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Performance Analysis of Leica Biosystems Monoclonal Antibody Programmed Cell Death Ligand 1 Clone 73-10 on Breast, Colorectal, and Hepatocellular Carcinomas

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Abstract: Programmed cell death receptor 1/Programmed cell death ligand 1 (PD-L1) checkpoint pathway is responsible for the control of immune cell responses. Immunotherapy using checkpoint inhibitors, such as anti–PD-L1 therapy, aids disease management and potentiates clinical outcomes. This study aimed to analyze the performance of the Leica Biosystems (LBS) USA FDA class I in vitro diagnostic monoclonal antibody (clone 73-10) to detect PD-L1 expression in breast, colorectal, and hepatocellular carcinomas compared with the class III FDA-approved PD-L1 detecting antibodies [SP263 (Ventana), 22C3 (Dako), and 28-8 (Dako)] using 208 unique tissue microarray–based cases for each tumor type. The interassay concordances between LBS 73-10 clone and other PD-L1 antibodies ranged from 0.59 to 0.95 Cohen kappa coefficient (K) and from 0.66 to 0.90 (K) for cutoff values of 1% and 50% tumor proportion score (TPS), respectively. The 73-10 clones showed inter-pathologist agreements ranging from 0.53 to 1.0 (K) and 0.34 to 0.94 (K) for cutoff values of 1% and 50% TPS, respectively. For the immune cell proportion score (IPS) using a cutoff of 1%, the Kappa coefficient of interassay concordances and inter-pathologist agreements ranged from 0.34 to 0.94. The 73-10 clone assay's sensitivity ranged from 78.3% to 100% (TPS $\geq$1%), 100% (TPS $\geq$50%), and 77.4% to 93.5% (IPS $\geq$1%), while its specificity was 97.9% to 100% (TPS $\geq$1%), 99.5% to 99.8% (TPS $\geq$50%), and 97.9% to 100% (IPS $\geq$1%). This exploratory evaluation of LBS 73-10 monoclonal antibody on a large set of breast, colorectal, and hepatocellular carcinomas showed the assay's technical performance is comparable to the FDA-approved companion/complementary diagnostics PD-L1 detection assays.

Key Words: PD-L1, clone 73-10, SP263, 22C3, 28-8, immunohistochemistry

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Programmed cell death receptor 1 (PD-1) and its ligand (PD-L1) play a regulatory role in immune cell signaling, such as modulating inflammation or an autoimmune reaction.[1,2]

PD-L1 is generally expressed on the surface of immune cells, including lymphocytes, dendritic cells, macrophages, and monocytes; however, the PD-L1 expressed on tumor cells binds to the endogenous PD-1, preventing PD-1/PD-L1 interaction and allows tumor cells to evade cell death, thereby supporting tumor cell proliferation.[3–8] Immunotherapies targeting the inhibition of the PD-1/PD-L1 receptor-ligand system have positively affected the clinical outcome of patients with cancer. Thus far the U.S. Food and Drug Administration (FDA) has approved 6 PD-1/PD-L1 checkpoint inhibitor therapies;[9,10] these include pembrolizumab, nivolumab, and cemiplimab for PD-1, and atezolizumab, avelumab, and durvalumab for PD-L1.

PD-L1 is widely used as a checkpoint inhibitor prognostic biomarker within the clinical setting.[11,12] For each of the approved drugs, there has been a consistent correlation between the drug response rate and the PD-L1 expression ascertained by the corresponding immunohistochemistry (IHC) assays.

FDA-approved companion/complementary diagnostic (US class III) PD-L1 IHC assays utilize 4 commercially available monoclonal antibodies including 22C3 (Dako), 28-8 (Dako), SP263 (Ventana), and SP142 (Ventana).[13,14] These assays are recommended to be used alongside an approved drug. The approved IHC assays

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employ a unique disease-specific scoring algorithm as dictated by the respective instructions for use (https://elabdoc-prod.roche.com/eLD/api/downloads/2456f3c8-2a9a-ea11-fc90-005056a71a5d?countryIsoCode=us, https://www.accessdata.fda.gov/cdrh_docs/pdf15/p150013c.pdf; https://www.agilent.com/cs/library/packageinsert/public/PD04163_rev_02_SK00521-5_IFU.pdf; https://www.rochebiomarkers.be/content/media/Files/PD-L1_SP263_package_insert.pdf). These clones are accredited for various indications, including non–small cell lung cancer (NSCLC), cervical cancer, urothelial carcinoma, esophageal cancer, esophageal squamous cell carcinoma, squamous cell carcinoma of the head and neck, and triple-negative breast cancer (TNBC).[13]

A novel 73-10 clone introduced by Dako demonstrated comparable PD-L1 expression to the other FDA-approved assays in NSCLC.[15] In addition, in a collaborative retrospective study Dako 73-10 clone showed encouraging results when compared with the approved in vitro diagnostics (IVDs), 22C3, 28–8, SP142, and SP263.[16] In the same study, SP142 showed lower sensitivity in detecting PD-L1 expressing tumor cells; hence it was not used as one of the comparators in this study.[16] Based on these results, Leica Biosystems (LBS) has developed its own 73-10 BOND Ready-to-Use PD-L1 antibody (PA0832).

