Peer Review File

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Reviewer A

Comment 1: The endpoint of the present study is the time to treatment discontinuation (TTD) that is defined as the period between clinical decision to initiate EGFR-TKI treatment and the clinical decision to end it. Is it mean the period between the initiation of administration of TKI and last day of the administration? TTD should be defined as the period between the initiation of administration of TKI and last day of the administration.

Reply 1: Thank you for the opportunity to clarify this point. We define TTD as the period (number of days) from the day of the consultation where initiation of treatment is decided upon until the day of the consultation where termination of treatment is agreed upon. These dates are the information that is available to us in our local quality assurance database. Because both drugs are administered as a daily tablet that can be both initiated and terminated from day to day, we believe that these dates correspond accurately to the period during which patients received the treatment. The half-life duration of Tarceva (36 hours) and Tagrisso (48 hours) means that patients would be drug-free within 1-2 weeks after cessation, depending on the treatment. This argument has been added to our Methods section.

This explanation raises the issue of pauses in treatment regime due to side effects, which we have added as a limitation in our discussion.

Changes in the text: We have added a few lines to our 'End points' section to elaborate on how TTD is defined in our study and why we believe it to be accurate. However, we have also added to our limitations that agreed-upon breaks in treatment regime due to side effects cannot be accounted for.

Comment 2: Authors compared the TTD of patient group subdivided by PD-L1 expression level by univariate analysis (log-rank test). However, the imbalance of patient characteristics is observed as below.

The proportion of patients with PS of ≥2
negative PD-L1 expression group: 10/52 (19.2%) vs positive PD-L1 expression group: 14/52 (26.9%)
negative and lower PD-L1expression group: 18/84 (21.4%) vs higher PD-L1 expression group: 6/20 (30.0%)

The proportion of patients with uncommon mutation

negative PD-L1 expression group: 8/55 (14.5%) vs positive PD-L1 expression group:14/54(25.9%)
negative and lower PD-L1expression group: 15/87 (17.2%) vs higher PD-L1 expression group: 7/22 (31.8%)

Therefore, multivariate analysis using Cox proportional hazard model should be conducted although authors rases the issue about proportional hazard assumption.
I think candidate of independent variables includes performance status, EGFR mutation status (common or uncommon mutation), comorbidities, and brain metastases.

Reply 2: We greatly appreciate this suggestion and have conducted the multivariate analysis using the Cox proportional hazard model. We firstly conducted a univariate analysis of the different independent variables and included those with a p-value of 0.05 or less in our multivariate model. The analysis shows that PD-L1 status is not significantly correlated to the time until treatment discontinuation. It did, however, show a significant influence of performance status, drug and EGFR mutation type (common vs uncommon).

Changes in the text: We have added a multivariate Cox proportional hazards analysis to our revised manuscript. We have now also included brain metastases at EGFR TKI baseline to our analysis of baseline characteristics.

Comment 3: Also, subset analysis of patient group of common mutation may be meaningful.

Reply 3: The authors agree that this is indeed meaningful, especially considering that our multivariate analysis shows significant impact of mutation subtype. We have conducted a subset analysis of TTD using the log rank test in patients with common mutations. This analysis yields a p-value of 0.66 and a Kaplan-Meier plot with less separation of our survival curves. Patients with uncommon mutations are known to respond poorly to EGFR TKI treatment, and the proportion of uncommon mutations is higher in the groups of low and high expression than in the negative group. This confounds the original results. We thank you for bringing this to our attention.

Changes in the text: We have added a subset analysis of patients with common mutations and added an interpreting section to our discussion.

Reviewer B

Comment 1: It is my honor to review the manuscript entitled "Tumoral PD-L1 does not predict time until treatment discontinuation in EGFR mutated NSCLC patients treated with EGFR-TKIs." submitted to the TLCR. The authors reviewed the NSCLC patients with mEGFR and showed that the expression of PDL1 did not influence the effect of EGFR TKIs.

