**Supplemental Table 1 – PD-L1 tumor proportion score concordance** **between cell blocks and lung tumor resections.**

|  |  |  |
| --- | --- | --- |
|  | **PD-L1 TPS (%)** | **Concordance, Cut-Off** |
| **Case**  | **Cell Block** | **Lung Tumor Resection** | **TPS ≥ 50%**  | **TPS ≥ 1%** |
| 1 | 0 | 0 | Yes | Yes |
| 2 | 0 | 1 | Yes | No |
| 3 | 2 | 0 | Yes | No |
| 4 | 0 | 0 | Yes | Yes |
| 5 | 0 | 0 | Yes | Yes |
| 6 | 5 | 3 | Yes | Yes |
| 7 | 1 | 0 | Yes | No |
| 8 | 1 | 0 | Yes | No |
| 9 | 0 | 0 | Yes | Yes |
| 10 | 0 | 0 | Yes | Yes |
| 11 | 0 | 0 | Yes | Yes |
| 12 | 5 | 0 | Yes | No |
| 13 | 30 | 20 | Yes | Yes |
| 14 | 27 | 25 | Yes | Yes |
| 15 | 0 | 0 | Yes | Yes |
| 16 | 0 | 0 | Yes | Yes |
| 17 | 0 | 0 | Yes | Yes |
| 18 | 23 | 0 | Yes | No |
| 19 | 8 | 0 | Yes | No |
| 20 | 0 | 0 | Yes | Yes |
| 21 | 15 | 0 | Yes | No |
| 22 | 0 | 0 | Yes | Yes |
| 23 | 0 | 1 | Yes | No |
| 24 | 0 | 0 | Yes | Yes |
| 25 | 0 | 0 | Yes | Yes |
| 26 | 0 | 0 | Yes | Yes |
| 27 | 0 | 1 | Yes | No |
| 28 | 0 | 0 | Yes | Yes |
| 29 | 0 | 0 | Yes | Yes |
| 30 | 0 | 1 | Yes | No |
| 31 | 0 | 0 | Yes | Yes |
| 32 | 93 | 58 | Yes | Yes |
| 33 | 100 | 100 | Yes | Yes |
| 34 | 67 | 55 | Yes | Yes |
| 35 | 53 | 83 | Yes | Yes |
| 36 | 98 | 94 | Yes | Yes |
| 37 | 50 | 55 | Yes | Yes |
| 38 | 98 | 98 | Yes | Yes |
| 39 | 85 | 10 | No | Yes |
| 40 | 69 | 20 | No | Yes |
| 41 | 65 | 4 | No | Yes |
| 42 | 28 | 60 | No | Yes |
| 43 | 0 | 55 | No | No |
| 44 | 85 | 93 | Yes | Yes |
| 45 | 0 | 0 | Yes | Yes |
| 46 | 0 | 0 | Yes | Yes |
| 47 | 23 | 50 | No | Yes |
| 48 | 0 | 0 | Yes | Yes |
| 49 | 5 | 0 | Yes | No |
| 50 | 0 | 0 | Yes | Yes |
| 51 | 83 | 85 | Yes | Yes |
| 52 | 1 | 0 | Yes | No |
| 53 | 0 | 0 | Yes | Yes |
| 54 | 0 | 10 | Yes | No |
| 55 | 0 | 8 | Yes | No |
| 56 | 0 | 0 | Yes | Yes |
| 57 | 2 | 0 | Yes | No |
| 58 | 40 | 80 | No | Yes |
| 59 | 0 | 0 | Yes | Yes |
| 60 | 0 | 0 | Yes | Yes |
| 61 | 0 | 0 | Yes | Yes |
| 62 | 0 | 0 | Yes | Yes |
| 63 | 8 | 30 | Yes | Yes |
| 64 | 30 | 60 | No | Yes |
| 65 | 0 | 0 | Yes | Yes |
| 66 | 0 | 0 | Yes | Yes |
| 67 | 28 | 50 | No | Yes |
| 68 | 0 | 0 | Yes | Yes |
| 69 | 1 | 0 | Yes | No |
| 70 | 1 | 0 | Yes | No |
| 71 | 0 | 0 | Yes | Yes |
| 72 | 95 | 90 | Yes | Yes |
| 73 | 55 | 70 | Yes | Yes |
| 74 | 40 | 0 | Yes | No |
| 75 | 0 | 0 | Yes | Yes |
| 76 | 0 | 0 | Yes | Yes |
| 77 | 0 | 1 | Yes | No |
| 78 | 8 | 1 | Yes | Yes |
| 79 | 0 | 10 | Yes | No |
| 80 | 100 | 100 | Yes | Yes |
| 81 | 0 | 0 | Yes | Yes |

