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Validated programmed cell death ligand 1 immunohistochemistry assays (E1L3N and SP142) reveal similar immune cell staining patterns in melanoma when using the same sensitive detection system

Kelly A Schats,[1,2] Emily A Van Vré,[1] Stefanie De Schepper,[1] Carolien Boeckx,[1] Dorien M Schrijvers,[1] Wim Waelput,[3,4] Erik Fransen,[5] Isabelle Vanden Bempt,[4] Bart Neyns,[6] Ingrid De Meester[2] & Mark M Kockx[4]

[1]*Department of Immunohistochemistry, HistoGeneX,* [2]*Department of Medical Biochemistry, University of Antwerp, Antwerp,* [3]*Department of Pathology, Universitair Ziekenhuis, Brussels,* [4]*Department of Molecular Pathology, HistoGeneX,* [5]*StatUa Center for Statistics, University of Antwerp, Antwerp, and* [6]*Medical Oncology, Universitair Ziekenhuis, Brussels, Belgium*

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Aims: Tumour cell and/or immune cell programmed cell death ligand 1 (PD-L1) expression is considered as a potential biomarker for anti-PD1 and anti-PD-L1 immunotherapy. Currently, different PD-L1 assays are used. This study aims to compare the staining patterns of two PD-L1 antibody clones in melanoma metastases and correlate them with PD-L1 mRNA expression.

Methods and results: The immunohistochemistry assays were optimized and validated independently on a Ventana Benchmark Ultra (Ventana Medical Systems Inc., Tucson, AZ, USA) (E1L3N) and XT (SP142), using the same detection system. In total, 46 melanoma metastases were stained with both validated immunohistochemistry assays. Stained slides were digitized for qualitative and semi-quantitative evaluation; done by pathologist and semi-automated software analysis. A subset of 21 melanoma metastases was selected for quantification of the PD-L1 mRNA expression. Accuracy and precision criteria were met for both assays. PD-L1 protein and mRNA expression showed remarkably good Spearman's coefficients of 0.90 (E1L3N) and 0.87 (SP142). Despite the remarkable correlation between both PD-L1 assays in expression patterns and quantification values ($\rho > 0.90$), E1L3N showed significantly more tumour cell staining than SP142.

Conclusions: E1L3N and SP142 IHC assays were optimized and validated successfully and independently for sensitive and accurate PD-L1 detection. Concordance was best for immune cell scoring, while E1L3N tended to detect more tumour cells. Determination of the clinically relevant cut-off values for immune cell versus tumour cell detection requires further research.

Keywords: E1L3N, immunohistochemistry, Nanostring, PD-L1, SP142

Address for correspondence: K A Schats, HistoGeneX NV, Prinses Josephine Charlottelaan 10, 2600 Antwerp, Belgium.
e-mail: kelly.schats@histogenex.com

Introduction

PROGRAMMED CELL DEATH LIGAND 1 (PD-L1)/ PROGRAMMED CELL DEATH 1 (PD-1) PATHWAY

PD-L1 (CD274, B7-H1) is one of the ligands that binds to the PD-1 receptor. In normal physiology, this PD-L1/PD-1 pathway plays an important role in the prevention of autoimmunity, as PD-L1 is a negative checkpoint molecule which dampens ongoing immune responses.[1,2] The ligand can be expressed by lymphocytes (T and B cells), antigen-presenting cells, endothelial cells and macrophages. The expression of PD-L1 is regulated by different mechanisms and can be induced by interferon (IFN)-γ. By contrast, PD-1 has a more narrow expression profile. It is found generally on T, B and natural killer (NK) cells after antigen recognition. More detailed information on PD-L1/PD-1 function, expression and regulation can be found in recent reviews.[2,3]

