Comparison of programmed death ligand 1 immunostaining for pancreatic ductal adenocarcinoma between paired cytological and surgical samples

Michael Muggilli¹, Donna Russell², Zhongren Zhou³

¹Department of Pathology and Laboratory Medicine, Oklahoma, ²Department of Pathology and Laboratory Medicine, University of Rochester, Rochester, ³Department of Pathology and Laboratory Medicine, Rutgers University, New Brunswick, New Jersey, United States.

INTRODUCTION

Pancreatic ductal adenocarcinoma (PDAC) is the third leading cause of cancer death in the United States, and the seventh leading cause of cancer-related death in both men and women worldwide.¹ The high mortality rate, with 5-year survival rates as low as 9%, illustrates the poor prognosis of PDAC.¹² Only 10–20% of PDAC are resectable, with the majority of diagnoses made only when non-surgical/neoadjuvant therapy is recommended. Treatment of PDAC without...
resection includes chemotherapy, radiotherapy, and palliative care. However, these treatments have not significantly improved the survival rates of PDAC patients.\(^2\) Given these low survival rates, new therapies for PDAC are urgently needed. Recent advances in Programmed death ligand 1 expression (PD-L1) immunotherapy have shown significant progress in the treatment of non-small cell lung carcinoma (NSCLC) and other cancers, which may improve outcomes in PDAC therapy as well.

The immune checkpoint programmed cell death protein 1 (PD-1) is expressed in tumor-infiltrating T-lymphocytes, B-lymphocytes, natural killer cells, monocytes, and dendritic cells. It is engaged by tumor-expressed PD-L1 and PD-L2, which increase the apoptosis of activated tumor-reactive T-cells and promote the growth of tumor cells \textit{in vivo}.\(^6\) Recently, PD-L1 antibodies in multiple clinical trials were used to treat many cancer types, including melanoma,\(^7\) NSCLC,\(^10-12\) hepatocellular carcinoma,\(^13\) esophageal cancer,\(^14\) and bladder cancer.\(^15,16\) Several studies also investigated the association of PD-L1 and prognosis of PDAC.\(^17-20\) With PD-L1 antibody E1L3N, PD-L1 expression was observed in 61.9% (26/42) of PDAC by fluorescent phosphor-integrated dot nanoparticles methods.\(^21\) Three meta-analysis data showed that PD-L1 expression was significantly associated with worse overall survival (OS) and positive lymph node metastasis.\(^18-20\) The percentages of PD-L1 expression ranged from 3% to 86%. All studies were based on surgical resection samples. However, in routine clinical practice, most PDAC cases do not have surgical biopsy or resection tissue for ancillary testing. Endoscopic ultrasound-guided fine-needle aspiration (FNA) of pancreatic tumors has become the standard method for diagnosing PDAC.\(^22\) Thus, frequently the only tissue available to evaluate PD-L1 expression comes from cytological samples, underscoring the urgency to standardize these samples for biomarker analysis.

Scoring standardization between the many studies and antibodies is similarly confounding. Various clinical trials use different definitions for scoring PD-L1 as positive. Tumor proportion score (TPS) has been used to evaluate PD-L1 expression with DAKO 22C3 antibody and immunohistochemistry (IHC) for lung cancer, which was based on evaluating the percentage of PD-L1 positive tumor cells relative to all viable tumor cells present. Of the 2222 NSCLS patients, 1475 (66.38%) had PD-L1 expression, including 623 (28.49%) cases with TPS ≥50% and 842 (37.89%) cases with TPS 1–49%.\(^23\) However, recently the new combined positive score (CPS) was developed to evaluate the PD-L1 expression in gastrointestinal cancers.\(^24\) CPS is the number of PD-L1 stained cells (tumor cells, lymphocytes, and macrophages) divided by the total number of viable tumor cells and multiplied by 100. Using CPS to evaluate PD-L1 expression in gastric/gastroesophageal junction tumor (GEJ), Kulangara \textit{et al.} reported that the prevalence of PD-L1 expression in patients with gastric/GEJ cancer was 57.6% (148 of 257 patients).\(^20\) To the best of our knowledge, no standard reporting criteria for scoring PD-L1 expression in pancreatic cytology samples have been published.