The primary objective of this study was to test LBS PD-L1 73-10 IHC assay, a class I IVD against the corresponding class III IVDs (Dako's 28-8, 22C3, and Ventana's SP263 assay) for assay performance comparison outside of any indicated disease states. The study was conducted to evaluate the performance of the 73-10 antibody as a potential diagnostic for further development of the clone.

All 4 assays were compared, and the percentage of PD-L1 positive tumor cells and tumor-infiltrating immune cells was assessed. Each expert scored the slides based on the PD-L1 expression in the tumor cells and infiltrating immune cells measured as tumor proportion score (TPS) and immune cell proportion score (IPS), respectively.

MATERIALS AND METHODS

Study Design

The study evaluated 208 unique Formalin Fixed Paraffin Embedded microarray tissue cores each for 3 indications (hepatocellular, colorectal, and breast carcinomas). Serial sections for each tissue microarray (TMA) core across the tumors were stained immunohistochemically with specific PD-L1 clones. Clone SP263 (Ventana PD-L1 antibody) stained cores on the BenchMark ULTRA platform (software v12.2), whereas clone 22C3 and 28-8 (both Dako PD-L1 antibodies) used Dako Autostainer Link 48 automated platform (software v4.0.3). LBS clone 73-10 (PA0832) used the BOND-III staining platform (software v5.1). Three board-certified pathologists scored antibody-stained tissue cores in all 4 assays. Staining and scoring of each case were performed at a single site (Ohio State University).

The patient details (demographics) of 208 cases across all 3 tumor types (breast, colorectal, and hepatocellular) are presented in Supplemental Table S1 (Supplemental Digital Content 1, http://links.lww.com/AIMM/A444).

The score for each case was assessed by evaluating the PD-L1 staining in tumor and immune cells. Each pathologist read 4 consecutive serial sections (for each of the 4 assays) of each TMA, that is, comprising the hepatocellular, breast, and colon carcinomas. Tumor staining was evaluated by calculating the TPS for 5 mutually exclusive categories: $<1\%$, 1% to 9%, 10% to 49%, 50% to 74%, and 75% to 100%. In addition, $\geq 1\%$ PD-L1 immune cell scoring was also evaluated for each tissue core by measuring the IPS. An appropriate positive control (tonsil or placenta tissue) was run with each assay.

TPS was analyzed both at 1% (TPS $\geq 1\%$) and 50% (TPS $\geq 50\%$) cutoff. These would be the tumor types showing 1% or 50% partial or complete PD-L1 membranous staining. A case was designated as positive if 2 or 3 readers (out of a total of 3 pathologists) scored the tumor cells having $\geq 1\%$ PD-L1 membranous staining and negative if there was $<1\%$ PD-L1 staining. The same approach was used for TPS $\geq 50\%$. In addition, the infiltrating immune cells expressing PD-L1 were identified by immune cell percentage score (IPS). The PD-L1 was negative if the IPS $<1\%$ and positive IPS $>1\%$. The immune cells were indicated as positive or negative based on the percent PD-L1 expression in the intra or peritumoral immune cells positioned between the tumoral and nontumoral tissue. The percentage of immune cells was evaluated as the proportion of PD-L1–positive immune cells occupying a tumor area. Therefore, a case would be defined as positive, if out of all the tumor-infiltrating immune cells, the proportion of PD-L1 expressing immune cells was $>1\%$.

Institutional Review Board waiver was obtained for this retrospective study before the study initiation. The study was conducted in full conformity with the principles set forth in The Belmont Report.

Study Endpoints

For each PD-L1 assay performed on breast, hepatocellular, and colorectal carcinomas, the primary analyses concordance of LBS 73-10 clone with Ventana's SP263, and Dako's 22C3 and 28-8 was inferred by calculating the positive percentage agreement, negative percentage agreement, and overall percentage agreement (OPA) with a 2-sided confidence interval for pairwise comparison using the Wilson score method.[17] The agreement rates were computed both for tumor cell positivity and immune cell positivity. For each stained case and assay, a majority score was calculated. These majority scores were compared to evaluate the agreement between the assays. Cohen kappa value (K) was evaluated for each pairwise agreement. The kappa values of <0, 0.01 to 0.20, 0.21 to 0.40, 0.41 to 0.60, 0.61 to 0.80, and 0.81 to 1.00 corresponded to the agreement of poor, slight, fair, moderate, substantial, and almost perfect, respectively.

K with a 2-sided CI and *P* value was also calculated to evaluate the inter-pathologist precision of 624 comparison pairs for each assay, indication, and tumor/immune cell percentage cutoff (208 cases x 3 pathologist comparisons). To assess surrogate assay sensitivity and specificity, the majority score of each case and assay across tested indications was calculated. Overall, there were 624 comparison pairs for each assay, indication, and tumor/immune score cutoff (208 cases x 3 pathologists/majority score comparisons).

