Research Article

PD-L1 testing in advanced stage lung cancer using cytology samples: Suitability and reporting issues. Comparison between two tertiary referral centers

Elshiekh Mohamed¹, Spinelli Manuela², Iles Sandra¹, Hopcroft Danielle¹, Trivedi Pritesh¹, Gupta Nandita¹, Bhagwat Priya¹ and Viola Patrizia*¹

¹Department of Cellular Pathology, NWLP Hosted by Imperial College Healthcare. NHS Trust, Charing Cross Hospital, London, UK
²Department of Pathology, Worcestershire Royal Hospital, Charles Hastings Way, Worcester, UK

Abstract

Background: Lung cancer is the most common cause of cancer-related death worldwide and unfortunately up to 80% of patients amongst newly diagnosed are inoperable therefore the cytological sample is often the only material available for diagnosis and assessment of molecular characteristics driving the treatment. Recently immunotherapy has shown promising results in tumors expressing Program Death Ligand 1 (PD-L1). The expression of PDL1 can routinely be detected by immunohistochemistry. However, the presence of several antibodies with different cut-off and the expression of this marker by normal immune cells are generating confusion in interpretation and the need for harmonization amongst pathologists.

Materials and methods: We assessed the suitability of 74 consecutive cell blocks from cytology samples for PDL1 testing and evaluate the concordance between two different antibodies (Ventana assay SP263 and Dako 223C pharmDx assay) and amongst different pathologists from two different tertiary referral center for thoracic pathology. The degree of agreement was measured by Fleiss K statistic (FKS) for categorical scores after dichotomization based on specified cutoffs. A review of discordant cases was also performed.

Results: Review of the slides stained with both antibodies showed substantial agreement within our department and moderate agreement with results from the other institution. Overall less than 10% of cases were deemed inadequate. Discordant cases showed a decreased amount of tumor cells, therefore, tumor heterogeneity could be responsible for the variation in the reading.

Conclusions: Our results show overall concordance between the two antibodies and the suitability of cytology material for PD-L1 testing.
Introduction

Lung cancer is the most common cause of cancer-related death worldwide with an incidence of 14% per year [1]. Non-small cell lung carcinoma accounts for more than 85% of cases leading to an overall 5 years survival rate around 18% [2,3]. About 80% of Non–Small Cell Lung Carcinomas (NSCLC) are diagnosed in an advanced stage and are therefore usually inoperable for locally advanced and/or metastatic disease. In these cases, surgical excision provides no or minimal benefit, thus diagnosis is often made with minimally invasive procedures such as transthoracic, TC–guided core biopsies, bronchoscopic biopsies, Endo–Bronchial Ultrasound–Guided Fine–Needle Aspiration (EBUS–FNA) or thoracentesis [4,5]. Therefore, in many patients, cytology is increasingly playing an essential role either diagnostic or prognostic role [6–10]. The premises seem to be good but, in contrast with Immunohistochemistry (IHC), which has already highly standardized and validated protocols, Immunocytochemistry (ICC) in clinical practice is still limited by lack of standardization and pre-analytical and analytical variability. Studies in the past few years have been compared ICC procedures versus the well-established IHC protocols, focusing on the selection of material (direct smears, monolayer preparations, cell blocks), use of different fixative agents, use of different pre-analytic platforms. [11,12] One of the larger comparison studies by Fowler in 2008 and Fisher in 2014 [7,10], confirmed that ICC on cytology can be as safe and reliable as IHC on histology, providing the use of appropriate techniques and strict adherence to quality protocols.

In the past few years a protein called Programmed Death receptor–1 (PD–1) and its ligands PD–L1 and PD–L2 have been identified as potential new markers, to guide the treatment of NSCLC.

