Assessment of Programmed Death–Ligand 1 (PD-L1) Immunohistochemical Expression on Cytology Specimens in Non–Small Cell Lung Carcinoma

A Comparative Study With Paired Surgical Specimens

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Key Words: Cytology; Cell block; Non–small cell lung carcinoma (NSCLC); Immunohistochemistry (IHC); Programmed death–ligand 1 (PD-L1); Effusion; Fine-needle aspiration; Immunotherapy

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ABSTRACT

Objectives: To evaluate whether non–small cell lung carcinoma (NSCLC) cytology specimens are reliable for programmed death–ligand 1 (PD-L1) immunohistochemical (IHC) testing.

Methods: Fifty-two cell blocks (CBs) with corresponding surgical pathology PD-L1 IHC testing were stained with a Dako PD-L1 pharmDX antibody (clone-22C3). Tumor cellularity was recorded as <100 or ≥100 cells. PD-L1 IHC was scored by percentage of tumor cells staining (<1%, ≥1%-49%, ≥50%) and compared between matched cases.

Results: Substantial agreement (κ = 0.63; 95% CI, 0.53-0.73) was reached between matched CB and surgical cases in CBs with ≥100 tumor cells compared to CBs with <100 tumor cells (slight agreement, κ = 0.19; 95% CI, 0.04-0.35). Overall, there was 67% agreement among paired cases (35/52 cases, κ = 0.51; 95% CI, 0.42-0.60).

Conclusions: CBs can be utilized for PD-L1 IHC testing, as illustrated by the 67% agreement between CB and surgical cases in our study. Disagreement is attributable to intratumoral heterogeneity and CB cellularity.

Lung cancer remains the leading cause of cancer deaths in the United States, with non–small cell lung carcinoma (NSCLC) accounting for most cases in both men and women.1 Patients with NSCLC often present at late stages. Chemotherapy and/or radiotherapy have historically served as first-line treatments for advanced disease but have offered limited benefit, with low response rates and low overall survival.2-3 Immune checkpoint inhibitors targeting the programmed cell death 1 (PD-1)/programmed death–ligand 1 (PD-L1) axis have emerged as promising therapeutic agents for this group of patients.4-6 PD-L1 immunohistochemistry (IHC) is the current predictive biomarker used to select patients for immunotherapy treatment. The efficacy of immune checkpoint inhibitors relies on the evaluation and scoring of PD-L1 expression by IHC.

To date, only patients with surgical pathology material have been included in clinical trials for PD-L1 immunotherapy. As a result, PD-L1 assays have been approved for testing on histologic specimens, with exclusion of cytologic material. However, approximately 70% of patients with lung cancer present at an advanced stage; therefore, cytology specimens may represent the initial or only material available for diagnosis and ancillary testing, often due to unresectable disease.7-8 It is precisely these patients who may receive the most benefit from immunotherapy.
The most recent National Comprehensive Cancer Network guidelines recommend that all patients with NSCLC be tested for PD-L1 expression.9 Over the past year, our laboratory has received increased requests for PD-L1 IHC evaluation on cytology specimens of patients with NSCLC. As a result, we performed an in-house validation and an investigational study to assess whether cytology specimens from patients with NSCLC are adequate and reliable for PD-L1 IHC evaluation. Furthermore, we compared PD-L1 scoring between paired surgical and cytologic specimens, as limited studies have investigated the level of concordance between surgical and cytologic PD-L1 scores.

Materials and Methods

Case Selection

The current study protocol was approved by the New York University Institutional Review Board. A retrospective review of the pathology database (Powerpath; Sunquest) was performed between January 1, 2016, and December 30, 2017, to identify NSCLC surgical biopsy and resection cases that had PD-L1 testing performed and reported. The concurrent or subsequent cytology preparations for each surgical pathology case yielded by our search were evaluated for (1) the presence of a formalin-fixed, paraffin-embedded (FFPE) cell block and (2) adequate tumor cellularity. Cases with fewer than 50 tumor cells were excluded from this study.

