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Prevalence and prognostic value of PD-L1 expression in molecular subtypes of metastatic large cell neuroendocrine carcinoma (LCNEC)

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ABSTRACT

Background: Pulmonary large cell neuroendocrine carcinoma (LCNEC) is a rare tumor with high mutational burden. Two subtypes of LCNEC are recognized, the co-mutated *TP53* and *RB1* group and the *TP53* and *STK11/KEAP1* group. We investigated PD-L1 and CD8 expression in a well characterized stage IV LCNEC cohort and compared expression in the two subtypes.

Methods: Immunohistochemical (IHC) analysis for PD-L1 and CD8 was performed on pathological reviewed pretreatment tumor samples for 148 stage IV LCNEC. Data about targeted next generation sequencing (TNGS) (*TP53, RB1, STK11, KEAP1)* and IHC for RB1 were available for most tumors. IHC staining for PD-L1 (DAKO 28-8) was performed and scored positive if tumors showed ≥1% membranous staining. CD8 was scored for intra-tumor T-cells and stromal cells.

Results: PD-L1 IHC expression data could be generated in 98/148 confirmed LCNEC samples along with RB1 IHC (n = 97) of which 77 passed quality control for TNGS. PD-L1 expression was positive in 16/98 cases (16%); 5 (5%) with ≥50%. PD-L1 expression was equal in *RB1* mutated and *RB1* wildtype tumors. None of *STK11* mutated tumors (n = 7) expressed PD-L1. PD-L1 expression was correlated with superior overall survival (OS), hazard ratio 0.55 ((95% Confidence Interval 0.31-0.96), p = 0.038). Intra-tumor CD8 was associated with PD-L1 expression (p = 0.021) and stromal and intra-tumor CD8 were correlated with improved OS (p = 0.037 and p = 0.026 respectively).

Conclusions: PD-L1 expression was positive in 16% of stage IV LCNEC tumors. This was independent of molecular subtype but associated with CD8 expression. In LCNEC patients with PD-L1 and/or CD8 expression superior OS was observed.

1. Introduction

Large cell neuroendocrine carcinoma (LCNEC) of the lung is an uncommon tumor, representing 1–3% of all types of lung cancer [1,2]. Although LCNEC shows hallmarks of non-small cell lung cancer (NSCLC), prognosis seems to be similar to small cell lung cancer (SCLC)

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with poor survival rates [1,3,4]. In LCNEC neuroendocrine morphology is required, and if present confirmation of neuroendocrine differentiation by immunohistochemistry (IHC) is necessary in the WHO 2015 classification [5]. Next generation sequencing (NGS) studies have identified two exclusive molecular subtypes of LCNEC. A subtype with inactivation of *TP53* and *STK11* and/or *KEAP1* genes, a second subtype with mutation of *TP53* and *RB1* (a hallmark of SCLC) [6–8]. These subtypes may be relevant for prognosis and response to therapy.

For stage IV LCNEC tumors, palliative chemotherapy is the treatment of choice. However, owing to the rarity of the tumor, no large randomized controlled trials concerning the most appropriate chemotherapy have been performed and currently both SCLC and NSCLC chemotherapy regimens are deemed appropriate. In a recent retrospective study, we showed relevance for the molecular subtyping. The study revealed that patients with LCNEC and wildtype *RB1* (NSCLC-like) had a longer overall survival (OS) when treated with NSCLC regime (platinum doublet with gemcitabine, docetaxel or paclitaxel) compared to SCLC regime (platinum-etoposide) or NSCLC regime containing pemetrexed. In contrast, no difference was observed in LCNEC cases with *RB1* mutation (SCLC-like) [9].

In NSCLC, PD-L1 expression has been reported in up to 60% of tumors and PD-1/PD-L1 targeted therapy with or without chemotherapy is standard of care in patients without EGFR or ALK mutation [10–15]. Approximately 30% of SCLC tumors are PD-L1 positive. However, due to insufficient data PD-1/PD-L1 targeted therapy for SCLC is so far only recommended in the National Comprehensive Cancer Network (USA) guideline as combination therapy [16–18]. Scarce data exist about PD-L1 expression in LCNEC, with prevalence of PD-L1 positivity reported in 9–32% of patients and conflicting results with respect to the prognostic relevance of PD-L1 [19–25]. Importantly, the majority of LCNEC studies evaluated surgically resected cases with non-metastatic disease whereas data on PD-L1 expression in metastatic (stage IV) disease is lacking. However, immunotherapy is of special interest in LCNEC since LCNEC has a high mutational burden (up to 11 mutations per Mb), and this may be related to response to immunotherapy [6,7,9,26–28].