RESULTS

Programmed Cell Death Ligand 1 Expression in Breast, Colorectal, and Hepatocellular Carcinomas

LBS 73-10 clone showed expected membranous expression of PD-L1 and was comparable to that seen with SP263 (Ventana), 22-C3 (Dako), and 28-8 (Dako) antibody clones in all cancer types (Figs. 1–4). The membranous PD-L1 expression was present in tumor cells, as well as in immune cells. The core dropouts and discordant cases were infrequent and comparable across all three TMA (Supplemental Table S2, Supplemental Digital Content 2, http://links.lww.com/AIMM/A445, and Supplemental Table S3, Supplemental Digital Content 3, http://links.lww.com/AIMM/A446). PD-L1 positivity was calculated for each assay and carcinoma type (Table 1). In breast carcinoma, for 1% and 50% TPS, as well as 1% IPS, the greatest positivity was exhibited by SP263 with the positivity of 13.3% (27/204), 1.0% (2/204), and 39.3% (81/206), respectively. Similarly, in colorectal carcinoma SP263 alongside 73-10 exhibited the highest TPS with a positivity rate of 3.4% (TPS $\geq$1%, 7/205) and 2% (TPS $\geq$50%, 4/205), and IPS 49.5% (103/208). For hepatocellular carcinoma, the SP263 score had the greatest TPS $\geq$1% (6.8%; 14/207) and IPS 25.0% (52/208), whereas 73-10 had the highest TPS $\geq$50% (3.8%; 8/208).

Interassay Analysis

Interassay agreement rate of LBS 73-10 clone was compared with SP263 (Ventana), 22-C3 (Dako), and 28-8 (Dako) antibody clones across all indications and was represented by Cohen kappa statistic (K) and point estimates positive percentage agreement, negative percentage agreement, and OPA. The TPS agreement for $\geq$1% PD-L1 expression was 0.59 (moderate) to 0.87 (almost perfect); for TPS $\geq$50%, the kappa value was 0.66 (substantial) to 0.85 (almost perfect). The immune cell staining was observed in all the 3 pairwise comparison analyses, which was represented as IPS $\geq$1%. The kappa value for IPS was 0.61 (moderate) to 0.78 (substantial). To compare the interassay analysis, the majority score (of PD-L1

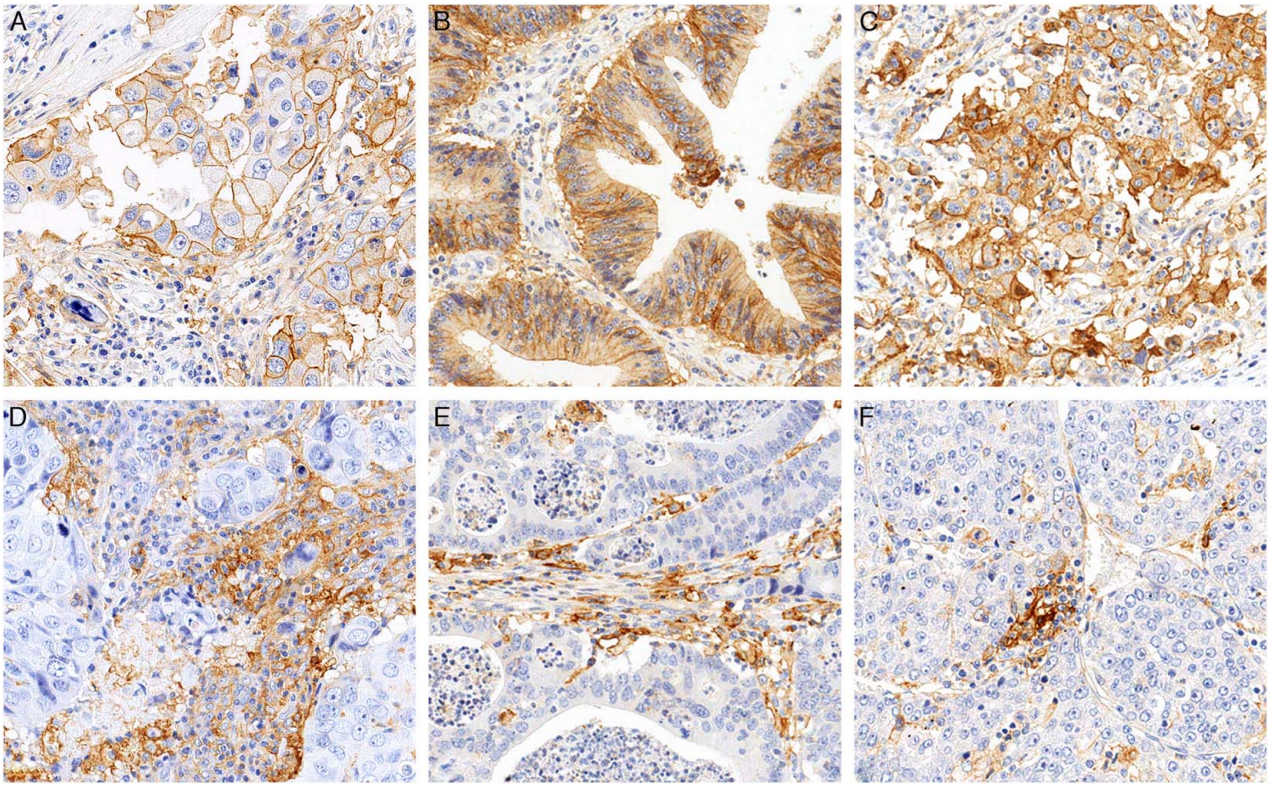

FIGURE 1. Programmed cell death ligand 1 (PD-L1) clone 73-10 expression in carcinomas of the breast (A and D), colon (B and E), and liver (C and F) in tumor cells (A–C) and in immune cells (D–F). Immunohistochemistry (IHC); original magnification x200. Same tissue microarray (TMA) cores were used to illustrate the pattern of PD-L1 expression with each of the antibodies.

