Original Article

Tumoral PD-L1 does not impact time to treatment discontinuation in EGFR mutated non-small cell lung cancer patients treated with EGFR tyrosine kinase inhibitor—a Danish cohort study

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Background: Not all non-small cell lung cancer (NSCLC) patients harboring epidermal growth factor receptor (EGFR) mutations respond equally to therapy with tyrosine kinase inhibitors (TKIs). Programmed death ligand-1 (PD-L1) has previously been speculated as a possible biomarker for treatment outcome because of its positive correlation with these mutations in cell lines, but clinical studies have yielded conflicting results. We investigate the predictive potential of this surface protein in relation to clinical benefit in patients as measured by time to treatment discontinuation (TTD).

Methods: We screened 516 Danish patients with EGFR mutations for inclusion based on a history of TKI treatment and a PD-L1 status that was assessed no earlier than three months prior to treatment initiation. Patients were stratified according to their expression of the potential biomarker as either negative (0%), low (1–49%) or high (≥50%). We employed the Kaplan-Meier method and the log rank test to test for a difference in treatment duration according to PD-L1 expression.

Results: We included 111 Danish patients. The median follow-up time from inclusion until death or censoring at the end of the study was 670 days (range, 32–1,664 days, 95% CI: 502–897 days). Fifty-seven patients (51%) categorized as PD-L1 expression negative, 32 (29%) as low and 22 (20%) as high. We tested for differences in treatment duration between the three groups. Our tests did not yield statistically significant P values.

Conclusions: In our cohort of 111 Danish patients with NSCLC harboring mutations in the epidermal growth factor receptor gene, expression levels of PD-L1 did not significantly impact the duration of clinical benefit from tyrosine kinase treatment.

Keywords: Non-small cell lung cancer (NSCLC); epidermal growth factor receptor (EGFR); tyrosine kinase inhibitor (TKI); programmed death ligand-1 (PD-L1); lung cancer

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Introduction

GLOBOCAN estimates that lung cancer accounted for 11.4% of new cancer diagnoses in 2020 (1). Its incidence has been surpassed by that of female breast cancer, but lung cancer remains the most common cause of cancer-related death (1). Non-small cell lung cancer (NSCLC) makes up more than 80% of cases (2,3) and the prognosis for stage IV NSCLC is poor (4,5). Treatment options for stage IV NSCLC are palliative, and the therapeutic strategy depends on clinical aspects (i.e., comorbidity and performance

status), molecular features including tumoral expression of programmed death ligand-1 and the presence of targetable oncogenic driver mutations such as mutations in the epidermal growth factor receptor (EGFR) gene. The EGFR gene encodes a transmembrane receptor tyrosine kinase. Mutations in the kinase coding region are dominated by exon 19 in-frame deletions and exon 21 substitutions that lead to constitutive activation of the receptor. This upregulates the downstream effects of cell proliferation, survival and motility, which results in cancer (6,7). A recent analysis estimated the prevalence of EGFR-mutations among patients with NSCLC to be 49.1% in Asia. In European patients, the prevalence is 12.8% (8). EGFR tyrosine kinase inhibitors (TKIs) target these mutations and improve the progression free survival (PFS) compared to chemotherapy (9). The third-generation drug Osimertinib is currently the standard of care for EGFR mutated NSCLC and is superior to previous variants including Erlotinib and Gefitinib (10). However, not all patients respond equally to EGFR TKIs, and resistance (either intrinsic or acquired) is considered inevitable. Predictive biomarkers for detecting these resistance mechanisms and optimizing the tailored therapy are wanted.

The surface protein programmed death ligand-1 (PD-L1) binds to the PD-1 receptor on T-lymphocytes and inhibits their cytotoxic function (11). Its expression by tumor cells is used as a biomarker for choosing immunotherapeutic treatment. Studies of EGFR mutated cell lines have found higher levels of PD-L1 compared to wild type cells and that activation of the EGFR pathway led to increase of PD-L1 (12-14). Known EGFR TKI resistance mechanisms including the T790M mutation have also been shown to increase the expression of PD-L1 (15). These relationships have led to speculations that PD-L1 levels might also be indicative of EGFR TKI outcome. Some clinical studies did find an elevated expression of PD-L1 in EGFR mutated patients as well (14,16,17), but several meta-analyses concluded the opposite (18-21). Results on the predictive value of PD-L1 for PFS on EGFR TKI treatment have also been ambiguous. Positive (17,22) and negative (23-26) correlations have been shown, and others yet find no impact (27,28). Our study contributes to this debate by testing for a correlation between PD-L1 levels and length of treatment in a European EGFR mutated cohort, whereas most previous studies have been conducted in Asia. By measuring treatment duration as a surrogate for clinical benefit rather than focusing on progression-free survival, we also believe that our end point, combined with the limited exclusion criteria of our cohort, better represents the clinical reality of EGFR TKI therapy. We present the following article in accordance with the REMARK reporting checklist (available at https://tlcr.amegroups.com/article/view/10.21037/tlcr-22-211/rc).

