# The One With Highest Tumor Proportion Score of Multiple Core Needle Biopsies From the Tumor Can Best Represent PD-L1 Status Estimated by Whole Surgical Specimen in Non-small Cell Lung Cancer

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**Research**

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# Abstract

**AIM**: The heterogeneity of PD-L1 status between core needle biopsies (CNBs) from one tumor was not 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.

**METHODS**: Random CNB was performed in surgical specimens from 170 consecutive Non-Small Cell Lung Cancer (NSCLC) samples. 51 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.

**RESULTS**: The heterogeneity of PD-L1 status between CNBs from one tumor was observed in 56.1% 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 biopsy specimen and the biopsy with most tumor volume were compared. At a cutoff of 1%, the concordance rates were 94.1%, 88.2%, 90.2%, 86.3%, 86.3% and 86.3%; At a cutoff 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.

**CONCLUSIONS:** 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% cutoffs.


# Introduction

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. PD-L1 is frequently expressed in many types of carcinomas [6, 9, 18], including NSCLC. PD-L1 allowed the cancer cells to evade the immune system in order to progress [15–17]. Pembrolizumab is an anti-PD-1 monoclonal antibody that was approved for treating advanced NSCLC based on PD-L1 expression in tumor cells (TCs). The KEYNOTE-010 trial demonstrated that pembrolizumab prolonged the overall survival of patients with a PD-L1 tumor proportion score(TPS) ≥ 1%[5], in which PD-L1was 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[2, 12]. Therefore, accurate evaluation of PD-L1 expression is necessary for patients with advanced NSCLC.

Due to the latest development of imaging and minimally invasive interventional procedures, a large population of patients with advanced NSCLC was pathologically diagnosed through core needle biopsy (CNB). Previous studies[1, 3, 7, 8, 10, 11] 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 one tumor. In clinical practice, to satisfy the needs of more biomarkers to be tested especially in NSCLC, pathologists usually encounter the situation that more than one CNB tissues were 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 previous studies [1, 10]. 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 study was to find out the best strategy for tissue choosing and scoring when more than one CNB were 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 was 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, USA) 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 specimen under 3 cm in diameter was 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).

# Immunohistochemistry and Scoring

Rehman et al[13] previously demonstrated that staining result of one block is enough to represent the entire tumor. So one representative block of each resection tumor were selected to perform PD-L1 assessment utilizing the DakoPharmDx 22C3 IHC assay on the Dako immunohistochemistry autostainer (Link 48)[14]. PD-L1 expression was observed blindly by two experienced pathologists (W.S. and X.Y.); discordant cases were reevaluated by a third pathologist (HY.W.). PD-L1 positive was defined as TPS ≥ 1%.

# Statistical Analysis

Statistical analysis was calculated using the SPSS software system (version 18.0, SPSS Inc., Chicago, IL, USA). The difference between categorical factors was assessed using Chi-square test and Fisher's exact test. P values from tests less than 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 all necrosis. 15 biopsies were excluded for no tumor cells in the biopsies. 10 randomly PD-L1 negative cases were included, too (Fig. 1). Finally, a total of 51 case (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 tumor cells were available for evaluation in all of biopsies. The patients included 30 men and 21 women ranging in age from 31 to 73 years and the median age was 60.5 years old. The median tumor size was 3.0 cm (range, 1.0-7.5 cm). The patient characteristics were shown in Table 1.

Table 1
The clinic-pathologic features of 52 patients

| Variables | N | % |
|---|---|---|
| Age(years) | | |
| <60 | 23 | 45.1 |
| ≥ 60 | 28 | 54.9 |
| Gender | | |
| 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 |

# 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 = 1–49%) and high expression(TPS ≥ 50%). The distribution of PD-L1 status was showed in Fig. 2. If 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%) which all of biopsies were concordant with the corresponding whole tumor resected specimen, while 28 cases (54.9%) which was at least one biopsy with discordant PD-L1 status compared with whole tumor resected specimen. The inter-biopsy heterogeneous of PD-L1 status was significantly observed in positive cases, regardless of PD-L1 high or low expression (Supplementary Table 1). 23 out of 41 (56.1%) positive cases show inter-biopsy heterogeneous in PD-L1 status (Supplementary table 1). The heterogeneity of PD-L1 status in biopsies from one case was not correlated with tumor size ($p$ = 0.925) and histologic subtypes ($p$ = 0.848) (Supplementary table 2 and Supplementary table 3). In tumors with diameter ≤ 2 cm, the inter-biopsy 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).

