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A retrospective cohort study of PD-L1 prevalence, molecular associations and clinical outcomes in patients with NSCLC: Results from the European Thoracic Oncology Platform (ETOP) Lungscape Project

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ABSTRACT

Introduction: The PD-L1 biomarker is an important factor in selecting patients with non-small cell lung cancer for immunotherapy. While several reports suggest that PD-L1 positivity is linked to a poor prognosis, others suggest that PD-L1 positive status portends a good prognosis.

Methods: PD-L1 positivity prevalence, assessed via immunohistochemistry (IHC) on tissue microarrays (TMAs), and its association with clinicopathological characteristics, molecular profiles and patient outcome- Relapse-free Survival (RFS), Time-to-Relapse (TTR) and Overall Survival (OS)- is explored in the ETOP Lungscape cohort of stage I-III non-small cell lung cancer (NSCLC). Tumors are considered positive if they have ≥1/5/25/50% neoplastic cell membrane staining.

Results: PD-L1 expression was assessed in 2182 NSCLC cases (2008 evaluable, median follow-up 4.8 years, 54.6% still alive), from 15 ETOP centers. Adenocarcinomas represent 50.9% of the cohort (squamous cell: 42.4%). Former smokers are 53.7% (current: 31.6%, never: 10.5%). PD-L1 positivity prevalence is present in more than one third of the Lungscape cohort (1%/5% cut-offs). It doesn't differ between adenocarcinomas and squamous cell histologies, but is more frequently detected in higher stages, never smokers, larger tumors (1/5/25% cut-offs). With ≥1% cut-off it is significantly associated with IHC MET overexpression, expression of PTEN, EGFR and KRAS mutation (only for adenocarcinoma). Results for 5%, 25% and 50% cut-offs were similar, with MET being significantly associated with PD-L1 positivity both for AC ($p < 0.001$, 5%/25%/50% cut-offs) and SCC ($p < 0.001$, 5% & 50% cut-offs and $p = 0.0017$ for 25%). When adjusting for clinicopathological characteristics, a significant prognostic effect was identified in adenocarcinomas (adjusted p-values: 0.024/0.064/0.063 for RFS/TTR/OS 1% cut-off, analogous for 5%/25%, but not for 50%). Similar results obtained for the model including all histologies, but no effect was found for the squamous cell carcinomas.

Conclusion: PD-L1 positivity, when adjusted for clinicopathological characteristics, is associated with a better prognosis for non-metastatic adenocarcinoma patients.

1. Introduction

Immunohistochemical detection of the Programmed death ligand 1 (PD-L1) protein is now an established biomarker test for the selection of patients for anti-PD1 and anti-PD-L1 immunotherapy in non-small cell lung cancer (NSCLC) [1–4]. It is important to know if a biomarker used to predict response to a therapy is also prognostic, as any prognostic effect could confound the apparent performance of the predictive biomarker. There have been varied reports of the prognostic significance of PD-L1 expression in NSCLC, with no clear consensus, often based on relatively small studies [5–9].

PD-L1 expression has also been related to a number of histopathological and molecular features in NSCLC, especially in adenocarcinoma. These features include higher grade histology in adenocarcinoma, assessed in various ways, evidence of epithelial-mesenchymal transition (EMT) and the upregulation or mutation of numerous genes including MET, PTEN, EGFR and KRAS. These associations are of interest not only to foster our knowledge of the biology of this important immune negative regulatory checkpoint, but also because there is evidence that some oncogenic signalling pathways, when activated, may intrinsically upregulate PD-L1 expression. The latter is important because it could represent a scenario where high PD-L1 expression is not immunologically active in suppressing a tumour-specific immune response [10].

The European Thoracic Oncology Platform (ETOP) established a large cohort of surgically resected NSCLC from 15 academic centres (Lungscape). This patient cohort is clinically fully annotated and has already been extensively studied with respect to clinical outcomes and extensive molecular analysis [11,12]. In this paper we present the associations between PD-L1 expression assessed using IHC and the post-operative survival outcomes for the patient cohort, as well as to the molecular biomarker data already held in the ETOP Lungscape database. The primary objective of this study is to determine PD-L1 prevalence, based on TMA IHC staining results, overall and according to specific histological subtypes, in a large cohort of NSCLC patients. In addition, we explore possible associations between PD-L1 status and patient, tumor & surgery characteristics, tumor molecular profiles and survival outcome.