PD–1 is a membrane immunoglobulin with a crucial role in regulating immune–mediated tissue damage. PD–1 acts as a membrane receptor with an immunomodulatory role in B and T cells, natural killer cells and macrophages, and as an essential regulatory factor in activated T cells [8]. Some studies demonstrated that the binding of PD–1 with specific ligands can block the T cell response to a tumor in different sites including the lung. Disruption of the PD–1/PD–L1 interaction results in activation of T cell immune response and this may play a crucial role in influencing tumor microenvironment and leading to downregulation and apoptosis of tumor-reactive T cells. Therefore, the block of PD–1/PD–L1 interaction leads to an increased response of T cells against tumor cells [3,13,14]. Several ligands to PD–1 have been identified and amongst them, PD–L1 is the most studied in lung cancer for its emerging role in tailoring the treatment of NSCLC. Monoclonal antibodies have been developed to target either PD–1 and PD–L1 and block the PD–1 receptor activity to allow T cells to attack tumor cells. Clinical trials with antagonists of PD–1 showed increased survival rates in patients with advanced, metastatic tumors including melanoma and NSCLC [8]. The study from Garaon et al is of central importance for pathologists as it proved that tumors which expression of PD–L1 in 50% or more of malignant cells have a significantly increased response to molecular therapy with Pembrolizumab [15], which has been also approved by US Food and Drugs Administration (FDA) for patients with at least 1% expression of PD–L1 [4].

For the last 30 years, the only therapeutic option for advanced/metastatic NSCLC was based on cytotoxic chemotherapy. Immunotherapy is used increasingly since studies proved the effectiveness of inhibitors that target PD–1 receptors such as Pembrolizumab, Atezolizumab, Nivolumab [13,15,16]. The expression of PD–L1 is evaluated with Immunohistochemical assay therefore it can be routinely detected. However, several clones are commercially available, each featuring different platforms and cut-offs; which can generate confusion and is urging the need for standardization, quality control, and harmonization amongst different laboratories and pathologists.

In this study, we tried to assess the suitability of cytology as material for PD–L1 testing and evaluated the concordance between two different antibodies and amongst pathologists in two different referring centers in reporting PD–L1 in cytology specimens.

Materials and methods

We retrieved 74 consecutive cell blocks from cytology samples and stained with VENTANA PD–L1 (SP263) Rabbit Monoclonal Primary Antibody, a rabbit monoclonal primary antibody produced against Programmed Death–Ligand 1 (PD–L1) also known as B7 homolog 1 (B7–H1) or CD274. B7 homolog 1, also known as PD–L1, is part of a superfamily of immune costimulatory molecules. These moecules play a crucial role in the so called “two–signal” model for lymphocyte activation. In this model, a lymphocyte requires two distinct signals in order for full activation to occur, the first provided by interaction of T Cell Receptor (TCR) on the lymphocyte with Major Histocompatibility Class (MHC) antigens on the Antigen–Presenting Cell (APC) and the second, costimulatory, is required to avoid an apoptotic or anergic response by the lymphocyte. The research to date suggests that the B7–H1/PD–L2/PD–1 interactions are involved in the negative regulation of some immune responses and may play an important role in the regulation of peripheral tolerance [17].

The SP263 antibody produces membranous and/or cytoplasmic staining, used in the detection of the PD–L1 protein in formalin–fixed, paraffin–embedded tissue. It was stained with BenchMark IHC/ISH Instruments. The BenchMark ULTRA system is a fully–automated immunohistochemistry and in situ hybridization slide staining system featuring 30 slides positions and and 35 reagent positions, which offers standardized IHC and ISH stainings [18].

NSCLC neoplastic cells labeled with the VENTANA PD–L1 (SP263) antibody were evaluated according to the producer instructions. The total percentage of tumor membrane signal intensities is visually estimated and used to generate the PD–L1 expression level. Tumor cell cytoplasmic staining is disregarded for determining PD–L1 expression. A PD–L1 IHC score is assigned by a trained pathologist based on his or her
The VENTANA PD–L1 (SP263) Assay guides immunotherapy decisions by identifying: NSCLC patients eligible for treatment with IMFINZI® (durvalumab), NSCLC patients eligible for treatment with KEYTRUDA® (pembrolizumab) and Non–squamous NSCLC patients most likely to benefit from OPDIVO® (nivolumab). All cases were reported by two trained cytopathologists with experience in thoracic pathology (PV and NG). Both negative and positive controls (tonsillar tissue) were included for each case.

All immunostained slides and matching hematoxylin and eosin–stained sections were reviewed, PD–L1 protein expression in NSCLC was determined by using Tumour Proportion Score (TPS), which is the percentage of viable tumor cells showing partial or complete membrane staining at any intensity.