# 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 cut-offs were set to 1% and 50%, respectively. Most of discordant cases manifested underestimated biopsy TPS compared with resection specimen (Supplementary table 4). 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 can help in decision-making? The present research showed that the length of tissue and tumor volume were not associated with the consistency between CNB and resection specimen (Supplementary table 5 and supplementary table 6) by Chi-square 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 TPS of the highest, mean, median, 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).

Table 2
Correlation of different TPS value from core needle biopsies and the concordance status

| | At a 1% cutoff | | | At a 50% cutoff | | |
|---|---|---|---|---|---|---|
| Criterions | 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% |

# 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.

$$Biopsy\ \ density = \frac{Number\ of\ biopsies}{Longest\ diameter\ of\ tumor}$$

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% cutoff, and the coincidence rate were consistently more than 90%. But, at a 50% cutoff, PD-L1 status of

cases with biopsy density ≥ 1 core/cm showed statistically higher coincidence rate compared with that of biopsy density<1 core/cm ($p = 0.017$) (Table 3) in Fisher's exact test. Therefore, in our suggestion, 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 more than 95%.

Table 3
The concordance status in different cutoffs and biopsy density

| | At a 1% cutoff | | | | At a 50% cutoff | | | |
|---|---|---|---|---|---|---|---|---|
| Biopsy density (core/cm) | discordant | concordant | coincidence rate | P value | discordant | concordant | coincidence rate | P value |
| <0.75 | 0 | 7 | 100% | 1.000* | 2 | 5 | 71.4% | 0.133* |
| ≥ 0.75 | 3 | 41 | 93.2% | | 3 | 41 | 92.3% | |
| <1 | 0 | 14 | 100% | 0.552* | 4 | 10 | 71.4% | 0.017*# |
| ≥ 1 | 3 | 34 | 91.9% | | 1 | 36 | 97.3% | |
| <1.5 | 2 | 24 | 92.3% | 1.000* | 5 | 21 | 80.8% | 0.051* |
| ≥ 1.5 | 1 | 24 | 96.0% | | 0 | 25 | 100% | |
| * Fisher's Exact Test | | | | | | | | |
| # $p<0.05$ | | | | | | | | |

# Discussion

Accurate evaluation of PD-L1 status is critical for 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 cutoff value set, such as 1% or 50% according to different treatment strategies.

The PD-L1 status of biopsies had been previously reported [1, 3, 7, 8, 10]. Some of the studies used the archival biopsies[3, 7, 8], while some studies were based on TMAs as surrogates of biopsy specimens[1, 10]. But, these materials (the 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 is most closely reflecting 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 showed between the CNBs from one tumor. It was observed in the cases with PD-L1 positive, regardless of PD-L1 high or low expression (Supplementary table 1), tumor size (Supplementary table 2) and histological subtypes (Supplementary table 3). The heterogeneity of PD-L1 status showed between the CNBs may be the main reason for the previous conflict results on using lung CNBs for PD-L1 evaluation.

In clinical practice, more than one tissue may be obtained during the percutaneous pulmonary biopsy. 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 the Munari's study[10]. In Munari's study, TMAs were used as surrogates of biopsy specimens. The maximum value appeared to better reflect PD-L1 expression on the whole sections, compared with mean, median and minimum values of biopsy. 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.