2. Methods

2.1. Study design

This is a retrospective cohort study of surgically resected, stage I-III NSCLC cases from the Lungscape cohort. Clinical and molecular data were obtained from the Lungscape iBiobank database (https://etopdata.etop-eu.org). Central review regarding completeness of mandatory clinical parameters and the 7th TNM staging exactitude was performed. The Lungscape database has extensive clinical data and molecular parameters on over 2400 surgically resected NSCLC, with follow-up greater than 3 years [11,12]. Tissue microarrays (TMAs) were prepared from tissue blocks held in participating institutes pathology archives, for each of the cases in the database, as previously described. These TMAs are held securely in safe storage in participating centres and provide freshly cut sections for ETOP Lungscape projects as required. The study was conducted according to Lungscape master and PD-L1 sub-study protocols; with adherence to country specific ethics and regulatory requirements and REMARK recommendations.

Four micron thick sections from the formalin-fixed, paraffin-embedded TMA blocks were cut in each participating centre, immediately prior to staining. PD-L1 IHC was carried out using the Dako 28-8 trial-validated assay according to the published protocol [13] using the commercially available diagnostic kit assay. Staining was carried out in three participating ETOP centres (Aberdeen, VUMC Amsterdam, Leuven), using the Dako Link 48 staining platform required by the trial-validated kit assay, after staff were trained in the technique and each centre had demonstrated adequate performance in an inter-laboratory quality assurance step (EQA) to assist in laboratory staining standardization [14]. Thereafter, stained TMA sections were returned to the participating centre of origin as appropriate. PD-L1 IHC was assessed and scored by participating pathologists after each had been trained in the scoring of the assay.

PD-L1 scores were made for each of the 4 TMA cores taken from each tumour. An overall score for each case was also given. Missing or un-assessable cores were also recorded. Scoring was based upon tumour cell membrane staining of any intensity and a minimum of 100 tumour cells were required to render a recorded score. Estimated actual tumour

cell proportion scores were recorded, normally to the nearest 10%, or a figure below 10% as appropriate. This allowed several thresholds (cut-offs) of PD-L1 tumour cell expression to be analysed. PD-L1 scores were electronically recorded on a standard proforma and returned to the ETOP Lungscape coordinating office where data were collated.

2.2. Statistical analysis

PD-L1 positivity prevalence is expressed as a percentage with a corresponding 95% exact binomial confidence interval (CI). Positivity cut-offs of 1%, 5%, 25% and 50% are considered.

Differences in clinicopathological characteristics by PD-L1 status are examined via Fisher's exact, Chi-square, Mantel-Haenszel or Mann-Whitney tests. Association of PD-L1 positivity with MET (clone SP44), ALK (clone 5A4), PTEN IHC (clone SP218), as well as several gene mutations is evaluated through Fisher's exact tests, overall and by histology subtype. These molecular data have been published elsewhere [12,15,16,17]. Assessment of homogeneity of the odds-ratios between molecular profiles and PD-L1 at each histology group was performed via the Breslow-Day statistic.

Clinical outcome is presented by relapse-free survival (RFS, time from surgery date to first relapse or death from any cause), overall survival (OS, time from surgery date to death from any cause); and time-to-relapse (TTR, time from surgery date to first relapse). If the corresponding event is not observed, the censoring date is the last day of follow-up. Observed differences in hazard are assessed using the log-rank test and are graphically depicted by Kaplan Meier curves. Follow-up time refers to time between enrollment and last contact date. The reverse censoring method is implemented in the median follow-up estimation.

PD-L1 effect on outcome is explored through Cox proportional hazard models, adjusting for clinicopathological variables of interest (gender, ethnicity, smoking history, age, adjuvant chemotherapy/radiotherapy, previous cancer history, performance status at diagnosis, stage, primary tumor localization, tumor size, histology, surgery year, technique and anatomy). To obtain the final models with significant outcome prognostic factors and corresponding hazard ratios (HRs) along with their 95% CIs, the backwards elimination method (removal $p \geq 0.10$) is used.

In all exploratory analyses, results with two-sided p-value ≤ 0.05 are considered significant. Analyses are performed overall and separately for the two primary histology groups: adenocarcinomas and squamous cell carcinomas.

Statistical analyses were carried out in SAS version 9.4 (SAS Institute, Cary, NC) and performed at the ETOP statistical center, FrontierScience Foundation-Hellas, Athens, Greece.

3. Results

3.1. Analysis cohorts

A total of 2402 retrospective cases from 15 centers have been captured in the ETOP iBiobank as of 16th November 2016. PD-L1 scores from TMAs is available for 2182 cases. This study cohort consists primarily of patients of Caucasian ethnicity (99.2%), males (64.1%), and former smokers (53.7%); with median age 66.3 years. Adenocarcinomas comprised 50.9% of the cohort; squamous cell carcinomas 42.4%. Further details of clinicopathological characteristics of the cohort, overall and for the two primary histologies are presented in Table 1 and Table S1.