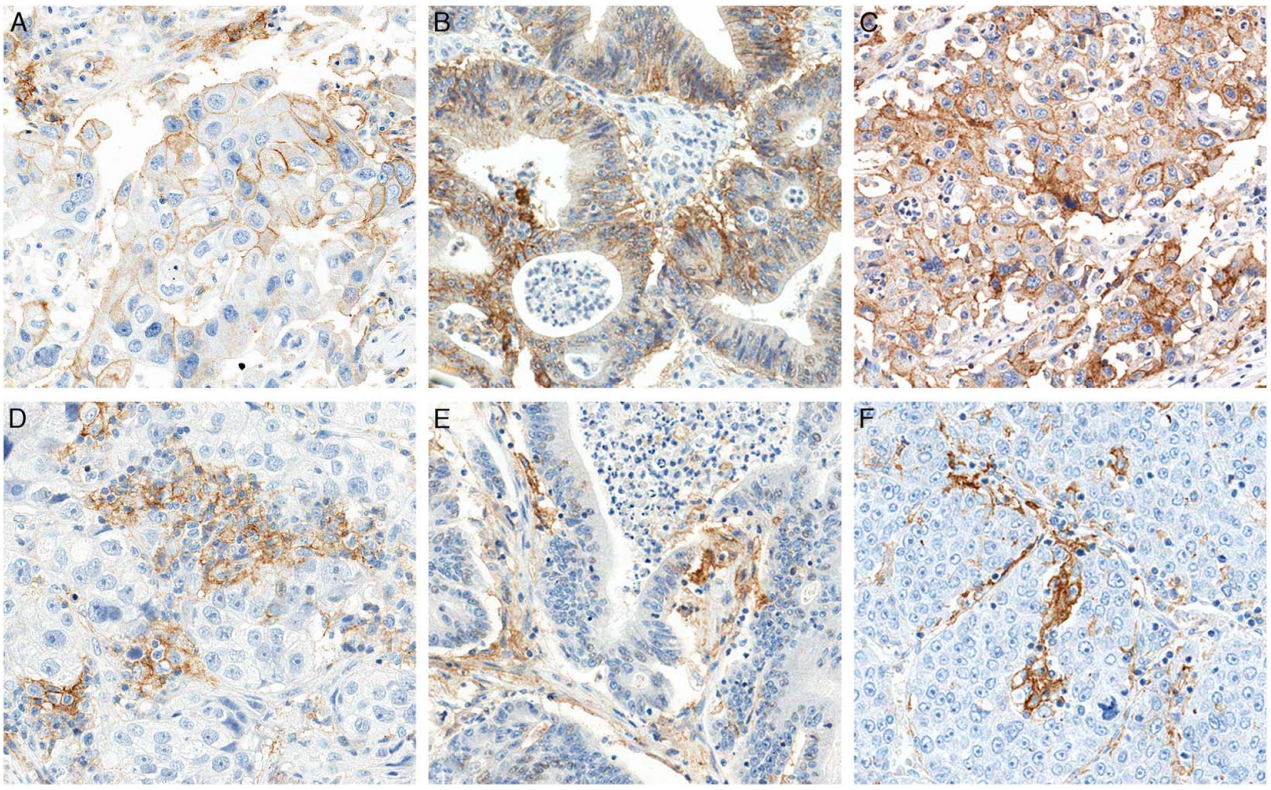

FIGURE 2. PD-L1 clone 22C3 expression in carcinomas of breast (A and D), colon (B and E), and liver (C and F) in tumor cells (A–C) and in immune cells (D–F). IHC; original magnification x200. Same TMA cores were used to illustrate the pattern of PD-L1 expression with each of the antibodies.

positive or negative) was inferred for both the comparator IHC assays and the 73-10 clone. The pairwise agreement between the assays showed comparable performance. The interassay kappa value and the corresponding OPAs are presented in Table 2. We performed detailed analysis of each assay comparison; 73-10 versus SP263, 73-10 versus 22C3, 73-10 versus 28-8, SP263 versus 22C3, SP263 versus 28-8, and 22C3 versus 28-8. (Supplemental Table S4, Supplemental Digital Content 4, http://links.lww.com/AIMM/A447; Supplemental Table S5, Supplemental Digital Content 5, http://links.lww.com/AIMM/A448; Supplemental Table S6, Supplemental Digital Content 6, http://links.lww.com/AIMM/A449; Supplemental Table S7, Supplemental Digital Content 7, http://links.lww.com/AIMM/A450; Supplemental Table S8, Supplemental Digital Content 8, http://links.lww.com/AIMM/A451; and Supplemental Table S9, Supplemental Digital Content 9, http://links.lww.com/AIMM/A452; Supplemental Table S10, Supplemental Digital Content 10, http://links.lww.com/AIMM/A453; Supplemental Table S11, Supplemental Digital Content 11, http://links.lww.com/AIMM/A454; and Supplemental Table S12, Supplemental Digital Content 12, http://links.lww.com/AIMM/A455).

