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 according to the TNM staging system, 7th edition.

Follow-up ended on September 30th, 2021. Incomplete
Patients diagnosed with EGFR mutated NSCLC from January 1st 2010 until September 30th 2020 from Herning, Aalborg or Aarhus: 516

No information on EGFR-TKI: 175

No information on PD-L1: 202
Invalid PD-L1: 27

Necessary information on PD-L1 status and EGFR TKI: 112

TTD <1 month: 1

Included: 111
Aarhus: 84; Herning: 27; Aalborg: 0

TTD censored: 26
Survival censored: 35

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 $\chi^2$-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%.

**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.
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
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) | 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) | NS | Not included |
| 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) | NS | No significant difference in PFS between when comparing all three groups (P value not listed) |
| 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) | NS, 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) | NS, 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 |

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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 external 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.

**Acknowledgments**

Funding: None.

**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

Data Sharing Statement: Available at https://tlcr.amegroups.com/article/view/10.21037/tlcr-22-211/dss

Peer Review File: Available at https://tlcr.amegroups.com/article/view/10.21037/tlcr-22-211/prf

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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**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. Ettinger DS, Wood DE, Aisner DL, et al. Non-Small Cell Lung Cancer, Version 5.2017, NCCN Clinical Practice Guidelines in Oncology. J Natl Compr Canc Netw 2017;15:504-35.
3. Duma N, Santana-Davila R, Molina JR. Non-Small Cell Lung Cancer: Epidemiology, Screening, Diagnosis, and Treatment. Mayo Clin Proc 2019;94:1623-40.
4. Asselain B, Barrière JR, Clarot C, et al. Metastatic NSCLC: Clinical, molecular, and therapeutic factors associated with long-term survival. Respir Med Res 2019;76:38-44.
5. Howlader N, Noone AM, Krapcho M, et al. SEER Cancer Statistics Review, 1975-2018, National Cancer Institute. Bethesda, MD. Available online: https://seer.cancer.gov/csr/1975_2018/
6. da Cunha Santos G, Shepherd FA, Tsao MS. EGFR mutations and lung cancer. Annu Rev Pathol 2011;6:49-69.
7. Ruiz-Cordero R, Devine WP. Targeted Therapy and Checkpoint Immunotherapy in Lung Cancer. Surg Pathol Clin 2020;13:17-33.
8. Melosky B, Kamhardt K, Hantschel M, et al. Worldwide Prevalence of Epidermal Growth Factor Receptor Mutations in Non-Small Cell Lung Cancer: A Meta-Analysis. Mol Diagn Ther 2022;26:7-18.
9. Greenhalgh J, Boland A, Bates V, et al. First-line treatment of advanced epidermal growth factor receptor (EGFR) mutation positive non-squamous non-small cell lung cancer. Cochrane Database Syst Rev 2021;3:CD010383.

10. Soria JC, Ohe Y, Vansteenkiste J, et al. Osimertinib in Untreated EGFR-Mutated Advanced Non-Small-Cell Lung Cancer. N Engl J Med 2018;378:113-25.

11. Juneja VR, McGuire KA, Manguso RT, et al. PD-L1 on tumor cells is sufficient for immune evasion in immunogenic tumors and inhibits CD8 T cell cytotoxicity. J Exp Med 2017;214:895-904.

12. Chen N, Fang W, Zhan J, et al. Upregulation of PD-L1 by EGFR Activation Mediates the Immune Escape in EGFR-Driven NSCLC: Implication for Optional Immune Targeted Therapy for NSCLC Patients with EGFR Mutation. J Thorac Oncol 2015;10:910-23.

13. Akbay EA, Koyama S, Carretero J, et al. Activation of the PD-1 pathway contributes to immune escape in EGFR-driven lung tumors. Cancer Discov 2013;3:1355-63.

14. Okita R, Maeda A, Shimizu K, et al. PD-L1 overexpression is partially regulated by EGFR/HER2 signaling and associated with poor prognosis in patients with non-small-cell lung cancer. Cancer Immunol Immunother 2017;66:865-76.

