Journal for ImmunoTherapy of Cancer

Longitudinal analysis of PD-L1 expression in patients with relapsed NSCLC

Nikolaus John,[1] Verena Schlintl,[2] Teresa Sassmann,[1] Jörg Lindenmann,[3] Melanie Fediuk,[3] Robert Wurm,[1] Philipp Douschan,[1,4] Martin Zacharias,[5] Lipika Kalson,[5] Florian Posch,[6] Gudrun Absenger,[2] Luka Brcic,[5] Philipp J Jost,[2,7] Angelika Terbuch [2]

To cite: John N, Schlintl V, Sassmann T, *et al*. Longitudinal analysis of PD-L1 expression in patients with relapsed NSCLC. *Journal for ImmunoTherapy of Cancer* 2024;**12**:e008592. doi:10.1136/jitc-2023-008592

► Additional supplemental material is published online only. To view, please visit the journal online (https://doi.org/10.1136/jitc-2023-008592).

Accepted 18 March 2024

For numbered affiliations see end of article.

Correspondence to
Dr Angelika Terbuch; angelika.terbuch@medunigraz.at

Luka Brcic; luka.brcic@medunigraz.at

ABSTRACT

Background The use and approval of immune checkpoint inhibitors for the treatment of non-small cell lung cancer (NSCLC) depends on PD-L1 expression in the tumor tissue. Nevertheless, PD-L1 often fails to predict response to treatment. One possible explanation could be a change in PD-L1 expression during the course of the disease and the neglect of reassessment. The purpose of this study was a longitudinal analysis of PD-L1 expression in patients with relapsed NSCLC.

Methods We retrospectively analyzed PD-L1 expression in patients with early-stage NSCLC and subsequent relapse in preoperative samples, matched surgical specimens and biopsy samples of disease recurrence. Ventana PD-L1 (SP263) immunohistochemistry assay was used for all samples. PD-L1 expression was scored based on clinically relevant groups (0%, 1%–49%, and ≥50%). The primary endpoint was the change in PD-L1 score group between preoperative samples, matched surgical specimens and relapsed tumor tissue.

Results 395 consecutive patients with stages I–III NSCLC and 136 (34%) patients with a subsequent relapse were identified. For 87 patients at least two specimens for comparison of PD-L1 expression between early stage and relapsed disease were available. In 72 cases, a longitudinal analysis between preoperative biopsy, the surgically resected specimen and biopsy of disease recurrence was feasible. When comparing preoperative and matched surgical specimens, a treatment-relevant conversion of PD-L1 expression group was found in 25 patients (34.7%). Neoadjuvant treatment showed no significant effect on PD-L1 alteration (p=0.39). In 32 (36.8%) out of 87 cases, a change in PD-L1 group was observed when biopsies of disease relapse were compared with early-stage disease. Adjuvant treatment was not significantly associated with a change in PD-L1 expression (p=0.53). 39 patients (54.2%) showed at least 1 change into a different PD-L1 score group during the course of disease. 14 patients (19.4%) changed the PD-L1 score group twice, 5 (6.9%) of them being found in all different score groups.

Conclusion PD-L1 expression shows dynamic changes during the course of disease. There is an urgent need for consensus guidelines to define a PD-L1 testing strategy including time points of reassessment, the number of biopsies to be obtained and judgment of surgical specimens.

WHAT IS ALREADY KNOWN ON THIS TOPIC

⇒ In non-small cell lung cancer without driver mutations, treatment decisions are based on PD-L1 expression. However, PD-L1 TPS fails to predict response in a relevant number of patients. PD-L1 expression can be induced on a cellular level and some authors found oncological treatment to influence PD-L1 expression. A possible alternative explanation for PD-L1 expression change is "misrepresentation" due to tumor heterogeneity and small biopsy size. To date, this topic is poorly addressed and there is no consensus guideline that defines PD-L1 testing.

WHAT THIS STUDY ADDS

⇒ Clinically relevant PD-L1 expression change was documented in a relevant number of cases. Contrary to previous findings, PD-L1 expression change was not significantly affected by oncological treatment. In selected patients with pronounced PD-L1 expression changes, we found significant tumor heterogeneity. Our study clearly shows that treatment with immune checkpoint inhibitors should not be restricted to certain PD-L1 expression levels without guidelines that define PD-L1 testing.

HOW THIS STUDY MIGHT AFFECT RESEARCH, PRACTICE OR POLICY

⇒ Our findings demonstrate that consensus guidelines for PD-L1 expression assessment are urgently needed. We suggest guidelines should address PD-L1 TPS validity in biopsies compared with surgical specimens with a number of recommended tissue blocks to be analyzed, and indication for rebiopsy in case of progression to minimize the effect of tumor heterogeneity on PD-L1 expression levels.

INTRODUCTION

Lung cancer is the leading cause of cancer-associated mortality in Western nations.[1] Non-small cell lung cancer (NSCLC) accounts for up to 85% of lung cancer.[2] Antibodies that target immune checkpoints, so-called immune checkpoint inhibitors (ICIs), have revolutionized the treatment of

NSCLC. The most important target for ICIs in NSCLC is the programmed death ligand 1/programmed death 1 (PD-L1/PD-1) immune checkpoint. High expression of PD-L1 in patients with metastatic NSCLC treated with ICIs is associated with higher progression-free survival (PFS) and overall survival (OS).[3 4] Therefore, the approval of ICIs for the treatment of metastatic NSCLC is often linked with PD-L1 expression. In early-stage NSCLC, recent approval of ICIs in the neoadjuvant and adjuvant setting is also dependent on PD-L1 expression. Nevertheless, approvals differ between the European Medicines Agency (EMA) and the Food and Drug Administration (FDA) of the USA. However, PD-L1 is not a reliable biomarker and often fails to predict response to treatment.[5] One possible explanation is the fact that PD-L1 expression in NSCLC is not static but seems to undergo dynamic changes.[5–9] Previous studies found that PD-L1 expression may be influenced by several factors including Interferon-γ and other cytokines release,[10–12] activation of oncogenic drivers[6 13] and specific drugs.[14] Some studies even showed that oncological treatment may alter PD-L1 expression.[8 9] Apart from PD-L1 expression change, PD-L1 misrepresentation due to tumor heterogeneity may also contribute to the discrepancy in measured PD-L1 expression.[15 16]

Few studies described PD-L1 expression change between preoperative biopsies and surgical specimens or resected tumors and disease recurrence, but no studies have investigated PD-L1 change over the full course of disease. Furthermore, due to the relatively low number of cases and conflicting results, there is still no consensus concerning PD-L1 alteration and reassessment of PD-L1 status. Therefore, we aimed to investigate PD-L1 expression changes and possible influencing factors from diagnosis of NSCLC above surgery to disease recurrence.

