Among Multiple Needle Core Biopsy Samples, the One with the Highest Tumor Proportion Score Best Represents the PD-L1 Status of the Whole Surgical Specimen in Non–Small Cell Lung Cancer

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Abstract: The heterogeneity of programmed death-ligand 1 (PD-L1) status between core needle biopsies (CNBs) from one tumor has not been well studied before. The current study attempts to find out the best index using multiple core biopsies from one tumor which can better reflect the actual PD-L1 status. Random CNB was performed in surgical specimens from 170 consecutive non–small cell lung cancer samples. Fifty-one cases (41 cases with PD-L1 positive and 10 cases with PD-L1 negative) and 216 matched CNBs were analyzed by DAKO 22C3 PharmDx Link 48 Autostainer. The PD-L1 status was compared between the surgical specimens and matched CNBs. Heterogeneity of PD-L1 status between CNBs from one tumor was observed in 56% of PD-L1 positive cases. Different tumor proportion score (TPS) statistical forms with regard to the highest, mean, median, weighted average TPS, as well as TPS showed by the longest weighted average TPS, as well as TPS showed by the longest

86.3%, respectively. The CNB with the highest TPS can best represent PD-L1 status estimated by whole surgical specimen. The highest TPS among the multiple biopsies is a robust evaluation of the PD-L1 status, but not mean TPS, at the 1% and 50% cut-offs.

Key Words: non–small cell lung cancer, PD-L1, biopsy, resection specimen

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The programmed death 1 (PD-1) and programmed death-ligand 1 (PD-L1) interaction acts as an immune checkpoint signal and is activated in many types of cancers, thus suppressing the function of activated T cells to identify and eliminate tumor cells (TC). PD-L1 is frequently expressed in many types of carcinomas including non–small cell lung cancer (NSCLC). PD-L1 allowed the cancer cells to evade the immune system in order to progress. Pembrolizumab is an anti-PD-1 monoclonal antibody that was approved for treating advanced NSCLC based on PD-L1 expression in TCs. The KEYNOTE-010 trial demonstrated that pembrolizumab prolonged the overall survival of patients with a PD-L1 tumor proportion score (TPS) ≥1%, in which PD-L1 was analyzed by immunohistochemistry (IHC). Moreover, for patients with a PD-L1 TPS ≥50%, pembrolizumab has been recommended in first-line therapy for its better efficacy and less toxicity than platinum-doublet chemotherapy. Therefore, accurate evaluation of PD-L1 expression is necessary for patients with advanced NSCLC.

Due to the latest developments in imaging and minimally invasive interventional procedures, a large population of patients with advanced NSCLC was pathologically diagnosed through core needle biopsy (CNB). Previous studies focused on the comparison of PD-L1 status between resection specimens and matched biopsies, but there were seldom studies about the heterogeneity of PD-L1 status between different biopsies from biopsy specimen and the biopsy with most tumor volume were compared. At a cut-off of 1%, the concordance rates were 94.1%, 88.2%, 90.2%, 86.3%, and 86.3%; At a cut-off of 50%, the concordance rates were 92.2%, 86.3%, 84.3%, 82.4%, 82.4%, and 86.3%, respectively. The CNB with the highest TPS can best represent PD-L1 status estimated by whole surgical specimen. The highest TPS among the multiple biopsies is a robust evaluation of the PD-L1 status, but not mean TPS, at the 1% and 50% cut-offs.
one tumor. In clinical practice, to satisfy the need for more biomarkers to be tested especially in NSCLC, pathologists usually encounter the situation that more than 1 CNB tissue was obtained during one intervention procedure. In this context, pathologists may be confused with which tissue could closely reflect the actual PD-L1 expression of the tumor. We suggested that the intratumoral heterogeneity of PD-L1 expression would also lead to the heterogeneity of PD-L1 status between the CNBs from the same tumor. Tissue microarrays (TMAs) were used as surrogates of biopsy specimens in some previous studies.\textsuperscript{13,15} TMAs could be an easy way to simulate bronchial biopsy while investigate PD-L1 heterogeneity within a certain block, but limited tumor tissue involved was unsuitable for the surrogates of CNBs. In this study, we simulated real CNBs using surgical NSCLC specimens. The aim of this study was to find out the best strategy for choosing and scoring tissue when more than 1 CNB was obtained from the same tumor, to closely reflect the PD-L1 expression status as determined on the resection specimen. In addition to get the optimal number of biopsies needed based on different clinical cut-off levels.

