Pathology & Oncology Research (2020) 26:2363–2370
https://doi.org/10.1007/s12253-020-00832-0

ORIGINAL ARTICLE

PD-L1 Expression in Small Cell and Large Cell Neuroendocrine Carcinomas of Lung: an Immunohistochemical Study with Review of Literature

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Received: 5 February 2020 / Revised: 10 April 2020 / Accepted: 26 May 2020 / Published online: 6 June 2020

Abstract

High-grade neuroendocrine tumors (HGNET) have distinctive tumor biology/behaviour. Newer modalities of treatment (immunotherapy) for them have been included in recent NCCN guidelines. Detection of programmed death receptor-ligand 1 (PD-L1) expression by immunohistochemistry have made easy identification of patients eligible for immunotherapy. We aimed to ascertain expression of PD-L1 on small cell and large cell neuroendocrine carcinomas of lung and review existing literature. Eighty-five cases of HGNET lung (primary/metastatic), were retrieved and reviewed. Immunostaining for PD-L1 using clone SP263 was done. Any amount/intensity of membranous staining of >= 1% tumor cells was cut-off for positivity. Previously published studies using *Google* and/*Pubmed* search engines were reviewed. Of 85 cases, 70 were small-cell lung cancer (SCLC), 11 large-cell neuroendocrine carcinoma (LCNEC) and 4 combined SCLC. Median age was 46.5 years with male preponderance. No PD-L1 expression was seen in 91.6% cases. The 7 positive cases were 4 LCNEC, 2 SCLC and 1 combined SCLC. The percentage positivity varied from 1–100%; lower percentage positivity was seen in SCLC. PD-L1 expression on immune cells was seen in 31.3% cases. Sixteen studies evaluating 1992 NET were found; E1L3N PD-L1 clone was commonly used clone. PD-L1 positivity was associated with better prognosis in most studies. There are only a few studies available in literature related to PDL1 expression in high grade neuroendocrine carcinomas of lung. In general, PD-L1 positivity is highly variable and seen in lower percentage of these tumors. With the recent approval of immunotherapy, biomarkers other than PD-L1 should also be investigated in these tumors.

Keywords Pulmonary high grade neuroendocrine tumors · PD-L1 · SP263 · Immunotherapy · Small cell lung carcinoma

Introduction

The 2015 World Health Organization (WHO) classification of the tumors of lung, pleura and thymus has classified the neuroendocrine tumors (NET) of the lung into preinvasive lesions, the carcinoids (typical and atypical), large cell neuroendocrine carcinoma (LCNEC) and the small cell lung carcinoma (SCLC) including the combined LCNEC and SCLC respectively [1]. Even though they are grouped together, the carcinoids differ significantly from the high grade LCNEC

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and SCLC in their biology and the overall behaviour of the tumor. Therefore, while the primary treatment for the carcinoids remain surgical resection, most of the high-grade lesions especially SCLC are inoperable at the time of diagnosis and require chemotherapy instead. While some cases respond to primary chemotherapy, most relapse within the first year of treatment, hence newer modalities including immunotherapy are being explored for such patients [2]. The recent NCCN guidelines have also incorporated immunotherapy in extensive-disease SCLC (SCLC-ED) and SCLC where recurrence was within six months of therapy [3].

The immune check-point inhibitors targeting the programmed death-1 (PD-1) receptor on immune cells and its ligand (PD-L1) on tumor cells have been the most sought-after drugs for immunotherapy in recent times. Their easy identification using immunohistochemistry (IHC) have made them more lucrative and many past and ongoing clinical trials have validated their safe and effective use in many malignancies [4]. In view of this, the aim of our study was to ascertain the expression of PD-L1 on high grade NET of lung. We also conducted a review of literature and compared our results with previously published studies on PD-L1 expression in NET of lung.