Of these 74 cases, 45 were previously sent to another institution for PD–L1 testing using 22C3 PharmDx assay (Dako). PD–L1 IHC 22C3 pharmDx is a qualitative immunohistochemical assay using Monoclonal Mouse Anti–PD–L1, Clone 22C3 intended for use in the detection of PD–L1 protein in formalin–fixed, paraffin–embedded (FFPE) non–small cell lung cancer (NSCLC) [20].

In our study two trained pathologists (TM and MRJ) assessed PD–L1 protein expression by using TPS. The sample was considered to have weak positive PD–L1 expression if TPS ≥ 1% and strong PD–L1 expression if TPS ≥ 50%. PD–L1 is considered negative from 0 to <1%. Weak positive from 1 to 49%, Strong positive >50% to 100%. Currently, PD–L1 IHC 22C3 pharmDx is indicated as an aid in identifying NSCLC patients for treatment with KEYTRUDA® (pembrolizumab) [15].

The test was performed with PD–L1 IHC 22C3 pharmDx TM assay (Dako), a qualitative immunohistochemical assay using Monoclonal Mouse Anti–PD–L1, Clone 22C3 intended for use in the detection of PD–L1 protein in formalin–fixed, paraffin–embedded (FFPE) non–small cell lung cancer (NSCLC), using EnVision FLEX visualization system on Autostainer Link 48.

Three cytopathologists from our institution (PB, NG, and PV) blindly reviewed all cases for both antibodies. Of notice, the two pulmonary pathologists underwent on–line training for the scoring of PD–L1 IHC for both antibodies on Tumour Cells (TCs) and Inflammatory Cells (ICs) as part of their routine reporting activity. The results were compared amongst readers and between the two institutions. The degree of agreement was measured by Fleiss’ statistic (FKS) for categorical scores after dichotomization based on specified cutoffs (<1%, 1–50%, and >50% partial or complete membrane positivity in tumor cells).

FKS scores of 0.81 or higher were considered near perfect, scores of 0.61 to 0.80 were considered substantial agreement, scores of 0.41 to 0.60 were considered moderate agreement, scores of 0.21 to 0.40 were considered fair agreement, scores of 0.01 to 0.20 were considered slight agreement whilst less than 0 was considered poor agreement [21].

Results

The majority of our samples were obtained through endobronchial ultrasound fine–needle aspiration (EBUS–FNA) of mediastinal lymph nodes (51.10%), pleural fluid aspiration (20%), and EBUS–FNA of supraclavicular/neck lymph nodes (17.80%). Bronchial washing/brushing and bronchoalveolar lavage accounted for the remaining 11.1%.

55.6% of patients were males, the median age was 64 years old (ranging from 38 to 83) and regarding the histological diagnosis 68.90% were Adenocarcinomas (ADCs), 17.80% were Non–Small Cell Carcinomas Non–Otherwise Specified (NSCC–NOS), 11.10% were Squamous Cell Carcinomas (SQCCs) and 2.20% were of cases showed other histology including neuroendocrine tumors.

Of those cases sent for testing 8.10% of total cases were deemed insufficient as having overall less than 100 neoplastic cells. 82.2% of cases stained with Ventana resulted in a 100% agreement at all different cut–offs (<1%, 1–50%, and >50% positive tumor cells). The intradepartmental agreement in classification measured by Fleiss’ kappa was 0.73, indicating substantial agreement, whereas comparison with the other institution showed moderate agreement (K=0.44). The agreement was calculated using the formulas as reported by McHugh [21]. The final value of K was 0.44 which, according to the abovementioned article should be interpreted as moderate agreement (range: 0.41 – 0.60).

All showed a variable number of macrophages staining positive at different intensities (Figure 1).

The discordant cases among the two institutions were reviewed (Figure 2) and clinical follow up recorded (Table 1). Review of H&E and PD–L1 stained slides showed that 4 out of 10 (40%) discordant cases, when restained with Ventana SP263, were just about meeting the adequacy criteria bearing approximately 100 cells. Overall only one case with a sufficient amount of material was reported negative at one institution.
Despite the recent approval of the PD-L1 IHC assays which allow Lymphoma Kinase (ALK), ROS-1 and more recently PD-L1 [22] such as Epidermal Growth Factor Receptor (EGFR), Anaplastic, the presence of actionable mutations in specific samples are routinely used for molecular testing including these patients are often already non-operable, various cytology response to PD-1 and PD-L1 inhibitors in advanced NSCLC. As of lung cancer [3].