Methods

Patients

Using our local Danish quality assurance database, we retrospectively identified patients with EGFR mutated NSCLC diagnosed between January 1st, 2010, and September 30th, 2020 that received Tarceva or Tagrisso as any line of therapy and whose course of EGFR TKI was predated by an assessment of tumoral PD-L1 no older than a maximum of three months. Routine testing of patient biopsies using the PD-L1 immunohistochemistry (IHC) assay 22C3 at our facility was introduced in 2016, and patients treated prior to this were generally excluded based on no available PD-L1 status. However, a small number of previously treated patients experienced disease relapse or progression after 2016, resulting in EGFR TKI treatment that was preceded by PD-L1 evaluation. These patients were included because the requirement was an available PD-L1 assessment at initiation of EGFR TKI rather than at original diagnosis. All patients were treated with Erlotinib or Osimertinib at outpatient clinics in the Danish cities Herning, Aalborg or Aarhus and received routine clinical care. Patients were excluded for any of the following reasons: (I) no EGFR TKI treatment received, (II) PD-L1 status unavailable or older than 3 months at the beginning of included EGFR TKI course (this was limited to 1 month if assessed during other systemic therapy) and (III) duration of treatment less than 30 days. Previous treatment with EGFR TKI did not result in exclusion if a new PD-L1 assessment was carried out prior to the investigated line of therapy. If any patients had received more than one line of EGFR TKI, we included them at the first treatment course that had a corresponding PD-L1 value. *Figure 1* shows the inclusion process as a flowchart. Smoking status and comorbidity were assessed at the time of NSCLC diagnosis. Remaining baseline characteristics related to the initiation of TKI treatment. Patients were categorized as M-stage 0 for no metastases, stage M1A for intrathoracic metastases or M1B for extrathoracic metases as according to the TNM staging system, 7th edition.

Follow-up ended on September 30th, 2021. Incomplete

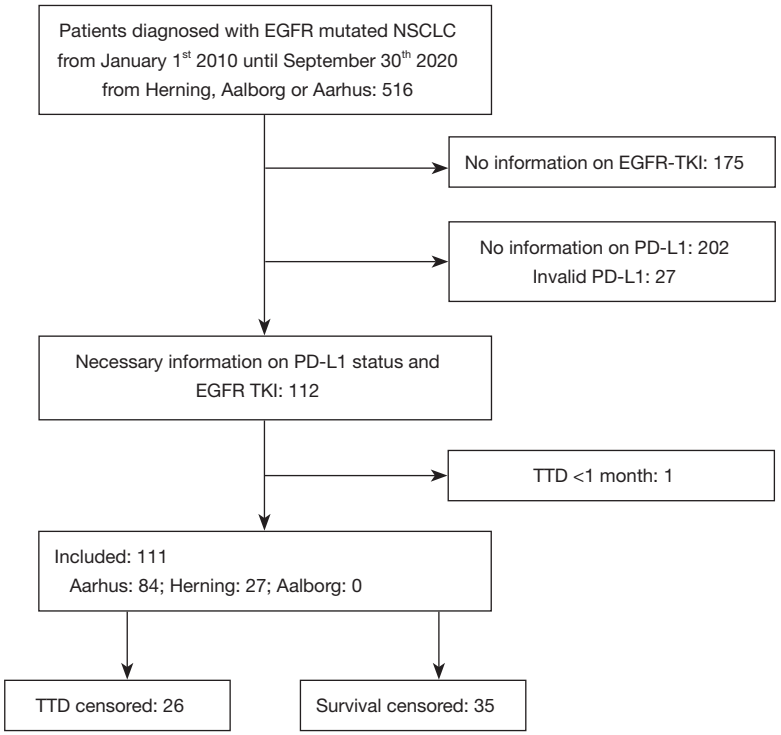

Figure 1 Flowchart depicting the inclusion process of patients. Aalborg, Herning and Aarhus are names of Danish cities. EGFR, epidermal growth factor receptor; NSCLC, non-small cell lung cancer; PD-L1, programmed death ligand-1; TKI, tyrosine kinase inhibitor; TTD, time to treatment discontinuation.

data on survival and treatment was censored 6 months after the last CT scan or by the end of our study period, whichever came first.

