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PD-1, PD-L1 and BIM as predictors of sentinel lymph node metastasis in primary cutaneous melanoma

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Letter to the Editor

Current risk stratification methods for primary cutaneous melanoma are based on clinicopathologic variables and are limited in their ability to identify patients at risk of metastasis (Morton et al., 2014). It has previously been shown that other ways of characterizing melanoma such as using the tumor cell adhesion marker integrin $\beta 3$ through

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DATA AVAILABILITY

Data used in this study are available upon request from AM.

CONFLICT OF INTEREST

The authors state no conflict of interest.

immunohistochemistry (IHC) (Hieken et al., 1996) or quantitative reverse transcription polymerase chain reaction (qRT-PCR) (Meves et al., 2015) are useful in discriminating between primary cutaneous melanomas that had or had not metastasized, for example, to sentinel lymph nodes (SLN). We sought to understand if other molecular markers like the PD-1/PD-L1 immune checkpoint are useful in the risk stratification of melanoma.

PD-L1 expression on tumor cells inhibits tumor rejection by engaging the PD-1 receptor on cytotoxic T cells, which inhibits T cell receptor function (Iwai et al., 2002). Here we tested the hypothesis that high PD-L1 and PD-1 expression in primary cutaneous melanoma biopsies correlates with tumor aggressiveness as defined by sentinel lymph node biopsy (SLNb) positivity within 90 days of diagnosis. We also tested whether BCL-2-interacting mediator of cell death (BIM) expression, a marker of PD-1 receptor engagement with PD-L1 (Dronca et al., 2016), correlates with SLNb positivity.

We utilized a cohort of 754 patients with thin and intermediate thickness primary cutaneous melanoma as previously reported (Bellomo et al., 2020) who underwent SLNb within 90 days of diagnosis at Mayo Clinic between 2014 and 2018 (see Table S1). Among the 754 patients, 128 (17.0%) had a positive SLNb. Clinicopathologic features were abstracted. We then used qRT-PCR to quantify gene expression for PD-1, PD-L1, BIM and integrin $\beta 3$. A statistical analysis was performed comparing qRT-PCR gene expression between patients with and without SLNb positivity (see Supplementary Text).

Based on univariate analysis, both BIM and integrin $\beta 3$ expression were significantly associated with SLNb positivity (Table 1). Integrin $\beta 3$ expression was previously shown to correlate with risk of SLN involvement (Arias-Mejias et al., 2020, Bellomo et al., 2020) and was quantified here as a positive control. For both PD-1 and PD-L1 expression there were no significant associations with SLNb positivity (Table 1). Although BIM was still significantly associated with SLNb positivity in a multivariable logistic regression model adjusted for Breslow depth and age (Table 1), the incremental gain in predictive ability as assessed by the area under the curve of a receiver operating characteristic was minimal between multivariable models including the two clinical risk factors alone versus with BMI expression; AUC, 0.78, 95% CI 0.74–0.82, for both models; Figure 1. This contrasts previous results for the same cohort where the predictive ability of a model that only considered Breslow depth with age was outperformed by a model which included cell adhesion-derived molecular variables including integrin $\beta 3$ in combination with Breslow depth and age (Bellomo et al., 2020).

In addition to the qRT-PCR analysis, we also evaluated PD-1 and PD-L1 expression by IHC in a subset of 90 patients from our 754 patient cohort (see Table S1). No significant relationship was detected between PD-1 and PD-L1 protein expression and SLNb positivity (see Table S2).

Previous research has shown that the integrin $\beta 3$ adhesion receptor, which plays a key role in cancer cell metastasis (Desgrosellier and Cheresch, 2010), is useful for primary cutaneous melanoma risk stratification (Bellomo et al., 2020, Hieken et al., 1996, Meves et al., 2015). This finding is informative since it provides a variable to use for predicting nodal metastasis

that is independent of the conventional clinicopathologic variables and can improve the predictive ability of SLN metastasis, e.g. in multivariable models (Bellomo et al., 2020). In addition, the confirmation of integrin $\beta 3$ expression correlating with nodal metastasis provides a comparison point when analyzing our PD-1/PD-L1 gene expression results.