In this study we evaluated the prevalence of PD-L1 expression in a large cohort of patients with well characterized and molecular profiled stage IV LCNEC. We furthermore investigated PD-L1 expression related to different mutational profiles (i.e. *RB1* mutation *vs. STK11/KEAP1* mutation) and to CD8 positive cells as a marker of immune system activity. We also studied the prognostic value of PD-L1 and CD8 expression in these LCNEC patients.

2. Material and methods

2.1. Patient and tissue selection

For this retrospective population-based study all data were retrieved from the Netherlands Cancer Registry and Netherlands Pathology Registry (PALGA) as described previously [29,30]. For all 232 stage IV LCNEC, diagnosed between 2003 and 2012 in the Netherlands on a pre-treatment sample, panel consensus pathology revision was performed as described earlier by three pathologists (ET, MdB & RvS) [31]. Samples were scored for neuroendocrine morphology (organoid nesting, palisading, rosettes or trabeculae), mitotic index, necrosis and neuroendocrine differentiation (positive immunohistochemistry (IHC) for at least one neuroendocrine marker). Diagnosis was confirmed in patients meeting the WHO-criteria [5]. An exception was made when strict WHO-criteria were not met, but the pathologists found it highly likely that LCNEC was the correct diagnosis, as described earlier [31,32]. In patients with panel consensus confirmed LCNEC (n = 148), targeted NGS was performed on tumor tissue from available FFPE tissue blocks for the genes *RB1*, *KEAP1*, *STK11* and *TP53*. Furthermore, IHC staining was executed for RB1 protein. Data concerning age, gender, OS, chemotherapy details and date of death or last day of follow-up were available and updated until 2015 [9].

The study protocol was approved by the medical ethical committee of the Maastricht University Medical Centre (METC azM/UM 14-4-043). The study is performed according to the Dutch "Federa, Human Tissue and Medical Research: Code of conduct for responsible use (2011)" regulations not requiring patient informed consent.

2.2. Immunohistochemistry

2.2.1. PD-L1

IHC staining for PD-L1 was performed with the monoclonal rabbit anti-PD-L1 clone 28-8 using the DAKO Autostainer Link 48 system with the PD-L1 IHC 28-8 pharmDx kit (DAKO, Agilent, USA) according to recommended protocols. Low pH target retrieval solution and Rabbit linker were used. Evaluation of the percentage tumor cells with partial or complete membranous staining was performed by EJS and BH. Tumor proportion score (TPS) was defined as the percentage of tumor cells with complete or partial membranous staining at any intensity. A TPS ≥ 1% was considered as positive. A distinction was made between PD-L1 + high (≥50%) and PD-L1 + low (1–49%).

2.2.2. CD8

DAKO C8/144B antibody was used for CD8 immunohistochemistry to stain T-cells on the DAKO autostainer link 48 system, high pH target retrieval was used. Samples were evaluated by two investigators (EJS and BH). CD8 density in tumor-associated stromal cells was arbitrary scored as negative, weakly positive, moderately positive or strongly positive. CD8 positive cells in the tumor were scored as negative, ≤1% or > 1%. When CD8 invasion was scored > 1% counting of CD8 positive cells was performed by evaluating three representative parts of the tumor with 200x amplification. Mean number of CD8 positive cells per mm^2 was calculated.

2.3. Mutational analysis

Targeted next generation sequencing had already been performed as described previously, covering the exons of *TP53*, *RB1*, *STK11* and *KEAP1* [9]. Immunohistochemistry was performed for RB1 with mouse antibody 13A10, with tonsillar tissue and tumor stromal cells as positive and negative controls, as reported before [9].

2.4. Statistics

All analyses were performed using SPSS (version 25 for Windows, Armonk, NY: IBM Corp.). Patient characteristics were evaluated with descriptive statistics. Correlation of PD-L1 expression with age, gender, mutational status (*TP53, RB1, STK11* and *KEAP1*) and IHC staining for RB1 and CD8 was investigated using the chi-square test. Median OS was evaluated by Kaplan Meier analysis and differences in survival were tested for significance with Log-Rank test ($P < 0.05$ was considered significant) for IHC for PD-L1, CD8 in the tumor, CD8 in stromal cells and RB1 and for mutation status of *RB1, STK11, KEAP1* and *TP53*. Multivariate cox-regression analysis included all factors with a significant impact (PD-L1 and CD8 in stromal cells), completed with the known prognostic factors age and gender. Results are presented as hazard ratios (HR) with 95% confidence intervals (CI).

3. Results

3.1. Patient characteristics

After selection of cases with sufficient tumor material for IHC staining, 98 pathology confirmed LCNECs treated with chemotherapy were stained for PD-L1, and 93/98 for CD8 (Table 1). The vast majority of those patients (85/98 for PD-L1 and 80/93 for CD8 respectively) fulfilled WHO criteria (Supplemental table A). For 97/98 cases RB1 IHC data were available and for 77/98 cases targeted NGS data for *TP53,*

Table 1
Expression of PD-L1 in LCNEC, patient characteristics and survival.