Materials and Methods

The study was of retrospective design and approved by the institute ethics committee [IEC-582/02.11.2018]. Eighty five cases of high grade neuroendocrine tumors of the lung (primary or metastatic), from June 2010 onwards, with available blocks, were retrieved from the archives of the department of pathology at the All India Institute of Medical Sciences, New Delhi. The hematoxylin-eosin (H&E) stained slides were reviewed to confirm the diagnosis. Sections were selected for immunohistochemistry.

Immunohistochemistry

Sections from selected blocks were subjected to IHC for PD-L1 (clone SP263, VENTANA Medical Systems, Inc) which was performed on VENTANA benchmark XT platform optimised with the OptiView DAB IHC Detection kit (VENTANA Medical Systems, Inc). Sections from term placenta were included as positive controls for endogenous PD-L1 expression (partial or complete membrane staining at any intensity of trophoblast lineage cells). The guidelines for interpretation of PD-L1 IHC has been adopted from the interpretation guide by VENTANA Inc. for PD-L1 (SP263) Assay Staining of Non-Small Cell Lung Cancer [5]. Any amount of membranous staining of tumor cells by SP263 at any intensity greater than background staining is considered positive. The membrane staining can be discontinuous, circumferential or basolateral. Any cytoplasmic staining was disregarded while ascertaining positivity [5, 6]. At least 100 viable tumor cells were assessed and the percentage of cells stained was determined (Tumor proportion score). We used 1% positive tumor cells as the cut-off for PD-L1 positivity [7]. The immune cells (IC) (lymphocytes and intra-alveolar macrophages) also demonstrate linear membranous, diffuse cytoplasmic, and/or punctate staining, but are not included in the scoring criteria using the SP263 clone. However, we tried to assess the percentage of tumors expressing PD-L1 in the IC also.

Results

Of a total of 85 cases (78 biopsies, 07 resections), the histological distribution was as follows: 70 SCLC, 11 LCNEC and 4 combined SCLC. The age of the patients varied from 28 years to 75 years (median and mean age: 46.5 years). There was a male preponderance (Male = 72; Female = 13), with Male:Female ratio of 6:1. Of the total 85 cases, 2 cases of SCLC had to be excluded due to insufficient tumor in the block noticed in IHC stained slides.

PD-L1 expression on tumor cells

91.6% (76/83) cases were negative for PD-L1 staining in the tumor cells. The seven (8.4%) positive cases (3 resections and 4 small biopsies) showed membranous staining in >= 1% tumor cells which include 36.3% (4/11) LCNEC (Fig. 1A,B), 25% (1/4) combined SCLC (Fig 1C) and 2.9% (2/68) SCLC (Fig. 1D)]. The tumor proportion score for PD-L1 in these seven cases has been depicted in Table 1.

PD-L1 Expression on IC

IC were present in 60.2% (50/83) of our cases. Histologically, the cases with IC were distributed as follows: SCLC 72% (36/50), LCNEC 20% (10/50) and combined SCLC 8% (4/50). 52% (26/50) were PD-L1 positive. Overall, 31.3% (26/83) of the cases had PD-L1 expressing IC (Fig. 1E, F). Histomorphologically, 23.5% (16/68) SCLC, 45.4% (5/11) LCNEC and 75% (3/4) combined SCLC had PD-L1 positive IC.

Review of Literature

After thorough internet search of the *Pubmed* and *Google* search engines, we could find only 16 studies evaluating the immunohistochemical expression of PD-L1 on NET of the lung. A summary of the review has been shown in Table 2. A total of 1992 pulmonary NET have been studied which include