In our study, we used both TPS and CPS to evaluate PD-L1 expression in paired cytological and surgical samples. We used surgical samples as the gold standard to evaluate PD-L1 expression in cytological samples.

**MATERIAL AND METHODS**

\textit{Study design}

**Paired cytological cell block and surgical samples**

Paired formalin-fixed cell blocks of cytological and surgical samples from the same patients with confirmed diagnosis of PDAC during 2010–2016 (\(n = 28\)) were found in the University of Rochester Medical Center Soft database. All the paired pancreatic FNA cytological and surgical resection samples were obtained within 1 month of each other. Both cell blocks from FNA cytological samples and resection tissues were fixed in formalin and paraffin embedded as routine standardized protocol in our laboratory [Figure 1]. The diagnosis was evaluated by MM and ZZ. The differentiation of PDAC was evaluated in surgical samples. For the cell block preparation, the one or two dedicated FNA passes were briefly washed in normal saline solution.
The samples were centrifuged to produce a concentrated cell button (5 min/2400 rpm), which was resuspended in 5 cc buffered formalin and centrifuged again (5 min/2400 rpm) to produce a fixed cell button. Slides containing the tissue sections of both the cytological cell blocks and surgical samples from the same patients were selected for immunostaining. All cytological cases in our study came from pancreatic FNA. No core biopsy was used. All patient identifiers were removed. This project was approved by Research Subjects Review Board at University of Rochester Medical Center.

PD-L1 IHC

PD-L1 IHC studies were performed on 4-μm thick sections of formalin-fixed and paraffin-embedded surgical tissues and cell blocks. After deparaffinizing and pretreating the tissue sections with PD-L1 pretreatment buffer at 99°C for 20 min, we applied ready-to-use mouse monoclonal antibody PD-L1 22C3 PharmDx IHC Kit (Dako, Carpinteria, CA, USA) following the manufacturer’s instructions. Appropriate positive and negative controls were evaluated by both Dako and our immunohistochemical laboratory control tissue.

TPS and CPS methods

The viable tumor cells showing partial or complete membrane staining at any intensity were defined as positive PD-L1 immunostain [Figure 2]. We used two methods to evaluate PD-L1 expression. TPS was based on evaluating the percentage of PD-L1-positive tumor cells relative to all viable tumor cells present in samples. The samples were considered to have PD-L1 expression if TPS ≥1%. CPS is the number of PD-L1 staining cells (tumor cells, lymphocytes, macrophages) divided by the total number of viable tumor cells and multiplied by 100. In surgical samples, PD-L1 positive inflammatory cells surrounding tumor cells were counted. However, in cell blocks it was difficult to evaluate the relationship between tumor cells and adjacent inflammatory cells. Therefore, we counted all PD-L1 positive inflammatory cells in cell blocks. We considered samples to have PD-L1 expression if CPS ≥1%. Based on our experience in evaluating PD-L1 expression in lung FNA samples, two cytopathologists counted cytological cell blocks and surgical samples with at least 100 tumor cells. If not in agreement, the two reviewers checked the slides again to reach a consensus.

Statistical analysis

The percentages of PD-L1-positive and PD-L1-negative immunostain in both cytological and surgical samples were calculated with CPS and TPS. Fisher’s exact test was used as appropriate to compare PD-L1 expression in both cytological and surgical samples. All statistical tests were two-sided. P < 0.05 was considered to be statistically significant.

RESULTS

PD-L1 expression in paired PDAC cytological and surgical samples using TPS

PD-L1 was expressed in nine out of 28 (32%) PDAC surgical samples and in nine out of 28 (32%) paired cytological samples [Figure 2 and Table 1]. Twenty-six out of 28 (93%) paired PDAC cases had correlating immunostain results, and only two cases (7%) showed PD-L1 expression discrepancy [Table 1]. The tumor cells showed positive PD-L1 in one cytological sample, but negative PD-L1 in the paired surgical sample. In one surgical sample, the tumor cells were positive for PD-L1, but in the paired cytological sample, the tumor cells were negative.

PD-L1 expression in paired PDCA cytological and surgical samples using CPS

With CPS, PD-L1 was expressed in 20 out of 28 (71%) PDAC surgical samples and in 16 out of 28 (57%) cytological samples [Figure 3 and Table 2]. Twenty out of 28 (71%) paired PDAC cases had concordant immunostain results, and eight out of 28 (29%) had non-concordant [Table 2]. In
seven cytological cases, the tumor cells and immune cells were negative for PD-L1 but positive in the paired surgical samples. In one cytological sample, the tumor cells and immune cells were positive for PD-L1 but negative in the paired surgical samples.

