Original Article

A Noninterventional, Multinational Study to Assess PD-L1 Expression in Cytological and Histological Lung Cancer Specimens

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BACKGROUND: The diagnosis of advanced lung cancer is made with minimally invasive procedures. This often results in the availability of cytological material only for subtype determination and companion diagnostic testing, with the latter being technically and clinically validated on histological material only. Thus, the primary objective of the MO29978 clinical study was to assess programmed death ligand 1 (PD-L1) protein expression on cytology samples as surrogates for histology samples in patients with lung cancer. **METHODS:** Formalin-fixed, paraffin-embedded histological samples and cytological cell blocks from 190 patients were analyzed with immunohistochemical assays using the rabbit monoclonal anti–PD-L1 antibody clones SP142 and SP263. PD-L1 expression was quantified on both tumor cells (TC) and tumor-infiltrating immune cells (IC). Overall concordance, sensitivity, specificity, and accuracy, with a 1% cutoff used for both assays, were assessed for PD-L1 expression on TC and IC. **RESULTS:** In non–small cell lung cancer histology and cytology samples measured with the PD-L1 (SP142) antibody (n = 173), the intraclass correlation coefficients were 0.40 and 0.06 on TC and IC, respectively. With SP142 and SP263, accuracies of 74.1% for TC and 51.9% for IC and accuracies of 75.2% for TC and 61.2% for IC, respectively, were reported. **CONCLUSIONS:** Overall, this study has demonstrated that PD-L1 analysis on TC is feasible in cytological material, but quantification is challenging. Tumor tissue should be preferred over cell block cytology for PD-L1 immunohistochemical analysis unless laboratories have validated their cytology preanalytical approaches and demonstrated the comparability of histology and cytology for TC PD-L1 results. *Cancer Cytopathol* 2020;128:928-938. © 2020 The Authors. Cancer Cytopathology published by Wiley Periodicals LLC on behalf of American Cancer Society This is an open access article under the terms of the Creative Commons Attribution-NonCommercial License, which permits use, distribution and reproduction in any medium, provided the original work is properly cited and is not used for commercial purposes.

KEY WORDS: cytology; histology; immunohistochemistry; lung cancer; programmed death ligand 1 (PD-L1); SP142; SP263.

INTRODUCTION

In the majority of patients, lung cancer is diagnosed at advanced, unresectable stages.[1] A diagnosis made through minimally invasive techniques results in the majority of patients' having only small tissue biopsies or cytology samples for subsequent histological and molecular subclassification.[2]

The standardization of the preparation of formalin-fixed, paraffin-embedded (FFPE) histological material from small biopsies allows for diagnostic assessments that are required for subsequent treatment decisions. However, wide variations in the types, preparatory methods, and diagnostic yields of cytology samples exist. The sample types depend on the accessibility of malignant lesions and include fine-needle aspiration (FNA) conducted with different needle sizes and imaging guidance, bronchial washing, bronchial brushing, bronchoalveolar lavage, sputum, and pleural effusion.[3] Samples can be prepared as conventional smears, liquid-based cytology, or cell blocks embedded in paraffin. The variability inherent to cytological material has resulted in its underuse for predictive immunohistochemistry (IHC). This variability includes the range of sample types and preparations, the difficulty of procuring cytology-specific controls, the use of alcohol-based fixatives that may interfere with results, and the uncertainty of test validation.[4] In addition, sample preservation also varies with the different types of formalin- or alcohol-based fixatives and storage (eg, air-dried or stained smears).[5]

The diagnosis of non–small cell lung cancer (NSCLC) not only requires the determination of the histopathological subtype (nonsquamous vs squamous) through the use of immunological markers (thyroid transcription factor 1 [TTF1] and p40) but also involves companion diagnostic assessments (eg, epidermal growth factor receptor [EGFR], ALK receptor tyrosine kinase [ALK], ROS proto-oncogene 1, receptor tyrosine kinase [ROS1], and programmed death ligand 1 [PD-L1]) that help to inform treatment decisions.[6-10] Although companion diagnostic tests have been approved for use with tumor tissue and even plasma, no test has been developed and approved for cytological material. Patients with only cytological material have always been excluded from pivotal clinical studies in NSCLC.[11] To date, small cell lung cancer (SCLC) does not require additional testing because of the absence of approved targeted therapies.

Since the approval of anti–PD-L1/programmed death 1 (PD-1) inhibitors (pembrolizumab, atezolizumab, nivolumab, and durvalumab) in NSCLC, PD-L1 has become an important predictive biomarker for guiding treatment decisions with anti–PD-L1/PD-1 therapies. Several PD-L1 immunohistochemistry (IHC) assays, including the Ventana PD-L1 SP142 and SP263 assays (Ventana Medical Systems, Inc) and the 22C3 pharmDx and 28-8 pharmDx assays (Dako/Agilent), have been developed as companion diagnostic tests with histological material.[12]

To date, several studies have explored the feasibility of measuring PD-L1 in cytological material. There is a high degree of agreement of tumor cell (TC) PD-L1 expression between histology and cell block cytology using PD-L1 22C3, E1L3N, and SP263 antibody clones.[13-22] Based on results from a feasibility study on cell lines that explored the various fixatives and preparation types,[23] this prospective, multinational study was aimed at determining which of the many sample types could be used to determine PD-L1 expression levels with the anti–PD-L1 antibody clones SP142 and SP263 in patients with lung cancer.

MATERIALS AND METHODS

Study Design and Objectives

The primary objective of this noninterventional, multinational study (MO29978; NCT03092739) was to assess PD-L1 expression on TC and tumor-infiltrating immune cells (IC) in cytological and histological samples prepared from patients with lung cancer with IHC using the SP142 antibody. For a robust evaluation of this objective, 2 readers were employed. Secondary objectives were 1) to explore the concordance of PD-L1–expressing TC and IC in histological samples in comparison with TC in cytological samples, 2) to explore the feasibility of different PD-L1 scoring algorithms for cytological specimens, 3) to explore the concordance and robustness of results related to PD-L1 expression by a second independent reading, and 4) to explore the feasibility of alternative diagnostic technologies. Protocol approval was obtained from the IRB/IEC for each site before participation in the study. The study design is provided in Figure 1.