Cytologic Preparation and Study Samples

Cell blocks were prepared using fine-needle aspiration (FNA) material placed in RPMI solution (Corning Cellgro). Effusion specimens were decanted into a conical tube and centrifuged to obtain a sufficient cell pellet. The supernatant fluid was removed. Cell blocks were further prepared by the plasma-thrombin method, fixed in 10% neutral buffered formalin for at least 6 hours, and embedded in paraffin, and sections were stained with H&E. Both surgical and cytologic specimens were diagnosed using the 2015 World Health Organization classification for surgical and cytologic specimens were diagnosed using.404 lung tumors. The 2015 World Health Organization classification for surgical and cytologic specimens were diagnosed using.

ded in paraffin, and sections were stained with H&E. Both neutral buffered formalin for at least 6 hours, and embed
ded in paraffin, and sections were stained with H&E. Both surgical and cytologic specimens were diagnosed using the 2015 World Health Organization classification for lung tumors.10 Immunohistochemical expression of protein markers including, but not limited to, thyroid transcription factor 1 (TTF-1), p40, p63, napsin-A, MOC-31, or BER-EP4 were performed, when necessary, to aid in the diagnosis of NSCLC.

In total, 52 cytology cases from 50 patients (two patients had two cytology specimens) with paired surgical specimens were selected for this study. Cytology samples were formalin-fixed cell blocks from pleural fluid (n = 20), lung FNA (n = 16), lymph node FNA (n = 14), bronchial brush (n = 1), and mediastinal FNA (n = 1).

PD-L1 Immunohistochemical Staining and Evaluation

Cell blocks of the cases selected for this study were retrieved and 4-μm serial sections were obtained. Unstained slides were used within the same week of serial sectioning for PD-L1 immunostaining. PD-L1 IHC expression was quantified using the PD-L1 clone 22C3 pharmDx kit (Dako/Agilent Technologies) and a Ventana BenchMark automated stainer (Ventana Medical Systems) on FFPE surgical and cell block sections. The Ventana platform was previously validated for surgical specimens in our laboratory by comparing the gold-standard (Keytruda test) scores with the same in-house NSCLC cases run on the Ventana platform with the OptiView detection kit, resulting in a correlation that was positive and significant (r = 0.88, P < 0.001). The sensitivity, specificity, positive predictive value, and negative predictive values were 92.3%, 100%, 100%, and 86.7%, respectively.11 Each PD-L1 run included positive and negative cell line controls provided by Dako. In addition, an in-house positive control (tonsil tissue) was included on every PD-L1 immunostained slide.

PD-L1 expression was evaluated using the tumor proportion score (TPS), defined as the percentage of viable tumor cells with partial/complete membranous staining at any intensity with respect to all viable tumor cells within the slide.12 The TPS results were then categorized as negative (TPS <1%), low positive (TPS ≥1%-49%), or high positive (TPS ≥50%). The following were not included in the TPS: cytoplasmic staining without any membranous staining, any staining in background cells (such as non-neoplastic cells including macrophages, hematopoietic cells, bronchial cells, and mesothelial cells), and any staining in necrotic cells.

PD-L1 expression in cytology samples was scored by two board-certified pathologists at a multiheaded microscope (A.S. and A.H.; a cytopathologist and a cytopathology fellow). The pathologists were blinded to the corresponding surgical specimen PD-L1 scores and were previously trained in PD-L1 evaluation by an in-house experienced pulmonary pathologist/cytopathologist (A.L.M.). All cases of PD-L1 score discordance between surgical and cytology samples were subsequently blindly reviewed by A.L.M.

All surgical specimens used for paired comparison had prior PD-L1 IHC performed with Dako PD-L1 pharmDx (clone 22C3), with TPS reported between January 2016 and December 2017 by a group of three pulmonary surgical pathologists. As part of

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the current study, these scores were also categorized as negative (<1%), low positive (≥1%-49%), and high positive (≥50%).

Statistics
Cohen’s κ statistic was calculated for the matched cytology and surgical pathology cases using IBM SPSS Statistics Version 23 (SPSS). The strength of association (agreement) was categorized as follows: 1.00, perfect agreement; 0.81 to 0.99, almost perfect agreement; 0.61 to 0.80, substantial agreement; 0.41 to 0.60, moderate agreement; 0.21 to 0.40, fair agreement; 0.00 to 0.20, slight agreement; and less than 0, poor agreement.

Results
A total of 52 paired specimens with a diagnosis of NSCLC were collected from 50 patients with a mean age of 68 years (range, 36-91 years). Diagnoses among patients included the following: pulmonary adenocarcinoma (n = 47), squamous cell carcinoma (n = 2), poorly differentiated NSCLC (n = 2), and NSCLC with sarcomatoid features (n = 1).