PD-L1 and EGFR

Testing for PD-L1 levels and EGFR mutations were done prior to EGFR TKI treatment and therefore blinded to the survival outcomes. Both tests were conducted separately from and prior to this register-based study, and data on results were found in our quality assurance database. Routine diagnostic work-up included testing for PD-L1 expression with immune histochemistry (IHC) using the 22C3 antibody (Agilent Cat# GE00621-2, RRID:AB_2833074) on FFPE biopsy specimens. PD-L1 expression was determined as the Tumor Proportion Score (TPS), the percentage of viable tumor cells that exhibited membrane staining at any intensity. We divided our patients into categories of negative (0%), low (1–49%) and high (50–100%) TPS.

EGFR testing of DNA from tissue biopsies was done routinely. Patients included before January 1st, 2018, were tested with the cobas EGFR Mutation Test (Roche Diagnostics). After this date testing was done with the CE-IVD approved NGS test (Oncomine Solid Tumor DNA and Fusion Transcripts kit (Life Technologies).

End points

Our primary end point was time to treatment discontinuation (TTD) defined as the number of days between the clinical decision to initiate EGFR TKI treatment and the clinical decision to end it. Tarceva and Tagrisso are administered as daily tablets that can be initiated and terminated with immediate notice. Their half-life durations are 36 and 48 hours, respectively, and patients would be drug-free within one or two weeks after ending treatment. We therefore believe that this interval corresponds well to the actual period of receiving the drug.

We chose TTD rather than PFS because treatment beyond progression is a common approach when treating NSCLCs with EGFR TKIs (29). Also, TTD is easier to determine as part of routine clinical care where image evaluation is less structured. We consider TTD a surrogate marker for duration of clinical benefit, and it has been

shown to correlate well with PFS determined by RECIST criteria (r=0.87) when considering treatment termination due to any cause (30). We defined termination due to progression (clinical or radiological) or death as events. Other reasons for treatment discontinuation resulted in censoring at the date of decision. Death was treated as an event rather than a competing risk because we consider death from other causes than cancer progression highly unlikely in this cohort. Switching from one EGFR TKI to another in the same line because of toxicity or change of clinical practice was considered a continuation of treatment. We finally examined whether PD-L1 was a prognostic biomarker for overall survival defined as time from the decision to initiate treatment until death.

Statistical analyses

All analyses were performed using STATA version 17.0. Distributions of dichotomized baseline characteristics were calculated using the χ^2-test. Equality of survivor functions was tested using the log rank test. We conducted both single variate analyses and a multivariate analysis using the Cox proportional hazards model. Tests were 2-sided and P values below 0.05 were considered significant.

Literature search

A systematic literature search was conducted in the PubMed database to find all clinical studies that evaluate TKI treatment outcome in patients stratified according to their levels of tumor PD-L1. A MeSH-term search strategy was created by combining the words NSCLC, EGFR, TKI and PD-L1, including relevant synonyms plus names of all commercially available EGFR TKIs. This search yielded 201 results in the PubMed database as of September 2020. Studies were eligible if (I) they included patients with advanced EGFR-mutated NSCLC, (II) patients received EGFR TKI as any line of therapy, (III) patients had available pre-treatment PD-L1 statuses and (IV) PFS in relation to PD-L1 status was an end point. We also inspected the reference lists of two metanalyses published in 2021 and included two studies from these, and 3 recent studies were included during an independent PubMed search carried out in May, 2022.

Ethical statement

The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013). Ethical approval of this study was not legally required as no biological material was collected (Danish Scientific Ethical Committees Act, paragraph 14.2). The study was conducted using pre-existing data readily available in our local quality assurance database (Aarhus Lung Cancer Registry). Patients were not contacted in relation to this study, and our findings did not impact their disease course in any way. The need for written consent was also waived due to the retrospective design.