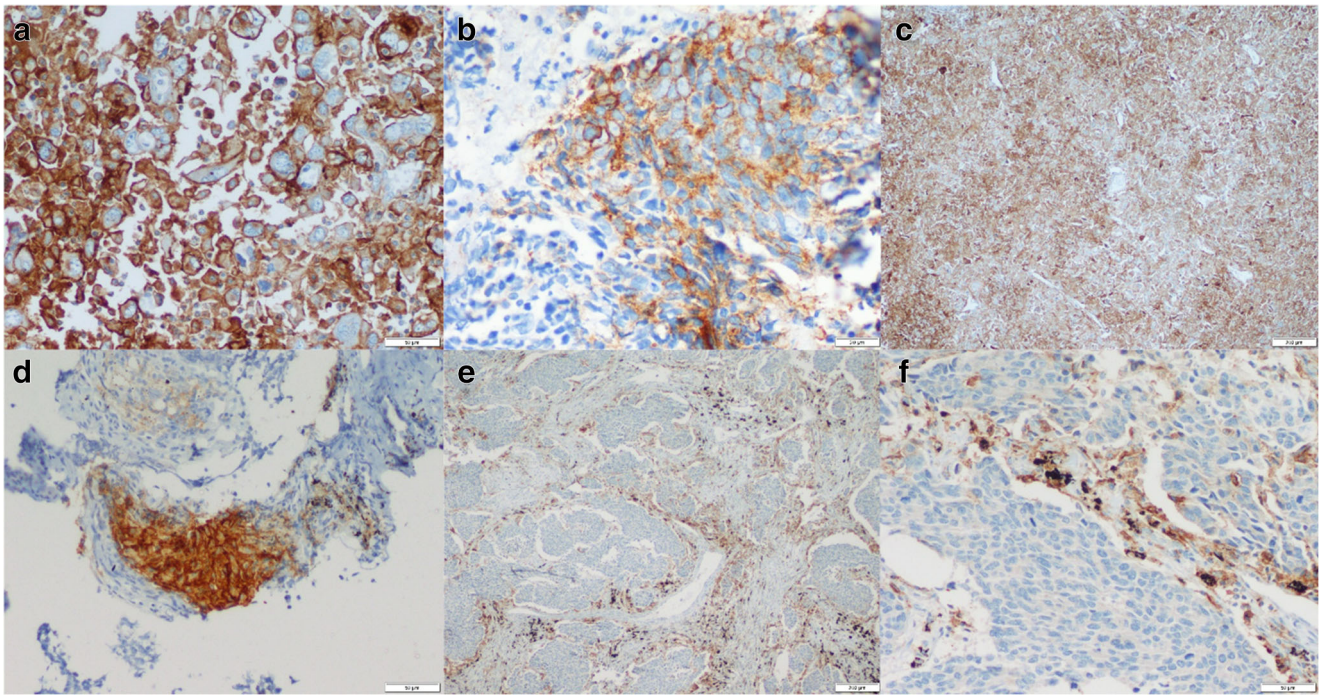

Fig. 1 PD-L1 expression in neuroendocrine carcinomas of the lung. (a, b) Diffuse membranous staining of tumors cells in two cases of Large Cell Neuroendocrine Carcinoma (LCNEC). (c) combined small cell carcinoma and (d) small cell carcinoma. (e) Immune cell staining of PD-L1 in a case of LCNEC which included lymphocytes, plasma cells as well as (F) alveolar macrophages. (Original magnification: a x 200, b x 400, c x 100, d x 40, e x 40, f x 200)

168 carcinoids (TC and AC), 1338 SCLC and 486 LCNEC. The PD-L1 clones used as well as the scoring criteria is varied across the studies. The E1L3N is the most commonly used clone [8–13]. Most of the studies have scored PD-L1 in both tumor cells as well as immune cells (IC) except in few where only tumor cells have been scored especially those using the E1L3N clone [8, 10–14]. Few studies have used the US Food and Drug Administration (FDA) approved clones for NSCLC [15–18]. Most of the studies have shown good survival characteristics when PD-L1 was positive in the IC as compared to tumor cells [16, 19–21]. Poor prognosis was associated with tumor cell PD-L1 positivity in some [21, 22].