	PD-L1 +	PD-L1-	p-value
LCNEC (n = 98)	16 (16%)	82 (84%)	–
1- < 50%	11 (11 %)	–	–
≥50%	5 (5%)	–	–
Age (median, range)	63 (37-74)	64 (34-82)	0.837[#]
Gender			
Male	10 (63%)	50 (61%)	0.909[#]
Female	6 (38%)	32 (39%)	
OS in months (95% CI)	8.9 (4.1-13.6)	6.6 (5.6-7.6)	HR 0.55 (0.31-0.96) p = 0.038[∞]

OS = overall survival; HR = hazard ratio.
[#] Chi-square test (for age group ≤65 and > 65).
[∞] Cox-regression including age and gender.

RB1, STK11 and *KEAP1* (Supplemental figure A). Median age at diagnosis of the 98 patients was 64 years (range 34–82 years). A total of 61% patients were male (Table 1). Chemotherapy included SCLC regimen (including a platinum component and etoposide) in 35% of patients, NSCLC regimen (including a platinum component with either gemcitabine, docetaxel or paclitaxel) in 44%, platinum-pemetrexed in 12% and 9% unspecified, respectively.

3.2. PD-L1 expression

Membranous staining of tumor cells for PD-L1 (≥1%) was observed in 16/98 (16%) LCNEC, staining was negative in 82/98 (84%) (Table 1). Positive staining included n = 5 (5%) LCNEC cases with ≥50% staining and n = 11 (11%) with 1–49% staining (Fig. 1). Outcome of PD-L1 expression was not associated with age or gender (Table 1). Subgroup analysis of the 85 patients with strict WHO-diagnosis was comparable to the results of the full cohort (Supplemental table A).

3.3. PD-L1 expression in molecular subgroups of LCNEC

The frequency of tumors positive for PD-L1 expression was equal in *RB1* mutated (SCLC-like) and *RB1* wildtype (NSCLC-like) LCNEC (n = 6 (17%) *vs.* n = 6 (15%), respectively, p = 0.842). All seven *STK11* mutated tumors were PD-L1 negative (p = 0.229). A higher frequency of PD-L1 positive LCNEC was observed in *TP53* wildtype tumors (*TP53* wildtype n = 5 (36%), *TP53* mutated n = 8 (12%), p = 0.043) (Fig. 2, Supplemental table B). Results were comparable for the subgroup of patients meeting WHO criteria (Supplemental table C).

3.4. CD8

Any intra-tumor CD8 staining was observed in 41/93 (44%) LCNEC and CD8 staining of > 1% was observed in 15/93 (16%) of LCNEC (Fig. 1, Supplemental table D). In LCNEC with CD8 count estimated at > 1%, CD8 counting exhibited a mean density of 142 cells/mm^2 (minimum 15 cells/mm^2, maximum 376 cells/mm^2) (Supplemental table E). Analysis of stromal tissue showed staining in 83/93 (89%) LCNEC; including n = 57 (61%) weak positive, n = 7 (8%) moderate positive, n = 19 (20%) strong positive (Supplemental table D). Intra-tumor CD8 expression and CD8 expression in tumor-adjacent stroma was associated, with 98% (n = 40) of samples positive in the tumor also being positive in stromal cells (p = 0.039). Expression of PD-L1 was associated with the presence of intra-tumor CD8 (p = 0.013) (Fig. 2, Supplemental table B). CD8 expression in both intra-tumor and stroma was comparable in *RB1* mutated (15/36, 42%) and *RB1* wildtype (20/