Abbreviations: PD-L1 – programmed death ligand; TPS – tumor proportion score

**Supplemental Table 2 – Comparison of continuous PD-L1 tumor proportion scores with cell block cellularity groups.**

|  |  |  |  |
| --- | --- | --- | --- |
| **Cellularity** | **< 200**  | **200-500**  | **> 500**  |
| **Observer** | **CP-1** | **CP-2** | **CP-1** | **CP-2** | **CP-1** | **CP-2** |
| Cases (n) | 27 | 28 | 25 | 32 | 29 | 21 |
| Intraclass correlation coefficient(95% CI) | 0.73 (0.49, 0.87) | 0.54 (0.22, 0.76) | 0.77 (0.55, 0.89) | 0.90(0.81, 0.95) | 0.95 (0.89, 0.97) | 0.98 (0.95, 0.99) |
| Pearson correlation | 0.73 | 0.56 | 0.77 | 0.90 | 0.95 | 0.98 |
| Kappa squared weights | 0.67 | 0.46 | 0.62 | 0.85 | 0.74 | 0.62 |

Abbreviations: PD-L1 – programmed death ligand, CP – cytopathologist, CI – confidence interval

**Supplemental Table 3 – Digital image analysis of PD-L1 immunohistochemistry in control tissues after CytoLyt® prefixation and delayed formalin fixation.**

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **Tissue type** | **Pre-analytic variable** | **Total proportion of positive cells, %** | **Proportion of strong positive, %** | **Proportion of moderate positive, %** | **Proportion of weak positive, %** | **H-score** |
| Tonsil | NBF | 88.44 | 64.67 | 23.30 | 0.46 | 241.08 |
| Tonsil | CytoLyt | 88.22 | 66.33 | 21.22 | 0.66 | 242.11 |
| Tonsil  | 1h Delay | 86.03 | 59.08 | 26.39 | 0.56 | 230.58 |
| Tonsil | 6h Delay | 91.48 | 58.95 | 31.48 | 1.06 | 240.86 |
| Tonsil | 18h Delay | 92.07 | 59.41 | 31.85 | 0.81 | 242.73 |
| Placenta | NBF | 51.66 | 41.23 | 10.10 | 0.32 | 144.23 |
| Placenta | CytoLyt | 40.41 | 33.82 | 5.26 | 0.33 | 114.31 |
| Placenta | 1h Delay | 52.34 | 41.51 | 10.46 | 0.38 | 145.81 |
| Placenta | 6h Delay | 49.76 | 31.73 | 17.57 | 0.46 | 130.79 |
| Placenta | 18h Delay | 52.63 | 38.56 | 13.62 | 0.44 | 143.37 |
| Placenta | 24h Delay | 52.81 | 39.05 | 13.47 | 0.30 | 144.38 |

Abbreviations: h – hours; NBF – 10 % neutral-buffered formalin

**Supplemental Table 4 – PD-L1 tumor proportion scores of dual-processed pleural fluids and endobronchial ultrasound-guided transbronchial needle aspiration specimens.**