PD-L1/PD-1 PATHWAY IN ONCOLOGY

Recently, the PD-L1/PD-1 pathway has become the focus of attention in oncology, as therapeutic benefits were discovered by blocking either the ligand (PD-L1) or its receptor (PD-1).[4,5] It became clear that this pathway is an important tumour immune-escape mechanism, as PD-L1 can also be expressed by tumour cells (TC). Clinical trials targeting the PD-L1/PD-1 pathway are ongoing and show promising results in different tumour entities.[6–10] Nivolumab and pembrolizumab were the first anti-PD-1 compounds receiving Food and Drug Administration (FDA) approval in melanoma (2014). More recently (2015), they received approval for additional therapeutic indications [either or both non-small-cell lung cancer (NSCLC) or kidney cancer]. In contrast to melanoma, the FDA has approved both anti-PD-1 compounds and PD-L1 immunohistochemistry (IHC) assays as, respectively, companion (22C3 Dako assay; Dako, Carpinteria, CA, USA) and complementary (28-8 Dako assay) diagnostics in NSCLC. However, assessing PD-L1 expression in tumour tissue is complicated by many challenges.[11] Differences between tumour entities, PD-L1/PD-1 compounds or therapy schedules might explain the variable predictive value of PD-L1 IHC. Furthermore, it is important to note that different IHC assays (i.e. different primary antibodies and assay conditions) and different PD-L1 IHC evaluation methods (i.e. different scoring methods and PD-L1 positivity cut-offs) complicate further the comparison between clinical studies. This might imply the application of a range of different PD-L1 IHC assays and scorings in order to determine eligibility to a specific anti-PD-1/PD-L1 compound.[12]

The aim of this study was to compare the performance and staining pattern of two in-house-developed PD-L1 IHC assays, using the commercially available E1L3N (Cell Signaling Technologies, Danvers, MA, USA) and SP142 (Spring Bioscience, Pleasanton, CA, USA) PD-L1 antibody clones. By applying different evaluation methods, a more extensive comparison of the two PD-L1 assays was endorsed.

Material and methods

TUMOUR TISSUE

All formalin-fixed paraffin-embedded (FFPE) samples (both validation and comparison) were obtained in accordance with the Helsinki Declaration of 1975 and approved by the hospital ethics committee (EC/PC/avl/2016.003 and CME 2010/266). They were all coded to protect the privacy of patients and blood donors.

PD-L1 IMMUNOHISTOCHEMISTRY

Two anti-PD-L1 IHC assays were developed using either clone E1L3N or clone SP142. The protocol of both assays is summarized in Table 1 and was optimized independently on 5-μm FFPE slides. Each run was qualified by including two tonsil tissue slides stained with a positive (E1L3N or SP142) and a negative (matched isotype DA1E; Cell Signaling Technologies) protocol.

PD-L1 IHC EVALUATION

The PD-L1 stained slides were digitized using a digital slide scanner (3DHISTECH, Budapest, Hungary). Qualitative evaluation was performed by comparing staining patterns in identical regions of serial slides. PD-L1 expression was evaluated quantitatively by two methods.

On one hand, semi-automated quantification was performed using Definiens tissue studio (Definiens AG, München, Germany). Using this software, an estimation is generated of the percentage area showing immunoreactivity for PD-L1 (referred to as the PD-L1-positive area), according to an in-house-developed algorithm. Serum and necrotic areas were excluded manually from analysis. As the software is unable to

Table 1. Summary of the staining protocol of both programmed cell death ligand 1 (PD-L1) immunohistochemistry assays

	SP142	E1L3N
Vendor	Spring Bioscience, Pleasanton, CA, USA	Cell Signaling Technologies, Danvers, MA, USA
Immunogen	Synthetic peptide from the C-terminus of human PD-L1 protein	
Species	Rabbit	
IHC platform	Ventana Benchmark XT	Ventana Benchmark Ultra
Heat-induced epitope retrieval (HIER)	CC1 (pH 8.5) (Ventana Medical Systems Inc., Tucson, AZ, USA)	
Incubation primary antibody	32 min	
Primary antibody concentration	Lot-dependent	Lot-dependent
Detection system	Optiview 3,3'-diaminobenzidine (DAB) detection with amplification (Ventana Medical Systems Inc., Tucson, AZ, USA)	

discriminate melanin pigment from 3,3′-diaminobenzidine (DAB), 17 tumour samples were excluded from the comparison experiment using the software analysis.