As expected, significant clinicopathological differences between adenocarcinomas and squamous cell carcinomas are detected. The squamous cell cohort includes more male patients, larger tumors, fewer never smokers, higher tumor stages and more pneumonectomies.

Median follow-up time for Lungscape PD-L1 cohort is 4.8 years (inter-quartile range (IQR): 3.7–6.2).

3.2. PD-L1 positivity prevalence in unselected NSCLC stage I-III tumor, overall and according to specific histological subtypes and stage

Out of the 2182 cases with non-missing PD-L1 TMA results, 2008 are evaluable ("Positive", "Negative"), while 174 are classified as "Not Evaluable". Using the 1% cut-off, PD-L1 positivity prevalence is estimated to be 43.4% (871/2008, 95% CI: [41.2, 45.6]). The respective results for 5%, 25% and 50% cut-offs are: 34.0% (683/2008, 95% CI: [31.9, 36.1]), 22.6%, 95% CI [20.8, 24.5] and 16.6% (334/2008, 95% CI: [15.0, 18.3]). Respective results by histological subtype are presented in Fig. 1A. No significant difference between adenocarcinoma and squamous cell histological subtypes is detected, for all cut-offs (Fig. 1A; Table S2). A significant association is detected between stage and PD-L1 status (for 1%, 5% and 25% cut-offs, $p = 0.048$, $p = 0.022$ and $p = 0.0077$ respectively), with PD-L1 positivity being more frequent in higher stages (Fig. 1B; Table 2; Table S2).

3.3. Baseline characteristics by PD-L1 status

PD-L1 positivity (1%, 5% and 25% cut-offs, but not 50%) is more frequently detected in never smokers, higher stages and in larger tumors (Table 2; Table S2). These associations are no longer significant when focusing on the adenocarcinoma cohort (any cut-off considered). With respect to squamous cell cohort, a significant association is detected only between PD-L1 status and smoking history for 1%, 5% and 25% cut-offs (respective p-values: < 0.001, 0.012, 0.0023), but not for 50%.

3.4. Association of PD-L1 positivity with molecular profiles

PD-L1 positivity (any cut-off considered) is more frequent in MET IHC positive cases overall as well as separately for adenocarcinomas and squamous cell carcinomas (all $p < 0.001$, Table 2, Table S3); to illustrate, for 1% cut-off, overall result of PD-L1 positivity: 60.9% in MET IHC positive vs. 38.0% in MET IHC negative, for adenocarcinomas:

Table 1
Clinicopathological characteristics of the PD-L1 Lungscape cohort, overall (N = 2182) and for adenocarcinoma (n = 1111) and squamous cell (n = 926) histology.

Characteristic	All patients (N = 2182)	Adenocarcinoma (n = 1111)	Squamous cell (n = 926)	p-value*
Gender – n (%)				
Male	1398 (64.1)	589 (53.0)	727 (78.5)	< 0.001
Female	784 (35.9)	522 (47.0)	199 (21.5)	
Smoking history – n (%)				
Current	689 (31.6)	350 (31.5)	296 (32.0)	< 0.001
Former	1171 (53.7)	558 (50.2)	531 (57.3)	
Never	230 (10.5)	157 (14.1)	64 (6.9)	
Unknown	92 (4.2)	46 (4.1)	35 (3.8)	
Stage (7th TNM classification) – n (%)				
Ia	526 (24.1)	311 (28.0)	186 (20.1)	0.0048
Ib	540 (24.7)	289 (26.0)	218 (23.5)	0.0066[f]
IIa	362 (16.6)	155 (14.0)	182 (19.7)	
IIb	268 (12.3)	108 (9.7)	140 (15.1)	
IIIa	446 (20.4)	231 (20.8)	182 (19.7)	
IIIb	40 (1.8)	17 (1.5)	18 (1.9)	
Histology- n(%)				
Adenocarcinoma	1111 (50.9)			
Squamous cell	926 (42.4)			
Large cell	76 (3.5)			
Adeno-squamous	36 (1.6)			
Combined-Mixed	27 (1.2)			
Sarcomatoid	6 (0.3)			

* Chi-square or Fisher's exact test for categorical variables, Mantel-Haenszel test for ordinal variables, (f) Categories Ia & Ib to I, IIa & IIb to II and IIIa & IIIb to III.