Surgical Pathology Samples
Of the 52 surgical cases, 36 came from direct sampling of the lung, including 27 surgical biopsy specimens and nine resection specimens; the remaining surgical tissue was obtained from other sites (16 cases). Small surgical biopsy specimens were procured by thorascopic biopsy (n = 11), computed tomography (CT)-guided core needle biopsy (n = 11), endobronchial ultrasound (EBUS)-guided core biopsies (n = 10), or ultrasound (US)-guided biopsy (n = 6). Excisional samples were lobectomy (n = 6), lymph node excision (n = 5), lung wedge resection (n = 1), lung segmentectomy (n = 1), and pleural excision (n = 1). All surgical specimens had at least 100 tumor cells.

Cytology Samples
Sixteen cytology specimens were directly obtained from the lung, 20 from pleural effusions, and 16 from other sites. Cytology specimens were procured by CT-guided FNA (n = 16), EBUS-FNA (n = 12), US-guided FNA (n = 2), palpable FNA (n = 1), bronchial brush (n = 1), or thoracentesis (n = 20).

Of 52 cell blocks, 38 (73%) cases had 100 or more tumor cells and 14 (27%) cases had fewer than 100 tumor cells. Cases with fewer than 100 tumor cells included five pleural fluid specimens, six lung FNAs, two lymph node FNAs, and one bronchial brush. Of the 38 cases with 100 or more tumor cells, 15 were pleural fluid, 10 were lung FNAs, 12 were lymph node FNAs, and one was a mediastinal mass FNA. A summary of cytology specimen type and cell block tumor cellularity is presented in Table 1.

PD-L1 Immunohistochemical Expression
Overall, PD-L1 IHC expression in cytology material was negative in 24 (46%) specimens, positive with low expression in 19 (37%) specimens, and positive with high expression in 14 (27%) specimens.
PD-L1 IHC expression in surgical pathology material was negative in 14 (27%) specimens, positive with low expression in 20 (38%) specimens, and positive with high expression in 18 (35%) specimens.

Agreement Between Paired Cytology and Surgical Pathology Specimens
As PD-L1 assessment on surgical pathology material is the established method of evaluation, the level of agreement between histology and cytology was examined. Agreement was defined as the concordance between the paired cytology-surgical specimens at the three expression levels: negative (<1% of tumor cells staining), low expression (≥1%-49%), and high expression (≥50%). Using this three-tiered categorization, there was agreement in 67% (35/52) of the cases, with κ = 0.51 (moderate agreement; 95% confidence interval [CI], 0.42-0.60).

Eleven of 17 cases were discordant due to PD-L1 scoring as negative on cytology but positive scoring on the paired surgical specimen. One of 17 study cases was discordant, scored as positive (low) on cytology but negative on the paired surgical specimen.

Table 1
Cell Block Tumor Cellularity by Cytology Specimen Type

<table>
<thead>
<tr>
<th>Cytology Specimen Type</th>
<th>Cases With &lt;100 Tumor Cells (n = 14), No. (%)</th>
<th>Cases With ≥100 Tumor Cells (n = 38), No. (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pleural fluid</td>
<td>5 (9.5)</td>
<td>15 (29)</td>
</tr>
<tr>
<td>Lung FNA</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CT guided</td>
<td>6 (11.5)</td>
<td>8 (15)</td>
</tr>
<tr>
<td>EBUS-TBNA</td>
<td>0 (0)</td>
<td>2 (4)</td>
</tr>
<tr>
<td>Lymph node FNA</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CT guided</td>
<td>0 (0)</td>
<td>2 (4)</td>
</tr>
<tr>
<td>EBUS-TBNA</td>
<td>1 (2)</td>
<td>8 (15)</td>
</tr>
<tr>
<td>US guided</td>
<td>1 (2)</td>
<td>1 (2)</td>
</tr>
<tr>
<td>Palpable</td>
<td>0 (0)</td>
<td>1 (2)</td>
</tr>
<tr>
<td>Bronchial brush</td>
<td>1 (2)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>EBUS-TBNA mediastinum</td>
<td>0 (0)</td>
<td>2 (4)</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>14 (27)</strong></td>
<td><strong>38 (73)</strong></td>
</tr>
</tbody>
</table>

CT, computed tomography; EBUS-TBNA, endobronchial ultrasound-guided-transbronchial needle aspiration; FNA, fine-needle aspiration; US, ultrasound.
The remaining five (29%) of 17 discordant pairs showed different levels of positive expression between the matched specimens: in three of the five instances, cytology showed low expression while the histologic sample showed high expression; in the remaining two instances, cytology showed high expression while the histologic sample showed low expression.

Interestingly, one patient had two pleural fluid cytology samples, one showing low PD-L1 expression (sample 27) and the other showing high expression (sample 44), with the corresponding surgical specimen showing high expression.