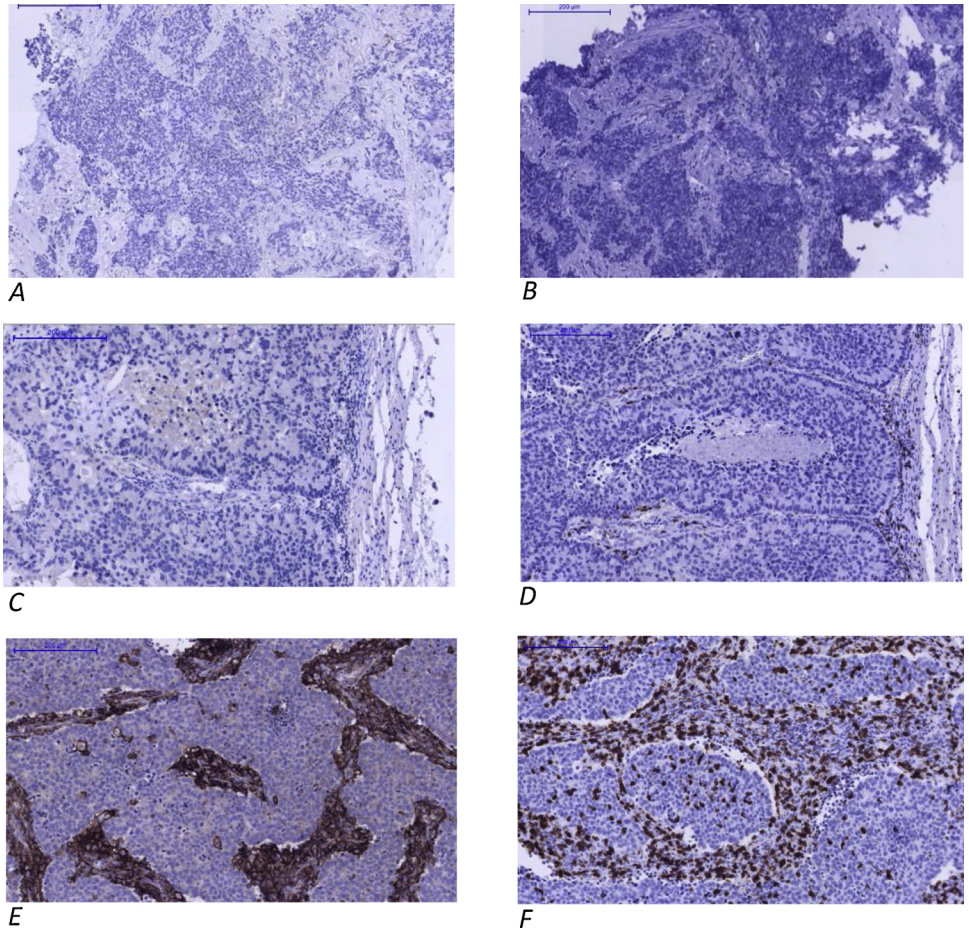


Fig. 1. Pathological slide overview of three patients with PD-L1 28-8 and CD8 staining. A) Patient 1; PD-L1 negative. B) Patient 1; CD8 negative. C) Patient 2; PD-L1 negative. D) Patient 2; CD8 stromal cells positive (weak), tumor cells negative. E) Patient 3; PD-L1 positive. F) Patient 3; CD8 tumor cells positive and stromal cells strongly positive.