Inter-pathologist Analysis

The 73-10 clone had comparable TPS and IPS agreement rates across all the 3 pathologists. For breast carcinoma cases, the 73-10 agreement rate for TPS $\geq 1\%$ was 0.70, TPS $\geq 50\%$ was 0.50, and IPS $\geq 1\%$ was 0.21. These endpoints were comparable to the other FDA-approved clones (SP263, 22C3, and 28-8).

For colorectal carcinoma TPS $\geq 1\%$ was 0.58, TPS $\geq 50\%$ was 0.92, and IPS $\geq 1\%$ was 0.68. Similar to the breast carcinoma, clone 73-10 agreement rates were comparable to other anti–PD-L1 clones. For hepatocellular carcinoma, the inter-pathologist agreement rates for 73-10 clones were 0.74 for TPS $\geq 1\%$, 0.91 for TPS $\geq 1\%$, and 0.76 for IPS $\geq 1\%$. 73-10 tumor and immune cell PD-L1 staining patterns were proportionate to the other clones. A summary of the inter-pathologist agreement is provided in Table 3. Detailed agreement analysis of each clone was also performed (Supplemental Table S13, Supplemental Digital Content 13, http://links.lww.com/AIMM/A456, Supplemental Table S14, Supplemental Digital Content 14, http://links.lww.com/AIMM/A457, and SupplementalTable S15, Supplemental Digital Content 15, http://links.lww.com/AIMM/A458).

Assay Sensitivity and Specificity

The surrogate assay sensitivity and specificity for the PD-L1 expression in tumor and immune cells were analyzed. To calculate the sensitivity and specificity for each assay, a majority score of the pathologists was used as a reference or truth. Each of the pathologists' score was

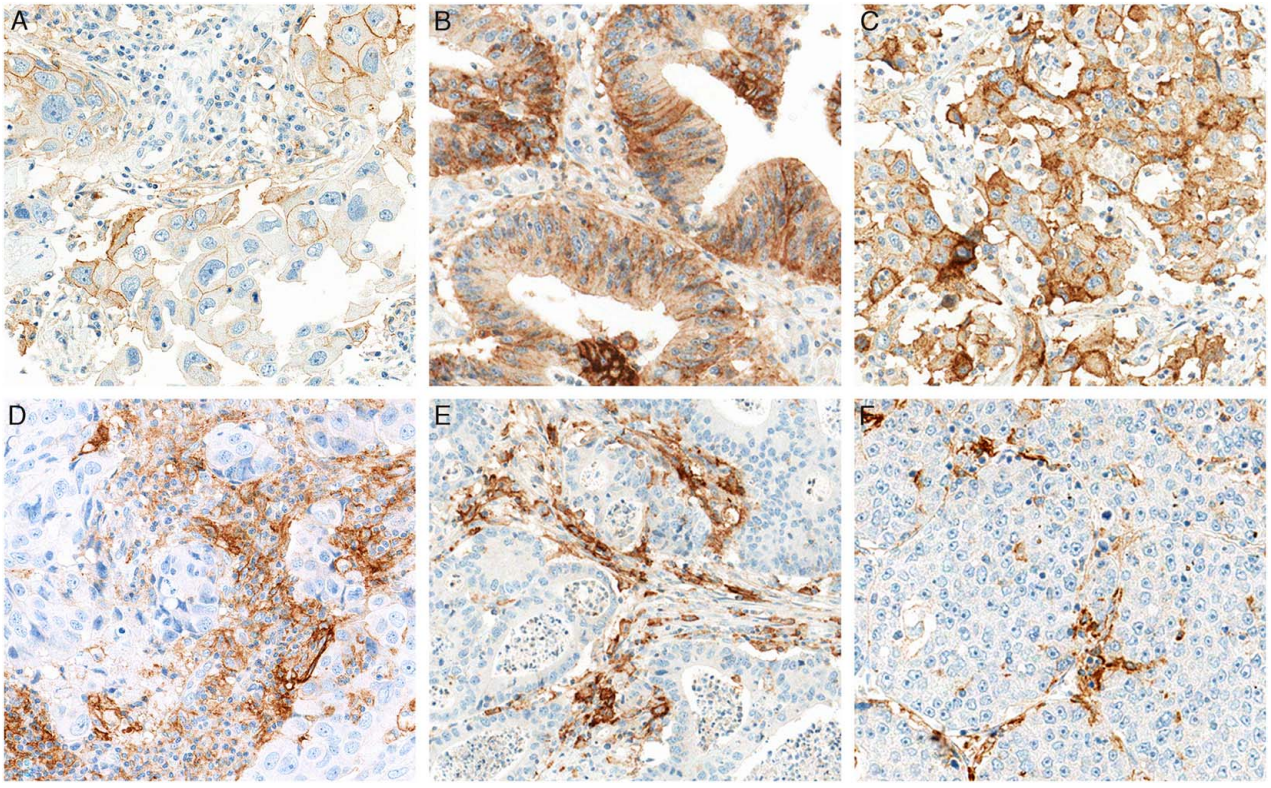

FIGURE 3. PD-L1 clone SP263 expression in carcinomas of breast (A and D), colon (B and E), and liver (C and F) in tumor cells (A–C) and in immune cells (D–F). IHC; original magnification x200. Same TMA cores were used to illustrate the pattern of PD-L1 expression with each of the antibodies.

compared with the majority score. Each assessment is represented as a 2-sided CI. The LBS 73-10 clone TPS $\geq$1% sensitivity was 78.3% for breast carcinoma and 100% for colorectal and hepatocellular carcinomas. The LBS 73-10 clone TPS $\geq$50% sensitivity was 100% across each cancer. The specificity was 100% each for breast and colorectal carcinomas, while 98.5% for hepatocellular carcinoma.