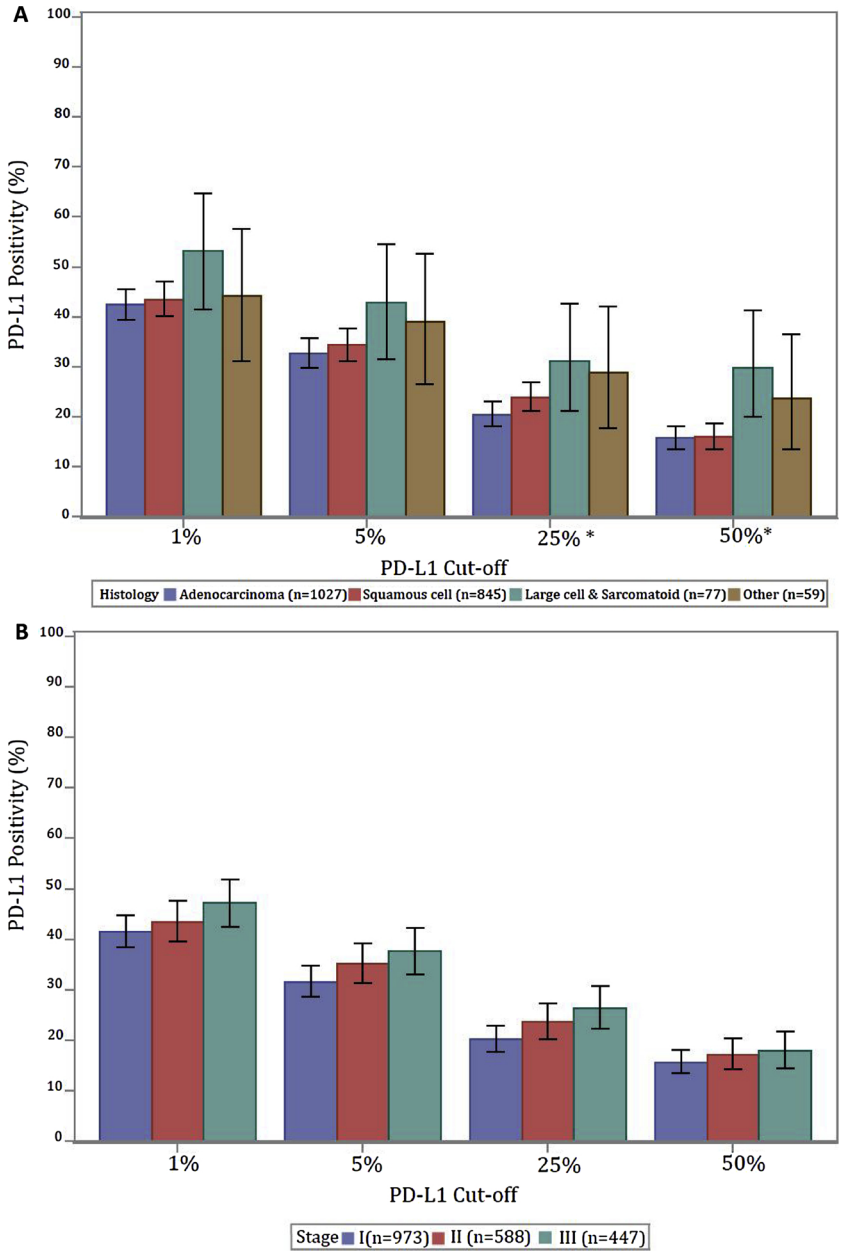

Fig. 1. A: Prevalence (%) of PD-L1 positive expression by histological subtype.
Note: A significant result is obtained for 25% and 50% cutoffs, between PD-L1 status and Histology. Respective p-values: 0.047 and 0.0083. B: Prevalence (%) of PD-L1 positive expression by stage category

60.8% vs. 33.2%, for squamous-cell carcinomas: 57.0% vs. 41.6%.

A significant relation is also observed between PTEN IHC status and PD-L1 (1%, 5% and 25% but not 50% cut-off), with PD-L1 positivity being more frequent in the PTEN No Loss subgroup; indicatively for 1% cut-off the PD-L1 positivity is 46.7% within the PTEN No Loss subgroup vs. 41.2% within the PTEN loss group (Table 2, Table S4). This association remains significant for specific cut-offs for the adenocarcinomas alone (1%, 5%) and the squamous-cell carcinomas (only for 25% cut-off).

Detailed results for KRAS and EGFR genes are presented in supplement (Tables S5-8). Neither PIK3CA, nor MET gene mutation are significantly associated with PD-L1. Association between PD-L1 positivity status and ALK IHC is examined only for adenocarcinoma patients, with no significant result found (for all four cut-offs all p-values NS; data not shown).

