

Prognostic implication of PD-L1 in early-stage non-small cell lung cancer: a retrospective single-centre study

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Summary

BACKGROUND: The prognostic role of programmed death-ligand 1 (PD-L1) expression in patients with localised and locally advanced non-small cell lung cancer has not been fully elucidated. This information could help to better interpret recent and upcoming results of phase III adjuvant or neoadjuvant anti-PD-1/PD-L1 immunotherapy studies.

METHODS: In a cohort of 146 patients with early or locally advanced non-small cell lung cancer treated with curative intent (by surgery or radiotherapy), we investigated the prognostic value of PD-L1 expression and its correlation with other biological and clinical features. PD-L1 expression was stratified by quartiles. Primary endpoints were overall and disease-free survival. We also analysed the prognostic impact of the presence of actionable mutations, implemented treatment modality and completion of the treatment plan. Neither type of patient received neoadjuvant or adjuvant immunotherapy or target therapy.

RESULTS: Of the 146 selected patients, 32 (21.9%) presented disease progression and 15 died (10.3%) at a median follow-up of 20 months. In a univariable analysis, PD-L1 expression $\geq 25\%$ was associated with significantly lower disease-free survival (hazard ratio [HR] 1.9, 95% confidence interval [CI] 1.0–3.9, $p = 0.049$). PD-L1 expression $\geq 50\%$ did not lead to disease-free survival or over-all survival benefits (HR 1.2 and 1.1, respectively; 95% CI 0.6–2.6 and 0.3–3.4, respectively; *not significant*). In a multivariate analysis, a stage $>I$ (HR 2.7, 95% CI 1.2–6, $p = 0.012$) and having an inoperable tumour (HR 3.2, 95%CI 1.4–7.4, $p = 0.005$) were associated with lower disease-free survival.

CONCLUSION: The population of patients with early-stage non-small cell lung cancer and PD-L1 expression $\geq 25\%$ who were treated with curative intent during the pre-immunotherapy era exhibited a worse prognosis. This finding provides justification for the utilisation of adjuvant immunotherapy in this subgroup of patients, based on the current evidence derived from disease-free survival outcomes. However, for patients with PD-L1 expression

$<25\%$, opting to wait for the availability of the overall survival results may be a prudent choice.

Introduction

Immune-checkpoint inhibitors, such as anti-programmed death-1 (anti-PD-1) and anti-programmed death-ligand 1 (anti-PD-L1) monoclonal antibodies, either alone or in combination with chemotherapy, are the backbone of modern treatment of metastatic non-small cell lung cancer that does not carry actionable mutations (such as EGFR, ALK, ROS1, BRAF or MET). These treatments have led to response and survival rates that were unattainable just a few years ago [1, 2]. Patients whose tumours express PD-L1 in at least 50% of cells are more likely to respond to compounds such as pembrolizumab, nivolumab or atezolizumab and to survive longer [3, 4]. Therefore, the prognostic value of PD-L1 in advanced disease depends on the availability of immunotherapy.

While the prognostic implication of PD-L1 tumour expression is well established in the metastatic setting, its significance remains unclear for stage I to III non-small cell lung cancer treated with curative intent [5–18]. In fact, the use of PD-1 or PD-L1 inhibitors in a neoadjuvant or adjuvant setting has been restricted to studies that are still in the late research phase or have only just begun publishing very early results [19, 20].

Thus, the prognostic and predictive significance of PD-L1 expression in the early-stage setting is currently receiving more attention, but the available information is limited [21].

Studies from the past decade have reported contradictory results, likely due to methodological flaws, such as lack of reproducibility of the PD-L1 expression assays, heterogeneity of disease stages, timing of specimen collection, inclusion of cases with actionable genetic alterations and heterogenous therapeutic interventions [14, 22, 23].

Several studies conducted in the pre-immunotherapy era, primarily in Asian countries, reported a negative correlation between increasing PD-L1 expression levels and survival in the early-stage setting [7–9, 24–27]. Contradictory

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results were observed among Caucasian patients, as some studies reported a positive correlation between increasing PD-L1 expression levels and survival [12–14].

The aim of this retrospective study was to investigate the prognostic significance of PD-L1 tumour expression in a homogeneous Swiss population of patients with non-small cell lung cancer in the early stages (I to III according to the TNM lung cancer staging system, 8th edition). PD-L1 was assessed using a standardised assay, and all cases were treated with curative intent, either with surgery, radiation therapy (including stereotactic body radiation therapy (SBRT) or conventional radiotherapy), chemoradiation or a combination of these modalities, in accordance with current standards [28, 29].

This information could be very useful in helping clinicians decide whether it is appropriate to include in therapeutic standards adjuvant treatment with atezolizumab or neoadjuvant treatment with nivolumab, based on the results of the IMpower010 and CheckMate 816 studies, respectively. Both studies showed clear benefits, in terms of disease-free survival in the first case and event-free survival in the second, especially for patients whose tumours had PD-L1 expression $\geq 50\%$ [19, 20]. Nevertheless, these results need to be explored further to establish the effect of these two immune-oncology strategies on overall survival.

Patients and methods

This study was conducted according to the STROBE (STrengthening the Reporting of OBservational studies in Epidemiology) Statement guidelines for observational studies [30].

Patient selection

Patients had to fulfil the following inclusion criteria: (a) diagnosis of adenocarcinoma, squamous cell carcinoma or undifferentiated carcinoma subtypes of the lung (neuroendocrine tumours, pure or mixed, were excluded) and (b)

resectable disease, stage I to III (TNM lung cancer staging system, 8th edition), as per a thoracic surgeon's assessment. In addition, patients had to have initiated a treatment with curative intent, with the main treatment method considered to be surgery, ablative radiotherapy or a multimodal approach. Completion of an adjuvant or neoadjuvant chemotherapy, according to current international standards, was not an inclusion criterion, but this information was thoroughly recorded to allow for its inclusion in the analysis. Tumour samples were assessed for PD-L1 expression and genetic aberrations.