On the other hand, semi-quantitative PD-L1 evaluation was performed by a qualified pathologist, blinded for the applied protocol, who scored the % PD-L1-positive TC and immune cells (IC), taking only the PD-L1 membrane staining (at any intensity) into account. TC scores are considered as all neoplastic cells; IC scores include lymphocytes, macrophages and dendritic cells. The pathologist's scorings on % TC and % IC were converted to PD-L1 positivity using a selection of cut-offs (Table 2). If any TC/IC IHC signal was observed samples were designated as positive, otherwise they were marked negative. To relate to published PD-L1 scores, seven commonly used cut-offs were applied on the % scorings of the pathologist. The cut-off from Taube *et al.*[10] (≥5% TC) was used on melanoma samples. The other cut-offs were applied on a range of other tumour entities. Two implemented cut-offs[9] were developed and published with IHC using the SP142 PD-L1 clone. The other five implemented cut-offs are used for IHC with PD-L1 antibodies other than E1L3N and SP142 (more specifically, clones 22C3, SP263, 5H1 and 28.8).

PD-L1 mRNA ANALYSIS

For each melanoma sample, tumour cell enrichment was performed by macrodissection of four FFPE sections (5 μm) prior to RNA extraction using the High Pure FFPET RNA isolation kit (Roche, Anderlecht, Belgium). Subsequently, the samples were analysed with the nCounter Pan Cancer Immune Profiling panel (Nanostring Technologies, Seattle, WA, USA) on a Nanostring Analysis System (Nanostring Technologies). The counts, generated per molecular 'barcode' (gene) by the nCounter system, were normalized for negative and positive controls as well as for the housekeeping genes present in the panel.

STATISTICAL ANALYSIS

All statistics were performed using MedCalc (version 12.3.00) and all plots were made with GraphPad (version 6.07). For the comparison study, the semi-quantitative scoring outcomes were evaluated using Spearman's correlation (ρ) and Wilcoxon's rank test (P-value 0.05 is considered significant). Spearman's correlations were also used to evaluate correlations between protein and mRNA expression (correlations above 0.85 are considered as strong).

Results

VALIDATION PD-L1 IHC

Both PD-L1 IHC assays were validated extensively. More information regarding the accuracy, repeatability and reproducibility of the IHC assays can be found in the Supporting information section (Tables S1-S2 and Figures S1-S2).

COMPARISON STUDY IHC ASSAYS

A comparison of staining pattern and scoring was performed on serial sections of 46 melanoma metastatic samples, which were stained for both validated E1L3N and SP142 PD-L1 IHC assays. Overall, the PD-L1 expression patterns were similar with E1L3N and SP142 IHC assays. The melanoma metastasis samples displayed either heterogeneous or homogeneous PD-L1 expression in which some samples showed clustering, whereas more dispersed PD-L1-positive cells could also be observed in some specimens. At the invasive margin of the tumour, one might even observe clustered or continuous PD-L1 interface activity. As illustrated in Figure 1A–H, some regions showed a good overlap in TC and IC staining,

Table 2. Level of concordance between E1L3N and SP142 immunohistochemistry (IHC) quantification, after conversion of the % tumour cells (TC) and % immune cells (IC) scoring to programmed cell death ligand 1 (PD-L1) positivity

(A)			E1L3N +	E1L3N −	% concordance (n = 46)
SP142	IC > 0	+	36	0	98
		−	1	9	
	TC > 0	+	21	0	91
		−	4	21	
	>50% TC[6]	+	0	0	100
		−	0	46	
	≥25% TC[7]	+	3	0	98
		−	1	42	
	≥ 1% TC or IC[6]	+	36	0	98
		−	1	9	
	≥5% TC[10]	+	15	0	87
		−	6	25	
	≥5% TC or IC[8]	+	20	1	87
		−	5	20	

(B)		E1L3N 0	E1L3N 1	E1L3N 2	E1L3N 3	% concordance (n= 46)
SP142						
Categories IC[9]						
0: < 1%	0	9	1	0	0	74
1: ≥ 1 < 5%	1	0	14	2	1	
2: ≥ 5 < 10%	2	0	3	1	1	
3: ≥ 10%	3	0	0	4	10	
Categories TC[9]						
0: < 1%	0	21	2	2	0	65
1: ≥ 1 < 5%	**1**	0	2	3	1	
2: ≥ 5 < 10%	**2**	0	0	2	8	
3: ≥ 10%	**3**	0	0	0	5	