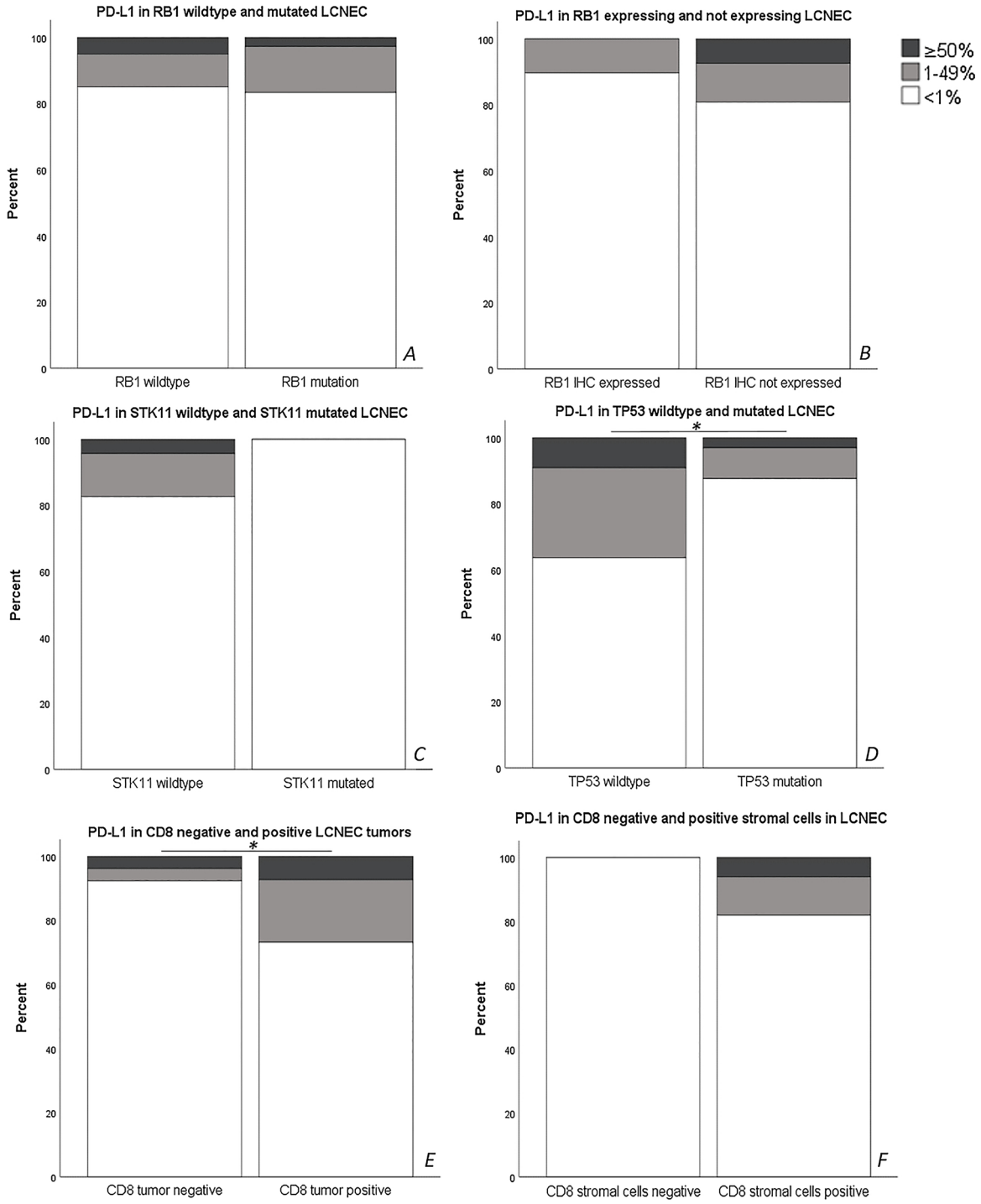
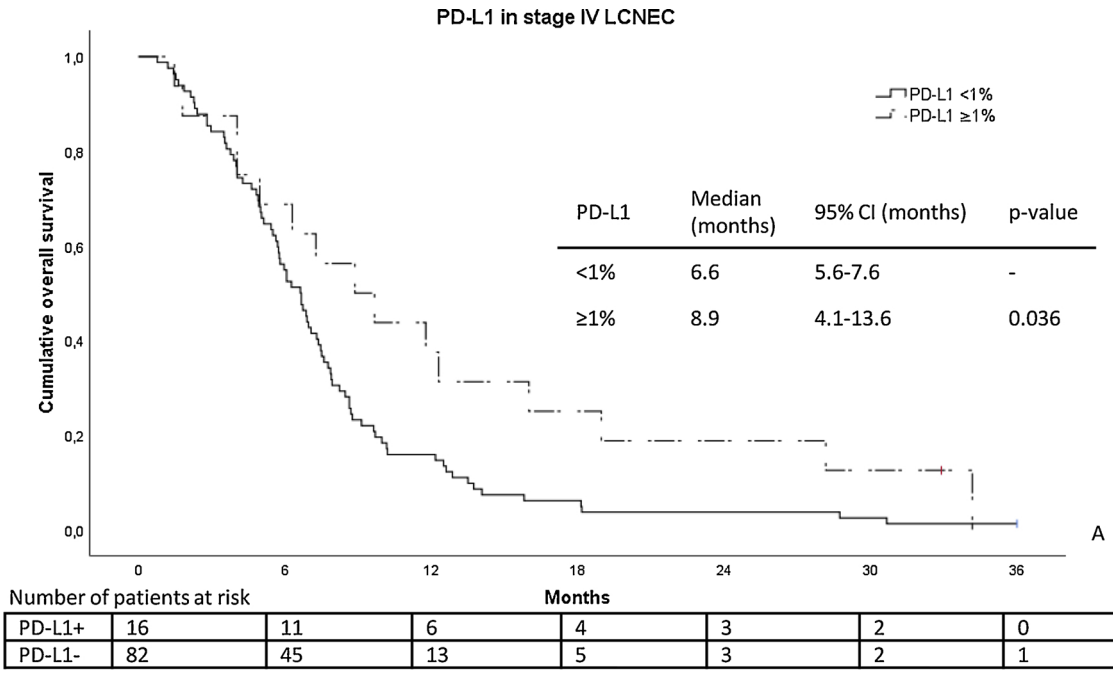


Fig. 2. PD-L1 expression in LCNEC patients: A) RB1 wildtype (N = 40) & mutated (N = 36) B) RB1 expressing (N = 29) & non-expressing (N = 68) C) STK11 wildtype (N = 69) & mutated (N = 7) D) TP53 wildtype (N = 11) & mutated (N = 65) E) CD8 non-expressing (N = 52) & expressing (N = 41) in T-cells in tumor F) CD8 non-expressing (N = 10) & expressing (N = 83) in stromal cells.

36, 56%) LCNEC (p = 0.238). All seven *STK11* mutated tumors had ≤1% intra-tumor CD8 staining (p = 0.332). Subgroup analysis of the patients with WHO-diagnosis was comparable to the full cohort results (Supplemental tables C & F).