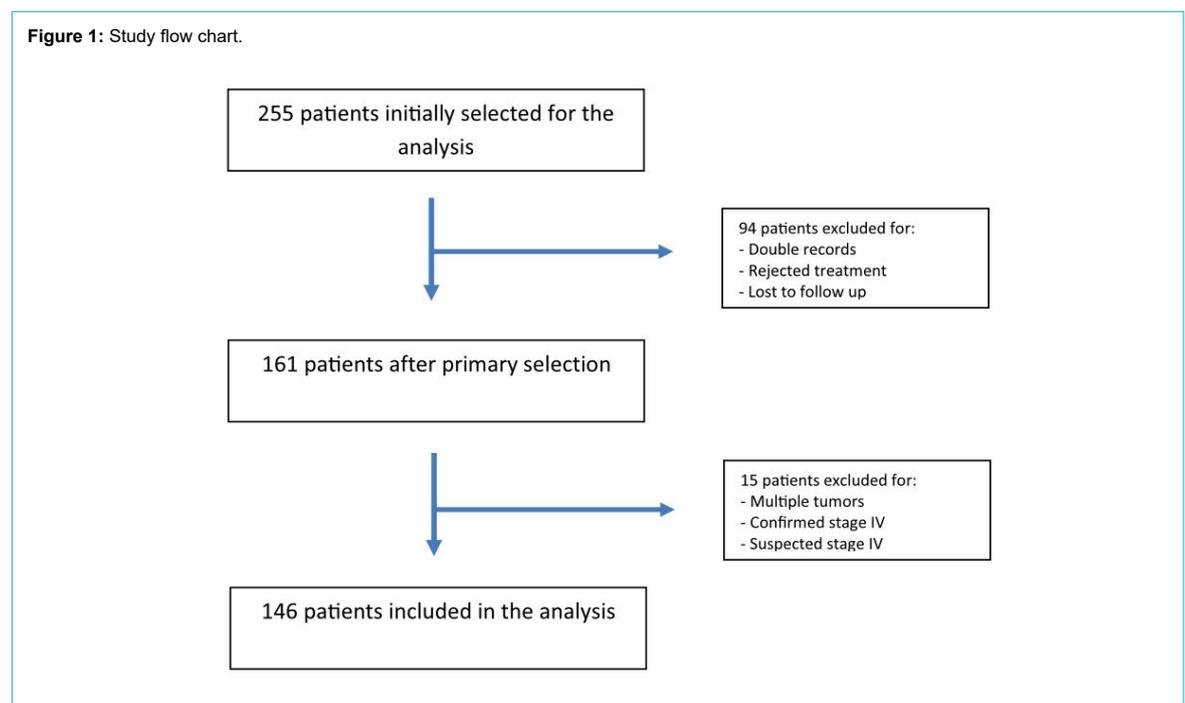
We retrieved data on 255 patients with localised, technically resectable non-small cell lung cancer who were treated with curative intent at the Oncology Institute of Southern Switzerland (IOSI) and/or at the Thoracic Surgery Department of Ente Ospedaliero Cantonale (EOC) in Canton Ticino, Switzerland. Among them, 146 patients, who were treated between 2016 and 2019, met all inclusion criteria and were included in the present analysis (figure 1). Histological or cytological diagnosis of non-small cell lung cancer was obtained for all patients either preoperatively, by transbronchial or transthoracic biopsy, or intraoperatively, with wedge resection and frozen section. PD-L1 expression and molecular characterisation were determined before any systemic treatment began.

Data collection and selection of variables

Clinical and pathological data were obtained from institutional medical records. Follow-up visits were scheduled according to our local policy, which is based on current international guidelines [31]. Participants were censored at the time of study completion.

We analysed the following variables: PD-L1 status at different levels of protein expression, gender, smoking status, completeness of the therapeutic plan, presence of driver mutations, histopathological subtypes, grade of differentiation, disease stage at diagnosis, systemic treatment at re-

Figure 1: Study flow chart.



lapse and type of treatment (i.e., tyrosine-kinase inhibitors, immunotherapy or chemotherapy). In addition, we evaluated other clinic-pathological prognostic factors, such as actionable alterations (EGFR, KRAS, BRAF, MET or HER-2 mutations or ALK, ROS1, NTRK or RET gene fusions), completeness of the treatment plan and the main modality of treatment received.

Assessment of PD-L1 expression

The immunohistochemical evaluation of PD-L1 was performed at the Institute of Pathology, EOC in Locarno, Switzerland, using the SP263 monoclonal rabbit anti-human antibody (Ventana/Roche, Ventana Medical Systems) on an automated instrument (Benchmark GX, Ventana/Roche) according to the standard protocol. The tumour proportion score (TPS) was used [32].

Assessment of genomic alterations

The presence of druggable genomic alterations was assessed in tumour biopsies from 107 of 109 cases with non-squamous histology, while no molecular analysis was performed on squamous cell tumours. Genomic DNA extraction was performed at the Institute of Pathology, EOC, Locarno, Switzerland, using the QIAamp DNA FFPE Tissue Kit (Qiagen, Chatsworth, CA, USA) and starting from three 8- μ m-thick serial sections of the selected FFPE tissue block. The extracted DNA was molecularly characterised using a next-generation sequencing approach. We employed the S5XL Ion Torrent (IOT) platform and used the Ion AmpliSeq Colon and Lung Cancer Panel v2 (Thermo Fisher Scientific, Waltham, MA, USA), which determines the mutational status of 22 genes, including those most relevant and most frequently mutated in non-small cell lung cancer (i.e., *EGFR*, *KRAS*, *BRAF* and *HER-2*).