Results

Our final cohort included 111 patients with EGFR mutated NSCLC. The median follow-up time from inclusion until death or censoring at the end of our study period was 670 days (range, 32–1,664 days, 95% CI: 502–897 days). Most patients were female (66%), had disseminated disease (72%) and were current or former smokers (60%). One hundred and five (95%) patients received EGFR TKI as their first palliative line (defined as treatment without curative potential), and all but one (99%) had adenocarcinoma histology. No expression of tumoral PD-L1 was found in 51% of our patients, while low and high expressions accounted for 29% and 20%. *Table 1* shows the distribution of dichotomized baseline characteristics in the three PD-L1 expression groups. None of the variables were unequally distributed.

PD-L1 and TTD

Median time until discontinuation of treatment was 502 days [range, 59–1,596 days (95% CI: 336–610 days)] for negative, 420 days [range, 32–1,549 days (95% CI: 210–653 days)] for low and 262 days [range, 42–884 days (95% CI: 82–573 days)] for high PD-L1 expression categories. We compared TTD between the groups using the Kaplan-Meier method as portrayed in *Figure 2* and tested for equality of survivor functions. Although visual assessment of the Kaplan-Meier plots indicates an initial shortening of clinical benefit for higher PD-L1, the log rank test did not result in a statistically significant difference.

To examine whether any of our cohort's baseline characteristics influenced the results, we chose to conduct a multivariate analysis using the Cox proportional hazards model as shown by *Table 2*. We first investigated each variable in a univariate model and included those with a

Table 1 Dichotomized baseline characteristics and their distribution

Variables	n (% of known)	PD-L1 categories			P value
		None	Low	High	
Gender					0.58
Female	73 [66]	38	19	16	
Male	38 [34]	19	13	6	
Age					0.78
Below 70	49 [44]	27	13	9	
70 or above	62 [56]	30	19	13	
M-stage					0.24
M0 or M1A	31 [28]	20	7	4	
M1B	79 [72]	37	25	17	
Smoking					0.57
Never	44 [40]	25	10	9	
Former/current	66 [60]	32	21	13	
PS					0.56
0/1	80 [77]	42	24	14	
2+	24 [23]	10	8	6	
Comorbidity					0.16
None	64 [58]	28	22	14	
Any	47 [42]	29	10	8	
Mutation					0.22
Del19/L858R	87 [80]	47	25	15	
Other	22 [20]	8	7	7	
Histology					0.62
Adenocarcinoma	109 [99]	55	32	22	
NOS	1 [1]	1	0	0	
Line of therapy					0.63
1st	105 [95]	54	31	20	
2nd or later	6 [5]	3	1	2	
Drug					0.34
ERL	64 [58]	33	21	10	
OSI or BOTH	47 [42]	24	11	12	
BM, baseline					0.50
No	92 [84]	48	28	16	
Yes	17 [16]	8	4	5	

P values for uneven distributions were calculated with the Chi square-test and considered significant when <0.05. No significant differences were found. M-stage, metastatic stage according to TNM, 7th edition; PS, performance status, NOS, not otherwise specified; ERL, Erlotinib; OSI, Osimertinib; BOTH, Erlotinib and Osimertinib consecutively; BM, brain metastases (at baseline of EGFR TKI initiation); PD-L1, programmed death ligand-1; EGFR, epidermal growth factor receptor; TKI, tyrosine kinase inhibitor.

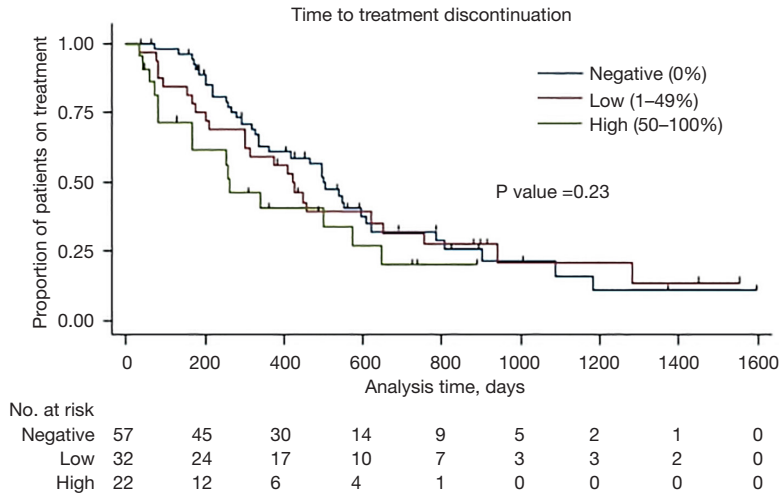

Figure 2 Kaplan-Meier plot showing TTD for three levels of tumoral PD-L1 expression. PD-L1, programmed death ligand-1; TTD, time to treatment discontinuation.