The discordant cases in our study are highlighted in Table 3. Image 1, Image 2, and Image 3 provide examples of concordant and discordant matched samples.

**Specimen Source and Type**

The PD-L1 scores of cytology specimens obtained directly from the lung (including both mediastinal mass and bronchial brush samples) were concordant with the PD-L1 scores of surgical pathology specimens in 15 (83%) of 18 paired samples, with $\kappa = 0.74$ (substantial agreement; 95% CI, 0.61-0.88). Among cytology cases procured from lymph nodes, there was agreement among eight (57%) of 14 paired cases, with $\kappa = 0.34$ (fair agreement; 95% CI, 0.16-0.52). Pleural effusions showed agreement among 12 (60%) of 20 paired samples, with $\kappa = 0.39$ (fair agreement; 95% CI, 0.25-0.53).

**Surgical Excisions vs Surgical Biopsies**

The cytology samples showed greater agreement with paired excisional samples (12/14 [86%], substantial agreement; $\kappa = 0.78$; 95% CI, 0.63-0.92) than with paired smaller surgical biopsy specimens (23/38 [61%], moderate agreement; $\kappa = 0.41$; 95% CI, 0.31-0.52).

**Cell Block Tumor Cellularity**

In cell blocks with 100 or more tumor cells, PD-L1 IHC was negative in 16 (42%) specimens, positive with low expression in seven (18%) specimens, and positive with high expression in 15 (40%) specimens. In cell blocks with fewer than 100 tumor cells, PD-L1 IHC was negative in eight (58%) specimens, positive with low expression in three (21%) specimens, and positive with high expression in three (21%) specimens. Among cell blocks with 100 or more tumor cells, there was substantial agreement ($\kappa = 0.63$; 95% CI, 0.53 to 0.73) with the corresponding surgical specimens; on the other hand, among cell blocks with fewer than 100 cells, there was only slight agreement with the corresponding surgical specimens ($\kappa = 0.19$; 95% CI, 0.04 to 0.35). See Table 4 and Table 5.

**Therapy Prior to Cytology PD-L1 Immunohistochemical Testing**

Thirty-six cytology samples were acquired concurrently with the paired surgical samples. Sixteen cytology samples were acquired after the surgical samples, with 12 of the 16 patients receiving cancer treatment in the interim. There was no difference in the level of agreement between patients who did and did not receive therapy. Among the patients who received therapy, there was agreement among eight (66%) of 12 paired samples, with $\kappa = 0.50$ (moderate agreement; 95% CI, 0.31 to 0.69), and among patients who did not receive therapy, there was agreement among 27 (67%) of 40 paired samples, with $\kappa = 0.51$ (also moderate agreement; 95% CI, 0.41 to 0.61).

**Quality Assurance on TPS**

After blinded review of discordant cases, the cytology PD-L1 scores matched between A.L.M. and the study pathologists, indicating that interpretive error was not a factor.

**Discussion**

With increased focus on targetable tumor biomarkers such as PD-L1, treating clinicians are increasingly...
<table>
<thead>
<tr>
<th>PD-L1 Score</th>
<th>Surgical Pathology</th>
<th>Cytology Specimen Type</th>
<th>No. of Tumor Cells on CB</th>
<th>PD-L1 Score*</th>
</tr>
</thead>
<tbody>
<tr>
<td>≥100</td>
<td>Positive (low)</td>
<td>NSCLC with lymph node biopsy</td>
<td>≥100</td>
<td>Positive (low)</td>
</tr>
<tr>
<td>≥100</td>
<td>Positive (high)</td>
<td>NSCLC with lymph node biopsy</td>
<td>≥100</td>
<td>Positive (high)</td>
</tr>
<tr>
<td>≥100</td>
<td>Positive (low)</td>
<td>NSCLC with lymph node biopsy</td>
<td>≥100</td>
<td>Positive (low)</td>
</tr>
<tr>
<td>≥100</td>
<td>Positive (high)</td>
<td>NSCLC with lymph node biopsy</td>
<td>≥100</td>
<td>Positive (high)</td>
</tr>
</tbody>
</table>

*PD-L1 Score: Positive (low) = 0-10%, Positive (high) = >10%
### Table 3 (cont)