IOT results were considered usable only when the target regions were covered by ≥ 300 reads, the depth values were $>2000\times$ and the uniformity was $>90\%$. The limit of detection of the variant allele frequencies was set between 2% and 5%. All the mutations demonstrated in the literature to be polymorphic and all the variants in intronic regions were excluded.

The presence of chromosomal alterations (gene fusions) in the ALK gene was determined using a fluorescence *in situ* hybridisation (FISH) assay on 4- μ m FFPE tissue sections treated with a Paraffin Pretreatment Kit II (Pretreatment Reagent VP2000, Abbott Molecular AG, Baar, Switzerland) and processed with the VP2000 automatic processor (Abbott Molecular AG) according to the manufacturer's instructions, using the ALK Break Apart FISH Probe Kit (Abbott Molecular Vysis, North Chicago, IL, USA). We evaluated FISH results following published criteria [33–35]. For FISH analyses, a minimum of 100 morphologically clear, non-overlapping nuclei from at least 8–10 areas were scored for each patient. Only experiments with $\geq 90\%$ hybridisation efficiency were considered. In order to avoid false positive/negative results, a cut-off of 15% was applied for considering a tissue positive for ALK gene fusion.

Sample size considerations and statistical methods

We related predefined outcome measures, overall survival and disease-free survival, to different levels of PD-L1 expression. Overall survival was defined as the time between the beginning of treatment and death from any cause, and disease-free survival was defined as the time between the beginning of treatment and disease relapse or death from any cause. The sample size was not predefined. However, with the currently expected 5-year overall survival rate of early or locally advanced non-small cell lung cancer ranging from 35–63% [36], our sample size of 146 patients would have a power of 88.95% to estimate a survival change from 50–63% with alpha set at 0.05 (two-sided test). Survival probabilities were calculated using the life table method, and survival curves were estimated using the Kaplan-Meier method; differences between curves were analysed using the log-rank test. Follow-up was calculated as median time to censoring using the reverse Kaplan-Meier estimator (i.e., by flipping the meaning of event (i.e., death) and censor of the standard Kaplan-Meier survival curve) [37]. Binomial exact 95% confidence intervals (95% CIs) were calculated for incidence rates. Continuous variables were expressed as median and interquartile range, and differences between groups were compared using the Mann-Whitney U test. Fisher's exact test was used to test for differences between frequencies of categorical data, as appropriate. Hazard ratios (HRs) and their 95% CIs in both univariable and multivariable analyses of prognostic factors were estimated using a Cox proportional hazards model. Multivariable analysis of clinical prognostic factors affecting either overall survival or disease-free survival was performed by a backward stepwise Cox regression starting from the variables with a significant impact on outcomes in the univariable analyses. The validity of the proportional hazard assumption was confirmed using Schoenfeld residuals, and the overall statistical significance of the Cox model was assessed using a likelihood-ratio test. P-values ≤ 0.05 (two-sided test) were considered to indicate statistical significance. Statistical analyses were conducted using STATA 16.1 statistical software (Stata Corp., College Station, TX, USA).

Ethical approval and consent to participate

The study was conducted in accordance with the Declaration of Helsinki, and the protocol was approved by the Ethics Committee of Canton Ticino (ProjectID 2021-01392).

Results

Patient characteristics

A total of 146 patients treated between 2016 and 2019 met all the inclusion criteria and were included in the analysis. Patient demographic and clinical characteristics are summarised in table 1. Cases were distributed according to the pathologic disease stage (or the clinical staging in cases of no surgery) as follows: *stage I*, 56.2%; *stage II*, 25.3% and resectable *stage III*, 18.5%. The percentages of male and female patients were 54.8% and 45.2%, respectively. Median age at diagnosis was 70 years (range 47–84). In terms of histology subtype, 23.3% of patients had a *squa-*

mous cell variant, 74.7% were classified as *adenocarcinoma* and 2% had *undifferentiated* histology. Data on tobacco use were available for 140 patients: 47.1% of patients were *current smokers*, 44.3% were *former smokers* and the remaining 8.6% of patients had *never smoked*. Patients received *surgery* (90%) or *radiotherapy* (10%) as the main upfront treatment. Treatments were classified as *complete* or *incomplete*, according to following standards: stage I, radical surgery; stage II, radical surgery and adjuvant chemotherapy; stage III, either radical surgery and adjuvant chemotherapy ± mediastinal radiation or neoadjuvant chemoradiation followed by radical resection or definitive chemoradiation. According to this rule, 72% of patients completed their treatment, and 28% did not, including 3 patients (2%) allocated to neoadjuvant chemotherapy who could not proceed to surgery. Information on presence or absence of driver mutations was available in 73% of cases.

Treatments for stages I and II were registered as *standard* (surgery) in 90.9% of the cases and *non-standard* (ablative radiation) in 9.1% of cases. PD-L1 expression status was available for 129 patients and was reported as following ranges: TPS <1% (44.2%), TPS 1–24% (27.9%), TPS 25–49% (7%), TPS 50–74% (13.2%) and TPS ≥75% (7.4%). In terms of cumulative frequencies, we found pos-

itive PD-L1 expression (>1%) in 55.8% of patients and strong positive PD-L1 expression (≥50%) in 20.9% (table 2). In terms of histology subtypes, positive PD-L1 expression was observed in 75% of squamous cell carcinomas and 47.8% of adenocarcinomas.