|  |  |  |  |
| --- | --- | --- | --- |
|  |  | **PD-L1 TPS, %** | **Concordance, cut-off** |
| **Case** | **Specimen type** | **Formalin-fixed** | **CytoLyt-fixed** | **TPS ≥ 50%**  | **TPS ≥ 1%** |
| 1 | EBUS-TBNA | 60 | 80 | Yes | Yes |
| 2 | EBUS-TBNA | 97 | 100 | Yes | Yes |
| 3 | PLFL | 50 | 80 | Yes | Yes |
| 4 | PLFL | 60 | 75 | Yes | Yes |
| 5 | EBUS-TBNA | 90 | 95 | Yes | Yes |
| 6 | PLFL | 63 | 65 | Yes | Yes |
| 7 | PLFL | 55 | 50 | Yes | Yes |
| 8 | PLFL | 70 | 55 | Yes | Yes |
| 9 | PLFL | 80 | 80 | Yes | Yes |
| 10 | EBUS-TBNA | 99 | 95 | Yes | Yes |
| 11 | EBUS-TBNA | 75 | 63 | Yes | Yes |
| 12 | PFLF | 65 | 90 | Yes | Yes |
| 13 | EBUS-TBNA | 100 | 98 | Yes | Yes |
| 14 | PLFL | 88 | 88 | Yes | Yes |
| 15 | EBUS-TBNA | 65 | 80 | Yes | Yes |
| 16 | EBUS-TBNA | 100 | 93 | Yes | Yes |
| 17 | PLFL | 75 | 80 | Yes | Yes |
| 18 | PLFL | 85 | 70 | Yes | Yes |
| 19 | PLFL | 93 | 100 | Yes | Yes |
| 20 | EBUS-TBNA | 100 | 100 | Yes | Yes |
| 21 | PLFL | 50 | 40 | No | Yes |
| 22 | PLFL | 5 | 3 | Yes | Yes |
| 23 | PLFL | 1 | 1 | Yes | Yes |
| 24 | PLFL | 30 | 25 | Yes | Yes |
| 25 | EBUS-TBNA | 20 | 8 | Yes | Yes |
| 26 | PLFL | 23 | 30 | Yes | Yes |
| 27 | PLFL | 15 | 1 | Yes | Yes |
| 28 | EBUS-TBNA | 2 | 5 | Yes | Yes |
| 29 | PLFL | 20 | 20 | Yes | Yes |
| 30 | EBUS-TBNA | 2 | 0 | Yes | No |
| 31 | EBUS-TBNA | 35 | 65 | No | Yes |
| 32 | EBUS-TBNA | 0 | 0 | Yes | Yes |
| 33 | PLFL | 0 | 0 | Yes | Yes |
| 34 | EBUS-TBNA | 0 | 0 | Yes | Yes |
| 35 | PLFL | 0 | 0 | Yes | Yes |
| 36 | PLFL | 0 | 0 | Yes | Yes |
| 37 | EBUS-TBNA | 0 | 0 | Yes | Yes |
| 38 | PLFL | 0 | 0 | Yes | Yes |
| 39 | PLFL | 0 | 0 | Yes | Yes |
| 40 | EBUS-TBNA | 0 | 0 | Yes | Yes |
| 41 | PLFL | 0 | 0 | Yes | Yes |
| 42 | PLFL | 0 | 0 | Yes | Yes |
| 43 | PLFL | 0 | 0 | Yes | Yes |
| 44 | EBUS-TBNA | 0 | 0 | Yes | Yes |
| 45 | EBUS-TBNA | 0 | 0 | Yes | Yes |
| 46 | EBUS-TBNA | 0 | 0 | Yes | Yes |
| 47 | EBUS-TBNA | 0 | 0 | Yes | Yes |
| 48 | PLFL | 0 | 0 | Yes | Yes |
| 49 | PLFL | 0 | 0 | Yes | Yes |
| 50 | PLFL | 0 | 0 | Yes | Yes |
| 51 | EBUS-TBNA | 0 | 1 | Yes | No |
| 52 | EBUS-TBNA | 0 | 3 | Yes | No |

Abbreviations: PLFL – pleural fluid, PD-L1 – programmed death ligand, EBUS-TBNA – endobronchial ultrasound-guided transbronchial needle aspiration

**Supplemental Table 5 – Digital image analysis of PD-L1 immunohistochemistry in a subset of dual-processed pleural fluids.**

|  |  |  |
| --- | --- | --- |
|  | **Percent Positive** | **H-Score** |
| **Case** | **Formalin-fixed** | **CytoLyt-fixed** | **Formalin-fixed** | **CytoLyt-fixed** |
| 6 | 27.6983 | 29.492 | 47.2274 | 53.4005 |
| 7 | 26.4819 | 29.3599 | 38.7094 | 39.3162 |
| 17 | 43.064 | 43.9248 | 80.46 | 86.4623 |
| 18 | 55.5556 | 48.53567 | 118.018 | 104.6249 |
| 22 | 19.7248 | 22.3844 | 42.9664 | 50.1217 |
| 24 | 15.7072 | 16.5574 | 26.8522 | 29.8361 |
| 26 | 18.3432 | 28.3019 | 27.2189 | 45.283 |
| 29 | 31.6746 | 33.59937 | 42.654 | 44.51736 |
| 38 | 2.22158 | 1.900818 | 4.16182 | 3.126987 |
| 39 | 3.24544 | 8.15287 | 5.22876 | 14.4586 |
| 42 | 3.12835 | 1.70709 | 4.96067 | 2.89867 |
| 43 | 1.46482 | 1.68914 | 2.23325 | 2.79631 |

**Supplemental Figure 1 – Difference in PD-L1 tumor proportion score between lung tumor resection (LTR) and cell block (CB) by time interval between specimens.**

****

**Supplemental Table 6 – Summary of cytology-histology comparative studies using PD-L1 22C3 IHC pharmDxTM with cell blocks**