MATERIALS AND METHODS

Study Cohort

The study cohort consisted of patients who had undergone lung cancer resection at the Peking University Cancer Hospital from March 2018 to June 2018. Patient demographics and clinical data were retrieved from the archives. We simulated the real CNB using the surgical specimens of NSCLC in vitro. First, the surgical resected specimen was fixed in 10\% neutral formalin, and then punctured using a full core biopsy instrument (ARGON, Frisco, TX) with a needle size of 18G. The number of biopsies acquired was based on the tumor size, defined as 1 to 2 biopsies per centimeter based on the longest axis of the tumor. Second, residue specimens under 3 cm in diameter were all sampled or at least 1 section per centimeter was sampled if the tumor diameter was over 3 cm. Finally, a total of 170 consecutive resection specimens were punctured, and totally 836 section blocks and 685 biopsies were collected. Tumor types were classified according to the 2015 WHO classification and staging was termed based on the TNM staging manual (AJCC, eighth edition).

IHC and Scoring

Rehman \textit{et al}\textsuperscript{16} previously demonstrated that staining result of one block is enough to represent the entire tumor. So, one representative block of each resection tumor was selected to perform PD-L1 assessment utilizing the DakoPharmDx 22C3 IHC assay on the Dako IHC autostainer (Link 48).\textsuperscript{17} PD-L1 expression was observed blindly by 2 experienced pathologists (W.S. and X.Y.); discordant cases were reevaluated by a third pathologist (H.W.). PD-L1 positive was defined as TPS $\geq 1\%$.

Statistical Analysis

Statistical analysis was calculated using the SPSS software system (version 18.0, SPSS Inc., Chicago, IL). The difference between categorical factors was assessed using $\chi^2$ test and Fisher exact test. $P$-values from tests $<0.05$ were considered as relative significant signal for further confirmation.

RESULTS

Patient Characteristics

There were 42 PD-L1 positive cases in 170 resected samples. The positive rate was 24.7\%. In 42 PD-L1 positive cases, one case was excluded for the matched biopsies were necrotic. Fifteen biopsies were excluded for no TCS in the biopsies. Ten randomly PD-L1 negative cases were included, too (Fig. 1). Finally, a total of 51 cases (41 cases with PD-L1 positive and 10 cases with PD-L1 negative) and 216 matched biopsies were included in this study. A minimum of 100 viable TCs were available for evaluation in all biopsies. The patients included 30 men and 21 women ranging in age from 31 to 73 years and the

![FIGURE 1. Flow diagram of case selection in this study. Note 1: in 170 resected lesions, there were 125 adenocarcinomas, 20 squamous cell carcinomas, 4 neuroendocrine carcinomas, 1 large cell carcinoma, 1 adenosquamous carcinoma, 3 sarcomatoid carcinomas, 1 lymphoepithelioma-like carcinoma, 10 metastatic tumors, and 5 other benign lesions. Note 2: the cases included squamous cell carcinomas, 4 neuroendocrine carcinomas, 1 large cell carcinoma, 1 adenosquamous carcinoma, 3 sarcomatoid carcinomas, lymphoepithelioma-like carcinoma. One block of each resection tumor was selected to staining PD-L1. Note 3: in 42 PD-L1 positive cases, 1 case was excluded for all of matched biopsies were necrosis. Fifteen biopsies were excluded for no tumor cells in the biopsies. Finally, a total of 51 case and 216 matched biopsies were included in this study. IHC indicates immunohistochemistry; PD-L1, programmed death-ligand 1.]
The median age was 60.5 years old. The median tumor size was 3.0 cm (range, 1.0 to 7.5 cm). The patient characteristics are shown in Table 1.