The number of patients with druggable genetic alterations was small; therefore, we could not perform a meaningful analysis. Classical epidermal growth factor receptor (EGFR) alterations, either exon 19 deletions or exon 21 point mutations (p.L858R), were present in 12.2% of patients; BRAF-V600 mutations in 1.87% and ALK rearrangements in 1.9%. KRAS mutations (including the G12C subtype, considered non-druggable at the time of data collection) were present in 38.7% of cases. TP53 inactivation was documented in 35.8% of patients. Other, less frequent genetic alterations are detailed in figure 2.

Survival outcomes

At a median follow-up time of 20 months (interquartile range 11–32), 32 patients (22%) experienced disease progression: 19 of them (61%) had a local progression, 7 had a distant relapse (23%) and 5 had both local and systemic disease progression. The median disease-free survival in

Table 1:
Baseline clinical characteristics.

Feature		n (%)
Age	<65 years	44 (30%)
	65–75 years	66 (45%)
	>75 years	36 (25%)
Sex	Male	80 (55%)
	Female	66 (45%)
Histologic type	Squamous	34 (23%)
	Non-squamous (adenocarcinoma)	109 (75%)
	Undifferentiated	3 (2%)
T stage	0 (minimally invasive adenocarcinoma)	2 (1%)
	1	63 (43%)
	2	47 (32%)
	3	24 (16%)
	4	10 (8%)
N stage	X	1 (0.7%)
	0	118 (81%)
	1	14 (10%)
	2	13 (9%)
TNM VIII stage	IA1	20 (14%)
	IA2	24 (16%)
	IA3	13 (9%)
	IB	25 (17%)
	IIA	10 (7%)
	IIB	27 (19%)
	IIIA	25 (17%)
	IIIB	2 (1%)
Smoking status	Current smoker	66 (47%)
	Former smoker	62 (44%)
	Never a smoker	12 (9%)
Main curative strategy	Chemotherapy	3 (2%)
	Surgery	126 (86%)
	Radiotherapy	13 (9%)
	Multimodal	4 (3%)
Immunotherapy at relapse	Yes	22 (15%)
	No	124 (85%)
Target therapy at relapse	Yes	3 (2%)
	No	143 (98%)

the entire cohort of 146 patients was 4.6 years (interquartile range 1.8–4.9), with 1-year and 2-year disease-free survival rates of 85% (95% CI 77–90) and 72% (95% CI 62–80), respectively.

Fifteen patients (10%) died, all due to lung cancer progression. The median overall survival was not reached, and 1-year and 2-year overall survival rates were 94% (95% CI 89–97) and 88% (95% CI 80–93), respectively.

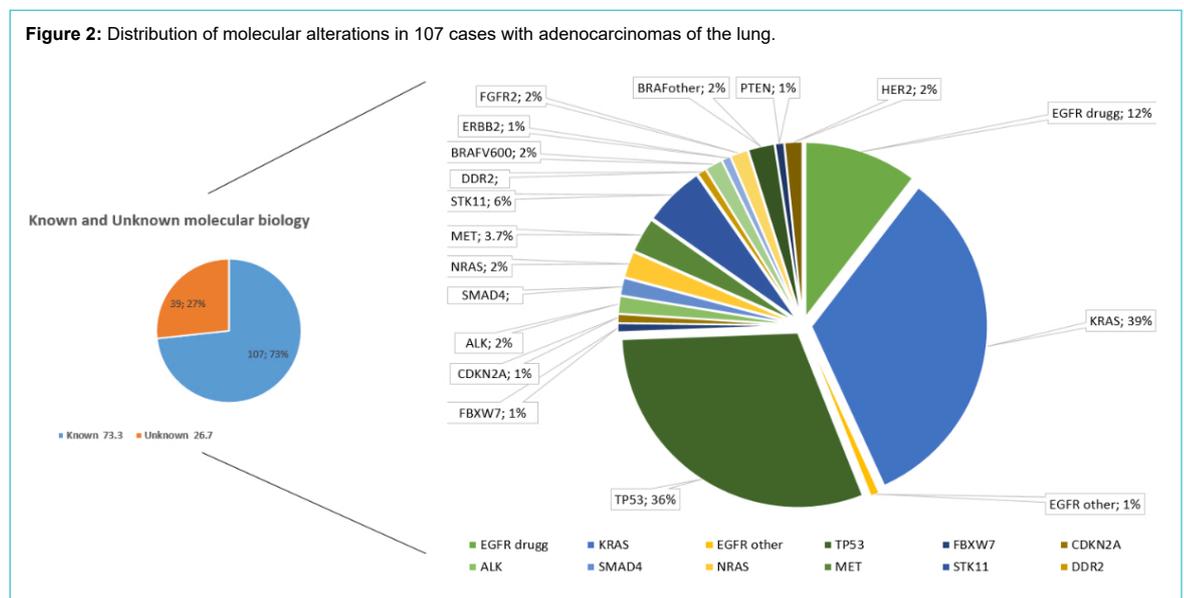
Univariable and multivariable analyses of prognostic factors

Univariable analyses of disease-free survival and overall survival are summarised in tables 3 and 4, respectively.

The sole clinical factor affecting overall survival was the type of main local treatment. Patients with inoperable tumours who had radiotherapy only had significantly poorer outcomes (HR 4.2, 95% CI 1.4–12.2) (figure 3).

Table 2:
Main histological and biological features.