3.5. Clinical outcome, overall and by PD-L1 status

Almost half of the patients are alive and disease free at their last follow-up (47.8%), while 32.3% of patients died due to disease. A total of 1135 (52.0%) RFS events, 849 (38.9%) TTR events and 991 deaths (45.4%) have been recorded. For the entire study cohort, no significant effect of PD-L1 on RFS, TTR and OS is found (univariate log-rank p-values NS, 1% cut off: Figures S1-2 and Fig. 2). Inference did not alter when adjusting for histology (stratified log-rank test p: 0.28, 0.27 and 0.31 respectively) or when considering alternative positivity cut-offs.

PD-L1 (1%) status is, however, a significant predictor of RFS, TTR and OS, for adenocarcinoma histology patients, when adjusting for age, performance status, stage tumor size, surgery anatomy, gender and ethnicity (adjusted p-values for 1% cut-off: 0.024, 0.064, 0.063; Fig. 3A; Table 2; Table S9). The corresponding hazard ratio (HR) estimations are: $HR_{RFS\ +\ vs.\ -} = 0.82$; 95% CI [0.69, 0.97], $HR_{TTR\ +\ vs.\ -} = 0.83$;

Table 2
Summary of significant results for the PD-L1 positivity.

	Cut-off point for PD-L1 positivity			
	≥1%	5 ≥ %	≥25%	≥50%
Prevalence of PD-L1 positive cases	43.4%	34.0%	22.6%	16.6%
More PD-L1 'positive' cases in the following groups:				
Higher stage; p[¥] =	0.048	0.022	0.0077	0.28
% PD-L1+ in I/II/III	41.5/ 43.5/47.2	31.7/ 35.2/37.6	20.2/23.6/ 26.4	–
Larger tumour; p[^] =	0.025	0.044	0.038	0.40
Median size (cm) in PD-L1 - vs +	3.5-3.6	3.5-3.7	3.5-3.8	–
Never smoker; p[*] =	0.013	0.055	0.019	0.19
% PD-L1+ in Cur/For/Nev	39.6/ 45.1/50.2	31.6/ 34.8/40.6	19.6/23.4/ 28.6	–
High MET IHC; p[*] =	< 0.001	< 0.001	< 0.001	< 0.001
% PD-L1+ in MET +/-	60.9/38.0	52.9/28.2	35.2/18.7	28.1/13.1
No PTEN loss (IHC); p[*] =	0.019	0.0052	0.031	0.062
% PD-L1+ in Loss/No Loss	41.2/46.7	31.0/37.2	20.5/24.8	–
KRAS mutation (all histologies)	0.092	0.086	0.045	0.064
% PD-L1+ in MD vs MND	–	–	26.0/21.2	–
KRAS mutation (adenocarcinomas)	0.022	0.055	0.017	0.17
% PD-L1+ in MD vs MND	45.5/37.5	33.9/27.6	23.9/17.2	–
Post-operative survival in Adenocarcinoma (adjusted Cox models)				
RFS; p =	0.024	0.023	0.076	0.36
$HR_{+ vs -}$ (95% CI)	0.82 (0.69, 0.97)	0.80 (0.67, 0.97)	0.82 (0.66,1.02)	–
TTR; p =	0.064	0.038	0.30	0.72
$HR_{+ vs -}$ (95% CI)	0.83 (0.68, 1.01)	0.80 (0.65, 0.99)	–	–
OS; p =	0.063	0.013	0.031	0.20
$HR_{+ vs -}$ (95% CI)	0.83 (0.69, 1.01)	0.77 (0.63, 0.95)	0.77 (0.60,0.98)	–

MD: Mutation detected, MND: Mutation not detected, HR: Hazard ratio.
(^): p-value based on Fisher's exact test; (¥): p-value based on Mantel-Haenszel test; (^): p-value based on Mann-Whitney test.

95%CI [0.68–1.01] and $HR_{OS\ +\ vs.\ -}$ = 0.83; 95%CI [0.69–1.01], indicating lower hazard for PD-L1 positive patients. This effect is preserved when considering the 5% and 25% cut-off (except for TTR with 25%) but not the 50% cut-off. Similar significant results are obtained for the overall model (including all histologies; Fig. 3B; Table S10), while no effect of PD-L1 (all cut-offs) on outcome is found for the squamous cell cohort.

The monotonicity of PD-L1 prognostic effect is further explored with the use of a three-level PD-L1 categorization (negative [< 1%], 1–25% and ≥25%). The respective adjusted Cox results for adenocarcinoma cohort and overall are presented in supplemental Tables S11-S12. In the adenocarcinoma cohort, for RFS and OS endpoints, the group of patients with PD-L1 expression ≥25% exhibits significantly better survival compared to PD-L1 negative (< 1%) patients: $HR_{RFS\ \{\geq 25\%\ vs.\ <\ 1\%\}}$ = 0.79; 95% CI [0.63, 0.98]; p = 0.035, and $HR_{OS\ \{\geq 25\%\ vs.\ <\ 1\%\}}$ = 0.75; 95%CI [0.58-0.96]; p = 0.024.