**Discussion**

The introduction of IHC for PD-L1 assessment put the pathologist in a crucial role in the diagnosis and management of lung cancer [3].

Quantification of PD-L1 is essential to assess patient eligibility for immunotherapy and to determine their possible response to PD-1 and PD-L1 inhibitors in advanced NSCLC. As these patients are often already non-operable, various cytology samples are routinely used for molecular testing including the presence of actionable mutations in specific biomarkers such as Epidermal Growth Factor Receptor (EGFR), Anaplastic Lymphoma Kinase (ALK), ROS-1 and more recently PD-L1 [22]. Despite the recent approval of the PD-L1 IHC assays which allow the test to be performed routinely, there are some issues. These include pre-analytic variability, different antibodies associated with different equipment not always present in all laboratories, different clones approved for different drugs and with different cut-offs for interpretation of positive results, the possibility of false positivity because of staining of immune cells, non-specific cytoplasmic staining in tumor cells and intratumoral heterogeneity which can affect low cellular samples. Generally speaking, immunohistochemistry is considered fast and reliable, providing quality control and standardization are ensured. The variability of samples and pre-analytic conditions must, however, be taken into account as many types of samples, including fluids, brushings, FNAs, direct smears, and cell blocks can be used in the different laboratory settings, as well as a different fixative and processing methods. Protocols for immunohistochemistry on cytological material are extremely variable and therefore standardization is essential for quality control, reproducibility, and consistency [6]. Many studies proved that many different cytology specimens from NSCLC are suitable for the determination of PD-L1 expression when compared with the results obtained with biopsies and surgical samples. For example, it has been investigated the rate of success in detection of PD-L1 on bronchoscopy samples, the concordance of PD-L1 expression in EBUS-TBNAs compared with the corresponding excision samples, the performance for FNAs and thoracacentesis material compared with histology samples, the correlation between cytology and histology in PD-L1 expression and concordance of interobserver agreement in the quantification of PD-L1 [3-5,22-24]. The results were promising: Skov et al studied 86 paired cyto-histological cases reporting an overall agreement between 85% and 95% [5]. Russell–Goldman et al. compared 56 cytology specimens with matching surgical samples and obtained a good concordance for TCs [3]. These data highlight the suitability for the use of cytological material for PD-L1 testing in agreement with our results. However discrepancy has also been reported when comparing different types of samples: Noll et al have reported a discrepancy in approximately 21% of cell–blocks, Skov et al. reported a disagreement of 15–5%, Kitazono et al. reported a discordance when comparing histology and cytology in approximately 10% of cases [2,5,23]. The main explanation given by all authors regarding the disagreement seen in those cases was tumor heterogeneity within histology samples. Therefore this stresses again the need for a reasonable amount of material when limited specimens (biopsy or cytology) are assessed for treatment purposes. We observed similar results when reviewing our cases since all discordant cases, although still adequate for assessment, showed a considerably less amount of tumor cells compared to the original slide. Hence we agree that tumor heterogeneity can explain the different outcomes. Despite PD-L1 testing in histology material that has long been approved in many tumors to select patients eligible for treatment with Pembrolizumab and Atezolizumab [16], cytology specimens have not been validated for PD-L1 testing yet [2,5,25]. The use of PD-L1 as a biomarker may be challenging because different drugs require different tests and of course not all laboratory have all different antibody clones and platforms. Currently, there are different assays available on the

Table 1: Follow-up of discordant cases among the two institutions.