### Heterogeneity of PD-L1 Expression Between the CNBs

In this study, we defined the PD-L1 status according to TPS: negative (TPS <1%), low expression (TPS =1% to 49%), and high expression (TPS ≥50%). The distribution of PD-L1 status is shown in Figure 2. If the TPS of paired samples were in the same interval, it was defined as concordance; if not, it was defined as discordance. In 51 case, there were 23 cases (45.1%) in which all of biopsies were concordant with the corresponding whole tumor resected specimen, while 28 cases (54.9%) in which there was at least one biopsy with discordant PD-L1 status compared with whole tumor resected specimen. The interbiopsy heterogeneous of PD-L1 status was significantly observed in positive cases, regardless of PD-L1 high or low expression (Supplementary Table 1, Supplemental Digital Content 1, http://links.lww.com/AIMM/A317). Twenty-three of 41 (56.1%) positive cases show interbiopsy heterogeneous in PD-L1 status (Supplementary Table 1, Supplemental Digital Content 1, http://links.lww.com/AIMM/A317). The heterogeneity of PD-L1 status in biopsies from 1 case was not correlated with tumor size ($P = 0.925$) and histologic subtypes ($P = 0.848$) (Supplementary Table 2, Supplemental Digital Content 2, http://links.lww.com/AIMM/A318 and Supplementary Table 3, Supplemental Digital Content 3, http://links.lww.com/AIMM/A319). In tumors with diameter ≤2 cm, the interbiopsy heterogeneity of PD-L1 expression was observed in 7 cases (41.2%), while 10 cases (58.8%) displayed the same PD-L1 status in homologous biopsies (Supplementary Table 2, Supplemental Digital Content 2, http://links.lww.com/AIMM/A318).

### Concordance of PD-L1 Status Between Resection Specimens and Matched CNBs

Of all the 216 biopsies involved, each one was regarded as an independent entity and compared with their corresponding resection specimen, the coincident rates of PD-L1 status were 82.4% and 83.3%, when cutoffs were set to 1% and 50%, respectively. Most of the discordant cases manifested underestimated biopsy TPS compared with the resection specimen (Supplementary Table 4, Supplemental Digital Content 4, http://links.lww.com/AIMM/A320). The sensitivity and specificity of the biopsy were 79.2% and 100% at a 1% cut-off, which were 73.8% and 89.0%, respectively, when cut-off was set to 50%.

### Value of Different TPS Base on Multiple CNBs From One Tumor in PD-L1 Evaluation

It can be observed that CNBs from one tumor could show different PD-L1 status in clinical practice and datum above reproduce this phenomenon. Pathologists may wonder which tissue was more suitable for assessing PD-L1 expression when more CNBs were available. Were there any features of the sample that could help in decision-making? The present research showed that the length

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**TABLE 1. The Clinic-pathologic Features of 52 Patients**

| Variables          | N (%) |
|--------------------|-------|
| Age (y)            |       |
| < 60               | 23 (45.1) |
| ≥ 60               | 28 (54.9) |
| Sex                |       |
| Male               | 30 (58.8) |
| Female             | 21 (41.2) |
| T stage            |       |
| 1                  | 20 (39.2) |
| 2                  | 24 (47.1) |
| 3                  | 6 (11.8)  |
| 4                  | 1 (2.0)   |
| N stage            |       |
| 0                  | 34 (66.7) |
| 1                  | 12 (23.5) |
| 2                  | 5 (9.8)   |
| Histologic subtype |       |
| Adenocarcinoma     | 33 (67.7) |
| Squamous cell carcinoma | 16 (31.4) |
| Lymphoepithelioma-like carcinoma | 1 (2.0) |
| Sarcomatoid carcinoma | 1 (2.0) |

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**FIGURE 2.** The distribution of programmed death-ligand 1 (PD-L1) status of 51 resected specimens and their matched biopsies (a total of 216). Each column in this figure represented 1 sample, and the squares meant PD-L1 status defined by its original tumor proportion score (TPS). The cases were continuously ranked by PD-L1 status of resected specimen. There were 10 negative, 22 low expression and 19 high expression cases in 51 resected specimens. In 216 biopsies, there were 75 negative, 67 low expression and 74 high expression. The PD-L1 status of biopsies were heterogeneous in positive cases, regardless of low and high expression.
of tissue and tumor volume were not associated with the consistency between CNB and resection specimen (Supplementary Table 5, Supplemental Digital Content 5, http://links.lww.com/AIMM/A321 and Supplementary Table 6, Supplemental Digital Content 6, http://links.lww.com/AIMM/A322) by \( \chi^2 \) test. We then compared different TPS statistical forms based on multiple tissues from one tumor to establish an index which can best represent its matched surgical specimen. These forms included the TPS of the highest, mean, median, and weighted average TPS; as well as TPS showed by the longest biopsy specimen and the biopsy with most tumor volume. Statistical analysis demonstrated that the highest TPS provided the best consistency, which was 94.1% at a 1% cut-off and 92.2% at a 50% cut-off, respectively (Table 2). These results indicated that the CNB with the highest TPS provided a better reflection on PD-L1 status of matched resection specimen (Fig. 3).