For the LBS 73-10 IPS $\geq$1% assay sensitivity was 77.4% for breast, 90.7% for colorectal, and 91.5% for hepatocellular carcinomas. The corresponding LBS 73-10 IPS $\geq$1% assay specificity was 82.4% for breast, 93.5% for colorectal, and 99.7% for hepatocellular carcinomas. The sensitivity and specificity of each assay are presented in Table 4. The 73-10 sensitivity and specificity were comparable to the other commercially available PD-L1 clones. Comprehensive sensitivity and specificity analyses of each assay were also performed (Supplemental Table S16, Supplemental Digital Content 16, http://links.lww.com/AIMM/A459, Supplemental Table S17, Supplemental Digital Content 17, http://links.lww.com/AIMM/A460, and Supplemental Table S18, Supplemental Digital Content 18, http://links.lww.com/AIMM/A461).

DISCUSSION

This is the first study that provides a performance assessment of the novel LBS 73-10 monoclonal antibody in comparison to the other commercially available PD-L1 clones on a large set of breast, colorectal, and hepatocellular carcinomas. The LBS 73-10 interassay and inter-pathologist agreement, sensitivity, and specificity are comparable to other commercially available antibodies (Ventana's SP263, Dako 22C3, and 28-8) across tested tumor types.

LBS 73-10 clone was previously utilized on a set of triple-negative breast carcinomas (TNBCs); however, the analyses were performed on a small sample size (62 tumor tissue cases).[18] This observational study performed a comprehensive examination of the LBS 73-10 PD-L1 antibody utilizing 208 unique cases for each of the disease states.

Use of 73-10 clone in Dako PD-L1 assay documented higher than 22C3 clone sensitivity without any significant correlation with outcomes of treated patients with NSCLC.[15] This is the first retrospective analysis that evaluated and compared LBS 73-10 antibody sensitivity and specificity in the tested disease states.

To the best of our knowledge, LBS 73-10 clone has not been tested on hepatocellular or colorectal carcinoma tumor tissue samples. This study explores the ability of the 73-10 clone to detect the expression of PD-L1 in the aforementioned tumor tissue types.

There is substantial evidence that an adjunct detection method in the tested indications may help in