Patient Samples

Samples were collected in a manner that was compliant with local ethics committee guidance and originated from patients diagnosed with NSCLC or SCLC. Patients had to have a pair of at least 1 cytological specimen and 1 histological specimen; both specimens must have originated from inside the thorax or the same extrathoracic site, been collected within a window of no more than 6 weeks, and been in compliance with the sample requirements for histological and cytological specimens as defined in this study (Fig. 1). Patients
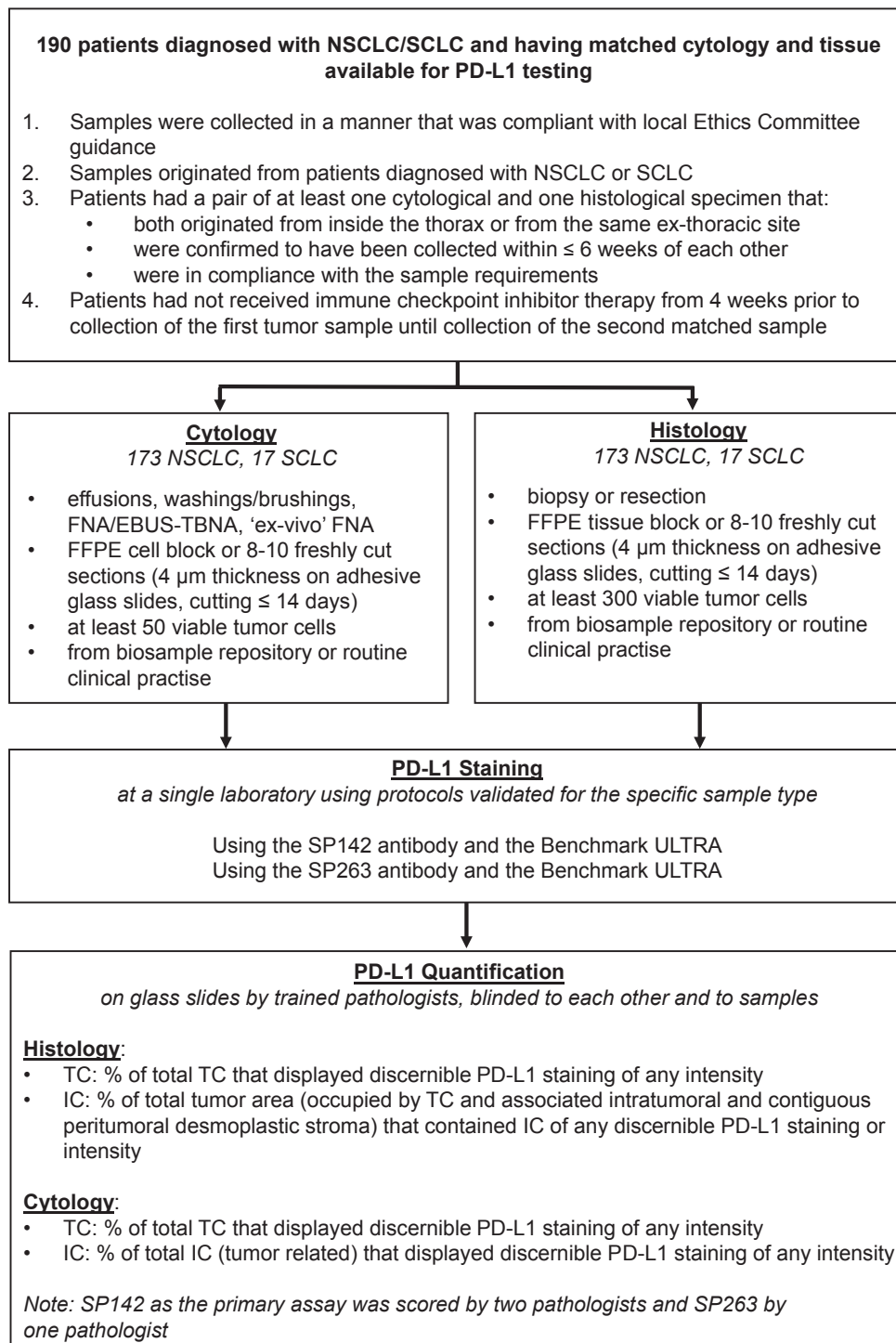
190 patients diagnosed with NSCLC/SCLC and having matched cytology and tissue available for PD-L1 testing

1. Samples were collected in a manner that was compliant with local Ethics Committee guidance
2. Samples originated from patients diagnosed with NSCLC or SCLC
3. Patients had a pair of at least one cytological and one histological specimen that:
   - both originated from inside the thorax or from the same ex-thoracic site
   - were confirmed to have been collected within ≤ 6 weeks of each other
   - were in compliance with the sample requirements
4. Patients had not received immune checkpoint inhibitor therapy from 4 weeks prior to collection of the first tumor sample until collection of the second matched sample

Cytology
173 NSCLC, 17 SCLC

- effusions, washings/brushings, FNA/EBUS-TBNA, 'ex-vivo' FNA
- FFPE cell block or 8-10 freshly cut sections (4 µm thickness on adhesive glass slides, cutting ≤ 14 days)
- at least 50 viable tumor cells
- from biosample repository or routine clinical practise

Histology
173 NSCLC, 17 SCLC

- biopsy or resection
- FFPE tissue block or 8-10 freshly cut sections (4 µm thickness on adhesive glass slides, cutting ≤ 14 days)
- at least 300 viable tumor cells
- from biosample repository or routine clinical practise

PD-L1 Staining
at a single laboratory using protocols validated for the specific sample type