Feature		(%)
Histology	MiA	2 (1%)
	Lepidic	13 (8%)
	Acinary	62 (43%)
	Mucinous	11 (8%)
	Micropapillary	5 (3%)
	Papillary	24 (17%)
	Cribriiform	1 (1%)
	Tubular	2 (1%)
	Solid	24 (17%)
	Unknown	2 (1%)
PD-L1 expression	<1	57 (39%)
	1–24	36 (25%)
	25–49	9 (6%)
	50–74	17 (12%)
	75–100	10 (6%)
	Unknown	17 (12%)
Druggable molecular alteration	Yes	22 (15%)
	No	85 (58%)
	Unknown	39 (27%)
KRAS mutation	Yes	41 (28%)
	No	65 (44%)
	Unknown	40 (27%)
EGFR mutation	Yes	13 (9%)
	No	94 (64%)
	Unknown	39 (27%)
PT53 mutation/deletion	Yes	38 (26%)
	No	69 (47%)
	Unknown	39 (27%)



Expression of PD-L1 in >25% of tumour cells was associated with lower disease-free survival (HR 1.9, 95% CI 1.0–3.9) (figure 4).

Other factors significantly affecting disease-free survival were the completion of standard treatment (HR 0.4, 95% CI 0.2–0.8), presentation with inoperable disease (HR 3.2,

Table 3:

Univariable analyses of factors that may affect disease-free survival.

Variable (n)	1-yr disease-free survival: % (95% CI)	2-yr disease-free survival: % (95% CI)	HR (95% CI)	p- value (Cox)
All patients (146)	94 (89–97)	88 (80–93)		
PD-L1 >25%	No (93)	87 (77–93)	1.0	0.049
	Yes (36)	73 (54–85)	1.9 (1.0–3.9)	
PD-L1 >50%	No (102)	79 (57–91)	1.0	0.604
	Yes (27)	83 (74–90)	1.2 (0.6–2.6)	
PD-L1 >50% or druggable mutation	No (59)	85 (69–93)	1.0	0.516
	Yes (45)	84 (71–92)	0.8 (0.3–1.7)	
Druggable mutation	No (85)	95 (67–99)	1.0	0.326
	Yes (22)	85 (75–92)	0.6 (0.2–1.7)	
KRAS mutation	No (65)	80 (63–90)	1.0	0.300
	Yes (41)	91 (80–96)	1.5 (0.7–3.4)	
Ever a smoker	No (12)	84 (75–89)	1.0	0.312
	Yes (128)	100 (NA–NA)	2.8 (0.4–20.6)	
Smoking status	Never (12)	100 (NA–NA)	1.0	0.358
	Former (62)	84 (71–91)	2.6 (0.3–19.6)	
	Current (66)	83 (70–91)	3.0 (0.4–23.1)	
Standard treatment completed	No (41)	90 (81–94)	1.0	0.012
	Yes (105)	73 (55–84)	0.4 (0.2–0.8)	
Main local treatment	Surgery (130)	86 (78–92)	1.0	0.003
	Radiotherapy (13)	67 (34–86)	3.2 (1.5–6.9)	
Stage	I (82)	94 (85–98)	1.0	0.003
	>I (64)	74 (61–86)	3.0 (1.4–6.2)	
Sex	Female (66)	98 (88–99)	1.0	0.177
	Male (80)	85 (74–92)	1.6 (0.8–3.2)	

NA: not available.

Table 4:

Univariable analyses of factors that may affect overall survival.

Variable ()	1-yr overall survival: % (95% CI)	2-yr overall survival: % (95% CI)	HR (95% CI)	p-value (Cox)
All patients (146)	94 (89–97)	88 (80–93)		
PD-L1 >25%	No (93)	96 (88–99)	1.0	0.087
	Yes (36)	88 (71–95)	2.4 (0.9–6.7)	
PD-L1 >50%	No (102)	94 (87–97)	1.0	0.874
	Yes (27)	92 (71–98)	1.1 (0.3–3.4)	
PD-L1 >50% or druggable mutation	No (59)	98 (87–99)	1.0	0.799
	Yes (45)	92 (78–97)	1.2 (0.3–4.1)	
Druggable mutation	No (85)	96 (88–99)	1.0	0.478
	Yes (22)	95 (67–99)	0.5 (0.06–3.8)	
KRAS mutation	No (65)	96 (87–99)	1.0	0.518
	Yes (41)	94 (79–98)	1.5 (0.4–5.8)	
Ever a smoker	No (12)	100 (NA–NA)	NA*	0.263 (Log-rank test)*
	Yes (128)	94 (87–97)	86 (77–92)	
Smoking status	Never (12)	100 (NA–NA)		0.191
	Former (62)	94 (84–98)	1.0*	
	Current (66)	93 (82–97)	2.0 (0.7–5.6)	
Standard treatment completed	No (41)	92 (80–99)	1.0	0.327
	Yes (105)	94 (87–98)	0.6 (0.2–1.7)	
Main local treatment	Surgery (130)	95 (89–98)	1.0	0.009
	Radiotherapy (13)	83 (48–96)	4.2 (1.4–12.2)	
Stage	I (82)	97 (88–99)	1.0	0.079
	>I (64)	91 (81–96)	2.8 (0.9–8.8)	
Sex	Female (66)	100 (NA–NA)	1.0	0.096
	Male (80)	89 (79–94)	2.6 (0.8–8.3)	

NA: not available.

* There are no deaths among non-smokers; hence, hazard for that group is zero, which means that the hazard ratio for any group that does have an event will tend to infinity. For this reason, the only meaningful HR that we could calculate is the one for the current smoker group vs the pooled groups of former and never smokers.

95% CI 1.5–6.9) and stage >I (HR 3.0, 95% CI 1.4–6.2) (figure 3).

The frequency of cases with PD-L1 expression >25% was similar in patients with stage I disease and those with higher stage disease (30% vs 26%, $p = 0.695$). Inoperability

was higher in patients with disease stage >I (69% vs 31%), but the difference was not statistically significant ($p = 0.074$). The rate of treatment completion was significantly lower in patients with disease stage >I (41% vs 96%, $p < 0.001$).