METHODS

Patients and study design

Since December 2015, tissue from advanced but also early-stage NSCLC patients was analyzed for driver mutations and PD-L1 expression at our institution. For this study, we, therefore, identified all consecutive patients who underwent surgery with curative intent for NSCLC (stages I–III) between December 2015 and December 2020 at the Medical University of Graz (figure 1). All patients who experienced disease relapse and underwent a rebiopsy until January 2023 were included in this study. Cases with synchronous distant metastatic disease or driver mutations that do not allow the use of ICIs in the first-line setting (EGFR, ALK, ROS, RET and NTRK) were excluded. Patients with BRAF and KRAS mutations were included in the analysis. Every patient had been discussed in a multidisciplinary tumor board. In cases with relapses within the lung in which a second primary could not be ruled out by clinical or histopathological criteria, a molecular analysis of the rebiopsy by next-generation sequencing was performed and this could conclusively rule out second primary lung tumors in all patients. We retrospectively obtained clinical data about age, sex, smoking status, Eastern Cooperative Oncology

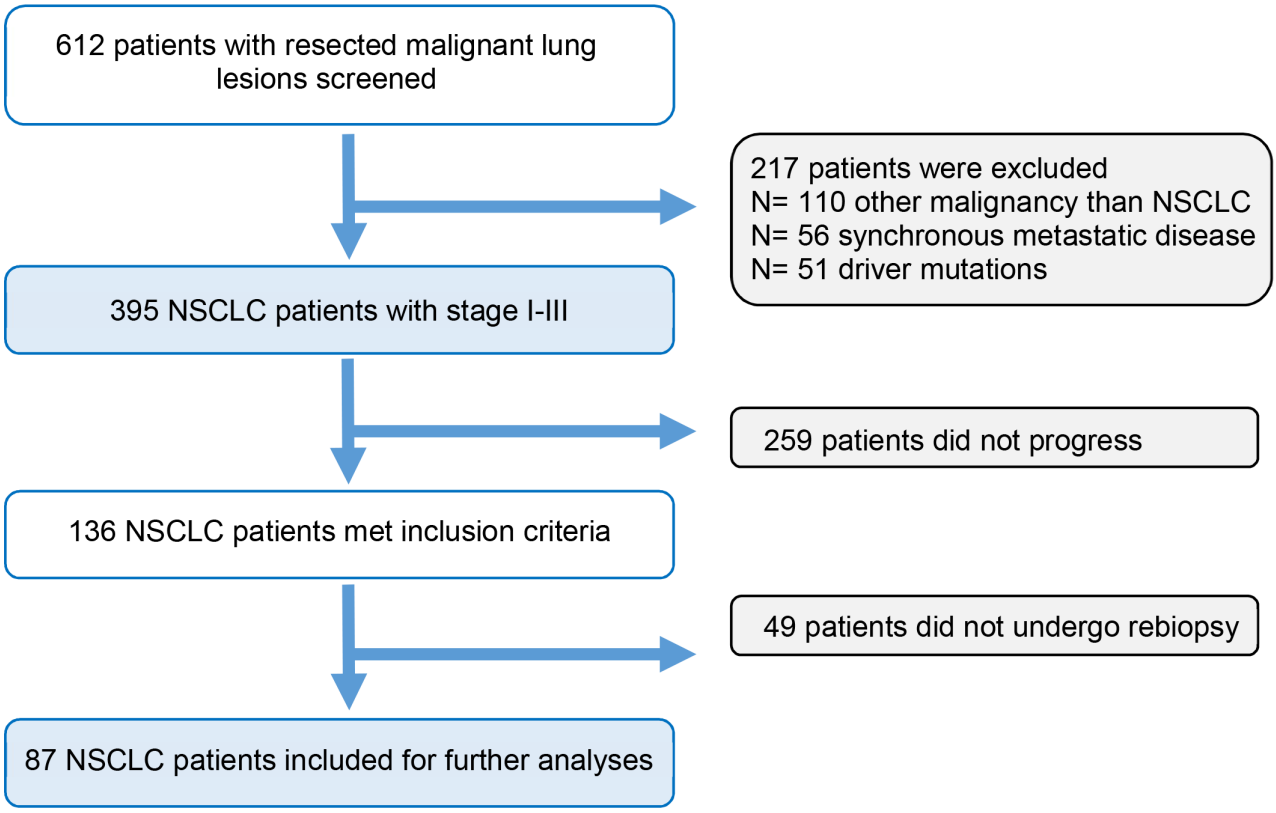

Figure 1 Consolidated Standards of Reporting Trials diagram and study design. NSCLC, non-small cell lung cancer.

Group (ECOG) performance status, histology, clinical preoperative and postoperative stage of disease, and neoadjuvant or adjuvant treatment modalities. PD-L1 expression from preoperative samples, matched surgical specimens and disease relapse was documented. If immunohistochemistry (IHC) for PD-L1 expression was not performed at all three time points, PD-L1 staining was performed retrospectively for this study. In all patients, Ventana PD-L1 (SP263) IHC assay was used for assessment of PD-L1 expression. Tumor specimens were scored in groups with negative PD-L1 expression, PD-L1 expression 1%–49% and PD-L1 high expression ≥50%, based on clinically used cut-offs.

Endpoints

The primary endpoint was the change in PD-L1 score group between preoperative samples, matched surgical specimens and relapsed tumor tissue.

Statistical analyses

All statistical analyses were performed with IBM SPSS Statistics V.28. Continuous variables were summarized with medians (25th–75th percentile), and count data as absolute frequencies (column %). The distribution of variables between samples with and without a change in the PD-L1 score group was compared with the χ^2 test. Missing data are reported and a complete case analysis was performed.