Using the SP142 antibody and the Benchmark ULTRA
Using the SP263 antibody and the Benchmark ULTRA

PD-L1 Quantification
on glass slides by trained pathologists, blinded to each other and to samples

Histology:
- TC: % of total TC that displayed discernible PD-L1 staining of any intensity
- IC: % of total tumor area (occupied by TC and associated intratumoral and contiguous peritumoral desmoplastic stroma) that contained IC of any discernible PD-L1 staining or intensity

Cytology:
- TC: % of total TC that displayed discernible PD-L1 staining of any intensity
- IC: % of total IC (tumor related) that displayed discernible PD-L1 staining of any intensity

Note: SP142 as the primary assay was scored by two pathologists and SP263 by one pathologist

FIGURE 1. Flowchart of the design of a noninterventional, multinational study to assess PD-L1 expression on cytological and histological NSCLC and SCLC specimens. EBUS-TBNA indicates endobronchial ultrasound–guided transbronchial needle aspiration; FFPE, formalin-fixed, paraffin-embedded; FNA, fine-needle aspiration; IC, tumor-infiltrating immune cells; NSCLC, non–small cell lung cancer; PD-L1, programmed death ligand 1; SCLC, small cell lung cancer; TC, tumor cells.

also must have not received immune checkpoint inhibitor therapy from 4 weeks before the collection of the first tumor sample until after the collection of the second sample. All samples were collected from material retained during routine clinical practice or from biosample repositories housed at participating sites.

Sample Processing and Staining Procedures

Cytological samples could have originated from pleural effusions, bronchial washing/brushing, FNA, or ex vivo FNA (see Supporting Table 1 for details of the ex vivo FNA biopsy procedure); they were prepared as FFPE cell blocks with 10% neutral buffered formalin used as either a fixative or a postfixative and were embedded in paraffin according to the local cell block protocol. Exposure to formalin and embedding in paraffin were mandated on the basis of prior research.[23] FFPE histological samples were obtained by biopsy or surgical resection. Blinding of both cohorts was performed so that histology and cytology samples could not be matched by the pathologists. Samples were sent for hematoxylin-eosin staining and PD-L1 analysis (Fig. 1). For details of the PD-L1 staining methodology, please see the supporting information.

Quantification of PD-L1 Staining

The following parameters were used for histological samples: PD-L1 expression on TC was indicated by the percentage of total TC that displayed discernible membranous PD-L1 staining of any intensity (minimum of 300 viable TC), and expression on IC was determined by the percentage of the total tumor area (occupied by TC and associated intratumoral and contiguous peritumoral desmoplastic stroma) that contained IC with discernible PD-L1 staining of any intensity. For cytological samples, PD-L1 expression on TC was indicated by the percentage of total TC that displayed discernible membranous PD-L1 staining of any intensity (minimum of 50 viable TC), and expression on IC was determined by the percentage of total IC that displayed discernible PD-L1 staining of any intensity. PD-L1 expression as determined with the anti–PD-L1 antibody clone SP142 was quantified by 2 independent pathologists. PD-L1 expression from anti–PD-L1 antibody clone SP263 expression was quantified by a single pathologist.

Statistical Analysis

This study was designed primarily to assess the concordance of PD-L1 SP142 staining samples for NSCLC, with SCLC samples analyzed separately in an exploratory manner. PD-L1 staining was also examined with IHC using the SP263 antibody. Results for TC and IC

data are described separately for each PD-L1 assay. An analysis of variance model was used to calculate intraclass correlation coefficients (ICCs) to summarize the intermethod variability (ie, the variability of PD-L1 quantification when performed on cytology vs histology) and the interreader variability for NSCLC samples examined with the SP142 antibody. This model included patient and method/reader as random effects. Sensitivity, specificity, and accuracy were determined with PD-L1 expression measured on histology samples as the reference and with a cutoff of ≥1% for TC or IC for both PD-L1 assays.

RESULTS

Samples (histology and cytology) from 190 patients with lung cancer (173 patients with NSCLC and 17 patients with SCLC) across 8 sites in 6 countries were analyzed for PD-L1 expression on TC and IC in this study (Figs. 1 and 2). Baseline characteristics and sample details are provided in Table 1. SCLC results are provided in Supporting Table 2. Overall, most samples for both histology and cytology were lung adenocarcinomas prepared as FFPE blocks and had an ischemia time of 1 to 8 hours, and they were mostly fixed for 6 to 48 hours in formalin. Most samples collected by washing or brushing were squamous cell carcinomas.

IHC Expression Using the SP142 Antibody

For the NSCLC samples, SP142 IHC TC and IC scoring was successful for 170 histology specimens and 165 cytology specimens. Among these specimens, 62 histology samples (36.5%) and 53 cytology samples (32.1%) were found to express PD-L1 on TC. PD-L1 expression on IC was observed for 96 of the 170 histology samples (56.5%) and for 22 of the 165 cytology samples (13.3%; Table 2). Among the 165 evaluable samples, 22 cytological specimens had <100 TC (pleural effusion, 1; FNA, 7; ex vivo FNA, 10; and brushings/washings, 4).

PD-L1 expression on TC according to cytological sample type is described in Figure 3A for NSCLC with the SP142 antibody. For histological samples, the percentage of tumors with PD-L1 expression (≥1%) on TC was highest in patients who had an FNA cytological sample and lowest for patients with a pleural effusion sample (Fig. 3A).