Table 1 Tumor proportion score in cases with PD-L1 positivity

PD-L1 positive case	Histologic subtype	Tumor proportion score
Case 1	SCLC	90%
Case 2	SCLC	2%
Case 3	Combined SCLC	84%
Case 4	LCNEC	50%
Case 5	LCNEC	57%
Case 6	LCNEC	72%
Case 7	LCNEC	100%

Discussion

NET of the lung form approximately 25% of primary lung neoplasms and SCLC is the commonest among them [25]. The low grade NET (TC and AC) are primarily resected and if there is loco-regional advanced disease, then they are administered somatostatin analogues whereas, high grade tumors are given chemotherapy upfront [25]. Due to the aggressive nature of high-grade NET and their propensity to recur despite therapy, newer modalities of treatment are being explored of which immunotherapy forms an important part and PD-1/PD-L1 inhibitors are currently the front runners of this group of drugs.

The tumor microenvironment plays an important role in modifying the tumor milieu and thereby impacting the anti-tumor immunity. The various components of this microenvironment are the IC, stromal cells, fibroblasts and the endothelial cells. Among the IC, the CD4 helper T-cells, CD8 + T-cells, NK cells, dendritic cells and the M1 macrophages are the anti-tumor inflammatory cells, whereas, the T regulatory cells (Tregs) marked by FOXP3, CD4 helper T-cells, M2 macrophages marked by CD163 + and the myeloid derived suppressor cells (MDSC) promote tumor growth [26, 27]. The anti-tumor T-cells, mainly the CD8 + cells, play a crucial role in maintaining adaptive immunity. Exhaustion of these T-cells by repetitive stimulation by tumor antigens leads to

Table 2 Review of literature of PD-L1 expression in Neuroendocrine tumors of the lung

Sr no	Author/year	No. of cases	Histology	Clone	Scoring criteria	Cut off	Percent positive	Survival correlation
1	Schultheis et al/2014 [8]	94 (61 pulmonary, 33 extrapulmonary)	SCLC	5H1 and E1L3N	Allred score	Not mentioned	TC 0% IC 17/92 (18.5%)	Not done
2	Ishii et al/2015 [23]	102	SCLC	Abcam	Not defined	> 5%	73/102 (71.6%)	Better prognosis if PD-L1 +
3	Fan et al/2016 [19]	80	Carcinoids 22 (27.5%) SCLC 48 (60%) LCNEC 10 (12.5%)	Abcam	SID (staining intensity distribution) (intensity X proportion)	>=3	T: 58.8% (47/80) IC: PD-1 stain	Good prognosis with both
4	George et al/2016 [9]	72 + 138	SCLC	E1L3N	Intensity x percent positive	> 3	4/210	Not done
5	Yu et al/2016 [18]	194	SCLC	SP142 and 28 − 8	TC tumor proportion score IC 0–3 (none, focal, moderate, severe)	> 1%	TC 40/194 (20.6%) IC 84/194 (43.3%)	No correlation with SCLC-LD Better in SCLC-ED
6	Takada et al/2016 [10]	40	SCLC	E1L3N, 28 − 8, SP142	Allred score 1% cut-off 5% cut-off	3 cutoffs used: Median of Allred score > 1% > 5%	Allred TCs 22.5–35% IC 42.5–50% > 1% TCs 20-32.5% ICs 40-52.5% > 5% TCs 15% ICs 37.5–40%	Not done
7	Kim et al/2017 [20]	192	SCLC 120 (62.5%) LCNEC 72 (37.5%)	B7-H1/PD-L1 antibody (R&D Systems)	Proportion of cells and intensity of stain assessed separately	> 1%	TC 29/192 (15.1%) IC 60/192 (31.3%)	Good prognosis with IC +
8	Miao et al/2017 [24]	83	SCLC	SP66, (Springbio, USA)	Intensity x percent positive	> 5%	TC 43/83 (51.8%)	Better prognosis if PD-L1 +
9	Inamura et al/2017 [11]	115	SCLC 74 LCNEC 41	E1L3N	Tumor cell percentage with membranous staining	TC > = 5%	TC 21% (25/115)	Better prognosis if PD-L1 +
10	Tsuruoka et al/2017 [12]	227	TC 20.3% (46) AC 2.6% (6) SCLC 30.4% (69) LCNEC 46.7% (106)	E1L3N	H-score (intensity x %positive)	1	TC 15/227 (7%)	Better prognosis with PD-L1 +
11	Yasuda et al/ 2018 [15]	39	SCLC	22C3	Tumor proportion score	1%	TC 2.5% (1/39)	Not done
12	Eichhorn et al/2018 [21]	76	LCNEC	SP263	H-score (intensity x %positive)	> 1	TC: 22.4% (17/76) IC: 36.8%	Poor prognosis if T+ Good prognosis if IC+
13	Kasajima et al/2018 [16]	242	TC 39 (17%) AC 18 (7%) LCNEC 58 (24%) SCLC 127 (52%)	22C3	TC Proportion of tumor cells stained IC mild, moderate, high	> 1%	TC 21 (12%) IC 73 (39%)	Good prognosis with IC +