<table>
<thead>
<tr>
<th>Sample No.</th>
<th>Age, y</th>
<th>Sex</th>
<th>Cytology Specimen Type</th>
<th>No. of Tumor Cells on CB</th>
<th>Surgical Pathology</th>
<th>PD-L1 Scorea</th>
<th>Surgical Pathology</th>
<th>PD-L1 Scorea</th>
</tr>
</thead>
<tbody>
<tr>
<td>41</td>
<td>M</td>
<td>61</td>
<td>Pleural fluid</td>
<td>≥100</td>
<td>Positive (low)</td>
<td>ADC</td>
<td>Positive (low)</td>
<td>ADC</td>
</tr>
<tr>
<td>42</td>
<td>M</td>
<td>60</td>
<td>Pleural fluid</td>
<td>≥100</td>
<td>Positive (low)</td>
<td>ADC</td>
<td>Positive (low)</td>
<td>ADC</td>
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<tr>
<td>43</td>
<td>F</td>
<td>91</td>
<td>EBUS-TBNA lymph node FNA</td>
<td>≥100</td>
<td>Positive (low)</td>
<td>ADC</td>
<td>Positive (low)</td>
<td>ADC</td>
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<tr>
<td>44</td>
<td>M</td>
<td>69</td>
<td>EBUS-TBNA lymph node FNA</td>
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<td>ADC</td>
<td>Positive (low)</td>
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<tr>
<td>45</td>
<td>M</td>
<td>57</td>
<td>EBUS-TBNA hilar lymph node</td>
<td>≥100</td>
<td>Positive (low)</td>
<td>ADC</td>
<td>Positive (low)</td>
<td>ADC</td>
</tr>
<tr>
<td>46</td>
<td>M</td>
<td>57</td>
<td>EBUS-TBNA hilar lymph node</td>
<td>≥100</td>
<td>Positive (low)</td>
<td>ADC</td>
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<td>ADC</td>
</tr>
<tr>
<td>47</td>
<td>M</td>
<td>57</td>
<td>EBUS-TBNA hilar lymph node</td>
<td>≥100</td>
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<td>ADC</td>
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<td>49</td>
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<td>63</td>
<td>EBUS-TBNA lymph node FNA</td>
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<tr>
<td>51</td>
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<td>61</td>
<td>Pleural fluid</td>
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<td>52</td>
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<td>61</td>
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<td>≥100</td>
<td>Positive (low)</td>
<td>ADC</td>
<td>Positive (low)</td>
<td>ADC</td>
</tr>
</tbody>
</table>

**Table 6.** A recent study of 41 paired NSCLC cases evaluated PD-L1 IHC expression in cytology smears and cell blocks in comparison to surgical pathology biopsy specimens. After the exclusion of cytology samples displaying low tumor cellularity, the authors showed a high concordance between paired specimens, particularly using smear preparations (36/37 smears and 31/38 cell blocks showed concordant expression). A study of 86 paired lung cancer cases showed a high correlation coefficient ($R^2 = 0.87$-0.89) and overall agreement of 85% and 94% when the cutoff for positivity was 1% and 50% PD-L1 expression, respectively. Other investigators have shown indirect evidence of similar PD-L1 expression between cytology and surgical pathology specimens by bulk case comparison.

Our study compared PD-L1 expression in matched NSCLC samples using the Dako 22C3 pharmDx antibody, the US Food and Drug Administration–approved companion diagnostic assay for treatment with pembrolizumab. Tumors displaying 50% or more PD-L1 expression and no *EGFR* or *ALK* aberrations qualify the patient for first-line therapy with this agent, while tumors with 1% to 49% PD-L1 expression and disease progression on platinum-based chemotherapy qualify the patient for second-line therapy. Paired agreement in this study was evaluated by following clinically relevant PD-L1 TPS tiers: negative (<1%), low expression (1%-49%), and high expression (≥50%). Agreement was achieved in 67% (35/52) of matched cases (moderate agreement; $\kappa = 0.51$; 95% CI, 0.42-0.60) when comparing PD-L1 testing in cytology with surgical pathology samples. Most discordant cases (11/17) were negative on cytology yet had low expression (7/11) or high expression (4/11) on the corresponding surgical specimen. Variability in the level of positive expression between paired samples was also a key reason for discordant results (5/17). This variability may pose a therapeutic dilemma in terms of whether the patient qualifies for first- or second-line pembrolizumab treatment.