Figure 3: Kaplan-Meier survival curves (DFS and OS) by main treatment modality and treatment completion. Kaplan-Meier curves for DFS (A) and OS (B) in the subgroup of patients treated with surgery (medically operable) vs radiotherapy (medically inoperable) in univariable analyses. Kaplan-Meier curves for DFS (C) and OS (D) for patients who completed treatment vs patients who did not complete treatment in univariable analyses. A positive significance on OS for the subgroup of patients who completed treatment was not reached. DFS: disease-free survival; OS: overall survival.

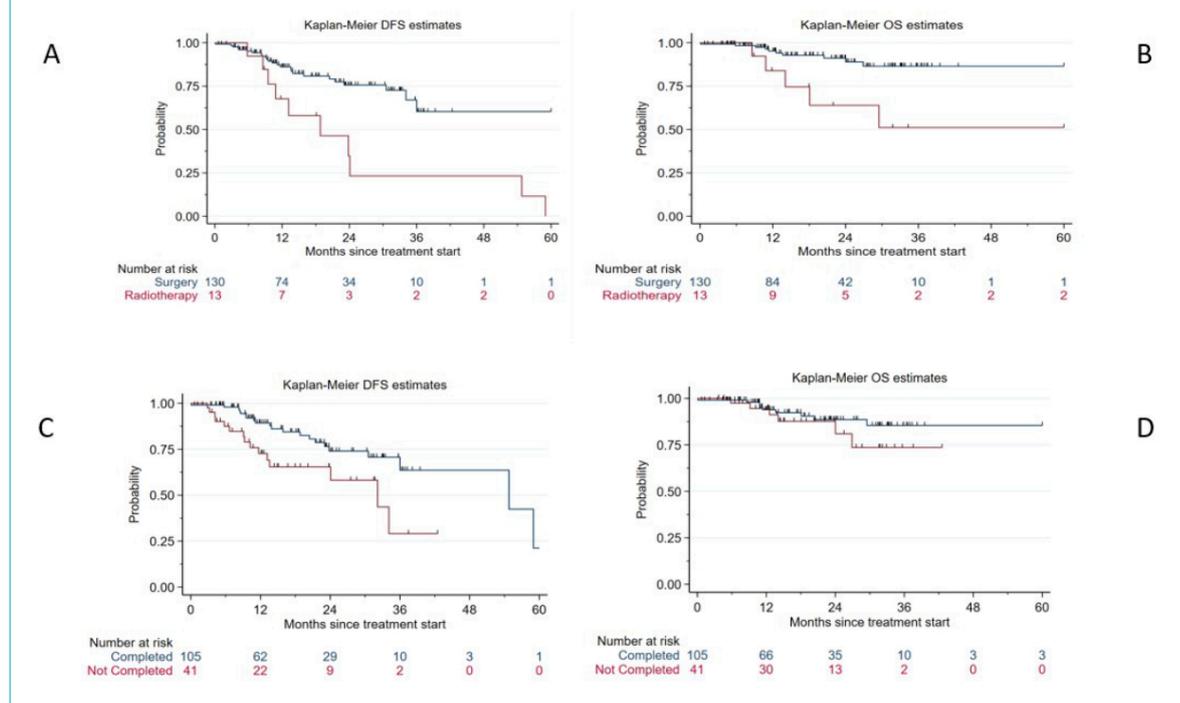
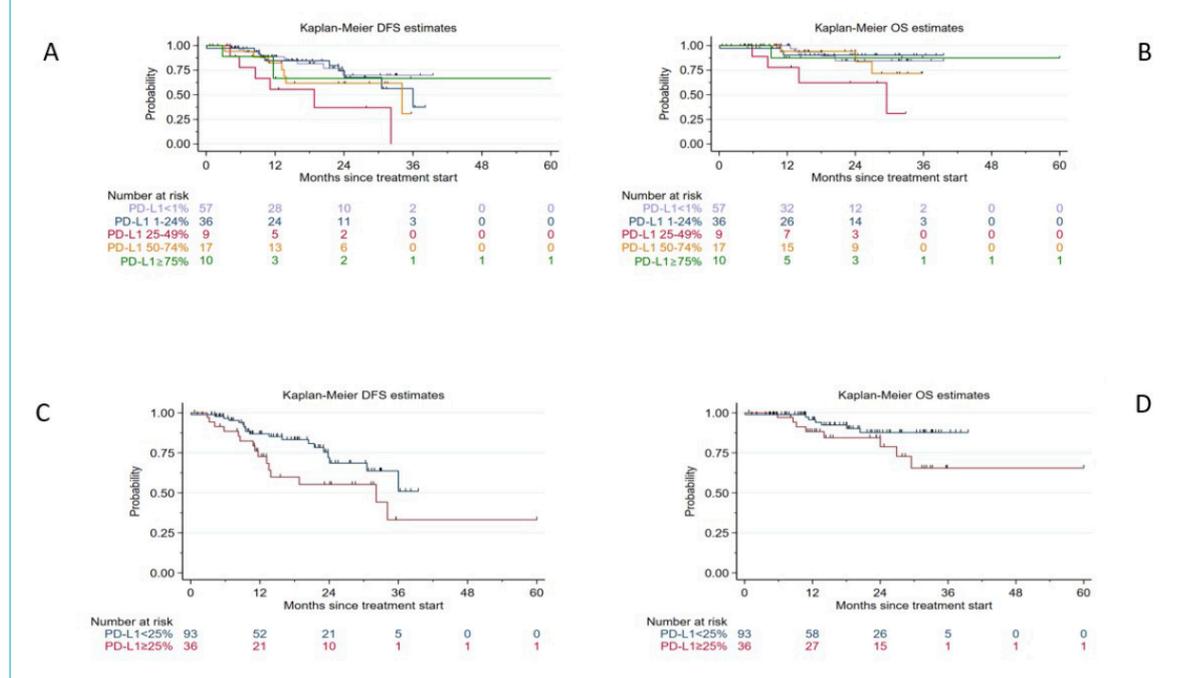