**Association of PD-L1 expression with the differentiation of PDAC**

The relationship between PD-L1 expression and tumor differentiation was analyzed in our study. We found that in both cytological and surgical samples [Table 3] well-differentiated tumor cases were negative for PD-L1 expression (0/8) with TPS. Fisher’s exact test showed that with TPS PD-L1 expression was significantly different among well- versus moderately- versus poorly-differentiated (P = 0.028) cancer samples in both cytological and surgical samples. The PD-L1 expression in the well-differentiated cancer samples was significantly lower than that in the moderately-differentiated samples (P = 0.0018) but not significantly different from the poorly-differentiated samples (P = 0.20). The PD-L1 expression in moderately-differentiated samples was also not significantly different from poorly-differentiated samples (P = 0.3742). With CPS, we found that the inflammatory cells in well-differentiated tumors showed a dramatically increased PD-L1 expression (6/8) from surgical samples with CPS but not from cytological samples (1/7) [Table 3]. Fisher’s exact test showed that PD-L1 positive cases were significantly different in three separate groups (well- vs. moderate- vs. poorly-differentiation) with CPS only in cytological samples (P = 0.004) but not in surgical samples (P = 0.724) [Table 3]. In cytological samples with CPS, the PD-L1 expression in well-differentiated samples was significantly lower than that in moderately-differentiated samples (P = 0.0022) but not significantly different from poorly-differentiated samples (P = 0.1189). Moderately-differentiated samples were also not significantly different from poorly-differentiated samples (P = 0.2898).

**DISCUSSION**

In our study, we evaluated the PD-L1 expression in cytological samples with paired surgical samples. Since there are no standard methods for determining PD-L1 expression in cytological samples, we used two common PD-L1 scoring systems: TPS and CPS. Using TPS, we found that the percentage of PD-L1 expression was excellently concordant (93%). with surgical samples from the same patients. Only two cases did not match. One was a PD-L1 negative case with a paired positive surgical sample in the cytological sample. It showed scant cellularity in cell block, barely reaching 100 tumor cells. The other PD-L1 negative case showed significant degeneration of tumor cells and
surrounding inflammatory cells. This might explain the false positive PD-L1 expression in cytological samples. However, PD-L1 heterogenic expression is a common phenomenon in pancreatic tumors and has been observed in other cases. Using CPS, the concordant rate between paired surgical samples and cytological samples was 71%. Seven PD-L1 negative cytological samples with paired positive surgical samples showed scant inflammatory cells, which might have caused the false negative.

PD-L1 expression has been reported in multiple clinical trials using various antibodies, including Dako 22C3,[26-27] Dako 28–8,[28,29] Ventana SP142,[30,31] and SP263.[32-33] The various clinical trials also used different definitions for calculating positive PD-L1 expression. TPS was used to evaluate Dako 22C3 antibody in a large randomized controlled trial for lung cancer. Of 2222 NSCLC patients, 1475 (66.38%) had PD-L1 expression, including 623 (28.49%) cases with TPS ≥50% and 842 (37.89%) cases with TPS ≤1–49%.[26] Among patients with at least 50% tumor cells expressed PD-L1, OS and progression-free survival were significantly longer with pembrolizumab than with docetaxel.[12] Subsequently, another scoring method, the CPS, was developed to evaluate PD-L1 expression.20 Using CPS to evaluate PD-L1 expression in gastric/GEJ tumors, other researchers reported that the prevalence of PD-L1 expression in patients with gastric/GEJ cancer was 57.6% (148 of 257 patients).[24] External reproducibility assessments demonstrated inter-pathologist overall agreement of 96.6% and intra-pathologist overall agreement of 97.2%. They concluded that CPS is a robust, reproducible PD-L1 scoring method that predicts response to pembrolizumab in patients with Gastric/GEJ cancer and is approved by the FDA.[24]

Therefore, in our study, we used both TPS and CPS to evaluate PD-L1 expression in PDAC. We found the PD-L1 positive expression with CPS was significantly higher compared to that with TPS (CPS vs. TPS: 71% vs. 32%; P = 0.0011) in surgical samples. In cytological samples, PD-L1 expression was higher with CPS than with TPS, but not significantly higher (CPS vs. TPS: 57% vs. 32%, P = 0.106). If CPS were used, more patients would qualify for clinical trial when surgical samples are not available. However, in cytological sampling, the disadvantage of CPS is the evaluation of inflammatory cell PD-L1 expression because many cytological samples lack significant inflammatory cell components in the cell block.