The heterogeneity of PD-L1 expression is variable in its scale and extent, so it's hard to accurate assess PD-L1 expression. Several studies have tried to quantify how many biopsy specimens of a NSCLC are required to provide accurate coverage of PD-L1 expression within a tumor[3, 10], most concluded that more biopsies were likely to provide greater accuracy. Haragan's study[4] showed that extensive sampling reduced the inaccuracy, but cannot eliminate it. In current study, the heterogeneity was found between CNBs obtained from the same surgical resected specimen. But more tissues did not improve the predictive accuracy, when the cutoff was set to 1%. The probability of accuracy was consistently more than 90% (Table 3). While in patients with PD-L1 high expression whose TPS ≥ 50%, the accuracy was associated with the quality of biopsy. The coincidence rare was only 71.4%, when the biopsy density was less than 0.75 core/cm, and increase to 97.3% when the biopsy density was more than 1 core/cm (Table 3). Patients with advanced NSCLC should be treated with pembrolizumab alone as first-line therapy if PD-L1 TPS ≥ 50%. Results of the current study suggested that insufficient quantity of CNB tissue may lead to underestimate of accurate PD-L1 status, which will allow more patients to benefit from immunotherapy. But the likelihood of getting more tissue may increase the risk of pneumothorax and hemorrhage. Our results showed that the tissues needed for accurate evaluation of PD-L1 status was correlated with the given cutoffs. According to different treatment purposes, if the concerned cutoff value is 1%, a few biopsies tissue could be enough. Nevertheless, if the concerned cutoff value is 50%, insufficient biopsies may lead to underestimation of PD-L1 status. It will provide great use for pathologists and oncologists in accurate evaluation of PD-L1 status of NSCLC patients.

Anti-PD-1/PD-L1 therapy has been recommended for the patients with unresectable NSCLC. We simulated real CNBs using resected NSCLC specimens in this study. The intratumoral heterogeneity of PD-L1 expression is ill-understood. Even detailed and extensive study in large series of tumors cases fails to reveal any particular pattern of this heterogeneity[4]. Therefore, we suggest that there is no difference between the unresectable and resectable NSCLC in the intratumoral heterogeneity of PD-L1 expression.

In conclusion, the inter-heterogeneity of PD-L1 status was found in the biopsies from the same tumor. The highest TPS of multiple biopsies made the most sense in the evaluation of the PD-L1 status. The quantity of core needle biopsies needed for accurate evaluation of PD-L1 status was correlated with the concerned cutoffs.

# Abbreviations

PD-1: programmed death 1; PD-L1: programmed death-ligand 1; CNBs: core needle biopsies; TPS: tumor proportion score; IHC: immunohistochemistry; TCs, tumor cells; TMAs, tissue microarrays; NSCLC, non-small cell lung cancer.

# Declarations

### Ethics approval and consent to participate

Ethical approval was obtained from the Ethics Committee Board of the Peking University Cancer Hospital and Institute, Key Laboratory of Carcinogenesis and Translational Research, Ministry of Education, Beijing, China (No. 2018KT88). Written informed consent was obtained from each patient.

### Consent for publication

Not applicable. All authors read and approved the final manuscript.

### Availability of data and materials

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

### Competing interests

The authors declare no conflict of interest.


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### Authors' contributions

Wei Sun and Xin Yang were responsible for planning, writing and critically reviewing the work. Changling Liu and Shanmei Liao were responsible for data analysis. Haiyue Wang was responsible for reviewing the pathology portions of the work. Dongmei Li was responsible for writing and critically reviewing the pathology portions of the work. Lixin Zhou, Di Hu, Xiaozheng Huang, Ling Jia, Qiang Kang, Qi Wu and Xinting Diao were responsible for perform the H&E and immunohistochemical staining. The PD-L1 IHC 22C3 pharmDx assay was provided by Agilent Technology.