4. Discussion

This study represents the largest cohort of cases so far published, stained for PD-L1, with associations described for clinical and molecular features and post-operative outcomes. Details of the post-operative outcomes and their association with clinicopathological parameters and other molecular findings have already been published [11,12,15,16]. These findings were largely in keeping with published literature, although the Lungscape cohort did not show any correlation with post-operative outcomes and any of the major molecular findings in the patients' tumours [12]. All staining was carried out using the trial-validated Dako 28-8 assay, which should assure consistent staining performance, and all pathologists were trained in reading PD-L1 IHC. Experience from the IASLC Blueprint project would suggest that this IHC assay is reliable and technically equivalent to two other commercial assays frequently used in clinical practice (the Dako 22C3 assay and the Ventana SP263 assay), and that inter-observer variability is low after training [18]. The data generated in this study are therefore likely to be robust, and relevant to more general current PD-L1 clinical practice.

Our finding of 56.6% of cases PD-L1 negative or < 1% expression and 16.6% expressing PD-L1 in over half of tumour cells is in line with previously published studies of surgically resected NSCLC [6,8,9]. Several published studies found fewer positive cases at all cut-off definitions but comparison is hampered by the widespread use of non-standardized, laboratory-developed PD-L1 IHC assays (LDTs) which almost inevitably will NOT show comparable performance to our trial-validated commercial assay.

Greater PD-L1 expression in more advanced surgically resected disease has been shown before [4,19], and in Stage 4 clinical cohorts, more cases tend to be in the > 50% cohort determined by the same or similar assays [20–22]. It is not clear why more advanced disease may be more likely to express more PD-L1. More advanced disease may be more antigenically diverse, eliciting an immune response, which becomes negated by negative immune checkpoint regulation as disease advances.

The literature shows no consensus on an association between smoking status and PD-L1 expression [23–28]. Again, data are confounded by heterogeneity of staining techniques and definitions of positivity. Given the known association between smoking history and response to anti-PD1 therapy [20–22], a positive association between smoking history and more PD-L1 expression might be expected. However, the latter cases were advanced disease and may not be comparable with our surgically resected cohort.

Several studies have also described a positive association between upregulation or abnormal expression of MET, other evidence of tumour epithelial-mesenchymal transition (EMT) or sarcomatoid NSCLC histology [29–31]. It is conceivable that such biological transformation could be associated with greater tumour immunogenicity and therefore adaptive upregulation of PD-L1. A similar argument could be made with respect to some of our findings with KRAS mutation. The unknown factor in this discussion is the possible contribution, in some cases, of intrinsic induction of PD-L1 upregulation driven by oncogenic signalling. This has been related to activity driven by JAK/STAT and PI3K signalling [10,32]. We found no correlation between PI3K mutations and PD-L1 expression, but given the possibility of signalling pathway crosstalk, single static gene status in individual tumours may not relate to pathway function. The negative correlation between PTEN loss and PD-L1 positivity is counter-intuitive to this argument, albeit that the PD-L1 expression differences were small. High levels of PD-L1 in a tumour may not necessarily reflect immunologically active ligand, suppressing an immune response. The identification of immunologically-relevant PD-L1 expression is clearly important but cannot be inferred from our data.

Several papers have described associations between PD-L1 expression, measured and defined in variable ways, and post-operative survival. Rather more literature favoured high PD-L1 expression as a poor prognostic factor, and this was supported by a meta-analysis of, then available, studies, all with relatively small case numbers [5–9,27,33–38]. Intuitively this seems logical, since immunologically active PD-L1 would negate anti-tumour immunity and promote tumour development. However, one of the largest studies published prior to this ETOP cohort, of 678 NSCLC cases [9], found that higher PD-L1 expression was associated with better post-operative outcomes. This study used a different PD-L1 IHC assay (a 22C3-clone based laboratory developed test) and defined 'high' PD-L1 as > 50% tumour cells positive,