**Quantity of CNB Required for Accurate PD-L1 Evaluation**

We used biopsies density to estimate quantity of CNB for objective assessing PD-L1 status with respect to resection specimen. Biopsy density = [Number of biopsies / Longest diameter of tumor].

Bigger biopsy density means more CNBs needed. In 52 resection specimens, the biopsy density was from 0.47 to 2.73 core/cm, and the median was 1.43 core/cm. As we suggested above, when more CNBs were involved in PD-L1 evaluation, the highest TPS was the best for accuracy evaluation of the PD-L1 status. So, the highest TPS was applied for this analysis. The results showed that the biopsy density was not associated with the concordance between CNB and surgical specimen at a 1% cut-off, and the concordance rate were consistently > 90%. But, at a 50% cut-off, the PD-L1 status of cases with a biopsy density \( \geq 1 \) core/cm showed a statistically higher concordance rate compared with that of biopsy density <1 core/cm (\( \chi^2 = 0.006 \) ) (Table 3) in the Fisher exact test. Therefore, in our suggestion, a biopsy density of 1 core/cm was a threshold for accurate assessment of PD-L1 status at a 50% cut-off, if we expected the coincident rate to be > 95%.

**DISCUSSION**

Accurate evaluation of PD-L1 status is critical for an appropriate treatment strategy chosen in advanced NSCLC. Our findings indicate that the PD-L1 status showed heterogeneous between CNBs in one tumor, and the highest TPS revealed by one of the multiple CNBs was the most suitable score to reflect the PD-L1 status of matched resection specimen. The quantity of CNB required was correlated with different cut-off value set, such as 1% or 50% according to different treatment strategies.

The PD-L1 status of biopsies has been previously reported,\(^{10-14}\) Some of the studies used archival biopsies,\(^{10-12,14}\) while some studies were based on TMAs as surrogates of biopsy specimens.\(^{13,15}\) These materials (archival biopsies and TMAs) were unsuitable for our study. This study focused on the heterogeneity of PD-L1 status between different CNBs in one tumor and their potential influence of PD-L1 evaluation in NSCLC. In this study, the actual surgical specimens were punctured for simulating CNB in vitro. Our design most closely reflects the actual procedure of core biopsy. It can obtain tissues in one tumor as much as possible, to satisfy the needs of study. The heterogeneity of PD-L1 status were shown between the CNBs from one tumor. It was observed in the PD-L1 positive cases, regardless of PD-L1 high or low expression (Supplementary Table 1, Supplemental Digital Content 1, http://links.lww.com/AIMM/A317), tumor size (Supplementary Table 2, Supplemental Digital Content 2, http://links.lww.com/AIMM/A318) and histologic subtypes (Supplementary Table 3, Supplemental Digital Content 3, http://links.lww.com/AIMM/A319). The heterogeneity of PD-L1 status shown between the CNBs may be the main reason for the previous conflicting results on using lung CNBs for PD-L1 evaluation. In addition, the positive rate was 24.7% (42/170) in this study. It was consistent with the previous studies, in which the positive rate was from 15.7% to 48.3%.\(^{10-13}\)

In clinical practice, more than one tissue may be obtained during percutaneous pulmonary biopsy. It is recommended to obtain 2 to 3 cores. Pathologists and clinicians may be confused with which tissue could closely reflect the actual PD-L1 expression of the tumor. Our results suggested that the tissue with the highest TPS were able to better reflect the PD-L1 status of resection specimen. The coincident rates were 94.1% and 92.2% at the 1% and 50% cut-offs, respectively. This result was consistent with Munari et al.'s\(^{15}\) study. In Munari and colleagues’ study, TMAs were used as surrogates of biopsy specimens. The maximum value appeared to better reflect

