TRANSLATIONAL LUNG CANCER RESEARCH

Peer Review File

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Review Comments

Dear authors, I have had the priviledge to review your interesting manuscript. I have some suggestions how to improve the presentation of your excellent findings for publication:
Outcome of patients treated with EGFR-TKI as a first-line systemic therapy:

Comment 1) PD-L1 expression was not correlated with behavioral or demographic features such as patient age, sex or smoking history (Table 2), the latter since the vast majority of patients were never-smokers (88.9%). What about the level of PD-L1 expression in common mutations, in Del19 versus L858R?

Reply 1) Thank you for the important comment. As noted in Table 2, PD-L1 expression was not correlated with oncogenic driver (Del19 versus L858R). As per reviewer's comment, we revised the manuscript as shown below. (see Page 8, Line 167-168)

Changes in the text 1) PD-L1 expression was not correlated with age, sex, smoking history, ECOG PS, presence of brain metastasis at diagnosis, type of EGFR mutation, or type of EGFR-TKI used (Table 2).

Comment 2) There were no statistically significant PD-L1 expression-related differences in the best treatment response of EGFR-TKI, based on RECIST 1.1 criteria (negative vs. weak vs. strong PD-L1 expression, p=0.133) (Table 2) – you should discuss this, since it implies that the level of PD-L1 expression is not important in best responders, so some other pathways are predominant…

Reply 2) Regarding the association between objective response rate and PD-L1 expression, we agree with your point that PD-L1 expression might not be an important factor in best responders. However, we have also observed numerically higher rate of primary resistance (PD) in strong PD-L1 expression than other weak or negative group. Therefore, we suppose that relatively lower rate of disease control in strong PD-L1 expression group might contributed to shorter PFS in our study population. We have revised the manuscript as shown below. (see Page 8-9, Line 186-188)

Changes in the text 2) However, the disease control rate (partial response + stable disease)

was numerically lower in strong PD-L1 expression group (81.3%) compared to those of negative (89.1%) and weak (97.3%) group.

Comment 3) Acquired resistance to first-line EGFR TKI treatment:
Re-biopsies were performed in 66 patients at progression, and the rate of T790M mutation between the three PD-L1 expression groups didn't differ significantly, but it would be good to analyze (1) the possible difference dependant on mutation subtype, Del19 vs L858R, and (2) the possible difference dependant on EGFR TKI applied.

Reply 3) We appreciate your constructive suggestion. As per reviewer's comment, we have reanalyzed the data and added the supplementary tables. (see Page 9, Line 196-197)

Changes in the text 3) The rate of T790M mutation was associated with neither subtype of EGFR mutation or pretreted EGFR-TKIs (supplementary table 4).

Supplementary Table 4-1. Comparison of T790M mutation between Exon 19 del and Exon 21 L858R.

T790M mutation detected from rebiopsy	Exon 19 del	Exon 21 L858R	p Value
No	11	14	0.569
Yes	21	20	

Supplementary Table 4-2. Comparison of T790M mutation between first-line EGFR-TKIs applied.

T790M mutation detected from rebiopsy	Gefitinib	Erlotinib	Afatinib	p Value
No	11	13	1	1.000
Yes	17	22	2	

Comment 4) Findings that among patients treated with third-generation EGFR-TKI (Osimertinib), the median PFS and the response based on RECIST 1.1 criteria differences were not statistically significant imply a need for additional discussion part regarding the efficacy of different EGFR TKIs in high PD-L1 expressors as well. Importantly, the efficacy of osimertinib for patients with EGFR-mutant NSCLC is not influenced by the level of tumor PD-L1 expression in the FLAURA trial: PFS remained unaffected by PD-L1 expression status as well.

So, related to this, also regarding the conclusion that strong PD-L1 expression in tumors might be a surrogate indicator of poor response to first-line EGFR-TKIs in NSCLC patients with sensitizing EGFR mutations, the authors should be more cautious when interpreting the data and rephrase this sentence.

Reply 4) Thank you for the important comment. As per reviewer's comment, we have added the additional comments in discussion part regarding the data from FLAURA trial and revised the manuscript as shown below. (see Page 11, Line 250-254 and Page 13, Line 315-317)

Changes in the text 4)

More recently, FLAURA trial (34) reported that, after first-line treatment with gefitinib or erlotinib, median PFS was 10.9 months in patients with PD-L1 expression <1%, while median PFS of 6.9 months was observed in patients with PD-L1 expression >1%, which was consistent with our results. However, interestingly, after first-line treatment with osimertinib, median PFS was unaffected by PD-L1 expression status.