(a) PD-L1 positivity (+)/negativity (−); (b) PD-L1 categories (0–3) for TC and IC according to Herbst *et al.*[9]

whereas other regions tended to show more TC membrane staining, using E1L3N clone (Figure 1I–P). Overall, both PD-L1 assays showed a good correlation (ρ = 0.95) with scoring by the pathologist and software analysis (Figure 2A,B). However, more detailed analysis of the pathologist's scoring revealed some differences in TC PD-L1 positivity (considered as > 0%). As illustrated in Table 2A, four samples were scored as 0% PD-L1 TC with SP142 whereas 1–5% TC staining was observed with E1L3N. Regarding IC positivity, there was only one sample with 1% IC by E1L3N and 0% with SP142. Because in the

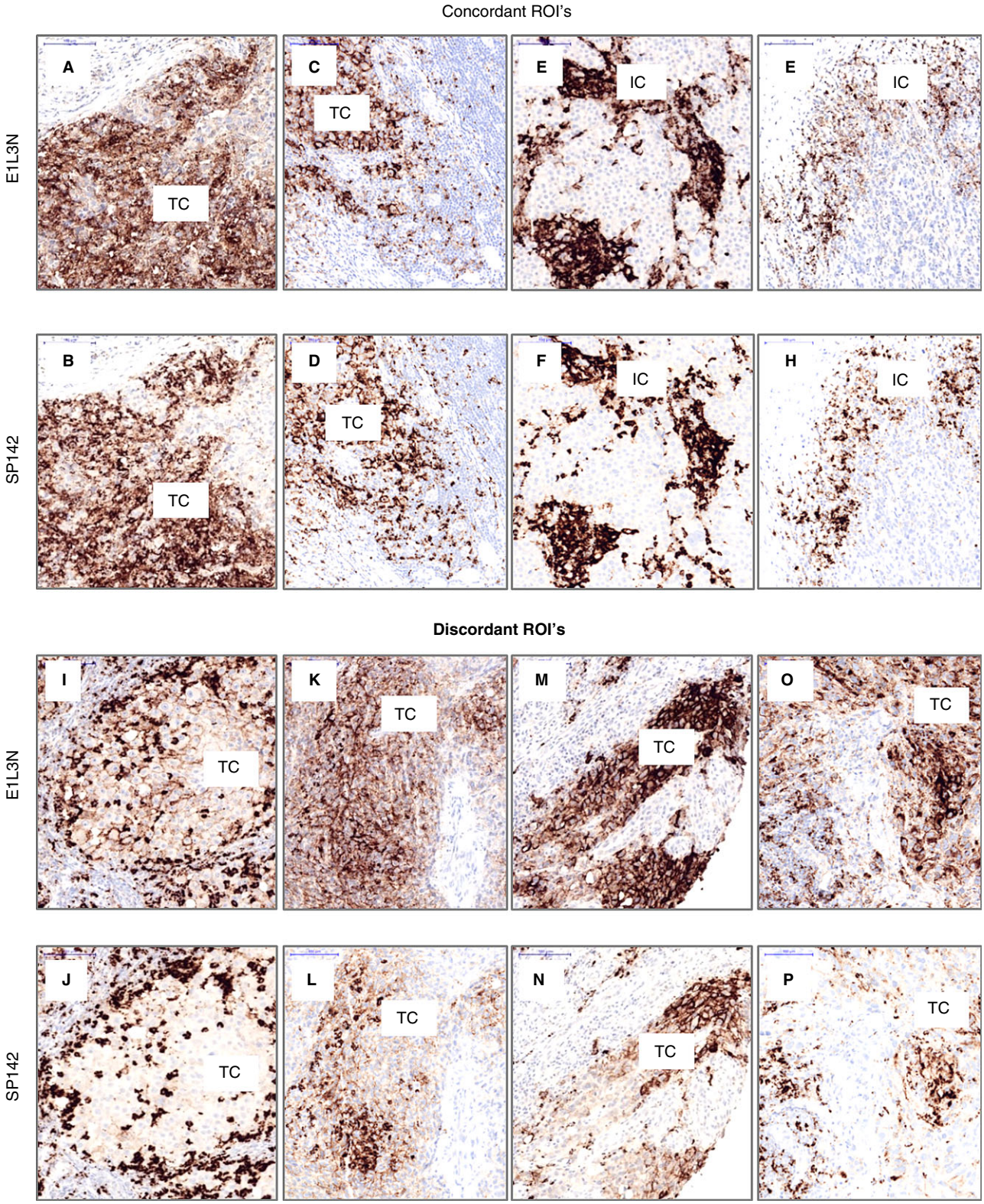

Figure 1. Comparison of two programmed cell death ligand 1 (PD-L1)-validated immunohistochemistry (IHC) assays using clone E1L3N and clone SP142 in melanoma metastases. **A–H**, concordant regions of interest (ROIs) in four different samples; **I–P**, discordant ROIs in four different samples. A single sample can comprise concordant (**C, D**) and discordant ROIs (**K, L**) for PD-L1 tumour cell (TC) staining (using both E1L3N and SP142 assays). The detection of PD-L1-positive immune cells (IC) (**E–H**) is similar in both assays.