First of all, the rationale for the primary hypothesis, the response to the EGFR-TKI would be related to the PDL1, is not based on the scientific knowledge, which inevitably lead negative finding.

Reply 1: We greatly appreciate your feedback. The authors agree that we have no knowledge of an existing scientific rationale behind the possible impact of PD-L1 on the effect of EGFR TKIs. However, we have conducted this study to investigate if such a relation exists and because others before us have shown conflicting results on the matter. Several articles (Soo et al, Su et al, Yoon et al, Yoneshima et al, Yang et al, Matsumo et al) have shown a significant correlation between PD-L1 levels and a shorter progression-free survival. Our study does not aim to elucidate the molecular pathways, but rather to contribute with data from a European cohort as nearly all conducted studies have included only Asian patients. We have adjusted our wording to include that there exists to molecular explanation, but that our study contributes to the debate by looking at a European cohort and studying an end point that we consider to be more clinically relevant than PFS in a time where treatment beyond progression is common.

Changes in the text: We have modified our introduction to emphasize how our study contributes to the existing results. We have also added that we are not aware of any existing molecular explanation for a possible correlation to our discussion.

Comment 2: This study is a retrospective, single arm, observational study. In addition to these limitations, the data used for this study was obtained from a single institution and the number of cases is limited to support the conclusion.

Reply 2: These points are relevant limitations to our study. We have revised our limitations paragraph to emphasize these points separately and increase transparency. We have also, based on the collected feedback from reviewers, elaborated on the issue of bias from the treating physician and added the point of pauses due to side effects. However, despite these limitations, we believe that our findings have merit because our study is European and contributes to the existing studies that have been conducted primarily in Asia. We also believe that the limited number of centers (2) is an advantage when looking to limit the bias of variability between treating physicians in deciding when to terminate treatment.

Changes in the text: We have revised our limitations section to increase transparency regarding these valid points.

Reviewer C

Comment 1: The authors reported a correlation between PD-L1 expression and EGFR-TKI efficacy in Danes, concluding that PD-L1 expression did not significantly correlate with the duration of EGFR-TKI treatment. They included patients whose PD-L1 expression was checked at diagnosis; although they included cases from 2010, the number of patients whose PD-L1 expression (22C3) was measured at diagnosis must be little between 2010 to 2015 because U.S. FDA approval of pembrolizumab was in 2015. Please show the number of cases by year.

Reply 1: Thank you for bringing to our attention that this needs clarification. We realise that we have incorrectly written 'PD-L1 assessment was done at diagnosis', which has been corrected to 'prior to therapy'.

Our study 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 palliative 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 22C3 at our facility was introduced in 2016, and patients treated prior to this were generally excluded from our cohort on the basis of 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 (either first palliative line or subsequent) that was preceded by PD-L1 evaluation. These patients were included because the requirement was an available PD-L1 assesment at initiation of EGFR TKI rather than at the time of diagnosis. This explanation has been added to our Methods section, and we hope that it suffices.

Changes in the text: We have modified our Methods sections under 'Patients', clarifying why we have chosen this inclusion period although 22C3 was not routinely used until 2016 at our facility.

Comment 2: If there are cases included that were not measured at diagnosis, they should be noted as such, and the approximate time from specimen collection to PD-L1 staining should be noted.

Reply 2: Our inclusion criterium was a PD-L1 status that had been carried out a maximum of 3 months prior to initiation of therapy. This did not necessarily correspond to the time of diagnosis. This fault has been corrected in our text, and we kindly refer to our reply to Comment 1. We believe that this short interval from PD-L1 to treatment initiation (3 months, or only 1 if another systemic therapy was ongoing at the time of evaluation) limits the influence of time and other treatments on PD-L1 expression.

Changes in the text: We have changed the wording of our 'PDL1 and EGFR' section and our abstract to correct this error on our part.

Comment 3: The small number of cases should be added to the limitation. Looking at the

Kaplan-Meier curve, the curve is divided by PD-L1 expression, although this is not significant. The difference may become significant as the number of cases increases.