There are several ongoing clinical trials for the treatment of PDAC.[34-39] In most trials, eligibility requirements mandate a tissue biopsy for biomarker testing.[35-37] However, the biomarker tests proven in trials have rarely been validated by tissue obtained by cytological methods. In routine practice, cytological samples have usually provided the diagnosis in more than half the patients with pancreatic cancer.[40] Recently, the College of American Pathologists has created guidelines for the validation of IHC on cytological preparations such as cell blocks, direct smears, and other methods before incorporating the antibodies into clinical practice.[41] In our study, we used paired cytological and surgical samples with diagnosis of PDAC to evaluate the CPS and TPS for PD-L1 expression. We found that the percentage of PD-L1 expression with TPS was excellently concordant (93%) with surgical samples from the same patients. Only two cases failed to match. One negative cytological case paired with a positive surgical sample showed scant cellularity in cell block, barely reaching 100 tumor cells. If clinical trials use TPS to evaluate the positive PD-L1 expression, the cytological samples should be similar to the surgical samples. However, the percentage of PD-L1 expression with CPS was only moderately concordant (71%) with paired surgical samples from the same patients. The major issue is the scant cellularity of inflammatory cells in cytological samples. If clinical trials use CPS to evaluate PD-L1 expression, the combined cytological sample and core biopsy should be considered to evaluate PD-L1 expression. Utilizing core needle biopsy for endoscopic pancreatic sampling could increase the yield of the tissue samples.[42,43] resolve the issue of inflammatory cell pauci-cellularity, and improve the correlation between PD-L1 expression in surgical and cytological samples calculated with CPS.

PD-L1 expression in PDAC has been published in many reports.[17,18,20,21] When the PD-L1 antibody E1L3N was used, PD-L1 was expressed in 61.9% (26/42) of PDAC by fluorescent phosphor-integrated dot nanoparticles methods.17 Univariate and multivariate analyses indicated that the PD-L1 expression was an independent, predictive, and poorly-prognostic factor in patients with PDAC.[17,18,20,21] When the PD-L1 antibody SP142 was used, PD-L1 was expressed in 34% of 252 PDAC cases.[37] In meta-analysis of the PD-L1 expression in PDAC, the PD-L1 varied from 3% to 86%.[19,20] In our study, with TPS, the PD-L1 positive rate was 32%, below the average of 45.7%. When we used CPS, our result was 71% in surgical samples, much higher than the average. The reason could be that previous studies counted only the positive PD-L1 tumor cells and not the positive PD-L1 inflammatory cells.

Based on our experience evaluating PD-L1 expression in lung FNA samples, we arbitrarily selected 100 tumor cells as the cutoff for evaluating PD-L1 expressions in cell blocks and surgical samples.[45] The number of paired surgical and cytological samples was limited by the scant cellularity in pancreatic FNA cell blocks. For future studies, the quality and quantity of pancreatic tumor cells in cell block or core biopsy need to be improved, with dedicated passes directly into fixative solution.

The association of PD-L1 expression in PDAC with clinicopathological features was studied.[17,18,20,21]
Meanwhile, the association of PD-L1 expression with the PDAC differentiation remains controversial. In one meta-analysis study, the high-level of PD-L1 expression was significantly associated with poorly-differentiated PDAC. However, in the other meta-analysis study, the pooled data indicated no significant correlation between PD-L1 expression levels and lymph node metastasis, distant metastasis, and/or differentiation. In our study, using TPS, no PD-L1 expression was observed in well-differentiated PDAC in both surgical and cytological samples. Our study showed the significant difference of PD-L1 expression among well-, moderately-, and poorly-differentiated PDAC cases in cytological samples using both TPS and CPS. However, in surgical samples, there was a significant difference among three differentiated groups with TPS, but not with CPS. This was due to the dramatically increased PD-L1 expression in inflammatory cells in well-differentiated PDAC groups. Only the moderately-differentiated group was significantly different from well-differentiated group with Fisher’s exact test. PD-L1 expression seems to significantly associate with moderately-differentiated PDAC.