RESULTS

Patient selection

A total of 612 patients who underwent surgery for malignant lung lesions between December 2015 and December 2020 were screened (figure 1). 217 patients met exclusion criteria due to other types of malignancy than NSCLC (n=110), metastatic disease at the time of surgery (n=56), and driver mutations not allowed in this study (EGFR, ALK, ROS, RET, NTRK, n=51). 136 out of the remaining 395 patients relapsed, of whom 87 patients underwent a rebiopsy and were included for further analyses (figure 1). All 87 patients had at least two specimens for comparison of PD-L1 expression between early stage (either preoperative sample or surgical specimen) and metastatic disease. In 72 (82.8%) cases, PD-L1 expression was available for all three time points (preoperative sample, surgical specimen and disease relapse). Of the remaining 15 cases, 13 patients did not undergo a preoperative biopsy or insufficient tissue for PD-L1 staining was left and two patients were deemed inoperable during surgery, therefore, no surgical specimen was available. These two patients received definitive chemoradiation and relapsed.

Patient characteristics

Baseline demographic and disease characteristics are detailed in table 1. The median age was 63 years (range 44–83). The majority (80%) had stages II and III disease at the time of surgery. 20 patients (23%) received

Table 1 Baseline characteristics of the study cohort (N=87)

	n (% miss.)	Summary estimate
Female gender	87 (0)	35 (40%)
Age at diagnosis (years)	87 (0)	63 [58–68]
Eastern Cooperative Oncology Group (ECOG) performance status	74 (15)	/
0 points	/	32 (43%)
1 point	/	37 (50%)
≥2 points	/	5 (7%)
Smoking status	67 (23)	/
Never	/	5 (7%)
Current or former	/	62 (93%)
Histology	87 (0)	/
Adenocarcinoma	/	53 (61%)
Squamous cell carcinoma	/	32 (37%)
Other	/	2 (2%)
Postoperative tumor stage	85 (2)	/
I	/	17 (20%)
II	/	36 (42%)
III	/	32 (38%)
Perioperative treatment	87 (0)	/
Neoadjuvant		20 (23%)
Adjuvant		29 (33%)
Both		4 (5%)
Time between surgery and rebiopsy ≥1 year	87 (0)	55 (63%)
Type of relapse	87 (0)	/
Local relapse	/	27 (31%)
Distant relapse	/	48 (55%)
Local and distant relapse	/	12 (14%)
Site of rebiopsy/surgical resection	87 (0)	/
Ipsilateral lung (ie, local recurrence)	/	23 (26%)
Mediastinal lymph nodes (ie, local recurrence)	/	16 (18%)
Brain	/	11 (13%)
Contralateral lung	/	9 (10%)
Extrathoracic lymph nodes	/	8 (9%)
Pleura	/	7 (8%)
Soft tissue		7 (8%)
Liver	/	3 (3%)
Bone	/	2 (2%)
Adrenal gland	/	1 (1%)

neoadjuvant treatment and 29 patients (33%) were treated in the adjuvant setting. No patient received perioperative treatment with ICIs as there was no approval at that time in this setting.

PD-L1 expression change between initial biopsy and surgical specimen

For the 72 cases with preoperative biopsies and matched surgical samples, we found a treatment-relevant group change in 25 patients (34.7%). 11 patients (15.3%) swapped into a higher PD-L1 score group, 14 patients (19.4%) changed into a lower group and 47 patients (65.3%) stayed in the same group. Preoperative tumor stage was stage I in 11 (15.7%), stage II in 27 (38.6%) and stage III in 32 (45.7%) patients. 16 (22.2%) out of the 72 patients received neoadjuvant treatment, 12 patients (75%) being treated with neoadjuvant chemotherapy, 3 (18.8%) with chemoradiation and 1 patient (6.3%) with SBRT who finally underwent surgery for disease progression. Response to neoadjuvant treatment was partial response in 11 (68.8%), stable disease in 3 (18.8%) and progressive disease in 2 cases (12.5%). We found no statistically significant influence of neoadjuvant treatment on the change in the PD-L1 score group between preoperative biopsy and matched surgical specimen (p=0.39, table 2). Also, the response to neoadjuvant treatment did not show a significant association with a change in the PD-L1 score group (p=0.99, table 2).

PD-L1 expression change between the surgical specimen and rebiopsy

For the 87 cases with rebiopsies of relapsed disease, specimens from surgery for previously early-stage disease were available in 85 cases. For the two patients who were treated with definitive chemoradiation, the initial diagnostic biopsy was taken for comparison of PD-L1 expression. When divided into the clinically relevant score groups of PD-L1 expression (0%, 1–49% and ≥50%), we found a treatment-relevant change in 32 patients (36.8%). 15 patients (17.2%) swapped into a higher PD-L1 score group, 17 patients (19.5%) changed into a lower group and 55 patients (63.2%) stayed in the same group. Interestingly, there was a significantly lower number of female subjects among patients with a change in PD-L1 expression (table 3). All other baseline characteristics including

Table 2 Characteristics of surgical samples and matched preoperative biopsies (n=72)

		No change in PD-L1 score group* (N=47)	Change in PD-L1 score group (N=25)	P value†
	n (% miss.)	Summary estimate	Summary estimate	
Female gender	72 (0)	19 (40%)	8 (32%)	0.482
Age at diagnosis (years)	72 (0)	64 [58–70]	60 [55–67]	0.404
ECOG performance status	62 (14)	/	/	0.573
0 points	/	19 (45%)	7 (35%)	/
1 point	/	21 (50%)	11 (55%)	/
≥2 point	/	2 (5%)	2 (10%)	/
Smoking status	55 (24)	/	/	0.999
Never	/	4 (11%)	1 (6%)	/
Current or former	/	34 (89%)	16 (94%)	/
Histology	72 (0)	/	/	0.449
Adenocarcinoma	/	26 (55%)	17 (68%)	/
Squamous cell carcinoma	/	19 (40%)	8 (32%)	/
Other	/	2 (4%)	0 (0%)	/
Preoperative tumor stage	70 (3)	/	/	0.999
I	/	7 (15%)	4 (17%)	/
II	/	18 (39%)	9 (38%)	/
III	/	21 (46%)	11 (46%)	/
Any neoadjuvant treatment	72 (0)	9 (19%)	7 (28%)	0.392
Best response to neoadjuvant treatment	16 (0)	/	/	0.999
Partial response	/	6 (67%)	5 (71%)	/
Stable disease	/	2 (22%)	1 (14%)	/
Progressive disease	/	1 (11%)	1 (14%)	/

*PD-L1 score group (PD-L1 negative, PD-L1 low (1%–49%), and PD-L1 high (50%–100%)).
†χ^2 test.