### TABLE 2. Correlation of Different TPS Value From Core Needle Biopsies and the Concordance Status

| Criteria                  | Discordant | Concordant | Concordance Rate (%) | Discordant | Concordant | Concordance Rate (%) |
|---------------------------|------------|------------|----------------------|------------|------------|----------------------|
| Longest linear extent     | 7          | 44         | 86.3                 | 9          | 42         | 82.4                 |
| Most tumor volume         | 7          | 44         | 86.3                 | 7          | 44         | 86.3                 |
| Average TPS               | 6          | 45         | 88.2                 | 7          | 44         | 86.3                 |
| Highest TPS               | 3          | 48         | 94.1                 | 5          | 47         | 92.2                 |
| Median TPS                | 5          | 46         | 90.2                 | 8          | 43         | 84.3                 |
| Weighted average TPS      | 7          | 44         | 86.3                 | 9          | 42         | 82.4                 |

TPS indicates tumor proportion score.
PD-L1 expression on the whole sections, compared with mean, median and minimum values of biopsy. Bigras and colleagues performed very creative research about this topic. In Bigras et al’s study, not only actual biopsy samples were compared with resected specimens, but also virtual samples were used to exhaustively model PD-L1 distribution and likely representation of actual results by a single real or virtual core as a function of sample size. Bigras et al’s results seem to indicate that high TPS in biopsies may occasionally misclassify the lesion when compared with larger samples. In Bigras et al’s report, the most prevalent misclassifications were false negatives. This most prevalent misclassification does not by itself explain the high overall percentage of misclassification. So, Bigras et al thought that there were others factors responsible for it, such as false positives. Our results were

FIGURE 3. Features under microscope of surgical resected specimen and matched biopsies (A, resection specimen, B, core needle biopsy 1, C, core needle biopsy 2, D, core needle biopsy 3). The intratumoral heterogeneity of PD-L1 expression was observed in resection specimen and tumor proportion score (TPS) was 70%. The matched biopsies from the same tumor showed different PD-L1 status, and TPS were 5%, 40%, and 95%, respectively. The biopsy with highest TPS was more representative for the TPS of surgical resection specimen.
in line with Bigras and colleagues’ report. In our report, most of the discordant cases manifested understimated biopsies TPS compared with resection specimen, but overestimated cases were also observed (Supplementary Table 4, Supplemental Digital Content 4, http://links.lww.com/AIMM/A320). Both false negative and false positive can lead to a misclassification of PD-L1 assessment. Extensive sampling is an effective method to reduce false negative. The highest TPS among CNB samples may raise a risk of false positive. But our results showed that the highest TPS provided the best consistency at the 1% and 50% cut-offs (Table 2). Moreover, in the clinical context, the main concern is to avoid missing patients who could benefit from an effective treatment. In advanced NSCLC, patients with the PD-L1 TPS ≥50% should be treated with Pembrolizumab alone as first-line. Our study was based on real punctured core tissue collected from the resection specimens to simulate the CNB, and included more values (weighted average TPS, TPS of the longest biopsy specimen, and TPS of the biopsy with most tumor volume). So, this result should be more objective and scientific.

Although tumor volume was not associated with the consistency between CNB and resection specimen (Supplementary Table 6, Supplemental Digital Content 6, http://links.lww.com/AIMM/A322). At present, the minimum number of vital carcinoma cells required to analyze the PD-L1 status in NSCLC by the Dako PharmDx PD-L1 kit 22C3 assay is 100. The calculation and reporting of tumor volume are an effective method to reduce false negative. The highest TPS among CNB samples may raise a risk of false positive. But our results showed that the highest TPS provided the best consistency at the 1% and 50% cut-offs (Table 2). Moreover, in the clinical context, the main concern is to avoid missing patients who could benefit from an effective treatment. In advanced NSCLC, patients with the PD-L1 TPS ≥50% should be treated with Pembrolizumab alone as first-line. Our study was based on real punctured core tissue collected from the resection specimens to simulate the CNB, and included more values (weighted average TPS, TPS of the longest biopsy specimen, and TPS of the biopsy with most tumor volume). So, this result should be more objective and scientific.

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