CONCLUSION

Our results showed that 93% of cytological PDAC cases were highly concordant with paired surgical samples using TPS to evaluate PD-L1 expression, which indicates that the cytological samples were useful for evaluation of PD-L1 expression in PDAC. With CPS, cytological samples were limited due to the scant inflammatory cells, with a moderate concordant rate of 71%. Extensive and widespread sampling of the pancreatic tumor and surrounding tissue, or combined FNA and core needle biopsies, may improve the detection of PD-L1 expression in cytological samples. In addition, PD-L1 expression was significantly associated with moderately-differentiated PDAC in both cytological and surgical samples except in surgical samples with CPS.

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COMPETING INTERESTS STATEMENT BY ALL AUTHORS

The authors made no disclosure.

ETHICS STATEMENT BY ALL AUTHORS

The authors made no disclosure.

LIST OF ABBREVIATIONS (In alphabetic order)

CPS – Combined positive score
IHC – Immunohistochemistry
PD-1 – Programmed cell death protein-1
PDAC – Pancreatic ductal adenocarcinoma
PD-L1 – Programmed death ligand 1
TMA – Tissue microarray
TPS – Tumor proportion score

EDITORIAL/PEER-REVIEW STATEMENT

To ensure the integrity and highest quality of CytoJournal publications, the review process of this manuscript was conducted under a double-blind model (authors are blinded for reviewers and vice versa) through automatic online system.

REFERENCES

1. Ferlay J, Soerjomataram I, Dikshit R, Eser S, Mathers C, Rebelo M, et al. Cancer incidence and mortality worldwide: Sources, methods and major patterns in GLOBOCAN 2012. Int J Cancer 2015;136:E359-86.
2. McGuigan A, Kelly P, Turkington RC, Jones C, Coleman HG, McCain RS. Pancreatic cancer: A review of clinical diagnosis, epidemiology, treatment and outcomes. World J Gastroenterol 2018;24:4846-61.
3. Petrelli F, Zaniboni A, Ghidini A, Ghidini M, Turati L, Pizzo C, et al. Timing of adjuvant chemotherapy and survival in colorectal, gastric, and pancreatic cancer. A systematic review and meta-analysis. Cancers (Basel) 2019;11:550.
4. Rangarajan K, Pucher PH, Armstrong T, Bateman A, Hamady Z. Systemic neoadjuvant chemotherapy in modern pancreatic cancer treatment: A systematic review and meta-analysis. Ann R Coll Surg Engl 2019;101:453-62.
5. Rebelatto TF, Falavigna M, Pozzari M, Spada F, Cella CA, Laffi A, et al. Should platinum-based chemotherapy be preferred for germline breast cancer genes (BRCA) 1 and 2-mutated pancreatic ductal adenocarcinoma (PDAC) patients? A systematic review and meta-analysis. Cancers (Basel) 2019;80:101895.
6. Dong H, Strome SE, Salomao DR, Tamura H, Hirano F, Flies DB, et al. Tumor-associated B7-H1 promotes T-cell apoptosis: A potential mechanism of immune evasion. Nat Med 2002;8:793-800.
7. Hua C, Boussemart L, Mateus C, Routier E, Boutros C, Cazenave H, et al. Association of vitiligo with tumor response in patients with metastatic melanoma treated with pembrolizumab. JAMA Dermatol 2016;152:45-51.
8. Ribas A, Hamid O, Daud A, Hodi FS, Wolchok JD, Kefford R, et al. Association of pembrolizumab with tumor response and survival among patients with advanced melanoma. JAMA 2016;315:1600-9.

9. Topalian SL, Szol M, McDermott DF, Kluger HM, Carvajal RD, Sharfman WH, et al. Survival, durable tumor remission, and long-term safety in patients with advanced melanoma receiving nivolumab. J Clin Oncol 2014;32:1020-30.

10. Garon EB. Current perspectives in immunotherapy for non-small cell lung cancer. Semin Oncin 2015;42 Suppl 2:S11-8.

11. Borghaei H, Paz-Ares L, Horn L, Spigel DR, Steins M, Ready NE, et al. Nivolumab versus docetaxel in advanced nonsquamous non-small-cell lung cancer. N Engl J Med 2015;373:1627-39.