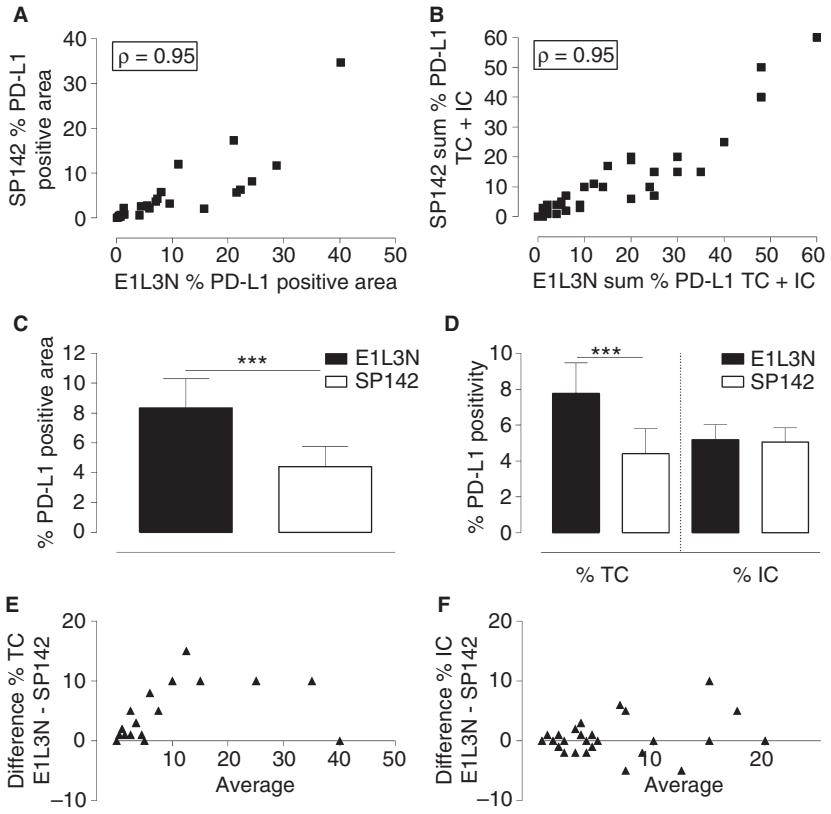

Figure 2. Correlation and difference in scoring between both assays (E1L3N and SP142) in 46 melanoma samples. There is a good correlation between both assays (SP142 and E1L3N) using % programmed cell death ligand 1 (PD-L1)-positive area by semi-automated computer analysis (**A**) and the sum of the tumour cells (TC) and immune cells (IC) scoring by a pathologist (**B**). The graph of the mean scorings (with standard error of the mean) illustrate a significant difference between E1L3N and SP142 (**C**, **D**). Scoring of % PD-L1 positivity in TC and IC by a qualified pathologist shows a higher scoring for the PD-L1 TC expression with E1L3N (21 of 46 samples) (**E**, **F**). ***$P < 0.001$ is significant (Wilcoxon's test).

literature many alternative cut-offs are used to determine PD-L1 positivity, concordance between both assays was investigated further using a selection of seven commonly used cut-offs. There was a concordance between both assays of >85% in five of seven applied cut-offs (Table 2A,B). When using the categories described by Herbst *et al.*[9] for IC and TC (Table 2B), concordance was 74% and 65%, respectively. Analysis of the semi-automated PD-L1-positive area scores revealed statistically significant higher PD-L1 values with E1L3N than SP142. Moreover, based on the scorings by the pathologist, it became clear that the significant difference is caused probably by a difference in TC staining (Figure 2C,D). Bland–Altman analysis of the pathologist's scoring also confirmed the systematic higher scorings for the PD-L1 TC expression with E1L3N in 21 of 46 cases (Figure 2E,F). An overview of the semi-quantitative PD-L1 scorings can be found in Table 3.