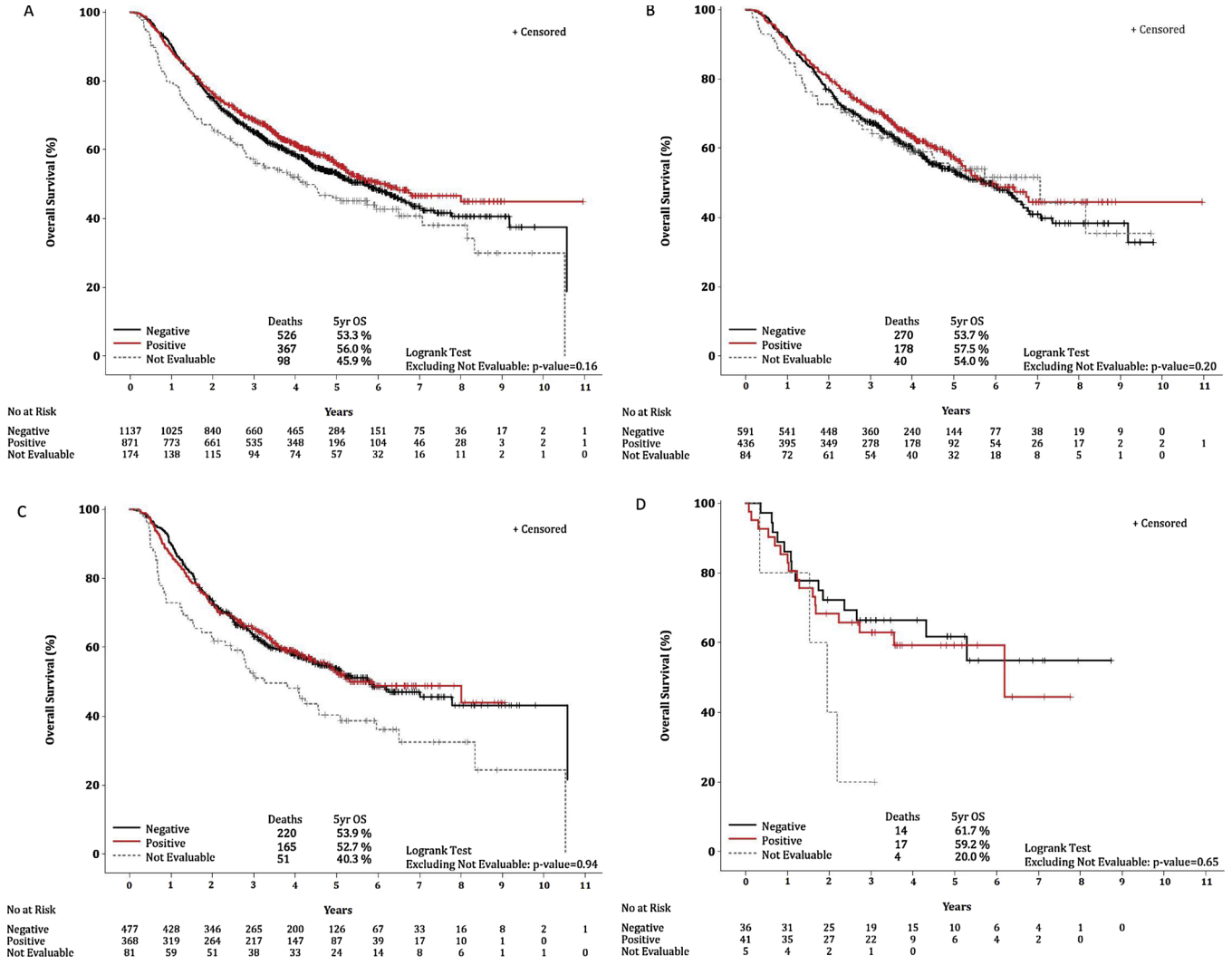

Fig. 2. A: Overall survival of PD-L1 status (1%) for all histologies.
Note: Not evaluable result based on a small subset of the total cohort (< 8.0%). B: Overall survival of PD-L1 status (1%) for adenocarcinoma histology. C: Overall survival of PD-L1 status (1%) for squamous cell histology. D: Overall survival of PD-L1 status (1%) for large cell and sarcomatoid histology (combined)

but also by H-score. Furthermore, this study found their overall positive association between higher PD-L1 expression and better post-operative outcome was preserved in their squamous and non-adenocarcinoma subgroups but not for adenocarcinomas, the opposite of our finding. It is much harder to rationalize why high PD-L1 expression may be a better prognostic factor, at least in adenocarcinoma, especially since it is associated with several clinicopathological features which are known to be poor prognostic features (higher stage, larger tumours, EMT, higher histopathological grade in adenocarcinoma etc) [23–31,33–38]. If this were a true biological effect, we would expect the finding to be preserved, and even stronger, when defining positivity by higher cut-offs such as 50%. This was not the case in our study. We note that the unadjusted for multiple-comparison p-values are reported, which if adjusted (for multiplicity) will not remain significant. The explanation for the effect noted in our adenocarcinoma cohort defined by > 1, 5% and > 25% expression cut-offs, and the difference between cohorts < 1% versus > 25% is elusive. It is conceivable, although purely speculative, that, in cohorts of patients defined by higher levels of PD-L1 there are relatively more cases where high PD-L1 is the result of intrinsic, oncogene-driven induction, and not adaptive immunity. This could represent a subset in the higher PD-L1 expressing cohort where PD-L1 expression is not immunologically active and therefore less likely to influence prognosis. In previous studies where data are available [6,8,9,27,33,34,37,38], half used whole sections and half used TMAs for analysis. A poor prognosis for PD-L1 'high' status was the conclusion in three of four studies in each case. The use of TMA, as in our study, with greater potential for variance in PD-L1 scoring due to sampling error, is unlikely to be an explanation for our findings, especially since our TMAs used four sample cores per tumour. This level of sampling has recently been shown to help offset sampling error due to heterogeneity of PD-L1 expression in NSCLC [39].