Reply 3: We agree and thank you for the suggestion. This is now stated in the revised manuscript. However, having performed our subgroup analysis on the suggestion of Reviewer A, resulting in a markedly lessened division of the curves, we believe some of the initial difference might stem from this confounding factor.

Changes in the text: We have added the point of our limited cases to our limitations.

Comment 4: Figure 2 should be tabulated.

Reply 4: Your feedback on Figure 2 is appreciated. Based on this and the feedback from Reviewer D regarding the issues of Figure 2 as well, we have chosen to remove it from the revised manuscript. Our intention was to make visualization of baseline characteristics distribution easy, but this does not seem to have been achieved. As Table 1 already contains adequate information on baseline characteristics, we deem this figure unnecessary.

Changes in the text: Figure 2 has been removed from the revised manuscript. We deem Table 1 to be sufficient, in accordance with Reviewer D.

Comment 5: Figures 3 and 4 should be C only.

Reply 5: We agree that figure C contains the necessary information. Figures 3 and 4 is now C only. Following this comment, we have chosen to conduct our multivariate analysis (in response to Reviewer A) based on only this comparison rather than the previous different groupings.

Changes in the text: Figure 3 and 4 have been modified and are now C only. The comparisons previously shown in A and B have been removed from the revised manuscript.

Comment 6: Table 1 should have only the left two and the right four columns (None, Low, and High).

Reply 6: Thank you. In accordance with our answer to Comment 5, this has been accommodated, and Table 1 has been adjusted in the revised manuscript.

Changes in the text: Table 1 has been modified in accordance with this suggestion.

Reviewer D

The manuscript by Dissing et. al. entitled "Tumoral PD-L1 does not predict time until treatment discontinuation in EGFR mutated NSCLC patients treated with EGFR TKI" aims to clarify the prediction power of PD-L1 in relation to clinical benefit in patients as measured by time to treatment discontinuation. In conclusion, the author found PD-L1 did not significantly impact the duration of clinical benefit of TKI treatments including TTD and OS. Generally, this study provided a conclusion result at least in patients from Denmark. However, it is still very controversial whether PD-L-1 can predict treatment efficacy of EGFR TKIs. The applicability of this study remains to be discussed. Several issues should be clarified.

Comment 1: What is time to treatment discontinuation?

Reply 2: Thank you for your thorough feedback. The need for clarification of this definition was also brought to our attention be reviewer A, and it has been specified in the revised manuscript. We kindly refer to our reply to Reviewer A, comment 1.

Changes in the text: We have added a few lines to our 'End points' section to elaborate on how TTD is defined in our study and why we believe it to be accurate. However, we have also added to our limitations that agreed-upon breaks in treatment regime due to side effects cannot be accounted for.

Comment 2: The author may think about that whether it is proper to use the term "palliative therapy" for EGFR TKIs.

Reply 2: The authors have considered this point and chosen to remain with our current wording. As treatment with EGFR TKI does not have a curative potential, we would consider it palliative by definition. We have added a definition to our revised manuscript.

Changes in the text: We have added our definition of 'palliative' as treatment without curative potential to our 'Results' section.

Comment 3: In the mention of Figure 1, it is better to draw how many NSCLC patients that 516 patients with mutant EGFR came from?

Reply 3: We agree with the feedback that it would be a good idea to show the number of NSCLC patients our cohort of 516 EGFR-mutated patients originated from. However, we are regretfully only able to provide the number of patients originating from Aarhus during this period, and because we also included EGFR-mutated patients from Herning (and Aalborg, although none made it to the actual cohort), we do not believe that this number would contribute meaningfully.

Changes in the text: No changes have been made.

Comment 4: Although treatment beyond progression is a common approach when treating NSCLC with EGFR TKIs, the decision of treatment discontinuation may be dependent on each clinical physician. How to eliminate this variation? The author may clarify it in this study.