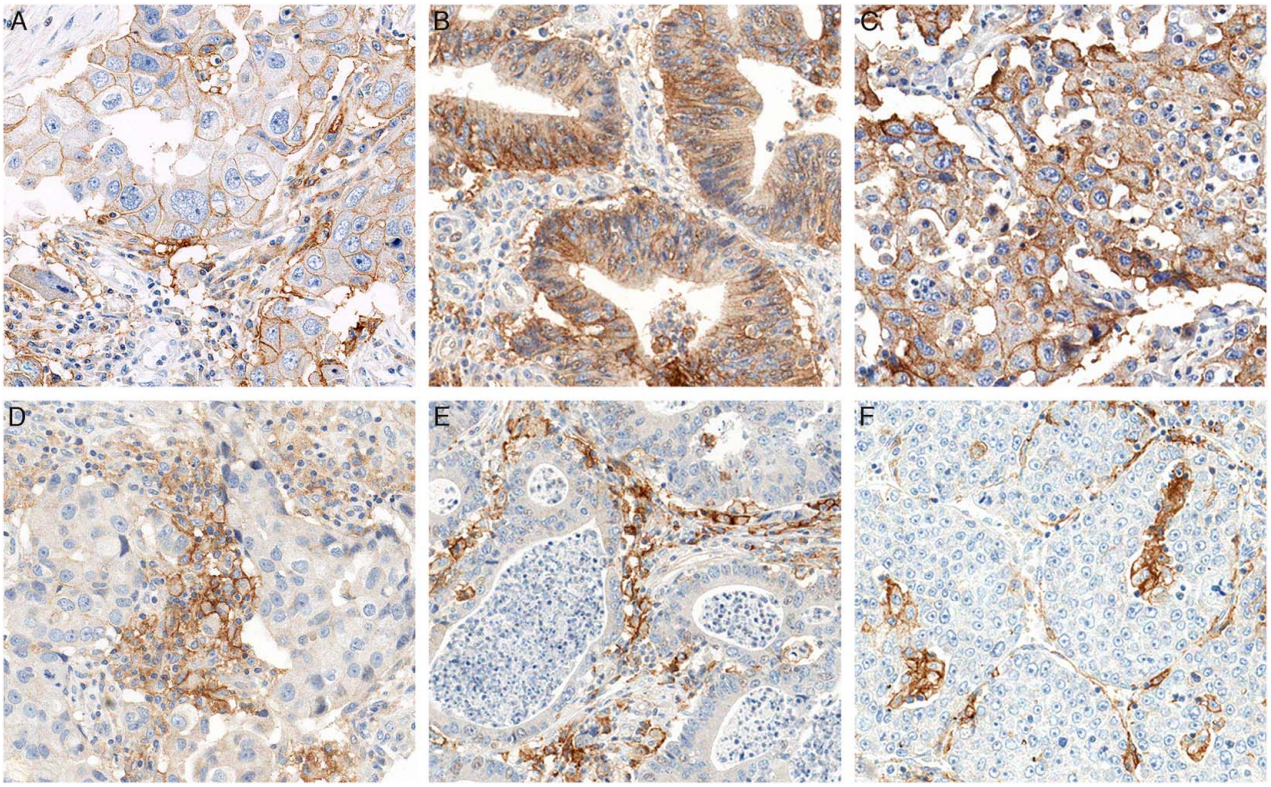

FIGURE 4. PD-L1 clone 28-8 expression in carcinomas of breast (A and D), colon (B and E), and liver (C and F) in tumor cells (A–C) and in immune cells (D–F). IHC; original magnification x200. Same TMA cores were used to illustrate the pattern of PD-L1 expression with each of the antibodies.

reporting the PD-L1 status in cancer patients;[19–21] therefore, this study compared the LBS clone 73-10 (PA0832) with the approved PD-L1 assays (Dako's 28-8 and 22C3, Ventana's SP263).

PD-L1 assay harmonization is an issue frequently faced by health care practitioners.[22] It has been demonstrated that some "fit-for-purpose" or laboratory-developed tests showed comparable outcomes to the

TABLE 1. Percentage PD-L1 Positivity

	PD-L1% positivity (positive cases/total cases)			
	73-10	**SP263**	**22C3**	**28-8**
Breast carcinoma				
TPS ≥ 1%	11.2 (23/205)	13.3 (27/204)	9.8 (20/205)	11.2 (23/205)
TPS ≥ 50%	0.5 (1/205)	1.0 (2/204)	0 (0/205)	0 (0/205)
IPS ≥ 1%	35.3 (73/207)	39.3 (81/206)	23.7 (49/207)	30.4 (63/207)
Colorectal carcinoma				
TPS ≥ 1%	3.4 (7/205)	3.4 (7/205)	1.5 (3/206)	2.4 (5/205)
TPS ≥ 50%	2.0 (4/205)	2.0 (4/205)	1 (2/206)	1 (2/205)
IPS ≥ 1%	33.2 (69/208)	49.5 (103/208)	30.3 (63/208)	26.4 (55/208)
Hepatocellular carcinoma				
TPS ≥ 1%	5.3 (11/208)	6.8 (14/207)	4.3 (9/208)	5.3 (11/208)
TPS ≥ 50%	3.8 (8/208)	3.4 (7/207)	2.4 (5/208)	2.9 (6/208)
IPS ≥ 1%	15.4 (32/208)	25.0 (52/208)	10.6 (22/208)	18.8 (39/208)

IPS indicates immune cell proportion score; PD-L1, programmed cell death ligand 1; TPS, tumor proportion score.

TABLE 2. Interassay Kappa Value Analysis and Corresponding Pairwise OPA Rate Analysis

	Kappa value								
Indication		73-10 vs SP263			73-10 vs 22C3			73-10 vs 28-8	
	≥ 1% TPS	≥ 50% TPS	≥ 1% IPS	≥ 1% TPS	≥ 50% TPS	≥ 1% IPS	≥ 1% TPS	≥ 50% TPS	≥ 1% IPS
Breast	0.82	0.66	0.71	0.87	NA	0.63	0.95	NA	0.69
Colorectal	0.76	0.75	0.61	0.59	0.66	0.67	0.66	0.66	0.66
Hepatocellular	0.79	0.79	0.71	0.90	0.76	0.75	0.81	0.85	0.78
	Interassay agreement; % agreement								
Indication		73-10 vs SP263			73-10 vs 22C3			73-10 vs 28-8	
	≥ 1% TPS	≥ 50% TPS	≥ 1% IPS	≥ 1% TPS	≥ 50% TPS	≥ 1% IPS	≥ 1% TPS	≥ 50% TPS	≥ 1% IPS
Breast carcinoma									
OPA (%)	96.1	99.5	86.4	84.5	99.5	84.5	99.0	99.0	86.4
Colorectal carcinoma									
OPA (%)	98.5	99.0	80.8	98.0	99.0	85.6	98.0	99.0	85.6
Hepatocellular carcinoma									
OPA (%)	97.6	98.6	90.4	99.0	98.6	94.2	98.1	99.0	93.8

IPS indicates immune cell proportion score; NA, not available; OPA, overall percentage agreement; TPS, tumor proportion score.

FDA-approved PD-L1 clones when tested for indications other than those approved by the regulatory agency.[22]

LBS 73-10 clone showed comparable interassay agreement where the kappa value agreement strength between all the combination clones ranged from substantial to almost perfect. The corresponding pairwise agreement rates demonstrated similar analyses across assays and tumors.

The variations in PD-L1 expression between studied carcinoma types may be attributed to the fact that PD-L1 expression varies significantly in different tumors.[23,24]

TPS and IPS scoring systems are also used in lung carcinoma for PD-L1 detection;[25,26] however, the PD-L1 expression analysis in lung tumor types for the tested clones was out of scope for this study.