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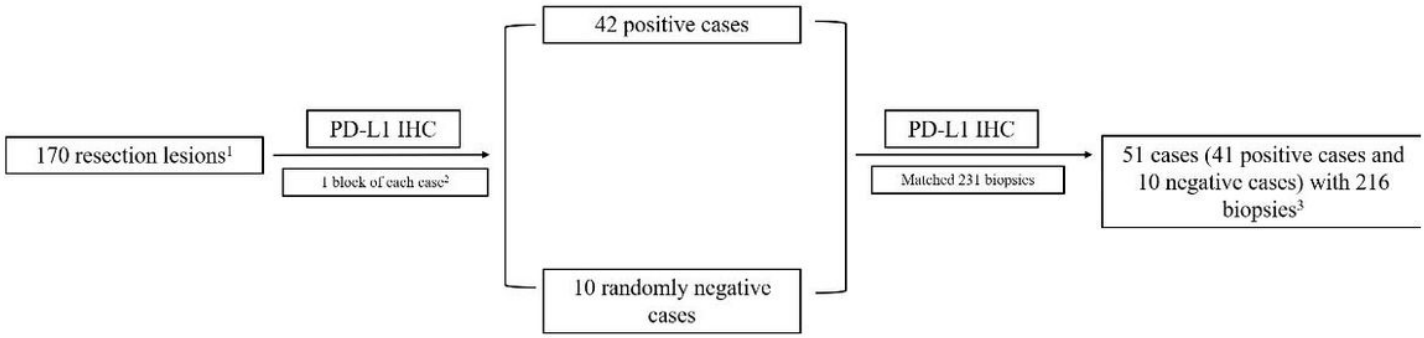
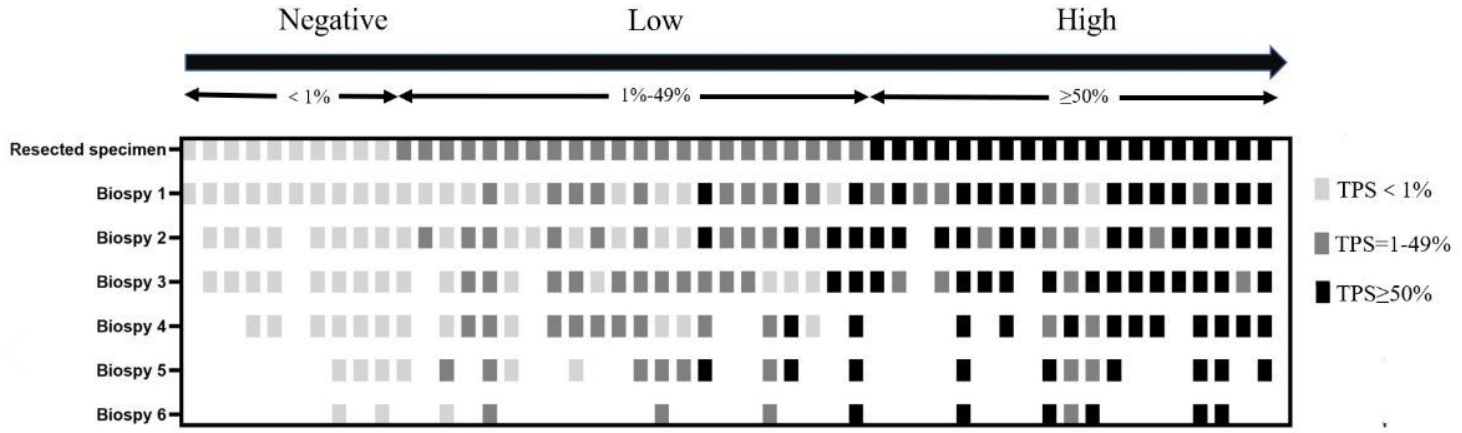
## Figures




## 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 were included adenocarcinoma, squamous cell carcinoma, large cell carcinoma, adenosquamous carcinoma, sarcomatoid carcinoma, lymphoepithelioma-like carcinoma. 1 block of each resection tumor was selected to staining PD-L1; Note 3: In 42 PD-L1 positive cases, one case was excluded for all of matched biopsies were necrosis. 10 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.




## Figure 2

The distribution of PD-L1 status of 51 resected specimens and their matched biopsies (a total of 216). Each column in this figure represented one sample, and the squares meant PD-L1 status defined by its original 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.