CORRELATION STUDY PD-L1 PROTEIN AND MRNA EXPRESSION

PD-L1 mRNA data were available for 21 of the 46 samples. The normalized PD-L1 mRNA counts (Table 3) were correlated with the PD-L1 protein expression as assessed by pathologist or software analysis. Both IHC assays showed good correlation with the PD-L1 mRNA expression data, as evident from a Spearman's correlation of > 0.85 (Figure 3).

Discussion

Currently, almost each anti-PD1 or PD-L1 compound is on track for the development of a proper PD-L1 IHC assay (including scoring method and PD-L1 positivity cut-off) to be used as a companion diagnostic. For pathology laboratories this means implementation of the different PD-L1 assays, running on different platforms and scoring with different methods. It can become even more complex when different cut-offs are advised per tumour entity. This will lead to an unsustainable situation in which a clinician needs to test a patient with all the different PD-L1 IHC assays (and cut-offs) in order to select an appropriate anti-PD-1/PD-L1 therapy. This rapid expansion in PD-L1 IHC tests, based on unique antibody clones, calls for a comparison experiment (as initiated already with the FDA blueprint proposal) using well-validated PD-L1 IHC assays (both laboratory developed and PharmDX kits).[13] The primary antibodies are generated with different immunogens

Table 3. Overall scores of E1L3N and SP142 immunohistochemistry (IHC) assays and the messenger RNA counts (mRNA) for the melanoma metastases

Comparison study							
	% TC*		% IC*		% PD-L1-positive area		mRNA
Sample no.	E1L3N	SP142	E1L3N	SP142	E1L3N	SP142	Normalized counts
7	40	40	8	10	28.7	11.7	//
8	40	30	8	10	24.3	8.2	250.38
17	40	40	20	20	40.2	34.7	122.07
21	30	20	10	5	21.0	17.3	124.11
18	20	5	10	10	8.0	5.8	//
20	20	5	4	5	22.2	6.3	135.87
23	20	5	5	10	5.9	2.2	//
25	20	10	10	10	15.7	2.1	240.9
29	20	5	5	10	7.0	3.7	251.03
39	20	5	5	2	/	/	120.29
24	15	5	20	10	/	/	//
13	10	5	20	15	21.5	5.7	130.13
30	10	5	4	5	/	/	//
34	10	2	10	4	9.5	3.2	//
12	5	2	10	15	7.3	4.3	//
16	5	5	15	15	11.1	12.0	41.5
19	5	2	4	2	/	/	13.8
27	5	5	1	2	/	/	12.12
28	**5**	**0**	1	2	/	/	//
33	**5**	**0**	4	3	1.3	2.2	40.89
36	5	4	15	15	/	/	//
14	3	2	3	5	/	/	56.97
15	**2**	**0**	2	1	/	/	//
26	2	1	10	10	4.4	2.6	//
45	**1**	**0**	1	1	0.2	0.1	1
1	0	0	0	0	/	/	//
2	0	0	5	5	4.1	0.6	//
3	0	0	2	4	0.4	0.5	1
4	0	0	4	4	0.7	0.6	//
5	0	0	1	1	/	/	10.47
6	0	0	0	0	/	/	//

Table 3. *(Continued)*

Comparison study							mRNA
	% TC*		% IC*		% PD-L1-positive area		Normalized
Sample no.	E1L3N	SP142	E1L3N	SP142	E1L3N	SP142	counts
9	0	0	0	0	0.1	0.0	//
10	0	0	0	0	0.0	0.0	//
11	0	0	10	10	5.5	2.8	//
22	0	0	1	0	0.2	0.1	//
31	0	0	1	3	1.4	0.8	4.57
32	0	0	1	1	/	/	1.3
35	0	0	0	0	/	/	//
37	0	0	5	4	0.7	0.3	//
38	0	0	1	1	0.2	0.1	1
40	0	0	0	0	/	/	1.04
41	0	0	2	2	/	/	//
42	0	0	0	0	0.0	0.0	//
43	0	0	0	0	0.4	0.2	//
44	0	0	1	1	/	/	1
46	0	0	0	0	0.0	0.0	//

(*) Semi-quantitative scoring by pathologist of % PD-L1 positive tumour cells (TC) and immune cells (IC). (/) Samples not semi-automatically analysed (% PD-L1-positive area) due to the high melanin content; (//) samples not analysed on the Nanostring platform. All samples are shown in descending order of E1L3N % TC scores. Samples shown in bold type are the discordant samples when applying 0% TC cut-off.