Inter-pathologist agreement rate was proportionate across clones. The immune cell PD-L1 agreement rate in breast carcinoma for the 73-10 clone was not very high; however, a similar trend was observed in other comparator assays (K ranged from 0.16 to 0.22 across assays; Table 4) as well. Variability in IPS in different tumor types is well documented;[27,28] therefore, this result is in alignment with prior observations. The sensitivity and specificity of the 73-10 clone were also akin to the comparators. Out of the 3 endpoints (TPS ≥ 1%, TPS ≥ 50%, and IPS ≥ 1%), there was significantly higher discordance for IPS ≥ 1% across all assays. The higher IPS discordance/variability could be due to the immune cell assessment not being used as a regular clinical parameter for detection than the corresponding tumor cell analyses for checkpoint inhibitor scoring within the tumor microenvironment.[16,23]

The use of TMAs instead of the whole tissue sections may be responsible for fewer data points for TPS ≥ 50% for each assay across tumors.[29–31] While all endpoints were evaluated by pathologists at a single site, future studies may warrant evaluating the 73-10 clone with the comparator assays with a higher number of experts across multiple study sites.

It is important to note that this study did not explore the performance of this clone for any of the currently FDA-approved assays for NSCLC, cervical cancer, urothelial carcinoma, squamous cell carcinoma of the head and neck, esophageal squamous cell carcinoma, and TNBC with the commercially available class III PD-L1 assays. Furthermore, this study does not claim that the assay can be used as a laboratory-developed test, companion, or complementary diagnostic test. These study results are preliminary. It may be worthwhile to test this clone in a prospective clinical study and assess its clinical utility in a well-designed clinical trial. Although we have compared the technical performance of the assay with other FDA-approved clones, we cannot comment on the clinical outcome results using the 73-10 clone. The treatment intervention value of an assay may be realized if appropriately tested as per the guidelines set forth by the regulatory agencies (FDA or Clinical Laboratory Improvement Amendments, also known as CLIA).[32] In summary, through this study, the technical assay

TABLE 3. Inter-pathologist Agreement Rate

	Inter-pathologist agreement (Kappa value)		
Assay	**≥ 1% TPS**	**≥ 50% TPS**	**≥ 1% IPS**
		Breast carcinoma	
73-10	0.70	0.50	0.21
SP263	0.63	0.66	0.22
22C3	0.68	NA*	0.20
28-8	0.58	NA*	0.16
		Colorectal carcinoma	
73-10	0.58	0.92	0.68
SP263	0.58	0.92	0.82
22C3	0.38	0.75	0.78
28-8	0.52	0.86	0.65
		Hepatocellular carcinoma	
73-10	0.74	0.91	0.76
SP263	0.80	0.90	0.83
22C3	0.54	0.75	0.70
28-8	0.69	0.94	0.82

*The value could not be calculated due to insufficient tumor or other similar unforeseeable reasons.

IPS indicates immune cell proportion score; NA, not available; TPS, tumor proportion score.

TABLE 4. Surrogate Assay Sensitivity and Specificity

		Breast carcinoma		Colorectal carcinoma		Hepatocellular carcinoma	
Assay	**Cutoff**	**Sensitivity (%)**	**Specificity (%)**	**Sensitivity (%)**	**Specificity (%)**	**Sensitivity (%)**	**Specificity (%)**
73-10	TPS 1%	78.3	100	100	97.9	100	98.5
	TPS 50%	100	99.5	100	99.8	100	99.7
	IPS 1%	77.4	82.4	90.7	93.5	91.5	97.9
SP263	TPS 1%	75.3	99.8	100	97.6	100	98.6
	TPS 50%	83.3	99.8	100	99.8	100	99.7
	IPS 1%	80.1	80.2	94.8	96.4	92.2	98.5
22C3	TPS 1%	78.3	99.8	100	97.7	87.5	98.1
	TPS 50%	NA	99.8	100	99.7	86.7	99.7
	IPS 1%	76.9	83.3	94.1	95.6	92.2	97.3
28-8	TPS 1%	88.4	95.9	100	97.8	100	98.3
	TPS 50%	NA	98.2	100	99.8	100	99.8
	IPS 1%	76.1	81.7	93.3	92.3	93.9	98.0

IPS indicates immune cell proportion score; NA, not available; TPS, tumor proportion score.

performance of 73-10 clone for PD-L1 detection was shown within the tested disease states.

Since this was an exploratory study where statistical acceptance criteria (pass/fail criteria) were not defined, future studies incorporating statistical rigor, using whole tissue sections would eliminate the current limitations.

CONCLUSION

Evaluation of LBS 73-10 monoclonal antibody on a large set of carcinomas showed that its technical performance is comparable to the clones utilized in FDA-approved companion/complementary diagnostics PD-L1 detection assays when testing breast, colorectal, and hepatocellular carcinomas for PD-L1 expression, albeit in a nonclinical setting. The 73-10 clone had similar patterns of expression in the tumor and immune cells and good concordance with 22C3 (Dako), 28-8 clone (Dako), and SP263 (Ventana) clones across all endpoints and indications. Similar tumor and immune cell scoring, and comparable inter-pathologist agreement, sensitivity, and specificity of the assays lend supportive data as to the potential use of the LBS 73-10 clone.

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