| Case # | Dako 223 pharma | Adequacy >100 cells | Ventana sp263 | Adequacy >100 cells | Treatment | Outcome |
|--------|-----------------|---------------------|--------------|---------------------|-----------|---------|
| 1      | Strong positive | Yes                  | Weak positive| Yes                  | Pembrolizumab | Alive   |
| 2      | Weak positive   | Yes                  | Weak positive| Yes                  | Osimertinib | Deceased|
| 3      | Strong positive | Yes                  | Negative     | Yes                  | Pembrolizumab | Deceased|
| 4      | Strong positive | Yes                  | Weak positive| Yes                  | Pembrolizumab | Alive   |
| 5      | Negative        | Yes                  | Strong positive| Yes                | Erlotinib   | Alive   |
| 6      | Weak positive   | Yes                  | Negative     | Yes                  | No treatment| Deceased|
| 7      | Strong positive | Yes                  | Weak positive| Yes                  | Pembrolizumab | Alive   |
| 8      | Weak positive   | Yes                  | Negative     | Yes                  | Pembrolizumab | Deceased|
| 9      | Strong positive | Yes                  | Weak positive| Yes                  | Pembrolizumab | Deceased|
| 10     | Strong positive | Yes                  | Weak positive| Yes                  | Pembrolizum  | Alive   |

and strong positive at the other, however, the patient was also bearing EGFR mutation and was treated with Erlotinib.
market, including the 22C3PharmDx assay for Pembrolizumab, the 28–8PharmDx assay, and SP263 Ventana for Nivolumab, the SPI2 Ventana assay in combination with Atezolizumab. As each PD–L1 assay is developed independently in different factories, each of them has different staining characteristics and this could hamper their interchangeability from a practical point of view. These issues prompted several study groups to assess the comparability of the different assays and their interchangeability, especially because not all laboratories can afford to buy new machines for each different assay [22]. All the studies published showed similar results in terms of comparability and interchangeability amongst the assays examined, with SP263, 22C3, and 28–8 sharing comparable uses and staining properties [5,26–30]. Interestingly, Tourous et al recently obtained similar results on a large series of cell blocks, the same cytology material examined in our study [31].

Moreover, there would be interpretation issues as different antibodies have different cut-offs causing interobserver variability; following the experience and skills of the reporting pathologist. We showed an intradepartmental substantial agreement that corroborates that interpretation of PD-L1 should be reserved for expert pathologists with a special interest in cytology/thoracic malignancy or well trained in the interpretation of PD-L1. The main issue is the distinction between positive malignant cells and pulmonary macrophages which can stain for this marker. Given the absence of architecture in cytosin or cell blocks, the correct identification of stained macrophages becomes a crucial pitfall. It also becomes of relevant importance when the value is borderline for a certain cut-off which is driving therapeutic decision.

Our results confirm those previously published showing good suitability of cytology material for this test and an overall good intradepartmental concordance of PD–L1 evaluation when a specialist or trained pathologists report PD–L1 at different cut-offs. Of note in our study, two pathologists were fully trained to report PD–L1 whilst the third pathologist is an expert cytopathologist who did not take any training. In our study, we showed we also compared the staining of two different antibodies on the same cytological material and the comparison showed only moderate agreement. This lower degree of agreement can be easily explained with loss of cellularity in the samples and can be therefore still be considered acceptable.

Our work confirmed the suitability of cytology material for PD–L1 testing, with results that are in line with previously published studies. Our results also demonstrated overall concordance between the two antibodies used (Ventana clone SP263 and Dako clone 22C3). We also highlight the importance of the cellularity present in the sample as well as the need for fully trained pathologists or at least highly specialized cytopathologists in reporting these cases as staining of non-neoplastic cells can affect the final score.

References

1. de Groot PM, Wu CC, Carter BW, Munden RF (2018) The epidemiology of lung cancer. Transl Lung Cancer Res 7: 220-233. Link: https://bit.ly/2K1ipPA

2. Noll B, Wang WL, Gong Y, Zhao J, Kalhor N, et al. (2018) Programmed death-ligand 1 testing in non-small cell lung carcinoma cytology cell block and aspirate smear preparations. Cancer Cytopathol 126: 342-352. Link: https://bit.ly/36atv1

3. Russell-Goldman E, Kravets S, Dahlberg SE, Sholl LM, Vivero M (2018) Cytologic-histologic correlation of programmed death-ligand 1 immunohistochemistry in lung carcinomas. Cancer Cytopathol 126: 253-263. Link: https://bit.ly/36ae6eC