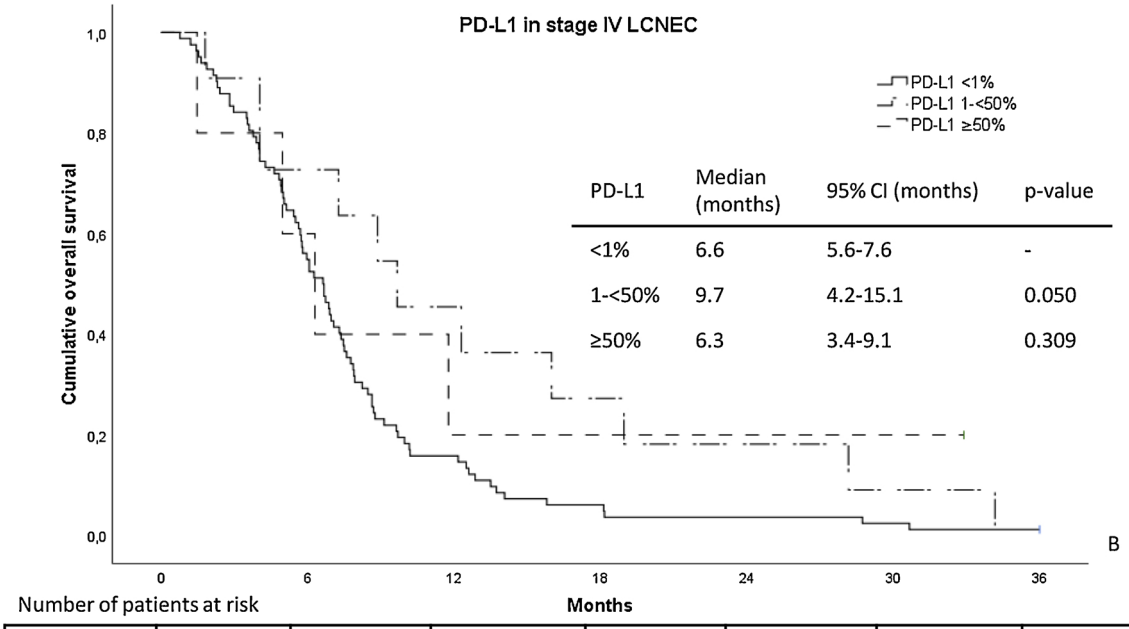
3.5. Survival

Median OS was 8.9 months (95% confidence interval (CI) 4.1–13.6 months) for patients with PD-L1+ tumors and 6.6 months (95% CI 5.6–7.6 months) for PD-L1- tumors (HR 0.55, 95% CI 0.31-0.96, p = 0.038). No difference in survival in PD-L1+ high (≥50%) or low (1–49%) was observed (Fig. 3). Positive staining of intra-tumor CD8 was associated with improved OS compared to negative staining (7.9 months and 5.8 months, HR 0.62 (95% CI 0.40-0.94, p = 0.026). Also, positive CD8 staining in stromal cells was correlated with a longer OS (6.9 months vs. 4.0 months, HR 0.49 (95% CI 0.25-0.96), p = 0.037) and a trend was seen for improved survival with a higher CD8 density in stromal cells (Supplemental figure B). Results were comparable for the subgroup of patients achieving strict WHO criteria (Supplemental figures C & D). No differences were found in OS for IHC RB1 or *RB1,*



Number of patients at risk

PD-L1+	16	11	6	4	3	2	0
PD-L1-	82	45	13	5	3	2	1



Number of patients at risk

PD-L1+ <50%	11	8	5	3	2	1	0
PD-L1+ ≥50%	5	3	1	1	1	1	0
PD-L1-	82	45	13	5	3	2	1

Fig. 3. (a)Overall survival for PD-L1 negative and positive tumors in stage IV LCNEC. (b)Overall survival for PD-L1 negative and positive tumors in stage IV LCNEC, subdivided in low (< 50%) and high (≥50%) PD-L1 expression.

TP53, STK11 and *KEAP1* mutation. Cox-regression included PD-L1, CD8 in stromal cells, age and gender and revealed HR 0.64 (95% CI 0.36–1.16, p = 0.141). CD8 in the tumor exhibited intersecting lines in the survival curve and was therefore excluded from cox-regression. Stratification for this factor revealed non-significant improved OS in PD-L1 positive tumors in both subgroups (Supplemental figure E).

4. Discussion

PD-L1 expression in pre-treatment samples of LCNEC patients with metastatic disease has not yet been reported; there is scarce information on PD-L1 expression in local disease. In this unique series of metastatic LCNEC we found PD-L1 staining (≥1%) in up to 16% of cases using the DAKO 28-8 IHC antibody. Hence, based on PD-L1 expression, combination therapy including PD-L1 targeted therapy might be a successful extension of current therapy for LCNEC patients. However, this requires further clinical evaluation.

The PD-L1 staining in LCNEC is comparable to reported values in SCLC, but distinctly lower than in NSCLC [12–14,16,18]. Several studies have recently provided a similar prevalence of PD-L1 staining in early stage LCNEC (9% (n = 58), 10.4% (n = 106), 16.7% (n = 72) and 22.4% (n = 76) (Table 2) [19–22]. However, three smaller studies

Table 2
Overview of literature of PD-L1 expression in LCNEC patients.