The agreement of histology with cytology was evaluated on a continuous scale. With the SP142 antibody, the ICCs for histology versus cytology in NSCLC were 0.40 (95% CI, 0.26-0.52) for TC and 0.06 (95% CI, –0.06
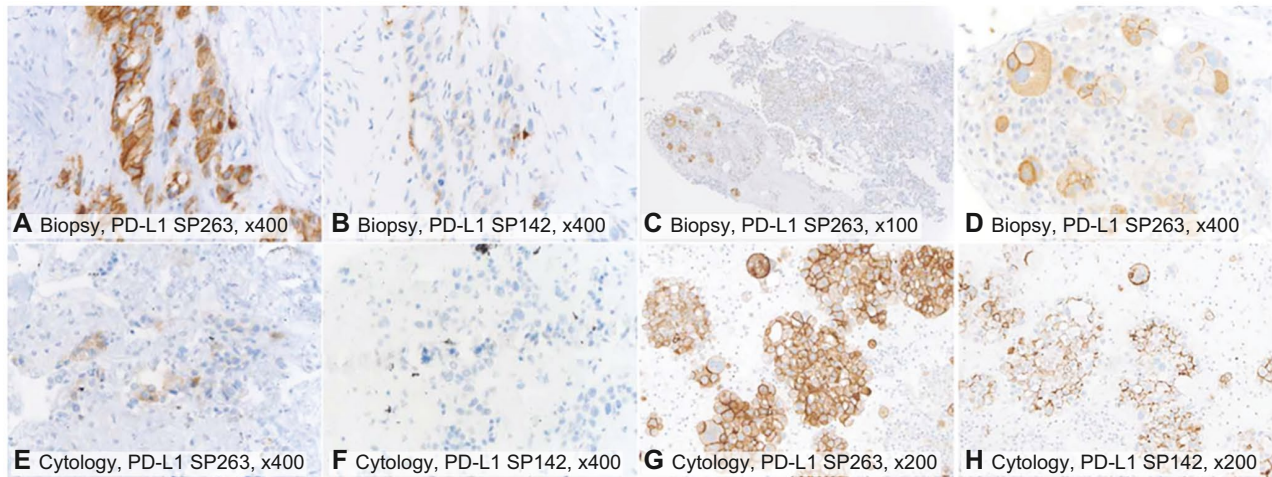
FIGURE 2. Examples of PD-L1 immunohistochemistry staining on (A-D) biopsies and (E-H) cytological cell block specimens using (A,C-E,G) the SP263 antibody and (B,F,H) the SP142 antibody, with brown membranous staining indicating PD-L1 positivity. (A,B,E,F) Case 1: SP263 stained a higher proportion of tumor cells than SP142 and was lower in the fine-needle aspiration cytology than the biopsy. (F) There was no staining by SP142 in cytology. (C,D,G,H) PD-L1 staining was heterogeneous in the biopsy ([C,D] SP263) but homogeneous in the cytology of the pleural effusion ([G] SP263 and [H] SP142). The magnifications were (A,B,D-F) ×400, (G,H) ×200, and (C) ×100. PD-L1 indicates programmed death ligand 1.

to 0.20) for IC (Fig. 4A). ICCs for histology versus cytology of the second independent reading were 0.23 and 0.17, respectively. Agreement was also evaluated by applying a 1% cutoff to both PD-L1 TC and IC scoring and thereby using PD-L1 as a binary variable to account for the challenges of performing an absolute quantification on cytological material. For this assay, specificity was high for both TC (84.2%) and IC (94.3%); however, sensitivity was low, especially for IC (19.6%; Table 3). These results were confirmed by the second pathologist's reading. Interreader agreement data are presented in Supporting Figure 1. In addition, post hoc analyses to assess the agreement between artificial ex vivo FNA samples and routinely performed cytological sample types (Supporting Table 3) and between cytology samples with <100 TC and cytology samples with ≥100 TC (Supporting Table 4) were completed.

IHC Expression Using the SP263 Antibody

PD-L1 (SP263) TC scoring was successful for 169 histology samples and 157 cytology samples, and IC scoring was successful for 168 histology samples and 157 cytology samples. PD-L1 staining on TC was present for 80 of 169 histology samples (47.3%) and for 59 of 157 cytology samples (37.6%). PD-L1 IC staining was observed for 125 of 168 histology samples (74.4%) and for 79 of 157 cytology samples (50.3%). TC staining using the

SP263 antibody resulted in a higher percentage of samples with PD-L1 expression in comparison with SP142 (Table 2).

PD-L1 expression on TC according to cytological sample type is described in Figure 3B for NSCLC with the SP263 antibody. For histological samples, the percentage of tumors with PD-L1 expression (≥1%) on TC was lowest when the cytology sample type was a pleural effusion (Fig. 3B).

The agreement of histology with cytology on a continuous scale was also evaluated with the SP263 antibody. Scatterplots of histology and cytology on TC and IC are shown in Figure 4B. The ICCs for cytology versus histology were 0.56 (95% CI, 0.43-0.65) for TC and 0.10 (95% CI, −0.04 to 0.25) for IC. When a comparison of histology and cytology was performed with a 1% cutoff applied to both PD-L1 TC and IC scoring, the specificity was 86.1% for TC and 69.2% for IC. Sensitivity was comparable for TC (63.5%) and IC (58.4%), and accuracy was 75.2% for TC and 61.2% for IC with a 1% cutoff (Table 3).

DISCUSSION

This study is the largest fully blinded, prospective analysis to comprehensively assess PD-L1 expression by using 2 different PD-L1 antibodies (SP142 and SP263) in lung histology and cell block cytology samples. For the SP142 antibody,

TABLE 1. Baseline Characteristics of Histological and Cytological Samples From Patients With Lung Cancer