Many factors play a role in the successful adaptation of IHC biomarkers originally validated on histologic specimens to cyto logic preparations. These include antibody clone used, platform selected, cell fixative used, cytology preparation (cell block vs smears), staining of background cells, and the subjective nature of the IHC evaluation. With regard to assessment of PD-L1 IHC, the cellularity of the sample, heterogeneous expression

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**Note:**

- $\kappa$ indicates the level of interobserver agreement.
- CI denotes the 95% confidence interval.
- TPS stands for tumor proportion score.
- PD-L1, programmed death–ligand 1; NSCLC, non–small cell lung carcinoma; S, subsequently acquired cytology sample; S (Tx), subsequently acquired cytology sample with the patient receiving treatment prior to cytology.
of PD-L1 within the same tumor, and type of pathology specimen (surgical/cytology vs biopsy/excision) are additional variables that should be considered.\(^\text{19,20}\)

In our study, eight (47%) of the discordant cases had cytology samples with fewer than 100 tumor cells, with only slight agreement with the corresponding surgical specimens ($\kappa = 0.19$; 95% CI, 0.04-0.35). Cases with 100 or more tumor cells had higher agreement ($\kappa = 0.63$; 95% CI, 0.53-0.73). While low tumor cell count is less than optimal and may lead to a false-negative PD-L1 expression, our study showed that cases with a tumor cell count less than 100 may display positive PD-L1 expression and therefore may not be the sole limiting factor. Skov and Skov\(^\text{13}\) previously reported no statistical changes in paired results after exclusion of cytology specimens with fewer than 100 tumor cells.

Intratumoral heterogeneity has been reported to have an impact on the detection of targetable biomarkers,\(^\text{21}\) with studies showing marked variability in PD-L1 staining within a single tumor.\(^\text{19,20}\) Five study cases showed varied positive (low or high) expression levels between surgical and cytology specimens. This variation is likely attributable to tumor heterogeneity. One of the cases in our study\(\text{Image 4}\) displayed high positive PD-L1 expression on both surgical pathology and cytology and low expression on a second cytology specimen. Upon review of the PD-L1 stained surgical pathology slide from this case, heterogeneous PD-L1 expression within the tumor was indeed noted.

\textbf{Image 1} Lung adenocarcinoma specimens collected from a 60-year-old woman (sample 5). \textbf{A}, Cell block prepared from pleural fluid (H&E, ×10). \textbf{B}, Programmed death–ligand 1 (PD-L1)–stained cell block section with less than 1% expression (×10). \textbf{C}, Thorascopic pleural biopsy (H&E, ×20). \textbf{D}, PD-L1–stained concurrent surgical biopsy specimen with less than 1% expression (×20).
Tumor heterogeneity has also been investigated as a possible reason for discordant PD-L1 expression between small surgical pathology biopsy specimens and excisional samples.22,23 Similarly, agreement was found to be higher in our study when comparison was made between cytology and paired surgical pathology excisional samples ($\kappa = 0.78; 95\% \text{ CI, } 0.63-0.92$) vs cytology with paired smaller surgical biopsy specimens ($\kappa = 0.41; 95\% \text{ CI, } 0.31-0.52$). Furthermore, as previously mentioned, most discordant cases in our study underrepresent PD-L1 surgical pathology scores. This trend was further explored to show that in paired cases with negative cytology PD-L1 staining and positive surgical pathology PD-L1 staining, more than half (6/11) of the surgical cases had PD-L1 levels of 10\% or less.

Timing of sample acquisition was also examined as a possible source of disagreement between paired samples, as prior studies have reported altered, either diminished or inducible, PD-L1 expression levels in chemotherapy- and/or radiotherapy-treated tumors.24,25 Investigation of paired samples showed that four (23.5\%) of 17 discordant study cases were not acquired concurrently, with the four patients receiving treatment prior to the acquisition of the cytology sample. When evaluating concordant study cases for temporal lag in cytology acquisition, eight (23\%) of 35 patients received treatment, with the samples acquired subsequent to the surgical pathology/treatment-naive material. Discordance in our study was likely not attributable to treatment prior to PD-L1 testing.

**Image 2** Metastatic lung adenocarcinoma specimens collected from the lymph node of a 64-year-old woman (sample 39). **A**, Computed tomography–guided fine-needle aspiration cell block (H&E, ×40). **B**, Programmed death–ligand 1 (PD-L1)–stained cell block section with 50\% or more expression (×40). **C**, Excisional lymph node specimen (H&E, ×40). **D**, PD-L1–stained concurrent surgical excision with 50\% or more expression (×40).
Metastatic lung adenocarcinoma specimens collected from the supraclavicular lymph node of a 54-year-old man (sample 24). 