Table 2 (continued)

Sr no	Author/year	No. of cases	Histology	Clone	Scoring criteria	Cut off	Percent positive	Survival correlation
14	Wang et al/2018 [22]	159	TC 35 AC 2 SCLC 94 LCNEC 28	SP142	% positive	TC > = 5% IC > 1%	TC & IC 72/159 (45.3%) TC 26/159 (16.3%) IC 63/159 (39.6%)	Poor prognosis with PD-L1 +
15	Ohtaki et al/2018 [13]	95	LCNEC	E1L3N	0 ≤ 1%, 1 = 1–5%, 2 = 6–10%, 3 = 11–25%, 4 = 26–50%, 5 ≥ 51%	TC Score > = 1	TC 70/95 (74%)	Better prognosis if PD-L1 +
16	Yoshimura et al/2019 [17]	44	SCLC	28 − 8, 22C3, SP263	Tumor proportion score	1% TC staining for clones 28 − 8 and 22C3 and 25% TC staining for clone SP263	TC 11/44 (25%)	No difference in PFS

LCNEC: Large Cell Neuroendocrine Carcinoma, T: Tumor cell, IC: Immune cell, SCLC: Small cell carcinoma lung, TC: Typical Carcinoid, AC: Atypical Carcinoid, SCLC-LD: Small cell carcinoma lung limited disease, SCLC-ED: Small cell carcinoma lung extensive disease, PFS: progression free survival

development of inhibitory receptors an important one of which is PD-1 along with its ligand (PD-L1) [26]. Use of immunotherapeutic drugs to check these immune check point receptor interactions with their ligands help in boosting the anti-tumor immunity of the individual [28]. Among the NET of the lung, the T-cell mediated anti-tumor immunity has been reported to be effective against the high grade tumors especially the SCLC, whereas the carcinoids have been found to be relatively "immune desert" tumors [16]. Recent evidences have suggested anti-PD-1 therapy to be beneficial in cancers with high mutation burden and SCLC being such a malignancy, drugs blocking the PD-1/PD-L1 pathway are bound to be effective [2, 29]. Further, the active role of involvement of the immune system in the pathogenesis of SCLC is evident by the high incidence of paraneoplastic disorders associated with this tumor. These disorders develop due to immune response against same antigens expressed by tumor and neurons [2, 20, 30, 31]. Therefore, SCLC being an immunogenic tumor, immunotherapy is a hope for these tumors. The NCCN guidelines have also recently added PD-L1 inhibitor atezolizumab as the first line drug along with carboplatin and etoposide for SCLC-ED. Further, PD-1 inhibitor pembrolizumab has been recommended in all cases with relapse within 6 months of therapy [3]. Studies evaluating PD-L1 expression in LCNEC are even fewer.