	Histology, No. (%)				Cytology, No. (%)		
	NSCLC	SCLC	Total		NSCLC	SCLC	Total
Total	173 (100.0)	17 (100.0)	190 (100.0)	Total	173 (100.0)	17 (100.0)	190 (100.0)
Location of lesion				Location of lesion			
Thorax	146 (84.4)	9 (52.9)	155 (81.6)	Thorax	134 (77.5)	6 (35.3)	140 (73.7)
Bronchus	27 (15.6)	8 (47.1)	35 (18.4)	Lymph node	8 (4.6)	4 (23.5)	12 (6.3)
				Pleura	3 (1.7)	—	3 (1.6)
				Other	28 (16.2)	7 (41.2)	35 (18.4)
Lung cancer subtype				Lung cancer subtype			
Small cell carcinoma	—	17 (100.0)	17 (8.9)	Small cell carcinoma	—	17 (100.0)	17 (8.9)
Adenocarcinoma	98 (56.6)	—	98 (51.6)	NSCC favor adenocarcinoma	92 (53.2)	—	92 (48.4)
Squamous cell carcinoma	58 (33.5)	—	58 (30.5)	NSCC favor squamous cell carcinoma	43 (24.9)	—	43 (22.6)
Adenosquamous carcinoma	3 (1.7)	—	3 (1.6)	NSCC NOS	20 (11.6)	—	20 (10.5)
Large cell carcinoma	4 (2.3)	—	4 (2.1)	Squamous cell carcinoma	15 (8.7)	—	15 (7.9)
Other	10 (5.8)	—	10 (5.3)	Other	3 (1.7)	—	3 (1.6)
Method of sample collection				Method of sample collection			
Resection	84 (48.6)	1 (5.9)	85 (44.7)	FNA	50 (28.9)	7 (41.2)	57 (30.0)
Biopsy	89 (51.4)	16 (94.1)	105 (55.3)	Pleural effusion	21 (12.1)	1 (5.9)	22 (11.6)
				Washing/brushing	33 (19.1)	2 (11.8)	35 (18.4)
				EBUS-TBNA	7 (4.0)	3 (17.6)	10 (5.3)
				Ex vivo FNA	62 (35.8)	—	62 (32.6)
Ischemia time				Ischemia time			
1-8 h	108 (62.4)	6 (35.3)	114 (60.0)	1-8 h	97 (56.1)	6 (35.3)	103 (54.2)
Unknown	65 (37.6)	11 (64.7)	76 (40.0)	Unknown/missing	76 (43.9)	11 (64.7)	87 (45.8)
Fixation time				Fixation time			
<6 h	28 (16.2)	1 (5.9)	29 (15.3)	<6 h	28 (16.2)	1 (5.9)	29 (15.3)
6-48 h	80 (46.2)	5 (29.4)	85 (44.7)	6-48 h	79 (45.7)	5 (29.4)	84 (44.2)
Unknown	65 (37.6)	11 (64.7)	76 (40.0)	Unknown	66 (38.2)	11 (64.7)	77 (40.5)
Fixative				Fixative			
10% neutral buffered formalin	173 (100.0)	17 (100.0)	190 (100.0)	10% neutral buffered formalin	113 (65.3)	6 (35.3)	119 (62.6)
				CytoLyt	58 (33.5)	11 (64.7)	69 (36.3)
				Cytofix	1 (0.6)	—	1 (0.5)
				PreservCyt	1 (0.6)	—	1 (0.5)
				Postfixative			
				10% neutral buffered formalin	158 (91.3)	16 (94.1)	174 (91.6)
				Other	—	1 (5.9)	1 (0.5)
				Missing	15 (8.7)	—	15 (7.9)
Type of sample sent for analysis				Type of sample sent for analysis			
Block	69 (39.9)	5 (29.4)	74 (38.9)	Block	71 (41.0)	5 (29.4)	76 (40.0)
Slide	104 (60.1)	12 (70.6)	116 (61.1)	Slide	102 (59.0)	12 (70.6)	114 (60.0)

Abbreviations: EBUS-TBNA, endobronchial ultrasound–guided transbronchial needle aspiration; FNA, fine-needle aspiration; NOS, not otherwise specified; NSCC, non–squamous cell cancer; NSCLC, non–small cell lung cancer; SCLC, small cell lung cancer.

TABLE 2. PD-L1 Expression Levels on TC/IC in the NSCLC Cohort

		SP142 Antibody, No. (%)				SP263 Antibody, No. (%)	
		First Evaluation		Second Evaluation		First Evaluation	
		Cytology (n = 165)	Histology (n = 170)	Cytology (n = 160)	Histology (n = 168)	Cytology (n = 157)	Histology (n = 168)
TC, %	PD-L1– (<1%)	112 (67.9)	108 (63.5)	136 (84.5)	129 (76.3)	98 (62.4)	89 (52.7)
	PD-L1+ (≥1%)	53 (32.1)	62 (36.5)	25 (15.5)	40 (23.7)	59 (37.6)	80 (47.3)
	1 to <5	32 (19.4)	26 (15.3)	10 (6.2)	16 (9.5)	23 (14.6)	28 (16.6)
	5 to <50	17 (10.3)	26 (15.3)	11 (6.8)	17 (10.1)	23 (14.6)	30 (17.8)
	≥50	4 (2.4)	10 (5.9)	4 (2.5)	7 (4.1)	13 (8.3)	22 (13.0)
IC, %	PD-L1– (<1%)	143 (86.7)	74 (43.5)	117 (73.1)	66 (39.3)	78 (49.7)	43 (25.6)
	PD-L1+ (≥1%)	22 (13.3)	96 (56.5)	43 (26.9)	102 (60.7)	79 (50.3)	125 (74.4)
	1 to <5	21 (12.7)	72 (42.4)	32 (20.0)	58 (34.5)	59 (37.6)	68 (40.5)
	5 to <10	1 (0.6)	17 (10.0)	8 (5.0)	32 (19.0)	15 (9.6)	41 (24.4)
	≥10	0 (0)	7 (4.1)	3 (1.9)	12 (7.1)	5 (3.2)	16 (9.5)