12. Fehrenbacher L, Spira A, Ballinger M, Kowanetz M, Vansteenkiste J, Mazieres J, et al. Atezolizumab versus docetaxel for patients with previously treated non-small-cell lung cancer (POPLAR): A multicentre, open-label, phase 2 randomised controlled trial. Lancet 2016;387:1837-46.

13. Kudo M. Immune checkpoint inhibition in hepatocellular carcinoma: Basics and ongoing clinical trials. Oncology 2017;92 Suppl 1:50-62.

14. Kudo T, Hamamoto Y, Kato K, Ura T, Kojima T, Tsushima T, et al. Nivolumab treatment for oesophageal squamous-cell carcinoma: An open-label, multicentre, phase 2 trial. Lancet Oncol 2017;18:631-9.

15. Jotte RM, Socinski MA, Reck M, Papadimitrakopoulou V, West HJ, Mok T, et al. PS01.53: First-line atezolizumab plus chemotherapy in chemotheraphy-naive patients with advanced NSCLC: A phase III clinical program: Topic: Medical oncology. J Thorac Oncol 2016;11:S302-3.

16. Sidaway P. Urological cancer: Atezolizumab: An alternative to cisplatin? Nat Rev Clin Oncol 2017;14:139.

17. Tessier-Cloutier B, Kalloger SE, Al-Kandari M, Milne K, Gao D, Nelson BH, et al. Programmed cell death ligand 1 cut-point is associated with reduced disease specific survival in resected pancreatic ductal adenocarcinoma. BMC Cancer 2017;17:618.

18. Gao HL, Liu L, Qi ZH, Xu HW, Wang WQ, Wu CT, et al. The clinicopathological and prognostic significance of PD-L1 expression in pancreatic cancer: A meta-analysis. Hepatobiliary Pancreat Dis Int 2018;17:95-100.

19. Hu Y, Chen W, Yan Z, Ma J, Zhu F, Huo J. Prognostic value of PD-L1 expression in patients with pancreatic cancer: A PRISMA-compliant meta-analysis. Medicine (Baltimore) 2019;98:e14006.

20. Zhuan-Sun Y, Huang F, Feng M, Zhao X, Chen W, Zhu Z, et al. Prognostic value of PD-L1 overexpression for pancreatic cancer: Evidence from a meta-analysis. Onco Targets Ther 2017;10:5005-12.

21. Yamaki S, Yanagimoto H, Tsuta K, Ryota H, Kon M. PD-L1 expression in pancreatic ductal adenocarcinoma is a poor prognostic factor in patients with high CD8(+) tumor-infiltrating lymphocytes: Highly sensitive detection using phosphor-integrated dot staining. Int J Clin Oncol 2017;22:726-33.

22. Shidham VB, Layfield LJ. Cell-blocks and immunohistochemistry. Cytojournal 2021;18:2.

23. Herbst RS, de Marinis F, Jassem J, Lam S, Mocci S, Sandler A, et al. PS01.56: IMpower110: Phase III trial comparing 1L atezolizumab with chemotherapy in PD-L1-selected chemotherapy-naive NSCLC patients: Topic: Medical oncology. J Thorac Oncol 2016;11:S304-5.

24. Kulangara K, Zhang N, Corigliano E, Guerrero L, Waldroup S, Jaiswal D, et al. Clinical utility of the combined positive score for programmed death ligand-1 expression and the approval of pembrolizumab for treatment of gastric cancer. Arch Pathol Lab Med 2019;143:330-7.

25. 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.

26. Herbst RS, Baas P, Kim DW, Felip E, Perez-Gracia JL, Han JY, et al. Pembrolizumab versus docetaxel for previously treated, PD-L1-positive, advanced non-small-cell lung cancer (KEYNOTE-010): A randomised controlled trial. Lancet 2016;387:1540-50.

27. Garon EB, Rizvi NA, Hui R, Leighl N, Balmanoukian AS, Eder JP, et al. Pembrolizumab for the treatment of non-small-cell lung cancer. N Engl J Med 2015;372:2018-28.