A, Palpable fine-needle aspiration cell block (H&E, ×10). 
B, Programmed death–ligand 1 (PD-L1)–stained cell block section with less than 1% expression (×10). 
C, Ultrasound-guided core biopsy specimen (H&E, ×10). 
D, PD-L1–stained concurrent surgical biopsy specimen with 50% or more expression (×10).

Table 4
PD-L1 Expression in Cytology Cell Blocks With ≥100 Tumor Cells

<table>
<thead>
<tr>
<th>Cell Blocks With ≥100 Tumor Cells</th>
<th>Surgical Pathology TPS Score</th>
<th>Proportion in Agreement, %</th>
<th>Cohen's κ (95% Confidence Interval)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Negative (&lt;1%)</td>
<td>Low Expression (≥1–49%)</td>
<td>High Expression (≥50%)</td>
</tr>
<tr>
<td>Cytology TPS score</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Negative (&lt;1%)</td>
<td>10</td>
<td>4</td>
<td>2</td>
</tr>
<tr>
<td>Low expression (≥1–49%)</td>
<td>1</td>
<td>4</td>
<td>2</td>
</tr>
<tr>
<td>High expression (≥50%)</td>
<td>0</td>
<td>0</td>
<td>15</td>
</tr>
<tr>
<td>Total, No. (%)</td>
<td>11 (29)</td>
<td>8 (21)</td>
<td>19 (50)</td>
</tr>
</tbody>
</table>

TPS, tumor proportion score.
Table 5
PD-L1 Expression in Cytology Cell Blocks With Fewer Than 100 Tumor Cells

<table>
<thead>
<tr>
<th>Cell Blocks With &lt;100 Tumor Cells</th>
<th>Surgical Pathology TPS Score</th>
<th>Proportion in Agreement, %</th>
<th>Cohen’s κ (95% Confidence Interval)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cytology TPS score</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Negative (&lt;1%)</td>
<td>3</td>
<td>3</td>
<td>2</td>
</tr>
<tr>
<td>Low expression (≥1-49%)</td>
<td>0</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>High expression (≥50%)</td>
<td>0</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>Total, No. (%)</td>
<td>3 (21)</td>
<td>7 (50)</td>
<td>4 (29)</td>
</tr>
</tbody>
</table>

TPS, tumor proportion score.

Table 6
Review of Reported Studies Comparing PD-L1 Immunohistochemistry Testing in Surgical and Cytology Samples

<table>
<thead>
<tr>
<th>Reference</th>
<th>Cytology Preparation Evaluated (Smear/CB)</th>
<th>Preservative/ Fixative Used</th>
<th>PD-L1 Antibody Kit Used</th>
<th>Minimum Cellularity on Cytology Preparations</th>
<th>Paired or Bulk Comparison</th>
<th>PD-L1 Scoring</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Noll et al, 2018²</td>
<td>Smear and CB</td>
<td>FNA material— RPMI CB-NBF (following 95% ethanol and 10% formalin mix)</td>
<td>22C3 pharmDX</td>
<td>≥100 tumor cells</td>
<td>Paired</td>
<td>Comparison by TPS%; considered concordant if TPS% results were identical; considered comparable if within 10% agreement; considered discordant if &gt;10% disagreement</td>
<td>23/38 CBs had identical paired TPS%, 8 had comparable TPS%, and 7 were discordant (1 false positive and 6 with variation in positive expression levels)</td>
</tr>
<tr>
<td>Skov and Skov, 2017¹³</td>
<td>CB</td>
<td>FNA material— saline CB-NBF (at least 18 hours)</td>
<td>28-8 pharm DX and 22C3 pharmDX</td>
<td>&lt;100 or ≥100 tumor cells</td>
<td>Paired</td>
<td>≥1%, ≥5%, ≥10%, and thereafter in 10% increments resulting in 11 categories</td>
<td>High correlation coefficient (R^2 = 0.87) to 0.89 and overall agreement of 85% and 94%, when the cutoff for positivity was 1% and 50% PD-L1 expression, respectively, with no change by exclusion of CBs with &lt;100 tumor cells (R^2 = 0.84) to 0.88</td>
</tr>
<tr>
<td>Heymann et al, 2017¹⁴</td>
<td>CB</td>
<td>FNA material— Cytolyt and/or NBF CB-NBF</td>
<td>22C3 pharmDX</td>
<td>≥100 tumor cells</td>
<td>Both</td>
<td>&lt;50 = negative; ≥50 = positive expression</td>
<td>Paired results: concordant expression in 4/5 cases (1 case excluded, considered unsatisfactory for evaluation) Bold results: CBs displayed a tendency for more positive expression than histologic specimens, with no statistically significant differences in expression between cytology and surgical pathology cases</td>
</tr>
<tr>
<td>Torous et al, 2018¹⁵</td>
<td>CB</td>
<td>FNA material— Cytolyt CB-NBF (range, 3-6 hours)</td>
<td>22C3 pharmDX</td>
<td>≥100 tumor cells</td>
<td>Bulk</td>
<td>&lt;1%, ≥1%-49%, ≥50%</td>
<td>No statistically significant differences in TPS% between cytology and surgical pathology cases</td>
</tr>
</tbody>
</table>