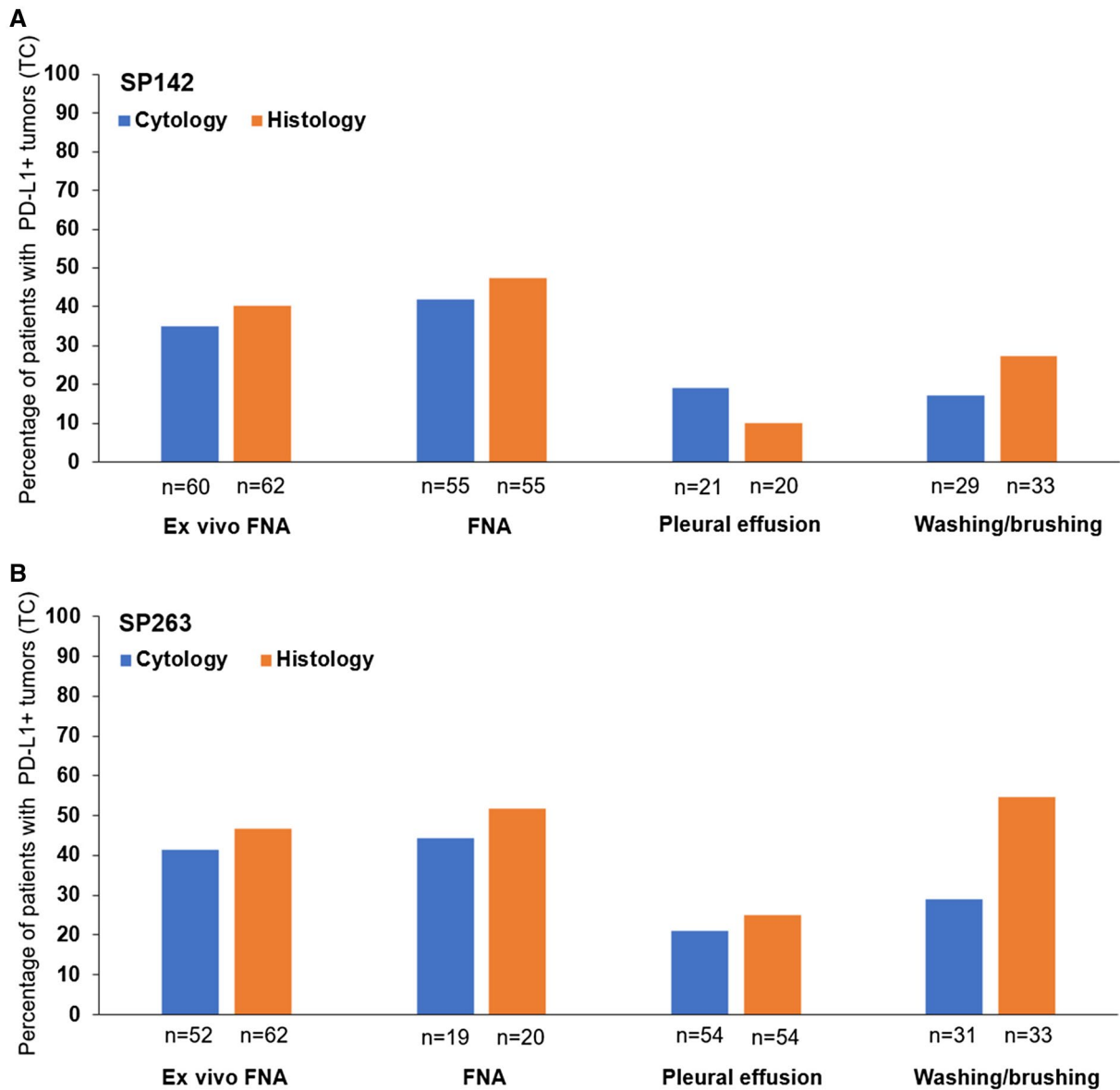Abbreviation: IC, immune cell; PD-L1, programmed death ligand 1; TC, tumor cell.

FIGURE 3. Histograms representing the percentages of patients with TC PD-L1 expression in histology and cytology samples according to the cytological sample type with (A) the SP142 antibody and (B) the SP263 antibody. FNA also included transbronchial needle aspiration. There were differences in the numbers of evaluable histology and cytology samples between the SP142 and SP263 results. Positive PD-L1 expression (PD-L1+) was defined as ≥1% of TC. FNA indicates fine-needle aspiration; PD-L1, programmed death ligand 1; TC, tumor cell.

this study has demonstrated an interreader agreement for TC scoring consistent with results from the Blueprint2 project in NSCLC.[13] IC scoring had considerably lower interreader agreement, which also confirms the finding from Blueprint2 that IC cannot be reliably quantified in NSCLC histology or cytology material.