28. Gettinger S, Herbst RS. B7-H1/ PD-1 blockade therapy in non-small cell lung cancer: Current status and future direction. Cancer J 2014;20:281-9.

29. Rizvi NA, Hellmann MD, Brahmer JR, Juergens RA, Borghaei H, Gettinger S, et al. Nivolumab in combination with platinum-based doublet chemotherapy for first-line treatment of advanced non-small-cell lung cancer. J Clin Oncol 2016;34:2969-79.

30. Dislich B, Stein A, Seiler CA, Kroll D, Berezowska S, Zlobec I, et al. Expression patterns of programmed death-ligand 1 in esophageal adenocarcinomas: Comparison between primary tumors and metastases. Cancer Immunol Immunother 2017;66:777-86.

31. Rebellato MC, Midha A, Mistry A, Sabalos C, Schechter N, Li X, et al. Development of a programmed cell death ligand-1 immunohistochemical assay validated for analysis of non-small cell lung cancer and head and neck squamous cell carcinoma. Diagn Pathol 2016;11:95.

32. Hirsch FR, McElhinny A, Stanforth D, Ranger-Moore J, Janssen M, Kulangara K, et al. PD-L1 immunohistochemistry assays for lung cancer: Results from phase 1 of the blueprint PD-L1 IHC assay comparison project. J Thorac Oncol 2017;12:208-22.

33. Buttner R, Gosney JR, Skov BG, Adam J, Motoi N, Bloom KJ, et al. Programmed death-ligand 1 immunohistochemistry testing: A review of analytical assays and clinical implementation in non-small-cell lung cancer. J Clin Oncol 2017;35:3867-76.

34. Pu N, Gao S, Yin H, Li JA, Wu W, Fang Y, et al. Cell-intrinsic PD-1 promotes proliferation in pancreatic cancer by targeting CYR61/CTGF via the hippo pathway. Cancer Lett 2019;460:42-53.

35. Feng M, Xiong G, Cao Z, Yang G, Zheng S, Song X, et al. PD-1/ PD-L1 and immunotherapy for pancreatic cancer. Cancer Lett 2017;407:57-65.

36. Burrack AL, Spartz EJ, Raynor JF, Wang I, Olson M, Stromnes IM. Combination PD-1 and PD-L1 blockade
promotes durable neoantigen-specific T cell-mediated immunity in pancreatic ductal adenocarcinoma. Cell Rep 2019;28:2140-55.e6.
37. Macherla S, Laks S, Naqash AR, Bulumulle A, Zervos E, Muzaffar M. Emerging role of immune checkpoint blockade in pancreatic cancer. Int J Mol Sci 2018;19:3505.
38. Tsukamoto M, Imai K, Ishimoto T, Komohara Y, Yamashita YI, Nakagawa S, et al. PD-L1 expression enhancement by infiltrating macrophage-derived tumor necrosis factor-alpha leads to poor pancreatic cancer prognosis. Cancer Sci 2019;110:310-20.
39. Ott PA, Bang YJ, Piha-Paul SA, Razak AR, Bennouna J, Soria JC, et al. T-cell-inflamed gene-expression profile, programmed death ligand 1 expression, and tumor mutational burden predict efficacy in patients treated with pembrolizumab across 20 cancers: KEYNOTE-028. J Clin Oncol 2019;37:318-27.
40. Cazacu IM, Chavez AA, Saftoiu A, Vilman P, Bhutani MS. A quarter century of EUS-FNA: Progress, milestones, and future directions. Endosc Ultrasound 2018;7:141-60.
41. Zhou F, Moreira AL. Lung carcinoma predictive biomarker testing by immunoperoxidase stains in cytology and small biopsy specimens: Advantages and limitations. Arch Pathol Lab Med 2016;140:1331-7.
42. Mohan BP, Shakhatreh M, Garg R, Asokkumar R, Jayaraj M, Ponnada S, et al. Comparison of franseen and fork-tip needles for EUS-guided fine-needle biopsy of solid mass lesions: A systematic review and meta-analysis. Endosc Ultrasound 2019;8:382-91.
43. Asokkumar R, Ka CY, Loh T, Ling L, San TG, Ying H, et al. Comparison of programmed death ligand 1 immunostaining for pancreatic ductal adenocarcinoma between paired cytological and surgical samples. Cytojournal 2021;18:28.