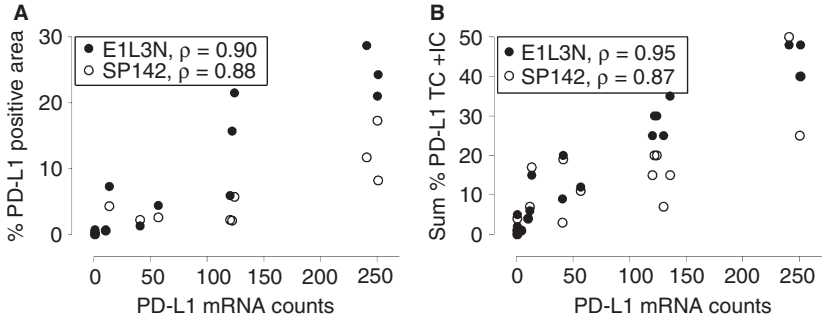

Figure 3. Correlation between programmed cell death ligand 1 (PD-L1) protein and mRNA expression in 21 melanoma samples. Spearman's correlation coefficients (ρ) are shown in the boxes. **A**, correlation between mRNA counts and % PD-L1-positive area (immunohistochemistry) evaluated by semi-automated computer analysis; **B,** correlation between mRNA counts and PD-L1 scoring by pathologist [sum of % PD-L1-positive tumour cells (TC) and immune cells (IC)].

leading to a unique epitope and, therefore, might cause different staining patterns. As well, the staining protocol can have a major influence on the IHC assay performance.[14]

This comparison study was set up with extensively validated IHC assays (E1L3N and SP142) using the same detection system (including amplification); only limited influences from differences in staining protocol

[staining platform, antigen retrieval buffer, heat-induced epitope retrieval (HIER) time] can be expected. Both assays were validated independently, aiming for the best sensitivity to noise ratio. Additionally, the PD-L1 protein expression (E1L3N and SP142 assays) was correlated with the PD-L1 mRNA expression (nCounter Pan Cancer Immune Panel; NanoString Technologies, Inc., Seattle, WA, USA). Remarkably good correlations were found for PD-L1 protein and mRNA expression. Other groups found no to weaker correlations but used different mRNA quantification methods [RNAscope and reverse transcription–polymerase chain reaction (RT–PCR)] (Table S3), as well as different methods for the assessment of PD-L1 protein expression (different IHC protocols, antibody clones and IHC quantification). Velchetti *et al.*[15] described a low Spearman's correlation coefficient between PD-L1 mRNA (RNAscope) and protein (clone 5H1) expression in lung cancer. In line with this observation, other groups described the weak/absence of correlation between PD-L1 mRNA [quantitative RT–PCR (qRT–PCR)] and protein (clones 5H1 or EPR1161) expression in renal cell carcinoma,[16] melanoma[17] and ovarian carcinoma.[18] One important advantage of the Nanostring technology is the applicability on FFPE samples (short RNA fragments).[19] The results of the Nanostring testing were confirmed in-house with RT–PCR and the technique was also tested for repeatability and reproducibility (data not shown). The thorough validation of both PD-L1 IHC and mRNA assays increases further the reliability of our observed correlation between PDL1 protein and mRNA in metastatic melanoma.

Both validated E1L3N and SP142 IHC assays revealed a good overlap in staining pattern and comparable scoring values. These results are different from some published comparison data. In a comparison experiment on 14 melanoma samples, using four different anti-PD-L1 clones (5H1, 28-8, SP142 and E1L3N), Sunshine *et al.*[20] showed a much lower correlation coefficient between E1L3N and SP142 compared to our study. McLaughlin *et al.*[21] compared the performance of SP142 and E1L3N by IHC and quantitative immunofluorescence (QIF) on 49 NSCLC specimens. Spearman's correlation between both assays, using QIF, was also much lower compared to the current study. It should be kept in mind that the comparison of E1L3N and SP142 IHC assays might reveal other conclusions in different tumour entities, while the current study focuses solely upon PD-L1 IHC in melanoma. Furthermore, it should be noted that PD-L1 is known as a dynamic marker with intratumour heterogeneity.[22] As serial slides were used for the comparison study, this biological variability was minimized as much as possible and is therefore not considered as a causative factor of the observed differences in this study. Moreover, the outcome of IHC comparison studies is, apart from primary antibody characteristics, affected largely by assay characteristics (staining platform, protocol) and quantification methods.