For the PD-1/PD-L1 immune checkpoint, our data shows no correlation of PD-1/PD-L1 expression in primary melanoma with SLN metastasis. The BIM marker showed a small significant difference in expression univariately, but when combined with the well-known predictive clinicopathologic variables of Breslow depth and age in a multivariable model, adding BIM did not do improve the performance of this model. In addition, when PD-1/PD-L1 and BIM expression competed for the inclusion into a multivariable model for the identification of primary melanoma at risk of nodal metastasis (Bellomo et al., 2020), the feature selection algorithm preferred genes with biological functions in cell adhesion and angiogenesis such as integrin $\beta 3$. These data indicate that the expression of the PD-1/PD-L1/BIM pathway in primary melanoma is not as closely linked to tumor cell metastasis as other biological systems.

This is an intriguing finding since PD-1/PD-L1 expression serves to inhibit the immune killing of neoplastic cells. In contrast to integrin adhesion which provides the sine qua non mechanical infrastructure for tumor cell intravasation and metastasis (Lämmermann et al., 2008), PD-1/PD-L1 interactions and BIM expression depend on the priming and activation of T cells. One potential reason that PD-1/PD-L1 and BIM expression did not associate with SLNb positivity is that the release of cancer cell antigens, cancer antigen presentation and T cell priming and trafficking is controlled by molecular processes unrelated to primary tumor cell migration and intravasation and may or may not occur in primary melanoma irrespective of its metastatic potential.

Our findings are supported by observations from other authors. For example, studies have shown marked heterogeneity of PD-L1 expression in primary tissue (Madore et al., 2015) and no significant association with survival in patients with metastasized melanoma (Gadiot et al., 2011). However, PD-L1 expression signaled a better response to anti-PD-1 therapy (Daud et al., 2016, Robert et al., 2015), especially if administered in combination with the anti-CTLA4 drug ipilimumab (Larkin et al., 2015). Because PD-L1 negative patients also respond to anti-PD-1 therapy albeit at a lower rate, PD-L1 expression testing is not always pursued in clinical practice (Patel and Kurzrock, 2015).

In conclusion, our study does not show a strong association between PD-1/PD-L1 and BIM expression with SLN metastasis in primary cutaneous melanoma. PD-1/PD-L1 and BIM expression may occur in primary melanoma irrespective of its metastatic potential. Once melanoma has metastasized, response to anti-PD-1 therapy is associated with PD-L1 expression.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Abbreviations:

BIM	BCL-2-interacting mediator of cell death
IHC	immunohistochemistry
PD-1	programmed cell death protein 1
PD-L1	programmed cell death 1 ligand 1
qRT-PCR	quantitative reverse transcription polymerase chain reaction
SLN	sentinel lymph node
SLNb	sentinel lymph node biopsy

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Figure 1. Receiver operative characteristic (ROC) curves for discriminating between SLNb positive and negative patients.

Table 1.

Ct Gene expression levels stratified by sentinel lymph node biopsy outcome

Molecular markers: Protein (<i>gene name</i>)	SLNb negative (N=626)	SLNb positive (N=128)	P value unadjusted[†]	P value adjusted[‡]
PD-L1 (<i>CD274</i>)			0.77	0.85
Mean (SD)	7.6 (1.2)	7.6 (1.5)		
Median (IQR)	7.7 (6.9, 8.5)	7.7 (6.8, 8.5)		
Range	(0.4–11.8)	(0.5–10.5)		
PD-1 (<i>CD279</i>)			0.06	0.09
Mean (SD)	7.6 (1.4)	7.9 (1.7)		
Median (IQR)	7.6 (6.6, 8.6)	8.0 (6.8, 9.1)		
Range	(1.5–12.6)	(1.7–11.7)		
BIM (<i>BCL2L11</i>)			0.004	0.04
Mean (SD)	6.3 (0.8)	6.1 (1.0)		
Median (IQR)	6.3 (5.8, 6.8)	6.0 (5.6, 6.6)		
Range	(2.0–8.9)	(1.2–9.6)		
Integrin $\beta 3$ (<i>ITGB3</i>)			<0.001	<0.001
Mean (SD)	6.9 (1.4)	5.6 (1.7)		
Median (IQR)	7.1 (6.1, 7.8)	5.7 (4.3, 6.9)		
Range	(2.4–10.8)	(1.1–10.2)		

[†]Comparisons between the SLNb negative and positive groups were evaluated using the two-sample t-test.

[‡]The association of each molecular marker with SLNb positivity was evaluated in a separate multivariable logistic regression model adjusted for patient age and Breslow depth.