4. Heymann JJ, Bulman WA, Swinarski D, Pagan CA, Crapanzano JP, et al. (2017) (PD–L1 expression in non-small cell lung carcinoma: Comparison among cytology, small biopsy, and surgical resection specimens. Cancer Cytopathol 125: 896–907. Link: https://bit.ly/39eV5PH

5. Skov BG, Skov T (2017) 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 [Internet]. 25: 453-459. Link: https://bit.ly/3a0oOSz

6. Schmitt F, Cochand-Proillet B, Toetsch M, Davidson B, Bondi A, et al. (2011) Immunocytochemistry in Europe: results of the European Federation of Cytology Societies (EFCS) inquiry. Cytopathology 22: 238-242. Link: https://bit.ly/3psDkZz

7. Fowler LJ, Lachar WA (2008) Application of immunohistochemistry to cytology. Archives of Pathology and Laboratory Medicine.

8. Sholl LM, Asner DL, Allen TC, Beasley MB, Borczuk AC, et al. (2016) Programmed Death Ligand-1 Immunohistochemistry (textemdash) A New Challenge for Pathologists: A Perspective From Members of the Pulmonary Pathology Society. Arch Pathol Lab Med 140: 341-344. Link: https://bit.ly/3aZbEoc

9. Schmitt FC (2007) Cells carry the clue for targeted treatment: a new horizon for cytopathology. Cytopathology 18: 275-277. Link: https://bit.ly/2kLDuI

10. Fischer AH, Schwartz MR, Moriarty AT, Wilbur DC, Souers R, et al. (2014) Immunohistochemistry Practices of Cytopathology Laboratories: A Survey of Participants in the College of American Pathologists Nongynecologic Cytopathology Education Program. Arch Pathol Lab Med 138: 1167-1175. Link: https://bit.ly/39gnUXM

11. Kirbis IS, Maxwell P, Fležar MS, Miller K, Ibrahim M (2011) External quality control for immunocytochemistry on cytology samples: A review of UK NEQAS ICC (cytology module) results. Cytopathology 22: 230-237. Link: https://bit.ly/2NqZ2Rv

12. Skoog L, Tani E (2011) Immunocytochemistry: an indispensable technique in routine cytology. Cytopathology 22: 215-229. Link: https://bit.ly/3oeKy2i

13. Intlekofer AM, Thompson CB (2013) At the Bench: Preclinical rationale for (CTLA)-4 and (PD)-1 blockade as cancer immunotherapy. J Leukoc Biol 94: 25-39. Link: https://bit.ly/3pbrL9c

14. Latchman Y, Wood CR, Chernova T, Chaudhary D, Borde M, et al. (2001) (PD–L2 is a second ligand for (PD–1 and inhibits T cell activation. Nat Immunol [Internet] 2: 261-268. Link: https://bit.ly/2LYKOFi

15. Garon EB, Rizvi NA, Hui R, Leighl N, Balmanoukian AS, et al. (2015) Pembrolizumab for the treatment of non-small-cell lung cancer. N Engl J Med 372: 2018-2028. Link: https://bit.ly/3IKWyXS

16. Weinstock C, Khozin S, Suzman D, Zang L, Tang S, et al. (2017) U.S. Food and Drug Administration Approval Summary: Atezolizumab for Metastatic Non–Small-Cell Lung Cancer. Clin Cancer Res 23: 4534-4539. Link: https://bit.ly/3iKWyXS

17. The B7 & CD28 superfamilies R&D Systems 2003 catalog. Link: https://bit.ly/3qM33g2

18. “Diagnostics.roche.com/global/en/products/instruments/benchmark-ultra.html#productinfo”
19. “Ventana PD-L1 (SP263) Assay Staining of Non-Small Cell Lung Cancer Interpretation Guide”. Link: https://bit.ly/3sTvMS0

20. PD-L1 IHC 22C3 PharmDxSK006; Link: https://bit.ly/2KKnFpz

21. McHugh ML (2012) Interrater reliability: The kappa statistic. Biochem Medica.

22. Stoy SP, Rosen L, Mueller J, Murgu S (2017) Programmed death-ligand 1 testing of lung cancer cytology specimens obtained with bronchoscopy. Cancer Cytopathol 126: 122-128. Link: https://bit.ly/369xogR