Table 3 Characteristics of surgical samples and rebiopsies (n=87)

		No change in PD-L1 score group* (N=55)	Change in PD-L1 score group (N=32)	P value†
	n (% miss.)	Summary estimate	Summary estimate	
Female gender	87 (0)	27 (49%)	8 (25%)	0.027
Age at diagnosis (years)	87 (0)	63 (58–68)	63 (56–68)	0.876
ECOG performance status	74 (15)	/	/	0.900
0 points	/	22 (45%)	10 (40%)	/
1 point	/	23 (47%)	14 (56%)	/
≥2 points	/	4 (8%)	1 (4%)	/
Smoking status	66 (24)	/	/	0.645
Never	/	4 (10%)	1 (4%)	/
Current or former	/	38 (90%)	23 (96%)	/
Histology	87 (0)	/	/	0.251
Adenocarcinoma	/	37 (67%)	16 (50%)	/
Squamous cell carcinoma	/	17 (31%)	15 (47%)	/
Other	/	1 (2%)	1 (3%)	/
Postoperative tumor stage	85 (2)	/	/	0.319
I	/	8 (15%)	9 (28%)	/
II	/	23 (43%)	13 (41%)	/
III	/	22 (42%)	10 (31%)	/
Any adjuvant treatment	87 (0)	17 (31%)	12 (38%)	0.529
Time between surgery and rebiopsy ≥1 year	87 (0)	37 (67%)	18 (56%)	0.304
Site of rebiopsy	87 (0)	/	/	0.548
Local recurrence	/	26 (47%)	13 (41%)	/
Distant recurrence	/	29 (53%)	19 (59%)	/

*PD-L1 score group (PD-L1 negative, PD-L1 low (1%–49%), and PD-L1 high (50%–100%)).
†χ^2 test.

performance status, smoking status and histology showed no significant association with a change in the PD-L1 expression group (table 3). In our cohort, 29 patients (33.3%) received adjuvant treatment. Two patients (6.9%) were treated with adjuvant chemoradiation, 6 (20.7%) with radiation and 21 (72.4%) with adjuvant chemotherapy. Adjuvant treatment was not associated with a statistically significant change in PD-L1 expression groups (p=0.53, table 3). The median time from surgery to rebiopsy was 15 months, with 75% of the cohort having an interval of at least 9.5 months and 25% of the cohort having an interval of at least 27 months between surgery and rebiopsy. When analyzing the influence of time to rebiopsy on PD-L1 expression change, we found no statistically relevant association between early and late relapse (p=0.30, table 3).

Longitudinal analysis of PD-L1 expression in patients with relapsed NSCLC

In 72 patients (82.8%), PD-L1 expression could be compared between preoperative samples, matched surgical specimens and biopsy of relapsed disease. 39 patients (54.2%) showed at least 1 change into a different PD-L1 score group during the course of disease. 14 patients (19.4%) changed the PD-L1 score group twice, 5 (6.9%) of them being found in all different score groups. We could not observe a significant trend of change for PD-L1 expression during the course of disease. In the 39 patients with at least one change of score group, 11 (28.2%) patients had a constant increase in PD-L1 expression whereas 12 (30.8) patients showed a constant decrease in PD-L1 expression. In 16 (41%) patients an increase of PD-L1 expression was followed by a decrease or vice versa (figure 2). When looking at the 33 patients who remained in the same PD-L1 score group for all three time points, 19 (57.6%) subjects belonged to the PD-L1 negative group, 6 (18.2%) patients to the groups with a score of 1%–49% and 8 (24.2%) patients showed a constant score of ≥50%.

To see if only small absolute percentage changes in PD-L1 expression were associated with a change in PD-L1 score group, we defined (for exploratory and analytical purposes) a “small” change as an absolute percent change in PD-L1

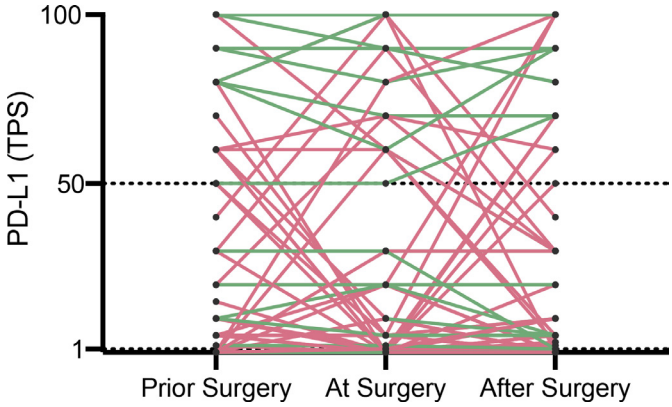

Figure 2 PD-L1 change of all matched samples (N=72). PD-L1 expression (TPS) in biopsy samples, surgical specimens, and rebiopsy was compared. Each line represents one patient. Green lines represent patients who stayed within in the same PD-L1 score group, red lines represent patients who changed the PD-L1 score group at least one time during the course of disease. TPS, Tumor Proportion Scores.

expression of ≤10%. Here, 21 out of 72 patients (29%) and 23 out of 87 patients (26%) hat a PD-L1 expression change of >10% from preoperative biopsy to surgery, or from either preoperative biopsy or surgery to recurrence, respectively (online supplemental figure 1). For the preoperative biopsy to surgery comparison, a change in PD-L1 category occurred in 9 (18%) out of 51 patients who had a ≤10% PD-L1 expression change, and in 16 (76%) out of 21 patients who had a >10% PD-L1 expression change, respectively (p<0.0001, see figure 3A). For the either preoperative biopsy or surgery to recurrence biopsy comparison, a change in PD-L1 category occurred in 14 (22%) out of 64 patients who had a ≤10% PD-L1 expression change, and in 18 (78%) out of 23 patients who had a >10% PD-L1 expression change (p<0.001, figure 3B). These data show that in a great propotion of cases the absolute percentage changes of PD-L1 expression were ≤10% (online supplemental figure 1), but that mainly the cases with an absolute percentage change in PD-L1 expression >10% were associated with a change in PD-L1 score group (figure 3).