When we compared NSCLC histology and cytology, agreement as per the ICC for TC or IC scoring could not be demonstrated with PD-L1 expression as a continuous variable. For a secondary analysis, a 1% cutoff was applied, and both assays had high specificity but lacked sensitivity. Thus, it may be possible to have false-negative results in cytological samples because of the low abundancy of IC or TC. In addition, despite being used in routine clinical practice, these cytological samples can be challenging to obtain. A requirement of ≥50 TC was chosen for cytological specimens because routinely obtained materials may contain <50 TC.
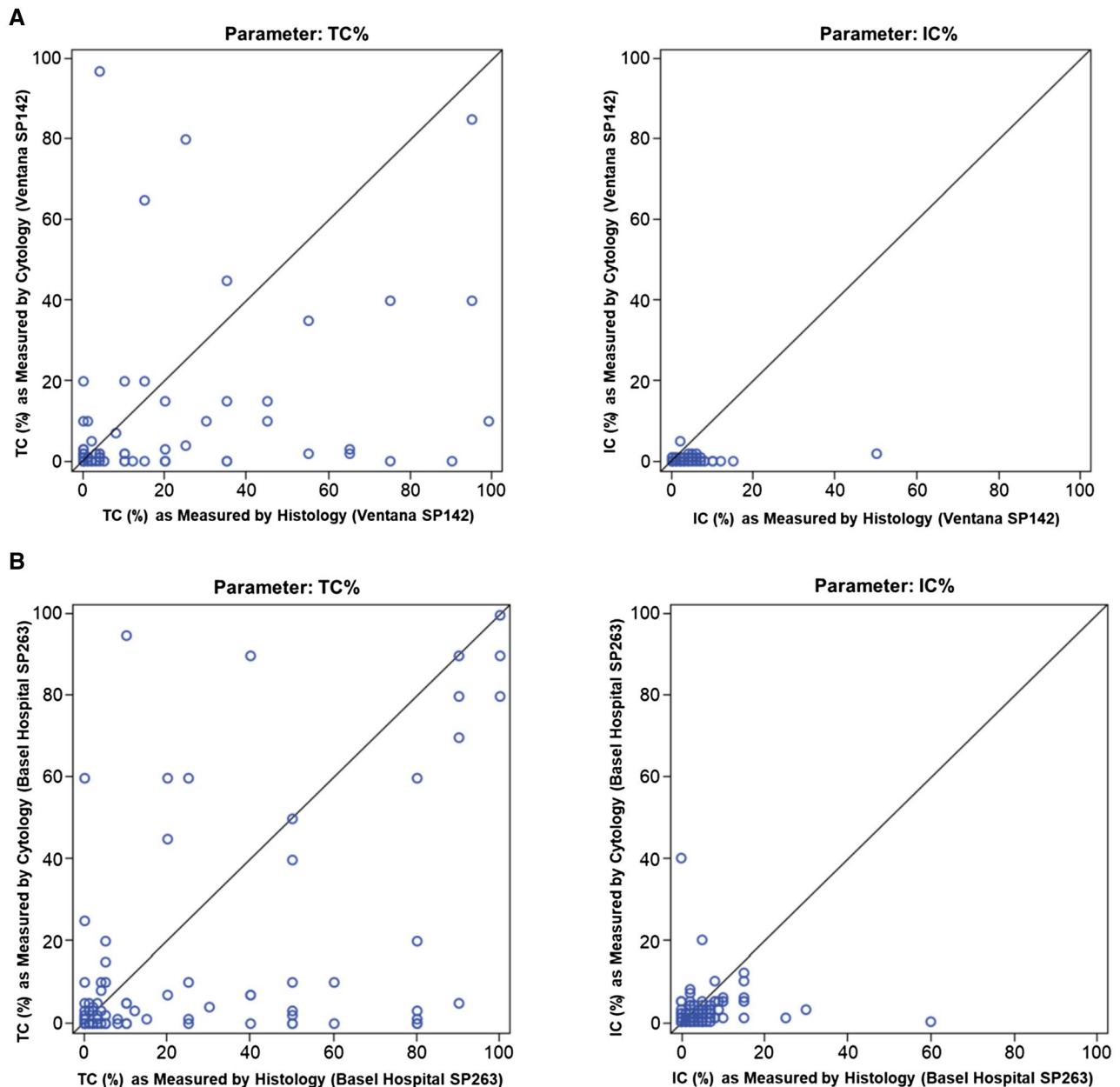
FIGURE 4. Scatter plots showing (A) the agreement of histology and cytology methods in TC and IC with the PD-L1 SP142 antibody and (B) the agreement of histology and cytology methods in TC and IC with the PD-L1 SP263 antibody. IC indicates immune cell; PD-L1, programmed death ligand 1; TC, tumor cell.

A subgroup analysis of cytological specimens with ≥100 TC demonstrated that accuracy and specificity were not dissimilar from those for specimens with ≥50 cells. However, sensitivity was lower in samples with 50 to 99 TC, and this suggests that a higher number of TC may be required to achieve better sensitivity in cytological specimens.

In addition, sensitivity was lower for the SP142 antibody than the SP263 antibody, and this confirmed the

results from Blueprint1.[20] In addition, some differences might be due to variances between the sample populations (Table 2).

IC scoring of cytological samples was challenging because differentiation of tumor-associated IC is not possible in samples that lack tissue context (eg, FFPE cell blocks from pleural effusions). Furthermore, samples from endobronchial ultrasound may be contaminated with non–tumor-associated lymphocytes. Taken together,

TABLE 3. Performance of PD-L1 Scoring Using Cytology Samples and Applying a 1% Cutoff (With Histology as the Reference)

SP142 Antibody: First Reader				SP142 Antibody: Second Reader				SP263 Antibody		
PD-L1 TC Status				PD-L1 TC Status				PD-L1 TC Status		
	Histology, No.				Histology, No.				Histology, No.	
Cytology	<1%	≥1%	Total	Cytology	<1%	≥1%	Total	Cytology	≥1%	Total
<1%	85	26	111	<1%	112	20	132	<1%	68	27	95
≥1%	16	35	51	≥1%	6	19	25	≥1%	11	47	58
Total	101	61	162	Total	118	39	157	Total	79	74	153
Performance	% (95% CI)			Performance	% (95% CI)			Performance	% (95% CI)	
Sensitivity	57.4 (44.1-70.0)			Sensitivity	48.7 (32.4-65.2)			Sensitivity	63.5 (51.5-74.4)	
Specificity	84.2 (75.6-90.7)			Specificity	94.9 (89.3-98.1)			Specificity	86.1 (76.5-92.8)	
Accuracy	74.1 (66.6-80.6)			Accuracy	83.4 (76.7-88.9)			Accuracy	75.2 (67.5-81.8)	
PD-L1 IC Status				PD-L1 IC Status				PD-L1 IC Status		
	Histology, No.				Histology, No.				Histology, No.	
Cytology	<1%	≥1%	Total	Cytology	<1%	≥1%	Total	Cytology	≥1%	Total
<1%	66	74	140	<1%	55	58	113	<1%	27	47	74
≥1%	4	18	22	≥1%	6	36	42	≥1%	12	66	78
Total	70	92	162	Total	61	94	155	Total	39	113	152
Performance	% (95% CI)			Performance	% (95% CI)			Performance	% (95% CI)	
Sensitivity	19.6 (12.0-29.2)			Sensitivity	38.3 (28.5-48.9)			Sensitivity	58.4 (48.8-67.6)	
Specificity	94.3 (86.0-98.4)			Specificity	90.2 (79.8-96.3)			Specificity	69.2 (52.4-83.0)	
Accuracy	51.9 (43.9-59.8)			Accuracy	58.7 (50.5-66.6)			Accuracy	61.2 (53.0-69.0)	