Figure 4: Kaplan-Meier survival curves (DFS and OS) for PD-L1 expression levels. In univariable analyses, PDL1 expression of $\geq 25\%$ was a significant poor prognostic factor for DFS but was not a significant predictor of OS. Kaplan-Meier curves for DFS (A) and OS (B) for PD-L1 expression for all cut-offs chosen for the analysis of our non-small cell lung cancer patient population and Kaplan-Meier curves for DFS (C) and OS (D) in patients with a PD-L1 tumour proportion score of $\geq 25\%$. DFS: disease-free survival; OS: overall survival.



In the multivariable analysis (stepwise Cox regression starting from the aforementioned variables that were significantly associated with outcomes in univariable analyses), having an inoperable tumour and stage >I remained significantly associated with lower disease-free survival (table 5).

Discussion

PD-1 belongs to the CD28 superfamily and is an inhibitory surface receptor that is expressed on activated T, B and natural killer lymphocytes and regulates their activation and expansion [38]. PD-L1, which belongs to the B7 family, is the main ligand of PD-1 and is frequently upregulated in several human malignancies, including lung cancer [12, 39]. The activation of the PD-1/PD-L1 signalling pathway can inhibit other pathways, such as RAS/MEK/ERK and PI3K/AKT, to suppress T cell proliferation [40]. In the tumour microenvironment, PD-L1 expression can induce depletion of infiltrating T lymphocytes, leading to a reduction in immune surveillance [41]. PD-L1 favours the upregulation of regulatory T cells and their negative regulatory functions; in addition, it inhibits the activity of effector T cells [42]. Consequently, PD-L1 expression helps cancer cells to avoid the antitumour immune response [43], while blocking PD-1/PD-L1 signalling restores this immune response against multiple tumour types, as demonstrated in recent clinical trials [44, 45].

These physiological mechanisms suggest that the expression of PD-L1 by tumour cells may lead to immune evasion and favour tumour growth.

Efforts have been made to determine the prognostic significance of PD-L1 expression in early-stage non-small cell lung cancer patients, but the results are highly heterogeneous.

First, several case series from the pre- and post-immunotherapy era, which assessed Asian and Caucasian patients with early-stage non-small cell lung cancer, were unable to demonstrate a consistent correlation between PD-L1 expression and overall survival or disease-free survival [21, 46, 47]. Second, a few other studies of Caucasian patients concluded that the expression of PD-L1 in tumour tissue correlated with a favourable prognosis [12–14, 48]. Finally, several studies of Asian patients suggested that PD-L1 expression was associated with a poor prognosis in terms of overall survival and/or disease-free survival [7–9, 24, 26, 27, 43]. There are many potential explanations for these discrepancies; for example, during the development of various commercially available immune checkpoint inhibitors (ICIs), different antibodies and platforms were used, along with different methodologies, target tumour material, scoring methods and thresholds of expression levels. Consensus could not be reached in these early

phases, and harmonisation and quality assessment efforts were conducted later. The Blueprint PD-L1 Immunohistochemistry Assay Comparison Project, an industrial-academic collaborative partnership to provide information on the analytical and clinical comparability of four PD-L1 immunohistochemistry assays used in clinical trials (22C3 and 28-8 manufactured by Dako, SP142 and SP263 manufactured by Ventana/Roche) investigated this issue [49]. This study indicated that, despite similar analytical performance of three (28-8, 22C3 and SP263) of four assays of PD-L1 expression, interchanging assays and cut-offs leads to “misclassification” of PD-L1 status of the same tumour sample. In an additional study by Huijuan Li and colleagues, no association was found between PD-L1 expression and survival when SP263, 22C3 and ab58810 were used, but a poor prognosis was evident if PD-L1 was assessed by SP142, E1L3N or 28-8 antibodies [47].

The most relevant finding of our analysis was that PD-L1 expression >25% was correlated with lower disease-free survival in a univariable analysis. Although this effect was not confirmed in the multivariable analysis, it may suggest the need for additional therapeutic interventions, especially for patients with tumours with PD-L1 $\geq 25\%$, in order to equalise survival outcomes with those of the more favourable group. In January 2022, atezolizumab became the first cancer immunotherapy available in Switzerland for adjuvant treatment of patients with early-stage non-small cell lung cancer. Its approval was based on the IMpower010 phase III study, which showed that adjuvant atezolizumab improved disease-free survival and reduced the risk of recurrence by 57% in the subpopulation of patients with PD-L1 $\geq 50\%$ and stage II–IIIA non-small cell lung cancer, compared with the best supportive care [19]. Data needed to assess overall survival in this study are not yet available; however, the Swiss regulatory authority Swissmedic, and, recently, the European Medicines Agency, considered this disease-free survival difference enough to deserve the aforementioned label. The Food and Drug Administration (FDA), in contrast, extended approval to patients with PD-L1 expression $\geq 1\%$.