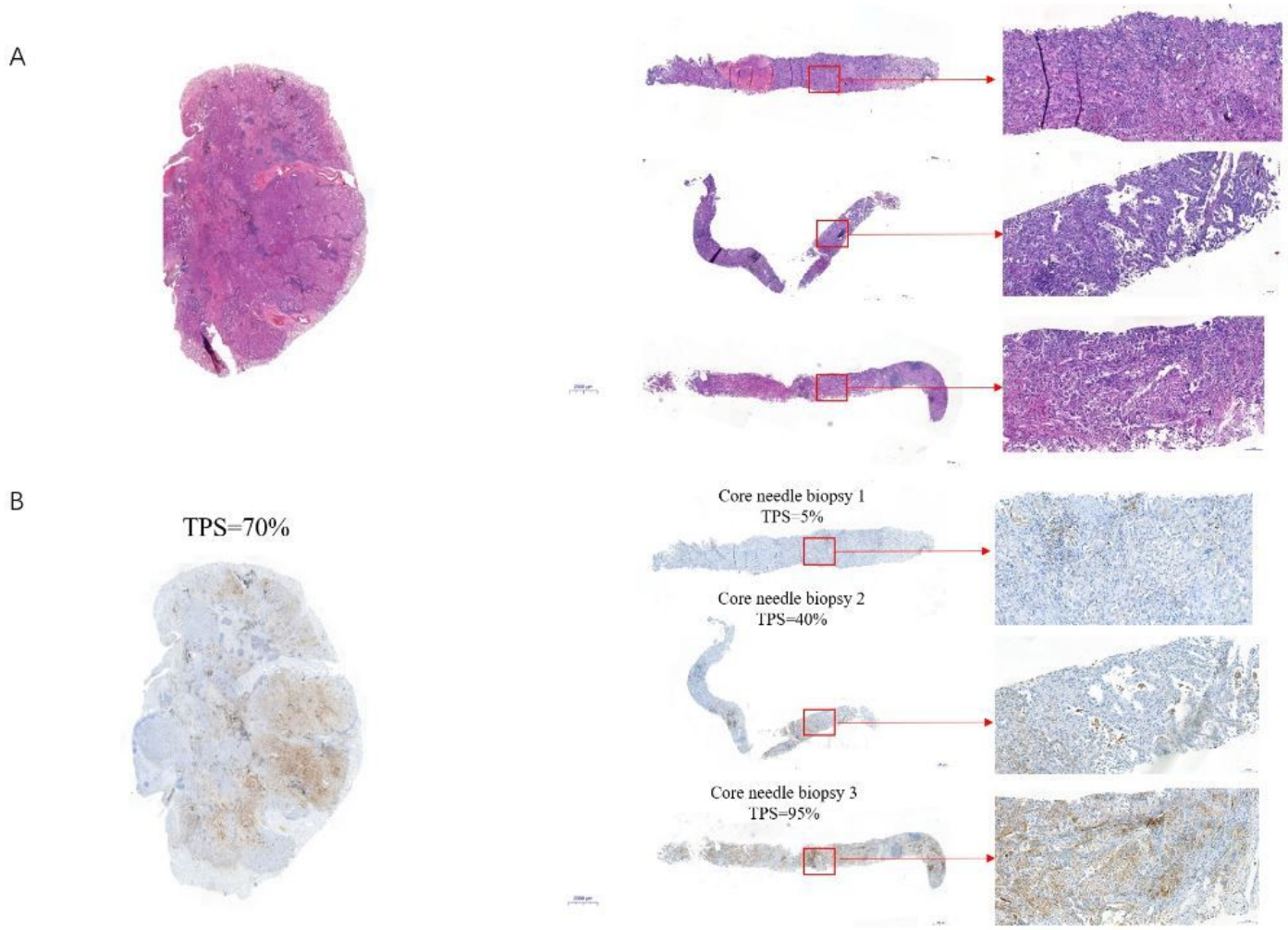



**Figure 3**

Features under microscope of surgical resected specimen and matched biopsies (A, HE slide, B, PD-L1 staining). The intratumoral heterogeneity of PD-L1 expression was observed in resection specimen, and 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.

## Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- Supplementarytable1.docx
- supplymentarytable2.docx
- supplymentarytable3.docx
- supplementarytable4.docx
- supplementarytable5.docx
- supplementarytable6.docx