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| Author (ref.) | Number of specimens (total) | Specimenpairs from same patient | Specimen pairs fromsame tumor site\* | Cytological preparation type, Fixative  | Paired surgical material  | TPS cut-off values |
| Skov (1)  | 174 | 87 | 84 | Smear/CB, 10% NBF | Resection, core biopsy, mucosal biopsy | ≥1%, ≥5%, ≥10%, ≥50% |
| Heymann (2) | 214 | 23 | 3 | CB, 10% NBF | Resection, core biopsy | ≥50% |
| Noll (3) | 82 | 41 | 34 | CB, 95% ethanol/10% NBF | Core biopsy | ≥50% |
| Torous (4) | 232 | 0 | 0 | CB, CytoLyt/formalin | Resection, core biopsy, transbronchial biopsy | <1%, ≥50% |
| Wang (5) | 1419 | 27 | Not specified | CB, CytoLyt/formalin or 10% NBF only | Resection, core biopsy, endobronchial biopsy, skin punch biopsy | ≥1%, ≥50% |
| Ilie (6) | 140 | 70 | Not specified | CB, 10% NBF | Bronchial biopsy | ≥1%, ≥50% |
| Xu (7) | 104 | 52 | Not specified | CB, 10% NBF | Resection, core biopsy | ≥1%, ≥50% |
| Sakata (8) | 122 | 61 | 21 | CB, fixative not specified | Resection | ≥1%, ≥50% |
| Hernandez (9) | 104 | 52 | 25 | CB, RPMI/10% NBF | Resection, core biopsy | <1%, ≥50% |
| Mei (10) | 265 | 0 | 0 | CB, 10% NBF | Resection, core biopsy | ≥1%,≥50% |
| Wang (11) | 265 | 34 | 16 | CB, CytoLyt/5% NBF | Core biopsy | ≥50% |
| Lou(our study) | 162 | 81 | 81 | CB,10% NBF | Resection | ≥1%,≥50% |

\* Pleural fluids excluded

1. Skov BG, Skov T. Paired Comparison of PD-L1 Expression on Cytologic and Histologic Specimens From Malignancies in the Lung Assessed With PD-L1 IHC 28-8pharmDx and PD-L1 IHC 22C3pharmDx. Appl Immunohistochem Mol Morphol. 2017;25(7):453-9.

2. Heymann JJ, Bulman WA, Swinarski D, Pagan CA, Crapanzano JP, Haghighi M, et al. PD-L1 expression in non-small cell lung carcinoma: Comparison among cytology, small biopsy, and surgical resection specimens. Cancer Cytopathol. 2017;125(12):896-907.

3. Noll B, Wang WL, Gong Y, Zhao J, Kalhor N, Prieto V, et al. Programmed death ligand 1 testing in non-small cell lung carcinoma cytology cell block and aspirate smear preparations. Cancer Cytopathol. 2018;126(5):342-52.

4. Torous VF, Rangachari D, Gallant BP, Shea M, Costa DB, VanderLaan PA. PD-L1 testing using the clone 22C3 pharmDx kit for selection of patients with non-small cell lung cancer to receive immune checkpoint inhibitor therapy: are cytology cell blocks a viable option? J Am Soc Cytopathol. 2018;7(3):133-41.

5. Wang H, Agulnik J, Kasymjanova G, Wang A, Jimenez P, Cohen V, et al. Cytology cell blocks are suitable for immunohistochemical testing for PD-L1 in lung cancer. Ann Oncol. 2018;29(6):1417-22.

6. Ilie M, Juco J, Huang L, Hofman V, Khambata-Ford S, Hofman P. Use of the 22C3 anti-programmed death-ligand 1 antibody to determine programmed death-ligand 1 expression in cytology samples obtained from non-small cell lung cancer patients. Cancer Cytopathol. 2018;126(4):264-74.

7. Xu H, Bratton L, Nead M, Russell D, Zhou Z. Comparison of programmed death-ligand 1 (PD-L1) immunostain for nonsmall cell lung carcinoma between paired cytological and surgical specimens. Cytojournal. 2018;15:29.

8. Sakata KK, Midthun DE, Mullon JJ, Kern RM, Nelson DR, Edell ES, et al. Comparison of Programmed Death Ligand-1 Immunohistochemical Staining Between Endobronchial Ultrasound Transbronchial Needle Aspiration and Resected Lung Cancer Specimens. Chest. 2018;154(4):827-37.

9. Hernandez A, Brandler TC, Zhou F, Moreira AL, Schatz-Siemers N, Simsir A. Assessment of Programmed Death-Ligand 1 (PD-L1) Immunohistochemical Expression on Cytology Specimens in Non-Small Cell Lung Carcinoma. Am J Clin Pathol. 2019;151(4):403-15.

10. Mei P, Shilo K, Wei L, Shen R, Tonkovich D, Li Z. Programmed cell death ligand 1 expression in cytologic and surgical non-small cell lung carcinoma specimens from a single institution: Association with clinicopathologic features and molecular alterations. Cancer Cytopathol. 2019;127(7):447-57.

11. Wang G, Ionescu DN, Lee CH, Hiruki T, Myers R, Shaipanich T, et al. PD-L1 testing on the EBUS-FNA cytology specimens of non-small cell lung cancer. Lung Cancer. 2019;136:1-5.