significant P value of 0.05 or less in our final model. PD-L1 level was not significantly associated with TTD in this analysis. High performance status and metastatic stage as well as uncommon mutations were negatively correlated with TTD, whereas being treated with Osimertinib showed a positive correlation to TTD. We finally conducted a subset analysis of patients harboring only the common EGFR mutations del19 or L858R. The resulting Kaplan-Meier plot is shown by *Figure 3* with a corresponding P value of 0.66.

PD-L1 and overall survival

We also examined whether PD-L1 levels impacted overall survival. The Kaplan-Meier plot is shown in *Figure 4*, and the log rank test yielded a statistically insignificant P value of 0.27.

Literature search

We compiled a list of previous studies and their conclusions in *Table 3* (17,22-28,31-36). Three studies from the two recent meta-analyses (37,38) were excluded for the following reasons: (I) the cohort was purposely constructed so more than half had primary resistance to EGFR TKI (39), (II) the study investigated post-TKI tissue samples (40) and (III) the study excluded patients with stage IIIB–IV disease (41). We included 14 studies that represent the existing results on pre-treatment tumoral PD-L1 as a predictive biomarker for EGFR TKI treatment. Thirteen studies were Asian, whereas only one was conducted on a Caucasian population. We registered the type of antibodies used and the cut-off values or grading systems to highlight differences in methodology. We focused on PFS or time on treatment as primary end points, and studies not including these were not listed. Finally, we investigated each study's definition of progressive disease to determine whether other studies have used TTD or variants thereof before us.

Discussion

This study of 111 NSCLC Danish patients treated with EGFR TKIs yielded no significant difference in TTD or OS according to PD-L1 levels. Our initial analysis did seem to visualize a trend towards shorter TTD for higher PD-L1 expression. However, we believe that the higher proportions of uncommon mutations in our 'low' and 'high' PD-L1 expression groups are responsible for a large part of this perceived difference. A subset analysis of patients harboring common EGFR mutations demonstrated even less impact of PD-L1 on TTD with a P value of 0.66.

The applicability of PD-L1 as a biomarker for EGFR TKI outcome is controversial. The authors of this article are not aware of any molecular rationale coupling the expression of PD-L1 to the effect of EGFR TKI treatment. However, the relationship between the two has already been examined in several previous studies with conflicting results (see *Table 3*). The heterogeneity between these conducted studies is large. Different types of antibodies are employed, and the lack of standardized cut-off values also results in

Table 2 Results of univariate analyses using the Cox proportional hazards model

Variable	Hazard ratio	Standard error	P value	95% CI
Univariate analyses				
PD-L1 category				
Negative	Reference			
Low	1.12	0.29	0.68	[0.67; 1.87]
High	1.70	0.53	0.09	[0.92; 1.13]
Performance status				
0 or 1	Reference			
2 or above	1.96	0.54	0.02	[1.14; 3.36]
Comorbidity				
None	Reference			
Any	0.94	0.22	0.80	[0.59; 1.50]
EGFR mutation				
Common	Reference			
Uncommon	2.85	0.82	0.00	[1.63; 5.00]
Drug				
Erlotinib	Reference			
Osimertinib or both	0.51	0.12	0.01	[0.31; 0.82]
Metastatic stage (TNM)				
0 or 1A	Reference			
1B	2.03	0.56	0.01	[1.18; 3.48]
Brain metastases				
No	Reference			
Yes	1.71	0.51	0.07	[0.95; 3.01]
Multivariate analysis				
PD-L1 category	1.29	0.22	0.13	[0.92; 1.79]
Performance status	2.04	0.59	0.01	[1.16; 3.58]
EGFR mutation	2.71	0.81	0.00	[1.50; 4.88]
Drug	0.47	0.12	0.00	[0.28; 0.79]
Metastatic stage (TNM)	1.68	0.50	0.08	[0.94; 3.01]

Independent variables with P values of 0.05 or less were included in the multivariate model. PD-L1, programmed death ligand-1; EGFR, epidermal growth factor receptor; TNM, Tumour, Node, Metastasis.