At present, there is no gold standard for PD-L1 evaluation that can be applied widely by pathology laboratories. Therefore, the definition of the PD-L1 IHC signal used in comparison studies is of great importance, as both membrane and/or cytoplasmic staining can be observed. Furthermore, several different cell types (TC/IC) can be considered within different PD-L1 scoring methods. As well as scoring by a pathologist of the % PD-L1 positively stained cells (TC and/or IC), some studies use histological scoring (H-score) to quantify the PD-L1 positivity.[23] The H-score system is based on the percentage of cells showing staining (0–100%) multiplied by their intensity (0: negative, 1: very weak, 2: moderate, 3: strong); the sum of this gives a scoring range of 0–300.[24] Others use software analysis for PD-L1 scoring.[15] To avoid bias inherent in certain quantification methods, the current study assesses PD-L1 IHC in different ways: pathologist scoring in combination with different cut-offs and semi-automated software analysis. Significantly higher values for E1L3N IHC were observed compared to SP142. Side-by-side comparison of identical regions of serial slides as well as pathologist scoring indicate clearly that the difference is caused by enhanced TC staining with E1L3N, while IC staining was comparable for both clones. As evident from the raw data, application of different cut-offs for PD-L1 positivity[6–10] show different levels of concordance between both IHC assays. Application of the cut-off categories for TC and IC from Herbst *et al.*[9] or the 5% TC cut-off[10] showed concordance levels <90%. This might have serious implications in clinical practice. It might be advisable to apply a negative cut-off on PD-L1 IHC assays. As illustrated in this study, there is a high correlation between both assays using a 0% cut-off.

This research can be extended to other relevant PD-L1 clones and might reveal equal performance of the assays in terms of selecting patients with absolutely no PD-L1 expression in TC and IC. Exclusion of these PD-L1-negative patients from anti-PD-1/PD-L1 therapy might be less dependent upon the applied validated IHC assay and more applicable in daily clinical practice. This finding indicates that a marker for therapy resistance in melanoma might be more realistic than a marker for response. Furthermore, both

patient groups classified with the 0% cut-off as PD-L1-positive (in TC and/or IC) or PD-L1-negative (for both TC and IC) will need further evaluation of immune parameters to achieve a better patient selection for immunotherapy. Each group will need a specific characterization of the tumour micro-environment; for example, evaluation of tumour infiltrating lymphocytes[25] and/or the expression of other checkpoint molecules. As reviewed by Ung *et al.*,[26] there is a growing need of multi-analyte biomarker testing, using different techniques, instead of mono-analyte testing.

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Conflicts of interest

K.S., E.V.V., S.D.S., C.B., D.S. and I.V.B. are employees and W.W. is a consultant for HistoGeneX NV. M.K. is CEO of HistoGeneX NV, which performs immunohistochemistry and molecular testing for pharmaceutical companies as part of (pre)clinical studies that evaluate new anticancer drugs. B.N. has consulting and/or advising roles at Bristol-Myers Squibb, Merck Sharp and Dohme, Roche, Novartis and Amgen. The authors have no other relevant affiliations or financial involvements with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the paper apart from those disclosed.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Data S1. Materials and methods.

Figure S1. Accuracy data programmed cell death ligand 1 (PD-L1) immunohistochemistry (IHC) assays.

Figure S2. A, Qualitative evaluation of precision data for E1L3N; B, qualitative evaluation of precision data for SP142.

Table S1. Semi-quantitative evaluation of the precision experiment of E1L3N (1A) and SP142 (1B). Scoring by pathologist: % PD-L1+ tumor cells (TC) and % immune cells (IC).

Table S2. Statistical evaluation of the precision experiment of both PD-L1 assays.

Table S3. RNA discussion: differences between the RNA analysis methods used for PD-L1 detection.