Reply 4: Thank you for raising this issue. We agree that the bias of the treating physician is the biggest disadvantage of using TTD rather than the more objectively measured RECIST criteria. Regretfully, there is no way for us to eliminate this variation entirely, and we have chosen to emphasize this issue in our paragraph on limitations in the revised manuscript. However, our study is a two-center study, and we believe that the variation in decisions among doctors from the same clinical departments will be smaller. So in this regard, our study design works to our advantage. Furthermore, all status scans at Aarhus University Hospital showing either unequivocal or debatable progression are reviewed at conferences between oncologists to ensure a standardized care, and we have added this point to our discussion as well.

Changes in the text: We have modified the text in our discussion of limitations to include these points.

Comment 5: Is it possible to draw a waterfall plot to correlate PD, SD, PR with PD-L1 categories?

Reply 5: Thank you for this suggestion. The authors have made the conscious decision to avoid the usage of image-based definitions of disease progression in this article. Our patients are all treated in routine clinical care where image descriptions are less standardized, and the status scans are often not described according to the RECIST criteria. It is therefore our opinion that the construction of such a waterfall plot would reintroduce the uncertainties that we have tried to eliminate by using TTD rather than PFS.

Changes in the text: No changes have been made to the manuscript.

Comment 6: Based on Figure 2, did 0, 1A, and 1B only refer to M stage or disease stage? I suggest the author to provide more comprehensive information.

Reply 6: Thank you for raising this point. Given the constructive criticism of Comment 7, combined with that of Reviewer C, comment 4, we have chosen to remove Figure 2 from our revised manuscript. You are correct in pointing out that Table 1 contains the necessary information on baseline characteristics. We have also clarified in our revised manuscript that 0, 1A and 1B refers to M-stage according to the TNM staging system, 7th edition.

Changes in the text: We have removed the original Figure 2 from our revised manuscript. We have also clarified that these numbers correspond to M-staging according to TNM, 7th edition in the 'Patients' section of Methods.

Comment 7: In Figure 2, the author should check whether there was any selection bias. There

were no male patients below 70 years, and no EGFR del19 in male (no other EGFR mutations in female), and no 2+ PS in female etc. The Figure 2 may be not a best way to illustrate baseline characteristics. Maybe Table 1 is sufficient.

Reply 7: The points raised here cannot be inferred from figure 2 as the colorization within each category is not related to the other categories. However, we conclude that Figure 2 is not optimal, and we have chosen to remove it. The necessary data on baseline characteristics can be found in Table 1.

Changes in the text: We have removed the original figure 2 from our revised manuscript. Also, Table 1 has been modified in compliance with advice from Reviewer C. We believe it contains the adequate information, as Reviewer D kindly points out.

Comment 8: In the mention of discussion, whether PD-L1 can be a significant and an applicable biomarker for TKIs treatments is still controversial due to many confounding factors. I think types of antibodies may not a good possible reason to explain this situation. Based on literatures in Table 2, it cannot support the issue of antibodies.

Reply 8: We agree with this criticism. We did not intend to imply that different antibodies account solely for the varying results of previous studies. We have adjusted the wording in our discussion to put less emphasis on antibodies and instead merely point out that the methodology varies.

Changes in the text: We have adjusted the wording of our discussion to put less emphasis on the difference in antibodies between the existing studies.

Comment 9: I would like to see more discussion about PD-L1 and treatment efficacy of EGFR TKIs based on biological function, signal transduction, and molecular mechanism.

Reply 9: To the best of our knowledge (and as pointed out by Reviewer B), there is no established scientific rationale for the coupling of PD-L1 and response to EGFR TKI treatment. We have added this clarifying statement in our discussion. Studies on cell-lines have shown that EGFR mutated cells express higher levels of PD-L1, and that the T790M mutation led to increased expression as well. However, studies in patients generally show the opposite, as concluded in several meta-analyses. These studies are already highlighted in our introduction. But we feel that a further exploration of the transduction pathways is beyond the scope of this clinical study that mainly aims to contribute to the debate by examining a European cohort in contrast to the existing studies that have all been conducted in Asia.

Changes in the text: We added that there exists no known rationale for a coupling between PD-L1 and EGFR TKI response to our discussion.