Author (year)	Number of patients	Number stage IV	Number of clinics	LCNEC confirmed (number of pathologists)	Antibody	PD-L1 cutoff value	PD-L1 positive tumors	Association PD-L1 and OS
Kasajima (2018)	53	3	10	Yes (5)	22C3	≥1%	9%	No effect
Tsuruoka (2017)	106	< 5*	1	Yes (1)	E1L3N	≥1%	10%	Better survival (HR 0.42 (95% CI 0.17-1.06, p = 0.067))
Kim (2018)	72	18	1	Yes (n/a)	B7-H1	≥1%	17%	No effect
Eichhorn (2018)	76	11	1	Yes (2)	SP263	≥1%	22%	Lower survival (p = 0.28)
Takada (2017)	15	0	1	n/a	SP142	≥5%	20%	n/a
Inamura (2017)	41	n/a	1	Yes (2)	E1L3N	≥5%	27%	Better survival (HR 0.44 (95% CI 0.1-1.3, p = 0.15))
Wang (2018)	28	0	1	Yes (2)	SP142	≥5%	32%	Lower survival (p = 0.459)

LCNEC confirmed = Pathology review performed within the scope of the study; OS = overall survival; HR = hazard rate; n/a = not available.
* 5 stage IV in SCLC (69) and LCNEC (106) combined.

revealed higher values of 20% (n = 15), 27% (n = 41) and 32% (n = 28) [23–25]. Besides the size of cohorts, the use of different IHC PD-L1 antibodies may explain differences in outcomes. We are the first to report the validated DAKO 28-8 antibody for staining in LCNEC. However, a blueprint study showed comparable results for usage of 22C3, SP263 and 28-8 in patients with NSCLC, whereas SP142 assay exhibited fewer stained tumor cells. No comparison was made for E1L3N and B7-H1 antibodies [33]. Therefore, our results should at least be comparable with studies using 22C3 or SP263 antibodies. No explanation for variation is found in different thresholds defining PD-L1 positivity (i.e. ≥ 5% instead of ≥1%), since higher values were found with higher thresholds (Table 2) [23–25].

Recently, upregulation of immune related pathways has been reported in an LCNEC subgroup with *TP53* and *RB1* mutation [7]. However, in this study PD-L1 and CD8 expression was similar in LCNEC with *RB1* mutated (SCLC-like) and *RB1* wildtype (NSCLC-like) tumors and although PD-L1 expression is known to be distinctly higher in NSCLC compared to SCLC, this is not reflected when evaluating molecular LCNEC subgroups. Consistent with previous reports of lower PD-L1 expression and lower response rates to PD-L1 targeted therapy in patients with co-mutated *KRAS* and *STK11* NSCLC, none of the seven *STK11* mutated samples in our study harbored PD-L1 expression and all had negative or limited (≤1%) CD8 staining. This might be due to the accumulation of neutrophils along with T cell suppressive effects and T cell exhaustion in *STK11* mutated tumors [34–39]. Since expression of CD8 positive cells in the tumor is associated with PD-L1 staining, this could clarify the reduced PD-L1 expression in *STK11* mutated tumors. Therefore, the effect of immunotherapeutic treatment might be reduced in *STK11* mutated LCNEC and this should be taken into account in future clinical trials.

So far, conflicting results were presented for deviating survival in tumors expressing PD-L1 in LCNEC. In this study, expression of any PD-L1 was correlated with a superior OS (8.9 vs 6.6 months). This is in accordance with previous reports by Inamura et al. and Tsuruoka et al. (Table 2) [20,24]. Contrary to our findings, Wang et al. reported a trend towards lower OS for total group of PD-L1 + pulmonary neuroendocrine carcinoma (p = 0.459). However, in multivariate analysis including clinical staging (I-III), PD-L1 was not an independent prognostic factor [25]. Also, a tendency to an inferior 5-year survival rate was revealed by Eichhorn et al. Nevertheless, despite a higher prevalence of PD-L1 staining in stage III and IV tumors, no multivariate analysis was reported. Therefore, the inferior survival might be related to a higher disease stage and not to PD-L1 expression by itself [22]. We included a more homogeneous population with only stage IV LCNEC, so our study is not affected by this confounding factor.