Tumor heterogeneity

To evaluate if tumor heterogeneity might be a possible explanation for the change in the PD-L1 score group, we reassessed the surgical specimens of the five patients who were found in all three score groups during their course of disease. A total of 6–8 slides per surgical specimen were investigated for PD-L1 expression. As expected, on different slides, and often also in one slide, there were areas of different PD-L1 expression, sometimes ranging from 0% to 100% (figure 4). By reassessing the surgical specimens with all available blocks instead of just one and scoring PD-L1 expression as the average of all slides, the PD-L1 result would have changed the score group in two patients.

DISCUSSION

In this study, we performed a longitudinal analysis of PD-L1 expression during the course of disease in NSCLC patients with metachronic, relapsed disease. To our knowledge, although there are similar studies exploring either the change in PD-L1 expression in the perioperative or in the

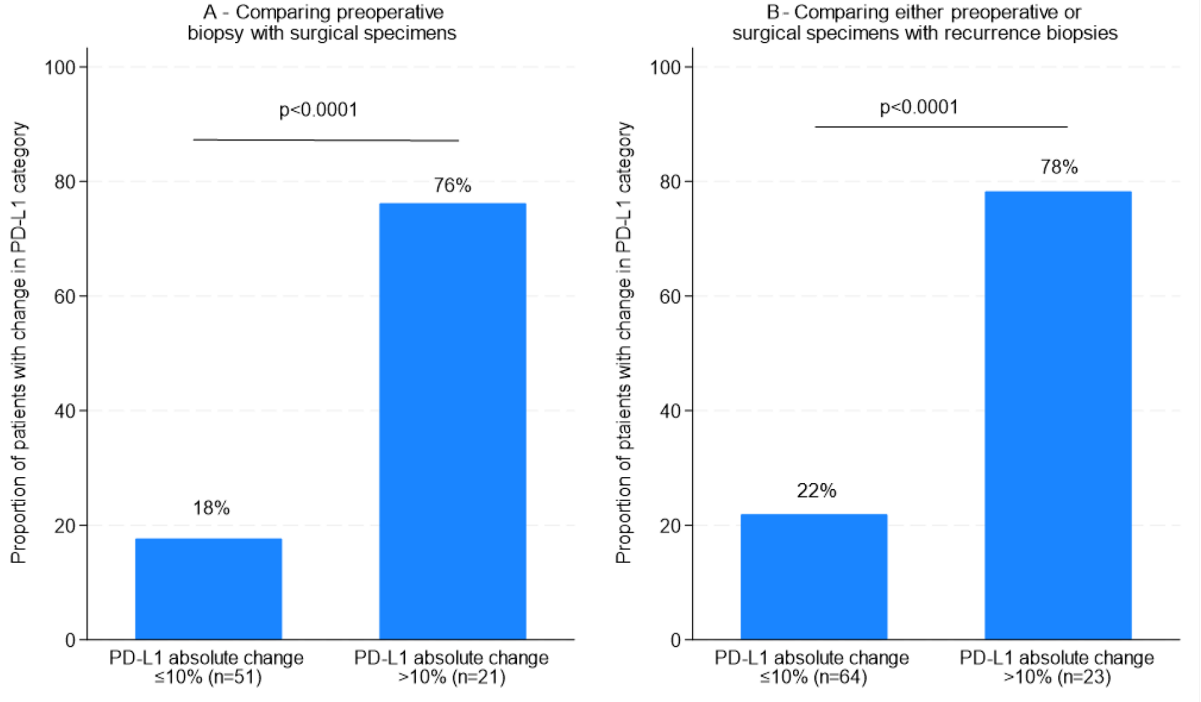

Figure 3 Association between an absolute percentage change in PD-L1 expression of ≤10 or >10% with a change in PD-L1 score group.

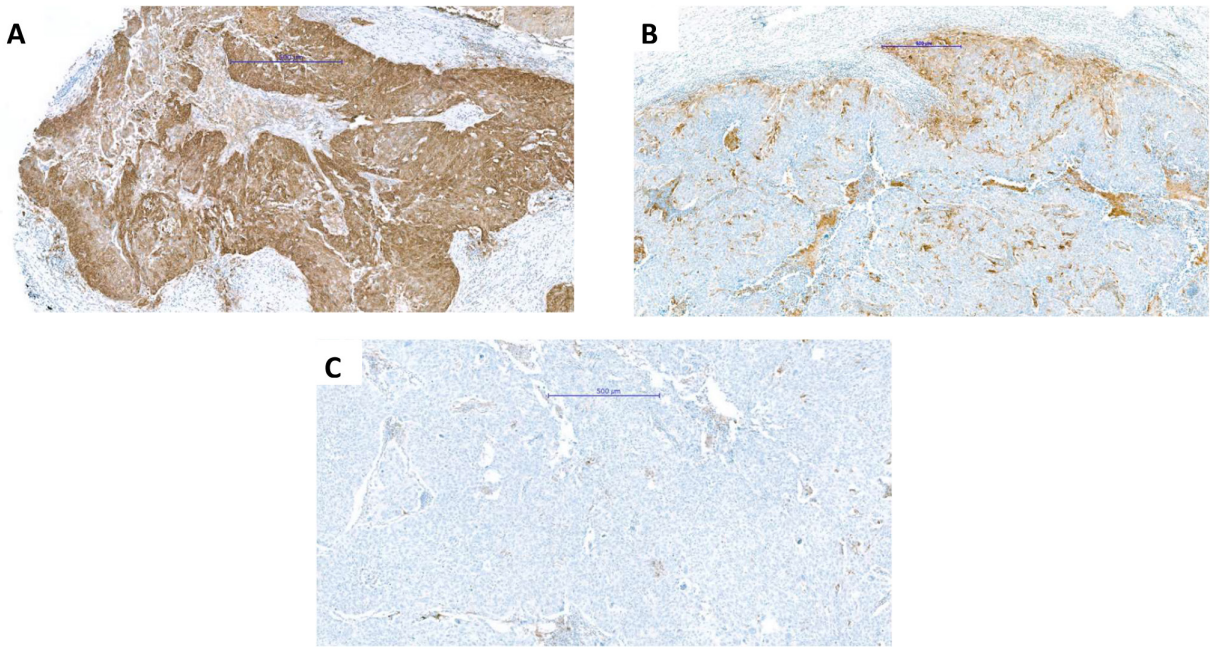

Figure 4 Heterogeneity of PD-L1 expression in the same tumor: tumor proportion scores ranging from 100% (A), over 20 (B) to <1% (C).

