.4-7.0)	2.0 (0.8-10.0)	3.5 (1.1-15.0)	<.001*
Mitoses/mm^2, median (range)	1 (0-6)	3 (0-12)	8 (0-32)	<.001*
Ulceration, % (n/N)	3% (1/40)	40% (10/25)	71% (15/21)	<.001†
Recurrence, % (n/N)	5% (2/40)	12% (3/25)	33% (7/21)	.021†
Distant Metastasis, %, (n/N)	0% (0/40)	4% (1/25)	14% (3/21)	.041†

*Kruskal-Wallis test. †Pearson Chi-square test.

Table 2. **Cox univariate regression analysis for 3-year DFS.**

Feature	Univariate HR (95% CI)	*P*-value
31-GEP Class 1B/2A	2.8 (0.5-17.2)	.253
31-GEP Class 2B	8.4 (1.7-40.7)	.008

Results

Figure 1. **DFS in patients with stage I-II CM.** Kaplan-Meier analysis demonstrated significant stratification of DFS. A Class 2B 31-GEP was associated with a significantly lower 3-year DFS than a Class 1A 31-GEP result.

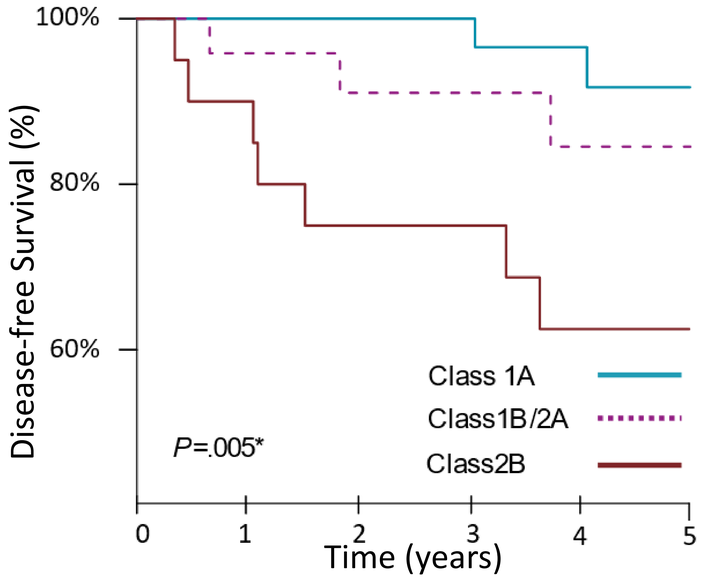

Table 3. **Recurrence rates and locations in patients with stage I-II CM.** Distant recurrence was more likely in patients with a Class 1B/2A or Class 2B 31-GEP result.

Population	3-yr DFS (95%CI)	Recurrences		
		All (% population)	Loco-Regional (% of events)	Distant (% of events)
Class 1A (N=40)	100% (100-100%)	2/40 (5.0%)	2/2 (100%)	0/2 (0%)
Class 1B/2A (N=25)	91.0% (79.9-100%)	3/25 (12.0%)	2/3 (66.7%)	1/3 (33.3%)
Class 2B (N=21)	75.0% (58.2-96.6%)	7/21 (33.3%)	4/7 (57.1%)	3/7 (42.9%)

*Log-rank test: X^2=10.4, degrees of freedom: 2.

Table 4**. Accuracy metrics for the 31-GEP. Stage I-II (N=86)**

Metric	%, (95% CI)	Likelihood Ratios
Sensitivity	77.8% (40.2-96.1%)	Positive:
Specificity	73.1% (58.7-84.0%)	2.9 (1.6-5.1)
PPV	33.3% (15.8-56.9%)	Negative:
NPV	95.0% (81.8-99.1%)	0.3 (0.1-1.0)

Class 1A was used as a negative result and Class 2B as a positive result. PPV: positive predictive value; NPV: negative predictive value.

CONCLUSIONS

- Significantly different risk profiles are maintained for Class 1A, 1B/2A, and 2B groups after a longer follow-up of 3.9 years,
- No distant metastatic events occurred in the Class 1A population.
- Patients with a Class 2B result had three times the odds of recurrence than those with a Class 1A result.
- The 31-GEP significantly differentiates recurrence risk in sentinel lymph node negative patients, which may allow for better resource allocation to patients at the highest risk of melanoma recurrence.

METHODS

- Primary tumors were tested with the 31-GEP from 86 patients with sentinel lymph node negative stage I-II CM staged according to the American Joint Committee on Cancer version 7 enrolled from 2015-2016 from five tertiary melanoma referral centres in Spain.
- The median follow-up time for the cohort was 3.9 years (compared to 2.2 years in a previously published report).
- Kaplan-Meier analysis and log-rank tests were used to compare 3-year disease-free survival (DFS).
- Univariate Cox analysis was used to determine the impact of a Class 2B vs. a Class 1A 31-GEP result on DFS prognosis.
- Prognostic accuracy was determined using Class 1A as a negative result and Class 2B as a positive result.

REFERENCES, FUNDING & DISCLOSURES

1. Gershenwald, *et al. CA Cancer J Clin.* 2017;67(6):472-492
2. Gerami P, et al. *Clin Cancer Res* 2015;21:175-83
3. Gerami P, et al. *J Am Acad Dermatol* 2015;72:780-5 e783
4. Zager JS, et al. *BMC Cancer* 2018;18(1):130
5. Gastman BR, et al. *J Am Acad Dermatol* 2019;80(1):149-157
6. Hsueh EC, et al. *J Hematol Oncol* 2017;10:152
7. Greenhaw B, et al. *Dermatol Surg* 2018;44(12):1494-1500
8. Cook RW et al. *Diagn Pathol*. 2018;13(1):13
9. Berger AC et al. *Curr Res Med Opin* 2016;32(9):1599-604
10. Dillon LD et al. *SKIN: J Cut Med* 2018;2(2):111-21
11. Schuitevoerder D et al. *J Drugs Dermatol* 2018;17(2):196-199
12. Farberg AS et al. *J Drugs Dermatol* 2017;16(5):428-431
13. Gershenwald et al. *CA Cancer J Clin* 2017;67(6):472-492
14. Hsueh EC, et al. *J Clin Oncol* 2016;34(15_suppl):9565
15. Greenhaw, et al. *JAAD* 2020;83(3):745-753
16. Podlipnik, et al. *JEADV* 2019:33(5):857-862

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