The PD-1/PD-L1 status of a patient is assessed by their immunohistochemical expression in the tumor tissue and the response of anti-PD-1/PD-L1 therapy has been reported to be higher in patients with high PD-1/PD-L1 expression [32]. Several clones of PD-L1 are available today some of which have been approved by the US FDA for use as companion or complementary diagnostics in various malignancies. We have utilised SP263 run on VENTANA Benchmark XT platform, which has been approved by the US FDA as a companion diagnostic for urothelial carcinoma (UC) [33] and in Europe, it is has been granted the Conformité Européenne (CE) mark for in vitro diagnosis when durvalumab is being used in NSCLC and UC, and when pembrolizumab or nivolumab are used in NSCLC [34]. The International Association for the Study of Lung Cancer (IASLC) recommended cut-offs for PD-L1 clone SP263 for Durvalumab therapy is expression in at least 25% of tumor cells and for Nivolumab the staining is sub-grouped as follows: less than 1%, 1–5%, 5–10%, and 10% or greater [7]. Nearly 8% of our cases had PD-L1 positivity in more than 1% of the tumor cells. The incidences of PD-L1 positive NET of lung across different studies have varied immensely. PD-L1 expression in SCLC has been found to be very low with amplification of its gene CD274 being found in only 1.9% (4/210) cases in the study by George et al. [14]. Studies based on IHC expression of PD-L1 in SCLC have reported variations in their frequencies. Its frequency has varied from 5.8% [12] to as much 71.6% [23]. Schultheis et al. in contrast have reported no expression of

PD-L1 in their cohort of 94 cases of pulmonary and extrapulmonary small cell carcinoma [8]. A recent study using the 22C3 clone of PD-L1 in SCLC, has reported approximately 12% of cases to be positive when a cut off of 1% was used and 3% cases positive when they increased the cut-off of to 50% [35]. Similarly, PD-L1 expressing LCNEC have ranged from 10.4% [12] to up to 100% [19]. A reason for this wide range of positivity of PD-L1 in high-grade NET of the lung can be the use of different clones of anti-PD-L1 for IHC along with variable cut-offs. Fan et al. have used the clone ab205921 by Abcam along with staining intensity distribution method [36] of scoring PD-L1 positivity and have demonstrated unusually high PD-L1 expression even in carcinoids. Similar clone has been used in the study by Ishii et al. [23] with demonstration of even higher PD-L1 expression in their cohort of SCLC. The high expression of PD-L1 in SCLC is in contradiction to most other studies and can be attributed to the unconventional clones of PD-L1 IHC used as well as the scoring methodology adopted [6]. The other studies [15, 21, 37] with relatively lower positivity including ours, have incorporated FDA approved clones and cut-offs for PD-L1 positivity. A study comparing three different FDA approved clones (28 − 8, 22C3 and SP263) did not show any significant difference in the tumor cell positivity, thereby reaffirming the use of only standardized clones for PD-L1 evaluation [17]. Low expression of PD-L1 has also been shown in neuroendocrine tumors of other sites [38]. An interesting observation was that all the studies with high PD-L1 expression (> = 50% positivity in tumor cells), were carried out in South-East Asian population especially of Mongolian origin (China and Japan) [13, 19, 23, 24]. So, an uncertainty of whether this variability can be associated to the race and ethnicity of the patients arises and may need deliberation in future.

In addition, the controversy of whether to include only tumor cells or IC or both while assessing PD-L1 expression is also not clear. Moreover, clones like E1L3N are expressed only on tumor cells [11–13] such that IC are not assessable in those cases. These discrepancies highlight the importance of the use of validated kits for PD-L1 assessment in tumors [39] as well as the need to follow universal guidelines for reporting. Nevertheless, despite the lack of consensus, various ongoing trials on SCLC such as the Checkmate 032 [37], Keynote 028 [40] and IMpower-133 [41] have advocated the use of anti-PD-L1 drugs in recurrent or SCLC-ED with significant difference in survival rates [42, 43]. The recommendations have formed part of the recent updated NCCN guidelines for treatment of SCLC as well as recurrent LCNEC of lung [3, 44]. Besides combinations with the standard chemotherapeutic drugs, trials are ongoing to evaluate the efficacy of combination with other non-chemotherapy agents [45].