metastatic setting, this is the first study comparing preoperative biopsies with matched surgical specimens for early-stage disease and subsequent rebiopsies from relapsed disease. We showed that PD-L1 expression in NSCLC shows dynamic changes throughout the course of disease in a relevant number of cases. PD-L1 expression in tumor cells is one of the most extensively studied predictive biomarkers in NSCLC. A vast number of publications found consistently that PD-L1 expression is associated with OS and PFS in patients who are treated with ICIs.[4 5 17–19] Therefore, treatment decisions and approvals of ICIs are based on PD-L1 expression cut-offs following the results of landmark trials for early-stage and metastatic NSCLC.[20–22] Atezolizumab, for example, gained approval for patients with resected NSCLC and a PD-L1 expression of at least one percent in the adjuvant setting by the FDA whereas the EMA-approved atezolizumab only for patients showing PD-L1 expression in ≥50% tumor cells of the surgical specimen.[22] Durvalumab was approved by the EMA as maintenance therapy for locally advanced stage III NSCLC with a PD-L1 expression of at least one percent following definitive chemoradiation based on the data of the PACIFIC trial.[23] In the palliative setting, clinical trials used different requirements for PD-L1 assessment. In the Keynote studies, treatment-naïve tumor samples from the diagnosis of metastatic disease were required for assessment of PD-L1 expression.[20 23–25] In the Checkmate studies, fresh or archival tumor samples had to be obtained within 3–6 months prior to enrolment.[26–28] In the PACIFIC trial, only archived tumor tissue was required for PD-L1 assessment, a recent biopsy was an optional requirement.[29] The same applied to the IMpower studies.[30 31] In the real-world setting, like in our study, we initially identified 136 patients with relapsed disease but only 87 patients (64%) underwent a rebiopsy. Sometimes tumors are difficult to access, and a biopsy is omitted for reasons like patient's safety or wish. However, in our study, 36.8% of patients changed the PD-L1 group when comparing the PD-L1 expression from surgery and subsequent relapsed disease. 18 patients had a PD-L1 expression ≥50% on surgical specimens whereas only 10 of them were in the high-expression group when biopsied for disease relapse. This leads to inconsistent treatment decisions as patients without a score ≥50% will be treated with a combination of chemotherapy and checkpoint inhibition instead of immunotherapy alone. The change in PD-L1 expression during the course of disease and the neglect of reassessment could be one explanation why we find patients who are thought to have PD-L1 negative tumors and still show an excellent response to checkpoint inhibition and on the other hand patients with high PD-L1 expression who do not respond to treatment with immunotherapy alone.

Another important factor that may explain differences in PD-L1 expression is tumor heterogeneity. Tumor evolution in lung cancer cell lines may lead to focally high expression in some and negative expression in other tumor areas.[15 32 33] Casadevall *et al* compared PD-L1 expression in separate areas of 94 non-squamous and 50 squamous cell carcinoma samples of the lung. Discordance was seen in 10% of the adenocarcinoma and 19% of the squamous cell carcinoma.[15] In our study, we reassessed the surgical specimens of the five patients who were found in all three different score groups during the course of disease, by analyzing all available tissue blocks. We found areas of significantly different PD-L1 expression within the same surgical sample. In two out of five patients, the overall result of PD-L1 expression would have been changed for clinical decision-making. This has major implications for the neoadjuvant, perioperative and adjuvant treatment decision-making. In the neoadjuvant setting, PD-L1 expression is determined by taking a biopsy.

Depending on the area sampled during biopsy, the PD-L1 result will vary significantly. Also, for adjuvant treatment decision-making, the PD-L1 analysis could vary depending on the area from which the diagnostic slide has been cut. Several studies compared PD-L1 expression in biopsy samples and surgical tissue. Ilie *et al* compared 160 patients with operable NSCLC on whole surgical tissue sections and matched lung biopsies. They found a high rate (48%) of discordance.[34] In all cases of PD-L1 disparity, biopsy underestimated PD-L1 expression when compared with surgical specimens. The accuracy of biopsies could be improved when at least six biopsies were performed and used for PD-L1 expression IHC.[34] The heterogeneity within the primary tumor might also explain different PD-L1 expressions in metastases (intertumoral heterogeneity). Several studies described pronounced discrepancies of PD-L1 expression when surgical specimens of the primary tumor were compared with locally lymph nodes and distant metastases.[16 33 35–38]

Tumor evolution and tumor-specific treatment might also lead to dynamic changes in PD-L1 expression during the course of disease. PD-L1 shows inducible expression by activation of NF-κB or IFN-γ secreted by infiltrating lymphocytes or irradiation.[12 39 40] In our study, perioperative treatment had no significant influence on PD-L1 expression. This is in line with a recent publication that investigated the effect of neoadjuvant platin-based chemotherapy on PD-L1 expression. By comparing 37 cases of NSCLC preneoadjuvant and postneoadjuvant chemotherapy, they found no statistically significant influence on PD-L1 expression.[41] However, our sample size might be too small and treatment modalities too heterogenous to conclude with certainty that neoadjuvant or adjuvant treatment has no effect on PD-L1 expression. Furthermore, even with an accurate PD-L1 expression analysis, response to checkpoint inhibition might vary due to patient-intrinsic factors such as age or the gut microbiota, insufficient tumor antigenicity, loss of major histocompatibility complex expression for antigen presentation, myeloid suppressor cells in the tumor microenvironment and tumor-intrinsic escape mechanisms as loss of interferon signaling and others.[42 43]

The main limitation of this study is its retrospective nature. As molecular testing evolved over the past years and patients were enrolled since 2015 the tested mutations varied. However, past 2019 molecular testing was performed using the Ion AmpliSeq Colon/Lung Cancer Panel V2 and Archer Fusion Plex Expanded Lung Panel in almost all cases which exceeds recent recommendations for molecular tests from the European Society for Medical Oncology. In 59 patients, testing of the RET and NTRK gene is missing. In one patient, NTRK, RET, HER2 and cMET are missing. Due to the scarce nature of these mutations, it seems rather unlikely that patients with these alterations were included in our final analysis. Furthermore, the current data do not allow a clear distinction between variations attributable to time, treatment or microenvironment conditions versus those arising from sample heterogeneity.