In conclusion, we demonstrated that PD-L1 strong expression predicts poor response to first- or second-generation EGFR-TKI in treatment naïve advanced NSCLC, and that PD-L1 expression may indicate underlying *de novo* resistance mechanism including JAK-STAT pathway.

Comment 5) Outcome of patients treated with immune checkpoint inhibitors following EGFR-TKI failure:There is a need for additional explanation in discussion: Lung adenocarcinomas with driver mutations from never-smokers have lower tumor mutational burden (TMB) than lung adenocarcinomas from smokers. Also the tumor microenvironment in lung cancer with driver mutations is considered a "cold tumor" based on lack of CD8+ tumor-infiltrating lymphocytes (TILs). Both low TMB and low CD8+ TILs are related to the limited efficacy of ICIs for these patients. However, high PD-L1 expression may represent a constitutive activation of PD-L1 signal and not a marker of adaptive immune response. Differential expression of PDL1 has been described across NSCLC with driver mutations, pointing to the importance of the genetic background in the PD-L1 expression: for example KRAS and MET alterations associated with higher tumor PD-L1 expression, opposite to EGFR mutations…
Although the autors have data on 18 patients only, the analysis regarding the subtype of EGFR mutation and efficacy of immune checkpoint inhibitors is lacking.

Reply 5) Thank you for raising the clinically important question. We agree with that more data regarding subtype of EGFR mutation status and ICI treatment are needed. As per reviewer's comment, we have analyzed regarding the subtype of EGFR mutation and efficacy of ICIs in

18 patients. However, there was only one partial response in our subjects and the difference in progression-free survival according to subtype of EGFR mutation was not observed as below table. Therefore, we have not found any important point in small subject number in our study and not included this additional analysis in the manuscript after discussion with our statistician.

subtype of EGFR mutation	median PFS	95% CI	p Value
Exon 19 del	1.400	0.692-2.108	0.264
Exon 21 L858R	6.267	0.000-16.386	

Comment 6) (Discussion part related to same topic): Please add some relevant comments regarding findings in ImPower 150 and 130 clinical trials, as well as in the publications of Hastings K et al. Annals of Oncology 2019, and Bai Y et al. PD-L1 expression and its effect on clinical outcomes of EGFR-mutant NSCLC patients treated with EGFR-TKIs. Cancer Biol Med. 2018.

Reply 6) We appreciate your constructive suggestion. As per reviewer's comment, we revised the manuscript as shown below. (See Page 11-12, Line 255-272)

Changes in the text 6)

One of the above-mentioned studies demonstrated the efficacy of immune checkpoint inhibitors in the treatment of EGFR-mutated PD-L1 high tumors, as these tumors are highly positive to CD8+ T cells (31,35); moreover, one of these patients showed PFS longer than 5 months following treatment with an immune checkpoint inhibitor (31). Although the level of PD-L1 expression did not seem to significantly affect PFS in EGFR-TKI-resistant patients that had been treated with immune checkpoint inhibitor, some patients with high tumor expression of PD-L1 did show more favorable outcomes, with PFS > 6 months, suggesting that subsets of PD-L1-high patients might benefit from treatment with immune checkpoint inhibitors. Therefore, although immunotherapy showed limited overall efficacy against EGFR-mutated lung adenocarcinoma (36,37), the specific effects of the immune check point inhibitors in patients with high PD-L1 expression may deserve further investigation.

In addition, immune checkpoint inhibitor in combination with platinum-based chemotherapy may be a possible therapeutic option in EGFR-TKI-resistant patients as the recent IMpower 150 trial reported that chemo-immunotherapy plus bevacizumab improved PFS when compared to chemotherapy alone in EGFR-mutant patients who had progressed on a EGFR-TKI therapy (38). The IMpower 130 trial failed to demonstrate a clinical benefit for the addition of immune checkpoint inhibitor to chemotherapy without bevacizumab in EGFR-

mutant NSCLC (39). Clinical benefit between immune checkpoint inhibitor alone and in combination with chemotherapy in EGFR-TKI-resistant patients is not yet studied well.

Comment 7) Please rephrase the sentence: Early cancer progression in patients with high PD-L1 expression was not due to the T790M mutation, since the rate of this mutation in EGFR-TKI resistant patients did not significantly differ depending on tumor PD-L1 expression. – Maybe: Early cancer progressors with high PD-L1 expression didn't have significantly more frequent T790M resistance mutation compared to those with low PD-L1 expression..or something similar

Reply 7) We appreciate your constructive suggestion. As per reviewer's comment, we revised the manuscript as shown below. (See Page 10, Line 235-237)

Changes in the text 7) Early cancer progressors with high PD-L1 expression didn't have significantly more frequent T790M resistance mutation compared to those with low PD-L1 expression.