different ways of stratifying PD-L1 expression. Although several studies categorize patients as positive or negative, the threshold varies between articles. Finally, criteria for inclusion of patients differ, exemplified by one study including ALK$^+$ patients in their PFS calculations (23), and by the variance in previous treatments and disease stages allowed. Our study contributes to the debate by including an all-European cohort of more than a hundred patients and by considering the end point of clinical benefit rather than progression-free survival in a time where treatment beyond

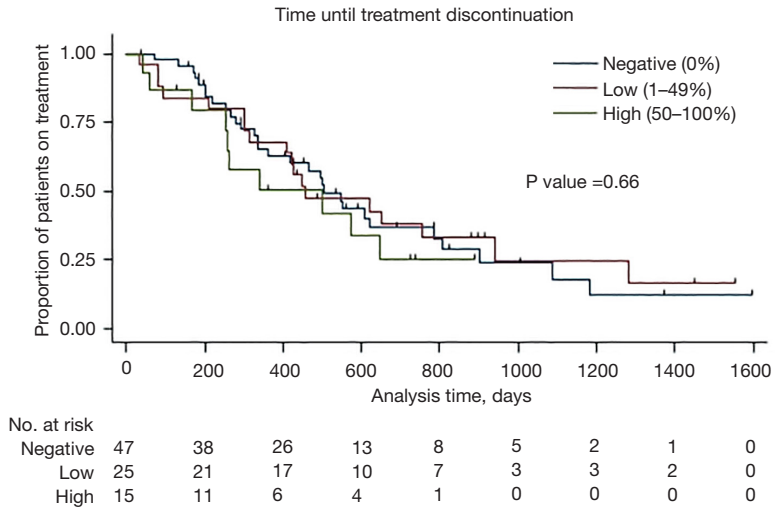

Figure 3 Kaplan-Meier plot showing time until treatment discontinuation according to levels of tumoral programmed death ligand-1 expression in a subset of patients harboring common EGFR mutations. Common mutations are either exon 19 deletions or L585R substitutions in exon 21. EGFR, epidermal growth factor receptor.

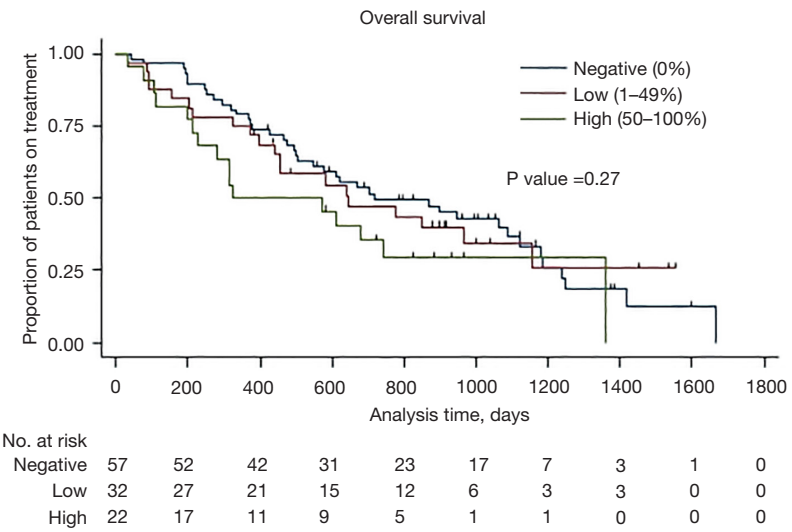

Figure 4 Kaplan-Meier plot showing overall survival according to tumoral PD-L1 expression levels. PD-L1, programmed death ligand-1.

progression is a common approach. To our knowledge, only one other study (36) has considered an end point that includes clinical evaluation as well as radiology.

Lan *et al.* (37) published a meta-analysis in 2021 which pools 12 studies examining the effect of PD-L1 on PFS. The adjusted analysis concluded no significant difference. However, patients were divided into only positive and negative. Several studies (16,24-26) suggest a worse outcome of EGFR TKI in patients with ≥50% tumoral PD-L1, an effect that might be diluted by pooling them with patients of 1–49% expression. Another meta-analysis conducted by Peng *et al.* found that higher PD-L1 expression is significantly associated with poorer PFS (HR 1.90, 95% CI: 1.16–3.10, P=0.011) (38). In conclusion, the topic of tumoral PD-L1 as a biomarker for PFS is still lacking standardized methods that could increase comparability.