In conclusion, we have shown that PD-L1 expression shows dynamic changes during the course of disease in patients with relapsed NSCLC. Tumor heterogeneity next to tumor evolution and transcriptional changes might be a possible explanation for this phenomenon. Treatment with ICIs should not be restricted to certain PD-L1 expression levels without consensus guidelines that define PD-L1 testing depending if tissue has been obtained by biopsy or surgery and when reassessment is necessary.

Author affiliations
[1]Division of Pulmonology, Department of Internal Medicine, Medical University of Graz, Graz, Austria
[2]Division of Oncology, Department of Internal Medicine, Medical University of Graz, Graz, Austria
[3]Division of Thoracic Surgery, Department of Surgery, Medical University of Graz, Graz, Austria
[4]Department of Internal Medicine, Marburg Lung Center, Giessen, Germany
[5]Diagnostic and Research Institute of Pathology, Medical University of Graz, Graz, Austria
[6]Division of Hematology, Department of Internal Medicine, Medical University of Graz, Graz, Austria
[7]BioTechMed-Graz Office, Graz, Austria

Contributors Conception and design: AT and LB. Acquisition of data: NJ, VS, JL, MF, MZ, LK and AT. Analysis and interpretation of data: FP, MZ and AT. Review and revision of the manuscript: all authors. Final approval of the manuscript: all authors. AT accepts full responsibility for the work and the conduct of the study, had access to the data, controlled the decision to publish and is guarantor for this work.

Funding The authors have not declared a specific grant for this research from any funding agency in the public, commercial or not-for-profit sectors.

Competing interests None declared.

Patient consent for publication Not applicable.

Ethics approval The study protocol conformed to the Declaration of Helsinki and was approved by the Ethics Committee of the Medical University of Graz (EK 33-174 ex 20/21).

Provenance and peer review Not commissioned; externally peer reviewed.

Data availability statement Data are available on reasonable request.

ORCID iD
Angelika Terbuch http://orcid.org/0000-0003-2918-1370

REFERENCES

1 Sung H, Ferlay J, Siegel RL, *et al*. Global cancer statistics 2020: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *CA Cancer J Clin* 2021;71:209–49.
2 U.S. Department of Health and. Lung and bronchus SEER 5-year relative survival rates, 2013-2019. SEER cancer Statistics. 2023. Available: https://seer.cancer.gov/statistics-network/
3 Gadgeel S, Rodríguez-Abreu D, Speranza G, *et al*. Updated analysis from KEYNOTE-189: pembrolizumab or placebo plus pemetrexed and platinum for previously untreated metastatic nonsquamous non-small-cell lung cancer. *J Clin Oncol* 2020;38:1505–17.