In conclusion, this is the largest study so far reported, relating PD-L1 IHC expression to various clinicopathological features of resected NSCLC. Several of the molecular associations are biologically plausible and, in some instances, in line with previously published work. The limited and questionable association between PD-L1 expression and relatively good post-operative outcome may not be a true biological effect.

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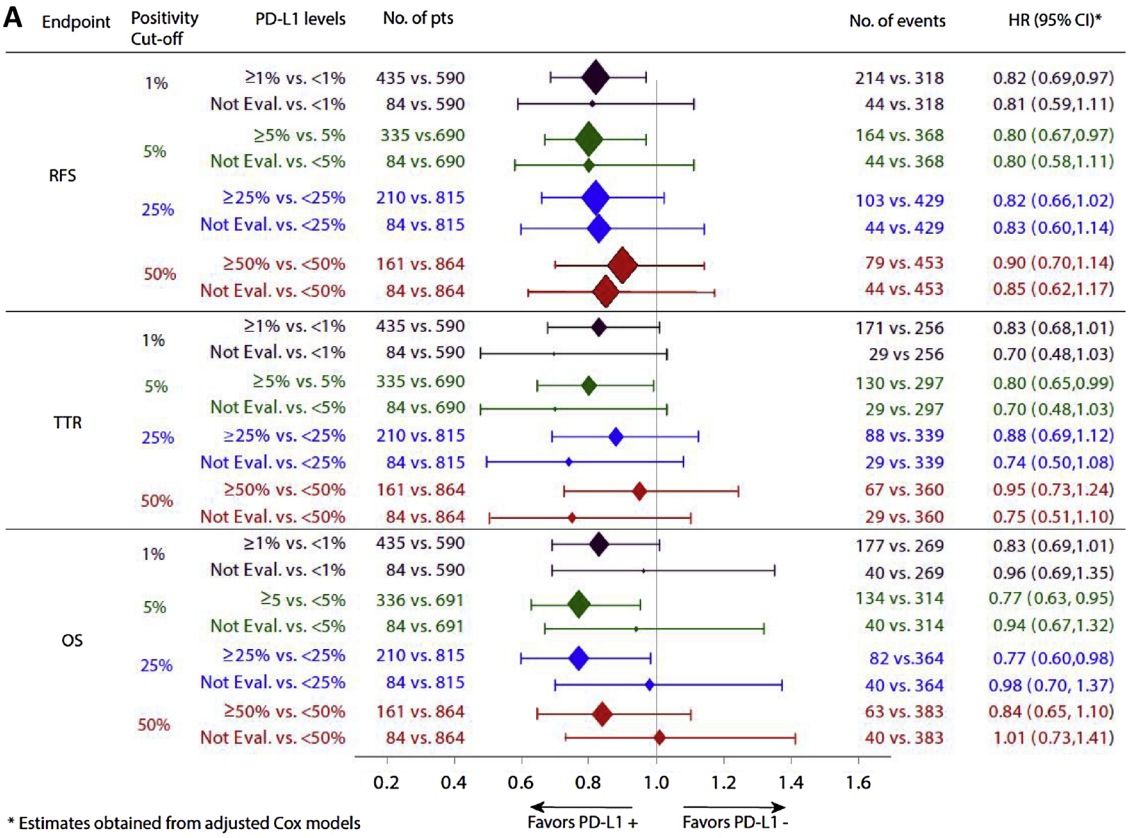

Fig. 3. A: Forest plot depicting PD-L1 (1%, 5%, 25% and 50% positivity cut-offs) effect on RFS, TTR, OS, adenocarcinoma patients only. B: Forest plot depicting PD-L1 (1%, 5%, 25% and 50% positivity cut-offs) effect on RFS, TTR, OS, all patients.

Conflict of interests

None to declare.

Appendix A

Lungscape 005 PD-L1

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Appendix B. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.lungcan.2019.03.012.

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