The various clones approved for PD-L1 detection (SP263, 22C3 and 28 − 8) in lung cancer are only validated for tumor cell counting and not for IC assessment. Clone SP142 is the only one which has been standardized for assessment of IC [46]. The SP263 clone stains the tumor cells better than the other clones and its propensity to stain the IC is still not validated [5, 47]. Ours was the only other study using this clone of PD-L1 on NET of lung besides that by Eichhorn et al. [21] where they have evaluated only cases of LCNEC. While analysing our cases, we noticed up to 60% of our cases harbouring IC of which a fair amount of (up to 31.3%) cases had easily discernible staining with the SP263 clone as compared to tumor cell staining in only 8.4% cases which hints towards immune privilege nature of these tumors. However, the number of cases being very few, this may not be a true representation of the frequency of PD-L1 on IC. Studies have shown high mutation burden to correlate significantly with PD-L1 positivity [20] and SCLC being one such tumor, immunotherapy has been seen to be beneficial in many such patients irrespective of their PD-L1 status [48–51]. Research and clinical trials have also shown that the predictive biomarkers used for immune check point inhibitors which have been approved for NSCLC cannot be applied for SCLC as both of these diseases have different molecular basis. There have been suggestions to ascertain different cut-off values and interpretations for PD-L1 and other biomarkers for SCLC [45].

The use of PD-L1 as a prognostic marker has also been controversial. Majority of the studies have demonstrated better prognosis in patients with higher number of PD-L1 positive immune cells in SCLC [16, 20, 21, 23, 31, 52, 53] as well as in non- small cell carcinoma of lung (NSCLC) [54]. Some studies have even shown tumor cell positivity in NET to be associated with better survival characteristics [11–13, 19, 23, 24]. Also, Eichhorn et al. [21], have demonstrated poor survival with tumor cell positivity in LCNEC and better prognosis in cases with IC expressing PD-L1. Therefore, multi-institutional studies with larger cohort of patients and using standardized clones for PD-L1 detection should be formulated to assess the exact role of PD-L1 expression on NET of lung. We also propose well defined criteria to be developed to assess PD-L1 on IC using all FDA approved clones.

Few limitations of our study which we can enumerate are as follows. Firstly, ours was a small study population with inclusion of biopsy specimens for evaluation of PD-L1 which may be affected by tumor heterogeneity. But this effect should not impact much as studies using tissue microarrays [55–57], which are equivalent to small biopsies, on different tumor types have demonstrated significant results. Having stated this, we would also like to reaffirm that most of the small biopsies were SCLC being invariably detected in advanced stages which are inoperable, making availability of resection specimens of these cases almost nil. This scenario is likely to be faced by most clinicians and therefore, PD-L1 assessment in this cohort of patients may have to be done only on biopsies. Secondly, due to the small sample size with very few PD-L1 positive cases, no survival analysis could be carried out.

Finally, we did not have clinical data on the responses to anti-PD-L1 immunotherapy of our cases as they were not prospectively followed up to evaluate their clinical outcomes.

In conclusion, neuroendocrine carcinomas of lung demonstrate PD-L1 positivity in very low percentages. Also, the lack of standardized guidelines for use of PD-L1 IHC in this subgroup of tumors may limit its reliability as a predictive biomarker however other immune biomarkers should also be investigated to draw substantial conclusions.

Author Contributions All authors contributed to the study conception and design. Material preparation, data collection and analysis were performed by [Prerna Guleria], [Sunil Kumar], Prabhat Singh Malik and [Deepali Jain]. The first draft of the manuscript was written by [Prerna Guleria] and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

Funding Information The work is funded by DST-SERB CRG/2018/003722.

Availability of Data and Material Not applicable

Compliance with Ethical Standards

Conflicts of Interest The authors declare no potential conflict of interest.

Ethics Approval The work has the approval of the Institutional Ethics Committee.

Consent to Participate Informed consent was taken from all patients included in the study.

Consent for Publication Not applicable

Code Availability Not applicable

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