Abbreviations: IC, immune cell; PD-L1, programmed death ligand 1; TC, tumor cell.

these data support the common practice to not report IC scores from cytological material.[4,13]

Overall, TC scoring was considered feasible. With a 1% cutoff, the reported overall agreement for both the SP142 and SP263 antibodies was lower than but comparable to the overall agreement, which has been shown to range from 82% to 97%.[4,15,17,21,24] These differences may be due to variations in the ways in which samples were obtained in different studies. Prior studies were mostly from a single site, and this allowed for more preanalytical standardization, local validation, and optimization of the IHC protocols for conditions at a single laboratory.

This study also included different cytological sample types, such as washings or brushings, effusions, small aspirates, and artificially generated ex vivo FNA.

Currently, it is unclear to what degree these types of cytological samples are suitable for optimal quantification of PD-L1 expression. Furthermore, cytological material included in this study was not completely representative of clinical practice because it contained a sizable fraction of artificial ex vivo FNA preparations taken from fresh resection specimens. Such specimens may be less homogeneous and less cellular than endobronchial ultrasound–FNA specimens in clinical practice. Although there is heterogeneity in tumor specimens in clinical practice and in this study, a subgroup analysis evaluating the sensitivity, specificity, and agreement of the NSCLC specimens with artificially generated ex vivo FNA samples from resection specimens did not result in outcomes that differed from the results of samples obtained in routine clinical practice.

This study had several other limitations. Because non–formalin-fixed samples require a different staining procedure, this study was restricted to formalin-exposed samples. Nevertheless, there is emerging evidence suggesting that PD-L1 testing may be applicable to ethanol-fixed, non–cell block cytology specimens.[22,24] Another contributor to variation could be that the histological material used in this study included both biopsy and resection specimens. Furthermore, no meaningful comparisons could be made between histology and cytology for the SCLC cohort because of the small number of samples collected and the overall low PD-L1 expression. Another limitation caused by the small sample size was the inability to address other expression cutoffs (eg, 25% and 50%) that might be clinically more relevant than the 1% cutoff.[25]

Overall, this study has demonstrated that qualitative PD-L1 analysis is feasible on almost all available cytological sample types when they are processed into cell blocks; however, absolute quantification remains a challenge because of the limited number of TC and IC, the absence of tissue context, and the risk of false-negative results. Rigorous local validation, quality control, and assurance of tumor content are critical at each laboratory to minimize false-negative results. Histological tumor specimens should always be preferred over cytology for PD-L1 assays unless laboratories have validated cytological processes for comparisons. With some limitations and risk of false-negative results, this study has demonstrated that PD-L1 expression on TC can be assessed qualitatively (applying a 1% cutoff); however, IC are unable to be robustly scored in cytology and thus should not be reported.

CONFLICT OF INTEREST DISCLOSURES

Lukas Bubendorf received a grant from Roche during the conduct of this study; a grant and personal fees from Bristol Myers Squibb; and personal fees from Boehringer Ingelheim, AstraZeneca, Pfizer, Bayer, AbbVie, and Eli Lilly. Esther Conde received personal fees from Roche and Pfizer and nonfinancial support from Roche, Pfizer, and MSD. Federico Cappuzzo received personal fees from Roche, AstraZeneca, Bristol Myers Squibb, MSD, Pfizer, Takeda, Eli Lilly, and Bayer. Hans-Ulrich Schildhaus received a grant from Roche during the conduct of this study and personal fees from Roche, Bristol Myers Squibb, and MSD. Isabel Esteban-Rodriguez received product and technical financing and nonfinancial support from Roche during the conduct of this study and personal fees from Roche, MSD, AstraZeneca, and Pfizer. Janine Feng, Jenny Devenport, Christine Boyiddle, Stefanie Morris, and Kerstin Trunzer are employed by Roche; Feng, Devenport, and Trunzer have received Roche stock. Keith M. Kerr received personal fees from Roche and Roche Diagnostics. The other authors made no disclosures.

AUTHOR CONTRIBUTIONS

Lukas Bubendorf: Conceptualization, funding acquisition, methodology, investigation, project administration, resources, supervision, writing–original draft, and writing–review and editing. **Esther Conde:** Resources and writing–review and editing. **Federico Cappuzzo:** Conceptualization, writing–original draft, and writing–review and editing. **Renata Langfort:** Investigation, resources, and writing–review and editing. **Hans-Ulrich Schildhaus:** Investigation, resources, and writing–review and editing. **Jiří Votruba:** Investigation, validation, and writing–review and editing. **Ángel Concha-López:** Resources and writing–review and editing. **Isabel Esteban-Rodriguez:** Investigation, resources, and writing–review and editing. **Janine Feng:** Investigation and writing–review and editing. **Jenny Devenport:** Data curation, formal analysis, project administration, visualization, and writing–review and editing. **Christine Boyiddle:** Methodology and writing–review and editing. **Stefanie Morris:** Writing–review and editing. **Kerstin Trunzer:** Conceptualization, investigation, methodology, supervision, visualization, writing–original draft, and writing–review and editing. **Keith M. Kerr:** Conceptualization, methodology, supervision, writing–original draft, and writing–review and editing.

DATA AVAILABILITY

Qualified researchers may request access to individual patient–level data through the clinical study data request platform (https://vivli.org/). Further details on Roche's criteria for eligible studies are available at https://vivli.org/members/ourmembers/. For further details on Roche's Global Policy on the Sharing of Clinical Information and how to request access to related clinical study documents, go to https://www.roche.com/research_and_development/who_we_are_how_we_work/clinical_trials/our_commitment_to_data_sharing.htm.

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