Our current study is the second of its kind to be conducted on a Caucasian population. D'incecco *et al.* found a significantly longer time to progression for positive compared to negative patients in an Italian cohort (17), but

Table 3 Existing literature on the topic of tumoral pre-treatment PD-L1 as a biomarker for PFS

Author, region, year	EGFR$^+$ patients	PD-L1 antibody	PD-L1 levels	PFS	Definition of progression	OS
Soo (23), Seoul, 2017	70 with available PD-L1	SP142	Continuous H-score	Shorter PFS for higher H-scores (P=0.017) Shorter PFS for 10% highest PD-L1 H-scores (P<0.001)	NS	No association between higher PD-L1 scores and OS (P=0.795)
Su (24), Guangdong, 2018	84 with available PD-L1	SP142	TPS of strong (TC ≥50% or IC ≥10%), weak (TC 5–49% or IC 5–9%) or negative (TC and IC <5%)	Shorter PFS for strong expression *vs.* weak/ negative (P<0.001)	NS	Not included
Yoon (25), Seoul & Busan, 2020	131	22C3	TPS of <1%, 1–49% or ≥50%	Shorter PFS for >50% *vs.* <1% (P=0.002) Shorter PFS for >50% *vs.* 1–49% (P=0.002)	RECIST 1.1	TPS ≥50% not associated with OS (P=0.181)
Yoneshima (31), Fukuoka, 2018	71, but pooled with 8 ALK$^+$	22C3	TPS of <1%, 1–49% or ≥50%	Shorter PFS for PD-L1 >1% *vs.* to <1% (P=0.016) No significant difference in PFS between when comparing all three groups (P value not listed)	NS	Not included
Kim (27), Seoul, 2020	66	SP263 + 22C3 + SP142	Positive/negative. Cut-off: ≥1% of viable tumor cells exhibited membrane staining	No significant difference in PFS for positive *vs.* negative (P=0.529)	NS	No difference in OS (P=0.150)
Tang (28), Guangzhou, 2015	99	EIL3N	Positive/negative. Cut-off: H-score of ≥5	No significant difference in PFS positive *vs.* negative (P=0.990)	NS	No difference (P=0.932)
Lin (22), Fuzhou, 2015	56	Ab58810	Positive/negative. Cut-off: mean H-score of all patients	Longer PFS for positive *vs.* negative (P=0.001)	NS, RECIST 1.1 for ORR/ DCR	Longer OS for positive patient (P=0.004)
Yang (26), Zhongzheng, 2020	153	22C3	TPS of <1%, 1–49% or ≥50%	Shorter PFS for ≥50% *vs.* 0% (P=0.009) Shorter PFS for ≥50% *vs.* 1–49% (P=0.044) Shorter PFS for ≥50% *vs.* 0–49% (P=0.007)	RECIST 1.1	No difference (P=0.605)
D'incecco (17), Italy (not further specified), 2015	54	Ab58810	Positive/negative. Cut-off: staining intensity of 2 in more than 5% of tumor cells	Longer time to progression for positive *vs.* negative (P=0.01)	NS	No difference (P=0.75)
Matsumoto (32), Osaka, 2019	52	28-8	High (≥50%) or low (0–49%)	Shorter PFS for high PD-L1 *vs.* low (P=0.0059)	RECIST 1.1	Not included
Kobayashi (33), Tokyo, 2018	32	Unclear	Positive/negative. Cut-off: staining intensity of 3 in more than 5% of cells	No significant difference in PFS for positive *vs.* negative (P=0.58)	NS	No difference

Table 3 (*continued*)

Table 3 (*continued*)

Author, region, year	EGFR$^+$ patients	PD-L1 antibody	PD-L1 levels	PFS	Definition of progression	OS
Chang (34), New Taipei & Yilan County & Yanchao District, 2021	114	22C3	TPS of <1%, 1–49% or ≥50%	No significant difference in PFS between groups (P=0.738)	RECIST 1.1	No difference (P=0.769)
Kang (35), Seoul, 2021	108	22C3, SP263	TPS of <1%, 1–49% or ≥50%	Significantly shorter PFS for strong *vs.* negative (P=0.001)	RECIST 1.1	Not included
Inomata (36), 2022, Toyama	49	22C3	Positive/negative. Cut-off: TPS of 1%	Significant impact of PD-L1 on time on treatment in adjusted analysis (P=0.022)	RECIST 1.1 or clinical judgment	Not included