23. Kitazono S, Fujiwara Y, Tsuta K, Utsumi H, Kanda S, et al. (2015) Reliability of Small Biopsy Samples Compared With Resected Specimens for the Determination of Programmed Death-Ligand 1 Expression in Non-Small-Cell Lung Cancer. Clin Lung Cancer 16: 385-390. Link: https://bit.ly/368FF4x

24. Sakakibara R, Inamura K, Tambo Y, Ninomiya H, Kitazono S, et al. (2017) (EBUS)-(TBNA) as a Promising Method for the Evaluation of Tumor PD-L1 Expression in Lung Cancer. Clin Lung Cancer 18: 527-534.e1. Link: https://bit.ly/2M26mkn

25. Sakakibara R, Inamura K, Tambo Y, Ninomiya H, Kitazono S, et al. (2017) EBUS-TBNA as a Promising Method for the Evaluation of Tumor PD-L1 Expression in Lung Cancer. Clin Lung Cancer 18: 527-534.e1. Link: https://bit.ly/3ogRcEW

26. Tsoa MS, Kerr KM, Kockx M, Beasley M-B, Borczuk AC, et al. (2018) (PD)-L1 Immunohistochemistry Comparability Study in Real-Life Clinical Samples

27. Marchetti A, Barberis M, Franco R, Luca G De, Pace MV, et al. (2017) Multicenter Comparison of 22C3 (PharmDx) (Agilent) and (SP)263 (Ventana) Assays to Test (PD)-L1 Expression for (NSCLC) Patients to Be Treated with Immune Checkpoint Inhibitors. J Thorac Oncol 12: 1654-1663. Link: https://bit.ly/3ocNA6T

28. Sughayer MA, Alnaimy F, Alshugayer AM, Qamhia N (2019) Comparison of 22C3 (PharmDx) and (SP)263 Assays to Test (PD)-L1 Expression in (NSCLC). Appl Immunohistochem Mol Morphol 27: 663-666. Link: https://bit.ly/2Yc4Zi0

29. Villaruz LC, Hunter KA, Kurland BF, Abberbock S, Herbst C, et al. (2018) Comparison of (PD)-L1 immunohistochemistry assays and response to (PD)-1/L1 inhibitors in advanced non-small-cell lung cancer in clinical practice. Histopathology 74: 269-275. Link: https://bit.ly/2LZVRhD

30. Takigawa N, Ochi N, Yamane H (2018) Histology versus cytology: (PD)-L1 testing in non-small cell lung cancer. Transl Lung Cancer Res [Internet]. 7: S225-S227. Link: https://bit.ly/3pf4xPH

31. Torous VF, Rangachari D, Gallant BP, Shea M, Costa DB, et al. (2018) (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 7: 133-1341. Link: https://bit.ly/3o9yc1C

Discover a bigger impact and Visibility of your article with Peertechz Publications

**Highlights**
- Signatory publisher of ORCID
- Signatory Publisher of DORA (San Francisco Declaration on Research Assessment)
- Articles archived in world’s renowned service providers such as Portico, CNKI, AGRIS, TDNet, Base (Bielefeld University Library), CrossRef, Scilt, J-Gate etc.
- Journals indexed in ICMJE, SHERPA/ROMEO, Google Scholar etc.
- OA-PHM (Open Archives Initiative Protocol for Metadata Harvesting)
- Dedicated Editorial Board for every journal
- Accurate and rapid peer-review process
- Increased citations of published articles through promotions
- Reduced timeline for article publication

Submit your articles and experience a new surge in publication services (https://www.peertechz.com/submission).

Peertechz Journals wishes everlasting success in every endeavours.

Copyright: © 2021 Elshiekh M, et al. This is an open-access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.

Citation: Elshiekh M, Spinelli M, Isles, S, Hopcroft D, Trivedi P, Gupta N, Bhagwat P, Viola P (2021) PD-L1 testing in advanced stage lung cancer using cytology samples: Suitability and reporting issues. Comparison between two tertiary referral centers. Ann Cytol Pathol 6(1): 001-006.
DOI: https://dx.doi.org/10.17352/acp.000022