The recently published CheckMate 816 randomised study assessing neoadjuvant nivolumab plus chemotherapy versus neoadjuvant chemotherapy alone reported similar results in favour of immunotherapy [20]. The median event-free survival was 31.6 months (95% CI 30.2 to not reached) with nivolumab plus chemotherapy and 20.8 months (95% CI 14.0–26.7) with chemotherapy alone (HR 0.63, 97.38% CI 0.43–0.91, $p = 0.005$). This greater event-free survival with chemo-immunotherapy compared to chemotherapy alone seemed to be due mainly to the subpopulation of patients with high PD-L1 expression ($\geq 50\%$). In addition, this study found that, at the first pre-specified interim analysis, the hazard ratio for death was 0.57 (99.67% CI 0.30–1.07) and was not significant. Upon

Table 5: Stepwise Cox regression of prognostic factors associated with disease-free survival.

Variable*	HR	95% CI	p-value (Wald test)
Stage >I	2.7	1.2–6.0	0.012
Inoperable tumour	3.2	1.4–7.4	0.005

N subjects = 126; n failures = 34; likelihood ratio test $p = 0.0003$.

* starting variables: completed treatment, stage >I, inoperable disease, PD-L1 >25%

the report of these event-free survival results, the FDA approved neoadjuvant nivolumab for stage II and III patients regardless the tumour's PD-L1 expression.

From the perspective of a clinical oncologist, it could be challenging to recommend adjuvant or neoadjuvant treatments based on a disease-free survival advantage alone, without a confirmed overall survival advantage. At present, due to the predominance of patients treated with ICIs for metastatic tumours, the potential long-term or permanent harm caused by these drugs may be underestimated. This possibility should be seriously considered before embracing perioperative treatments with anti-PD-1 or anti-PD-L1 agents as a standard of care. Jain and colleagues conducted a large retrospective pharmacovigilance study on cardiovascular adverse events associated with ICIs in US patients. They reported a high incidence of subacute or chronic cardiovascular toxicity, with rates of stroke 4.6%, heart failure 3.5%, atrial fibrillation 2.1%, conduction disorders 1.5%, myocardial infarction 0.9%, myocarditis 0.05%, vasculitis 0.05% and pericarditis 0.2% [50]. In addition, among patients who developed nephritis, use of ICIs was associated with a twofold to threefold higher risk of myocardial infarction (HR 2.03, 95% CI 1.25–3.31, $p = 0.004$), heart failure (HR 2.37, 95% CI 1.86–3.03, $p < 0.001$), conduction disorders (HR 3.06, 95% CI 2.17–4.3, $p \leq 0.001$), atrial fibrillation (HR 3.29, 95% CI 2.46–4.4, $p < 0.001$) and stroke (HR 1.75, 95% CI 1.39–2.2, $p < 0.001$). Additionally, the development of pneumonitis while on ICIs was associated with heart failure (HR 2.61, 95% CI 1.23–5.52), and the development of encephalitis was associated with conduction disorders (HR 4.35; 95% CI 1.6–11.87). Effects like these could theoretically counterbalance event-free survival or disease-free survival advantages, precluding the desired overall survival benefit. This possibility is even more relevant for subgroups of patients with a biologically better prognosis, as may be the case for those with PD-L1 expression $< 25\%$. By contrast, immediate adoption of perioperative immunotherapy could be justified for patients whose tumours express high levels of PD-L1 ($\geq 50\%$), given that both aforementioned randomised trials showed the greatest event-free survival and disease-free survival benefit, respectively, in this category.

Our patient population was relatively small but homogeneous in terms of diagnostic work-up and treatment allocation criteria and delivery. In addition, unlike in prior studies, PD-L1 was measured according to modern diagnostic and therapeutic standards.

Our results confirmed many of the principles determined by many years of research on lung cancer treatment. For example, both the impossibility of completing the treatment strategy and a disease stage $> I$ were associated with lower disease-free survival in univariable analyses. Undergoing radiotherapy as the main treatment modality was associated with lower disease-free survival and overall survival in univariable analyses, probably mostly due to a selection bias, since, at our institute, stereotactic body radiation therapy is offered to frail patients deemed unable to tolerate surgery. In the multivariable analysis, radiotherapy as the main treatment and a disease stage $> I$ correlated with lower disease-free survival. As mentioned above, analysing the treatment modality as a variable in a retrospective study may suffer from several biases, such as se-

lection of patients by operability or resectability, omission of pathological nodal evaluation in the case of stereotactic body radiation therapy and underrepresentation of the SBRT arm, among other characteristics [51–54]. Findings from single institutions have always showed mixed results, ranging from surgery being the best option [55] to similar outcomes for surgery and radiotherapy [51–53, 56]. Two studies on SEER-Medicare linked data found superior long-term outcomes of lobectomy compared with stereotactic body radiation therapy [57, 58]. A study of US veterans observed that patients who underwent surgery had a higher cancer-specific mortality rate than those who underwent SBRT [54]. In contrast, a meta-analysis by Zheng et al. [59] concluded that survival outcomes were not significantly different between the two groups after adjusting for age and operability parameters. In conclusion, based on our results and the aforementioned studies, we recommend, for very early stages, a surgical resection over SBRT whenever possible. Weaknesses of the present analysis include its retrospective nature, the relatively small number of patients and the rather short median follow-up period for a population with predominantly stage I or II disease (for which a longer follow-up period is needed to record a substantial number of events).

Conclusion

In our cohort of early-stage non-small cell lung cancer patients treated with curative intent, the population of patients with PD-L1 expression $\geq 25\%$ who were treated during the pre-immunotherapy era had a worse prognosis. This finding supports the use of adjuvant immunotherapy based on current evidence derived from disease-free survival outcomes. For the group of patients with PD-L1 expression $< 25\%$, it may be reasonable to wait for the overall survival data to be available before adopting perioperative immunotherapy as the standard of care.

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Potential competing interests

All authors have completed and submitted the International Committee of Medical Journal Editors form for disclosure of potential conflicts of interest. No potential conflict of interest was disclosed.

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