Correlation with OS for included studies is also listed, as well as antibodies, cut-off values and definition of progression for each study. PD-L1, programmed death ligand-1; PFS, progression free survival; OS, overall survival; TPS, tumor proportion score; TC, tumor cells; IC, immune cells; NS, not specified.

we believe that our study design has several advantages. Our cohort, which is twice as big, is stratified into three expression levels that are representative of current clinical practice when deciding between palliative treatments. We have also specified a time limit of 3 months from PD-L1 assessment to treatment initiation to avoid the influence of time and other therapies on expression levels. Finally, we chose to evaluate TTD rather than PFS because this date is easier to assess in routine clinical care and because we believe it to be a more relevant end point for this patient cohort. It is an accepted practice to continue EGFR TKI treatment beyond radiological progression if there is clinical benefit, and the routine radiological assessment lacks standardization. Our definition of TTD covers treatment discontinuation on basis of both radiology and clinical assessment. Of the studies in *Table 3*, only half have specified how 'disease progression' was defined and all but one of these relied on the RECIST criteria (version 1.1) without considering clinical progression. We believe that we are the first to evaluate the correlation between PD-L1 and duration of clinical benefit in European cohort of EGFR mutated NSCLC treated with TKIs.

Our study also has limitations. (I) Using TTD as our end point does rely on the subjective opinion of the treating physician because there is no general definition of clinical progression nor clinical benefit. This bias is difficult to eliminate entirely. However, 84 of our patients were treated at Aarhus University Hospital (AUH), and the remaining patients were treated in Herning. The small number of centers is an advantage in this regard because we believe that doctors from the same departments are more likely to follow the same principles in decision-making. At AUH, all status scans showing possible or definite progression are reviewed at conference meetings with senior staff to ensure a standardized treatment. (II) The study was done retrospectively and therefore subject to selection bias. (III) We only included patients from two institutions in Denmark. The majority (84/111) were included from Aarhus University Hospital. This limits the extern validity of our findings, but we also believe that it is an advantage in decreasing the influence of personal bias on decisions-making amongst doctors. (IV) Our database did not contain information on pauses during treatment. Patients experiencing adverse effects are sometimes taken off the drug until side effects lessen. We believe that TTD corresponds well to the time of receiving the drug, but we are not able to account for possible agreed-upon pauses that might influence this. (V) Although our cohort is of considerable size when comparing to the existing literature, the number of included patients is limited. (VI) As for any study on tumoral PD-L1, the heterogeneity of tumors remains a problem. McLaughlin *et al.* (42) demonstrated that different areas of the same tumor might stain as positive and negative. Biopsies used to evaluate PD-L1 are not necessarily representative of the entire tumor and the categorization of patients could in fact be due to chance. (VII) We have used the log rank test and the Cox proportional hazards analysis although our survival curves cross. This is a violation of the assumption of proportional hazards that the tests are based on, and we are aware that it

weakens the ability to detect an actual difference (43). We chose the log rank test because no standardized solution exists and because this is the common choice in cancer survival analyses. (VIII) We did not include other variables than PD-L1. Efficacy of EGFR TKIs might be modified by a variety of other factors including co-mutations (44,45) and tumor mutational burden (46).

Conclusions

We did not find a significant correlation between PD-L1 expression and length of treatment or OS in our Danish cohort. The clinical applications of these findings are limited, as PD-L1 is not used a biomarker for choosing EGFR TKI treatment in EGFR mutated NSCLC patients. However, our study contributes to the diverging results on this topic by focusing on a European cohort and using time until treatment discontinuation as an end point.

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Footnote

Reporting Checklist: The authors have completed the REMARK reporting checklist. Available at https://tlcr.amegroups.com/article/view/10.21037/tlcr-22-211/rc

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Conflicts of Interest: All authors have completed the ICMJE uniform disclosure form (available at https://tlcr.amegroups.com/article/view/10.21037/tlcr-22-211/coif). The authors have no conflicts of interest to declare.

Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013). Ethical approval of this study was not legally required as no biological material was collected (Danish Scientific Ethical Committees Act, paragraph 14.2). The study was conducted using pre-existing data readily available in our local quality assurance database (Aarhus Lung Cancer Registry). Patients were not contacted in relation to this study, and our findings did not impact their disease course in any way. The need for written consent was also waived due to the retrospective design.

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