In this study, a minority of samples (16%) had > 1% CD8 positive cells in the tumor, while higher amounts were seen in the stromal cells (89%). This may indicate that only a subgroup of LCNEC is an 'inflamed tumor', while the majority likely is 'immune excluded'. In those tumors, T-cell response is present, but T-cells do not seem to be able to penetrate the tumor. A positive correlation for intra-tumor CD8 expressing cells and PD-L1 expression was found. A correlation between PD-L1 expression and CD8 density in stromal cells has been reported previously [19,25]. In this study, both positive CD8 in T-cells in the tumor and in stromal cells were correlated with improved OS. In NSCLC patients, OS is also improved with increased CD8 T-cell infiltration in both tumor cells and stromal cells (HR 0.77 (95% CI 0.66-0.93) and HR 0.77 (95% CI 0.69-0.86), respectively) [40]. For LCNEC patients, Wang et al. detected an improved OS with a higher CD8 density in stromal cells (HR 2.77; 95% CI 1.29–5.93, p = 0.009), however, association with OS was not found for CD8 density in tumor cells [25]. Kasajima et al. found a correlation between CD8 density and higher immune cell infiltration, the latter resulting in a prolonged OS (37 vs 80 months, p = 0.03) [19]. Therefore, the improved OS we and others found in patients with PD-L1 expression might be partly due to a more active immune system in those patients, reflected by CD8. Although the tumor develops escape systems (i.e. PD-L1) to resist the immune system, this inhibition seems to be only partial, preserving beneficial effects in at least part of the patients. In multivariate cox-regression analysis in this study, including CD8 positive cells in stroma, PD-L1 was not an independent prognostic factor. However, sample sizes for this analysis were small with only 10 patients in the CD8 negative group.

This study has some limitations. First, data was collected retrospectively and therefore we could not obtain all clinical characteristics of patients, i.e. smoking history or WHO performance score. Furthermore, most pathologic diagnoses were performed on biopsy samples, whereas it is known that it is difficult to diagnose LCNEC according to WHO-criteria on biopsy specimen [5,32]. However, the main problem for LCNEC diagnosis on biopsy specimen is lack of sensitivity, and not a lack of specificity [32]. Subgroup analysis of the 85 patients with strict WHO-diagnosis was comparable to the results of full cohort (supplementary data). Another limitation is that we only established PD-L1 in tumor cells, not in stromal cells. However, former studies revealed a positive correlation between CD8 positive cells and PD-L1 expression in stromal cells, both as a measure of immune activity [19,25]. Therefore, CD8 can be considered as a reasonable alternative. PD-L1 28-8 clone is known to show some background staining, but we have taken this into account and only scored membranous staining as positive.

Several small case series have reported responses (duration of response up to 6 months) to PD-L1 monotherapy as second and later-line treatment in patients with LCNEC, irrespective of PD-L1 expression [41–43]. Furthermore, a response to nivolumab treatment was seen in few selected patients with SCLC having disease progression after at least one previous platinum-containing regimen [16]. Based on these studies, PD-L1 monotherapy might be suitable in LCNEC patients, irrespective of PD-L1 expression. However, owing to relatively low levels of PD-L1 expression and the high proportion of 'immune excluded' tumors with low CD8 and PD-L1 expression, combination with chemotherapy or another immunotherapy might be more appropriate. This is supported

by recent results in first line treatment of SCLC where a combination of chemotherapy and atezolizumab showed a significant survival benefit [44]. Another example of combination therapy is the improved response rate in SCLC patients treated with nivolumab and ipilimumab [26]. In the future, more investigations including prospective trials are necessary to reveal the effect of PD-L1/PD-1 inhibition in patients with LCNEC and the predictive value of PD-L1, CD8 and/or tumor mutational burden.

In conclusion, this is the largest study so far reporting PD-L1 expression in patients with well characterized stage IV LCNEC. Few patients had discernable PD-L1 expression, with 5/98 high expressers, independent of molecular subtype. Patients with PD-L1 expression had a better OS than PD-L1 negative patients. CD8 expression in T-cells in the tumor and stroma was correlated with PD-L1 expression and improved OS. These results question the role of single agent PD-(L)1 inhibition in metastatic LCNEC and call for combination strategies.

Conflict of interest statement

Drs. Hermans reports grants from Bristol-Myers Squibb, during the conduct of the study.

Dr. Derks reports grants from Bristol-Myers Squibb, during the conduct of the study; personal fees from IPSEN, Boehringher-Ingelheim, Pfizer and BMS, outside the submitted work;

Dr. Thunnissen reports personal fees from oncologica UK limited, outside the submitted work;

Dr. Groen reports grants to the institution from Eli Lilly, Merck, BMS, Pfizer and Cancer-ID European Subsidy, outside the submitted work;

Dr. Speel reports grants from Bristol-Myers Squibb, during the conduct of the study; grants from Astra Zeneca, Pfizer, Novartis and MSD, personal fees from AbbVie and Roche, outside the submitted work;

Dr. Dingemans reports grants from Bristol-Myers Squibb, during the conduct of the study; personal fees from Roche, BMS, Eli Lily, Takeda and Boehringer Ingelheim, outside the submitted work;

The other authors did not report conflicts of interests.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.lungcan.2019.02.022.

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