15. Peng S, Wang R, Zhang X, et al. PD-L1 expression predicts TKI response and better prognosis in a cohort of patients with EGFR-mutant lung adenocarcinoma. Lung Cancer 2015;88:324-30.

16. Zhang M, Li G, Wang Y, et al. PD-L1 expression in lung cancer and its correlation with driver mutations: a meta-analysis. Sci Rep 2017;7:10255.

17. Li D, Zhu X, Wang H, et al. Association between PD-L1 expression and driven gene status in NSCLC: A meta-analysis. Eur J Surg Oncol 2017;43:1372-9.

18. 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.
EGFR-mutant non-small cell lung cancer. Cancer Sci 2019;110:3244-54.

33. Kobayashi K, Seike M, Zou F, et al. Prognostic Significance of NSCLC and Response to EGFR-TKIs of EGFR-Mutated NSCLC Based on PD-L1 Expression. Anticancer Res 2018;38:753-62.

34. Chang CY, Lai YC, Wei YF, et al. PD-L1 Expression and Outcome in Patients with Metastatic Non-Small Cell Lung Cancer and EGFR Mutations Receiving EGFR-TKI as Frontline Treatment. Oncotargets Ther 2021;14:2301-9.

35. Kang M, Park C, Kim SH, et al. Programmed death-ligand 1 expression level as a predictor of EGFR tyrosine kinase inhibitor efficacy in lung adenocarcinoma. Transl Lung Cancer Res 2021;10:699-711.

36. Inomata M, Matsumoto M, Mizushima I, et al. Association of Tumor PD-L1 Expression With Time on Treatment Using EGFR-TKIs in Patients With EGFR-Mutant Non-small Cell Lung Cancer. Cancer Diagn Progn 2022;2:324-9.

37. Lan B, Wang Y, Wu J, et al. The predictive and prognostic effects of PD-L1 expression on TKI treatment and survival of EGFR-mutant NSCLC: A meta-analysis. Medicine (Baltimore) 2021;100:e27038.

38. Peng Z, Lin H, Zhou K, et al. Predictive value of pretreatment PD-L1 expression in EGFR-mutant non-small cell lung cancer: a meta-analysis. World J Surg Oncol 2021;19:145.

39. Hsu KH, Huang YH, Tseng JS, et al. High PD-L1 expression correlates with primary resistance to EGFR-TKIs in treatment naïve advanced EGFR-mutant lung adenocarcinoma patients. Lung Cancer 2019;127:37-43.

40. Kim TJ, 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.

41. Bai Y, Chen X, Hou L, et al. PD-L1 expression and its effect on clinical outcomes of EGFR-mutant NSCLC patients treated with EGFR-TKIs. Cancer Biol Med 2018;15:434-42.

42. McLaughlin J, Han G, Schalper KA, et al. Quantitative Assessment of the Heterogeneity of PD-L1 Expression in Non-Small-Cell Lung Cancer. JAMA Oncol 2016;2:46-54.

43. Li H, Han D, Hou Y, et al. Statistical inference methods for two crossing survival curves: a comparison of methods. PLoS One 2015;10:e0116774.

44. Roeper J, Falk M, Chalaris-Rißmann A, et al. TP53 co-mutations in EGFR mutated patients in NSCLC stage IV: A strong predictive factor of ORR, PFS and OS in EGFR mt+ NSCLC. Oncotarget 2020;11:250-64.

45. Hellyer JA, White MN, Gardner RM, et al. Impact of Tumor Suppressor Gene Co-Mutations on Differential Response to EGFR TKI Therapy in EGFR L858R and Exon 19 Deletion Lung Cancer. Clin Lung Cancer 2022;23:264-72.

46. Offin M, Rizvi H, Tenet M, et al. Tumor Mutation Burden and Efficacy of EGFR-Tyrosine Kinase Inhibitors in Patients with EGFR-Mutant Lung Cancers. Clin Cancer Res 2019;25:1063-9.