4 Reck M, Rodríguez-Abreu D, Robinson AG, *et al*. Updated analysis of KEYNOTE-024: pembrolizumab versus platinum-based chemotherapy for advanced non–small-cell lung cancer with PD-L1 tumor proportion score of 50% or greater. *J Clin Oncol* 2019;37:537–46.
5 Gibney GT, Weiner LM, Atkins MB. Predictive biomarkers for checkpoint inhibitor-based immunotherapy. *Lancet Oncol* 2016;17:e542–51.
6 Kim T-J, Hong SA, Kim O, *et al*. Changes in PD-L1 expression according to tumor infiltrating lymphocytes of acquired EGFR-TKI resistant EGFR-mutant non-small-cell lung cancer. *Oncotarget* 2017;8:107630–9.
7 Choe E-A, Cha YJ, Kim J-H, *et al*. Dynamic changes in PD-L1 expression and Cd8+ T cell infiltration in non-small cell lung cancer following chemoradiation therapy. *Lung Cancer* 2019;136:30–6.
8 Zens P, Bello C, Scherz A, *et al*. The effect of neoadjuvant therapy on PD-L1 expression and Cd8+Lymphocyte density in non-small cell lung cancer. *Mod Pathol* 2022;35:1848–59.
9 Sheng J, Fang W, Yu J, *et al*. Expression of programmed death Ligand-1 on tumor cells varies pre and post chemotherapy in non-small cell lung cancer. *Sci Rep* 2016;6:20090.
10 Zitvogel L, Galluzzi L, Kepp O, *et al*. Type I interferons in anticancer immunity. *Nat Rev Immunol* 2015;15:405–14.
11 Bai J, Gao Z, Li X, *et al*. Regulation of PD-1/PD-L1 pathway and resistance to PD-1/PD-L1 blockade. *Oncotarget* 2017;8:110693–707.
12 Jardim DL, Murugesan K, Elvin JA, *et al*. PD-L1 gene amplification and focality: relationship with protein expression. *J Immunother Cancer* 2023;11:e006311.
13 Yang C-Y, Lin M-W, Chang Y-L, *et al*. Programmed cell death-ligand 1 expression in surgically Resected stage I pulmonary adenocarcinoma and its correlation with driver mutations and clinical outcomes. *Eur J Cancer* 2014;50:1361–9.
14 Liang M, Yang H, Fu J. Nimesulide inhibits IFN-gamma-induced programmed Death-1-ligand 1 surface expression in breast cancer cells by COX-2 and Pge2 independent mechanisms. *Cancer Lett* 2009;276:47–52.
15 Casadevall D, Clavé S, Taus Á, *et al*. Heterogeneity of tumor and immune cell PD-L1 expression and lymphocyte counts in surgical NSCLC samples. *Clin Lung Cancer* 2017;18:682–91.
16 Saito Y, Horiuchi S, Morooka H, *et al*. Inter-tumor heterogeneity of PD-L1 expression in non-small cell lung cancer. *J Thorac Dis* 2019;11:4982–91.
17 Brody R, Zhang Y, Ballas M, *et al*. PD-L1 expression in advanced NSCLC: insights into risk stratification and treatment selection from a systematic literature review. *Lung Cancer* 2017;112:200–15.
18 Paver EC, Cooper WA, Colebatch AJ, *et al*. Programmed death Ligand-1 (PD-L1) as a predictive marker for Immunotherapy in solid tumours: a guide to immunohistochemistry implementation and interpretation. *Pathology* 2021;53:141–56.
19 Carbognin L, Pilotto S, Milella M, *et al*. Differential activity of Nivolumab, Pembrolizumab and MpdI3280A according to the tumor expression of programmed death-Ligand-1 (PD-L1): sensitivity analysis of trials in Melanoma. *PLoS One* 2015;10:e0130142.
20 Reck M, Rodríguez-Abreu D, Robinson AG, *et al*. Pembrolizumab versus chemotherapy for PD-L1-positive non-small-cell lung cancer. *N Engl J Med* 2016;375:1823–33.
21 Langer CJ, Gadgeel SM, Borghaei H, *et al*. Carboplatin and Pemetrexed with or without Pembrolizumab for advanced, non-squamous non-small-cell lung cancer: a randomised, phase 2 cohort of the open-label KEYNOTE-021 study. *Lancet Oncol* 2016;17:1497–508.
22 Felip E, Altorki N, Zhou C, *et al*. Adjuvant atezolizumab after adjuvant chemotherapy in Resected stage IB–IIIA non-small-cell lung cancer (Impower010): a randomised, Multicentre, open-label, phase 3 trial. *Lancet* 2021;398:1344–57.
23 Antonia SJ, Villegas A, Daniel D, *et al*. Overall survival with durvalumab after chemoradiotherapy in stage III NSCLC. *N Engl J Med* 2018;379:2342–50.
24 Gandhi L, Rodgríguez-Abreu D, Gadgeel S, *et al*. Abstract Ct075: KEYNOTE-189: randomized, double-blind, phase 3 study of Pembrolizumab (Pembro) or placebo plus Pemetrexed (Pem) and platinum as first-line therapy for metastatic NSCLC. *Cancer Research* 2018;78:CT075.
25 Paz-Ares L, Luft A, Vicente D, *et al*. Pembrolizumab plus chemotherapy for squamous non-small-cell lung cancer. *N Engl J Med* 2018;379:2040–51.
26 Carbone DP, Reck M, Paz-Ares L, *et al*. First-line Nivolumab in stage IV or recurrent non-small-cell lung cancer. *N Engl J Med* 2017;376:2415–26.
27 Paz-Ares L, Ciuleanu T-E, Cobo M, *et al*. First-line Nivolumab plus Ipilimumab combined with two cycles of chemotherapy in patients with non-small-cell lung cancer (Checkmate 9La): an international, randomised, open-label, phase 3 trial. *Lancet Oncol* 2021;22:198–211.
28 Vokes EE, Ready N, Felip E, *et al*. Nivolumab versus Docetaxel in previously treated advanced non-small-cell lung cancer (Checkmate 017 and Checkmate 057): 3-year update and outcomes in patients with liver metastases. *Ann Oncol* 2018;29:959–65.
29 Antonia SJ, Villegas A, Daniel D, *et al*. Durvalumab after chemoradiotherapy in stage III non–small-cell lung cancer. *N Engl J Med* 2017;377:1919–29.
30 Socinski MA, Jotte RM, Cappuzzo F, *et al*. Atezolizumab for first-line treatment of metastatic Nonsquamous NSCLC. *N Engl J Med* 2018;378:2288–301.
31 Nishio M, Barlesi F, West H, *et al*. Atezolizumab plus chemotherapy for first-line treatment of Nonsquamous NSCLC: results from the randomized phase 3 Impower132 trial. *J Thorac Oncol* 2021;16:653–64.
32 Bassanelli M, Sioletic S, Martini M, *et al*. Heterogeneity of PD-L1 expression and relationship with biology of NSCLC. *Anticancer Res* 2018;38:3789–96.
33 Haragan A, Field JK, Davies MPA, *et al*. Heterogeneity of PD-L1 expression in non-small cell lung cancer: implications for specimen sampling in predicting treatment response. *Lung Cancer* 2019;134:79–84.
34 Ilie M, Long-Mira E, Bence C, *et al*. Comparative study of the PD-L1 status between surgically Resected specimens and matched biopsies of NSCLC patients reveal major discordances: a potential issue for anti-PD-L1 therapeutic strategies. *Ann Oncol* 2016;27:147–53.
35 Terra SBSP, Mansfield AS, Vrana JA, *et al*. Heterogeneity of programmed death-ligand 1 expression in thymic epithelial tumours between initial specimen and synchronous or metachronous metastases or recurrences. *Histopathology* 2019;74:364–7.
36 Gowrishankar K, Gunatilake D, Gallagher SJ, *et al*. Inducible but not constitutive expression of PD-L1 in human Melanoma cells is dependent on activation of NF-KB. *PLoS One* 2015;10:e0123410.
37 Frank MS, Bødtger U, Høegholm A, *et al*. Re-biopsy after first line treatment in advanced NSCLC can reveal changes in PD-L1 expression. *Lung Cancer* 2020;149:23–32.
38 Lacour M, Lee SY, Rulle U, *et al*. The need of re-biopsy: increase in PD-L1 expression from initial stage to recurrence of non-small cell lung cancer. *Annals of Oncology* 2018;29:viii512.
39 Abiko K, Matsumura N, Hamanishi J, *et al*. IFN-Γ from lymphocytes induces PD-L1 expression and promotes progression of ovarian cancer. *Br J Cancer* 2015;112:1501–9.
40 Deng L, Liang H, Burnette B, *et al*. Irradiation and anti-PD-L1 treatment synergistically promote antitumor immunity in mice. *J Clin Invest* 2014;124:687–95.
41 Rojkó L, Reiniger L, Téglási V, *et al*. Chemotherapy treatment is associated with altered PD-L1 expression in lung cancer patients. *J Cancer Res Clin Oncol* 2018;144:1219–26.
42 Kalbasi A, Ribas A. Tumour-intrinsic resistance to immune checkpoint blockade. *Nat Rev Immunol* 2020;20:25–39.
43 Hack SP, Zhu AX, Wang Y. Augmenting anticancer immunity through combined targeting of angiogenic and PD-1/PD-L1 pathways: challenges and opportunities. *Front Immunol* 2020;11:598877.