CB, cell block; FNA, fine-needle aspiration; NBF, 10% neutral buffered formalin; PD-L1, programmed death–ligand 1; TPS, tumor proportion score.
on cytology material, with moderate agreement reached in both patients who were previously treated ($\kappa = 0.50$; 95% CI, 0.31 to 0.69) and those who were not ($\kappa = 0.51$; 95% CI, 0.41 to 0.61).

PD-L1 staining in effusion specimens may pose a diagnostic challenge due to PD-L1 nonspecific expression in macrophages, B cells, natural killer cells, and dendritic cells. This difficulty is augmented when tumor cells appear as discohesive cells among macrophages. In addition, to date, PD-L1 IHC assays have not been validated for decalcified tissue, and staining after decalcification should be avoided. One surgical pathology case in our study group consisted of metastatic adenocarcinoma to the rib. In adherence with the Dako manual, the rib specimen included in our study did not undergo decalcification prior to PD-L1 testing.

Last, of the different cytology samples in our cohort, those procured directly from the lung (either by CT-guided or EBUS-guided FNA) showed substantial agreement ($\kappa = 0.74$; 95% CI, 0.61-0.88) with PD-L1 expression in paired surgical specimens. Cytology samples obtained from lymph nodes and pleural effusions showed only fair agreement ($\kappa = 0.34$; 95% CI, 0.16-0.52 and $\kappa = 0.39$; 95% CI, 0.25-0.53, respectively). These results provide evidence...
that cytology material obtained directly from the lung may serve as the best sample for PD-L1 biomarker testing.

Limitations of our study include the relatively small sample size, which should be followed up with larger paired PD-L1 expression studies, limited number of squamous cell carcinoma cases for PD-L1 analysis, and the use of only one type of PD-L1 assay.

In conclusion, our study confirms that cytology cell blocks can be used for PD-L1 IHC evaluation. Discordance with surgical specimens may occur. Most of our discordant cases were negative on cytology with positive expression on the corresponding surgical specimen. Positive expression on cytology appears to be reliable compared with paired surgical specimens as illustrated in our study, whereas a patient with a negative PD-L1 cytology result may benefit from PD-L1 testing on repeat cytology or surgical material. Differences in scoring of surgical pathology and cytology samples are

Image 4 (cont) E, Excisional lymph node specimen (H&E, ×20). F, PD-L1–stained concurrent surgical excision showing heterogeneous PD-L1 expression among tumor cells with reported PD-L1 of 50% or more.

Image 5I Macrophages as a pitfall for programmed death–ligand 1 (PD-L1) interpretation in cytologic specimens. A, Cell block prepared from pleural fluid showing a tumor cell (center) among many scattered macrophages (H&E, ×40). B, PD-L1–stained cell block section revealed additional tumor cells on deeper sectioning showing strong membranous expression for PD-L1. Background macrophages also show membranous staining for PD-L1 at varying intensities (×40).
likely attributed to intratumoral heterogeneity and cell block cellularity. While cases with a tumor cell count less than 100 may lead to missing some PD-L1 positive cases, cases with fewer than 100 tumor cells that do show positive PD-L1 staining have proven to be reliable. Surgical pathology excisional samples showed greater agreement with cytology material than was observed with surgical pathology biopsy specimens, reinforcing prior reports that smaller diagnostic material (surgical pathology or pathology biopsy specimens, reinforcing prior reports with cytology material than was observed with surgical pathology excisional samples showed greater agreement in cases with fewer than 100 tumor cells that do show positive PD-L1 expression. Smaller diagnostic material may lead to discordant PD-L1 expression levels compared with larger excisional tissue. PD-L1 IHC on cytology cell blocks should be considered in the evaluation of PD-L1 biomarker testing as a reliable method. Further studies with larger cohorts may shed light on how to best use these specimens for PD-L1 analysis to broaden the accessibility of testing for patients with NSCLC.

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References