Peer Review File

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Reviewer A

Comment 1: Introduction: Page 3/Line 102:
Can you explain more "30% of naive NSCLC" developed first resistance..." Do you mean harbor or developed? after wich treatement do mutations appear?
I found 30% so high percentage.
Reply 1: We agree that the phrasing of the sentence is quite misleading. We thank the reviewer for pointing this out. We initially meant to convey “harbor” and pertain “primary resistance" of treatment naive cohort according to the cited study by Bai et al. 2018 (doi: 10.20892/j.issn.2095-3941.2018.0223), which was corroborated by high frequency of other driver mutations discussed in review articles enumarated in the in-text citation. Bai et al. concluded that 30% of the initial cohort from different clinical studies that they cited had no beneficial response to TKIs, which signify primary resistance. However, the author did not mention what specific treatment interventions were used, what confounding mutations were present, and what constituted the initial cohort that qualified them within the “30% resistant population”. Upon deeper investigation of the literatures, we found that the studies were heterogenous in terms of population, sample size, type of treatments, and presence of other driver mutations. Additionally, the clinical endpoints of the trials were variable, no agreement among PFS, OS, and ORR. Therefore, we decided to omit this statement to prevent disclosing potentially inaccurate frequency.
Changes in the text: We rephrased the sentence to focus on the involvement of T790M mutations in acquired drug resistance instead. We also provided statistical data on the occurrence of T790M mutation. Please see Introduction (Page 4, Lines 86-87).

Reviewer B

Comment 2: Could you propose/discuss a possible mechanism to explain this counterintuitive result on PFS and OS specifically which is particularly clear in Exon 21 patients?
Reply 2: We thank the reviewer for this valuable comment. We agree for the need to provide explanation about the counterintuitive result on PFS and OS of patients with different EGFR-sensitizing mutations in our cohort. One possible reason for the differential PFS and OS observed in our study would be the presence of distinct concomitant mutations in exon19(del) and exon21L858R, as well as in EGFR-mutants with different PD-L1 status (negative, low, and high). Good examples to elucidate this would be from the retrospective studies by Liang et al. 2020 (doi:10.2147/CMAR.S255967) and Li et al. 2022 (doi:10.1186/s12935-022-02488-z). Liang et al found that the frequencies of mutations in other driver genes are different in exon19(del) and exon21L858R. KRAS is one of the oncogenes that is known to be enriched for driver mutations in exon21L858R compared to exon19(del). Additionally, Li et al. found that PD-L1 negative EGFR-mutants have higher KRAS mutation compared to PD-L1 positive

(combined low and high) NSCLC. Collectively, these findings could provide explanation to the observed variation in the survival of our EGFR-mutant NSCLC cohort.

Changes in the text: We added a section expounding on the involvement of concomitant mutations in the differential outcomes of EGFR-mutant NSCLC with varying PD-L1 expression. Please see Discussion (Page 10, Lines 332-339). We also appended the bibliography for the additional references (38-41).

Comment 3: In the exon 19, the insufficient number of patients in the PD-L1 neg Filipino cohort (NR) could mislead in the interpretation of the results of the 1 year PFS rate. Indeed, the TCGA results show that the OS is independent of the PD-L1 status (1492 d vs 1516 d) and the PFS fig2a shows the same. What could explain the differential effect of PD-L1 expression status depending on the type of EGFR activating mutations on OS (Fig 1d) when both mutations destabilise the inactive conformation of EGFR, trigger increased receptor activity through a similar mechanism, and might trigger similar downstream effects?

Reply 3: We agree that at the molecular level, the oncogenic function of exon19(del) and exon21L858R may work similarly. The two EGFR-activating mutations destabilize the inactive form of the EGFR kinase domain and eventually render the same signaling activity through a similar pathway. However, many studies suggest that the oncogenic drivers that lead to the acquisition of EGFR mutations may vary in NSCLC (doi: 10.3389/fonc.2020.01256). Additionally, due to loss of genomic instability in many cancer types like NSCLC, there are a number of primary and secondary genetic alterations that are distinct between exon19(del) and exon21L858R, which could result in the differential outcomes of a particular group of EGFR-mutant. In short, EGFR-sensitizing mutations is not the only oncogenic drivers that dictates the treament outcomes of patients. A possible explanation for the differential outcomes observed in EGFR-mutant NSCLC with relation to PD-L1 expression is the presence of distinct concomitant mutations, as described above in Reply 2. These differential concomitant mutations could influence other oncogenic pathways. In fact, we previously found that the crosstalk between EGFR and PD-L1 pathways could be triggered by PI3K-mediated signaling through TTF-1 upregulation (Luna et al. 2023; doi:10.1016/j.lungcan.2022.12.015). Indeed, PIK3CA, a component of the PI3K complex was found to be amplified in NSCLC and correlated positively with PIK3CA-activating mutations (Qiu et al. 2021; PMCID: PMC8263631). PIK3CA mutations have been found to be enriched in non-responsive NSCLC with exon21L858R compared to exon19(del) (Liang et al. 2020; doi:10.2147/CMAR.S255967). PIK3CA mutation is a negative prognostic factor in NSCLC (Qiu et al. 2021; PMCID: PMC8263631). In return, PI3K signaling was found to downregulate PD-L1 expression in NSCLC (Quan et al. 2022; doi:10.7150/jca.77619). Collectively, these findings support the differential survival outcomes of patients with different EGFR-sensitizing mutations and PD-L1 expression in our cohort.

Changes in the text: The previously added discussion on concomitant mutations were appended with discussion pertaining to the differential PIK3CA profile of patients with different EGFR mutations and PD-L1 expression. Please see Discussion (Page 10, Lines 339-348). We also appended the bibliography for the additional references (42-43).

Comment 4: It is not clear to me why in fig2b the median PFS of exon 21 patients is only of 207d in the high PD-L1 TPS (not that different from the PD-L1 neg of 163d) while it appears much better? Same for OS fig 2d. If the data are correct, how do you explain the good prognostic of exon 21 with positive PD-L1 (1%) would show a better correlation?
Reply 4: We thank the reviewer for pointing out potential error in the calculation of Logrank p value. We understand that the median survival days of 207 and 163 seems to be quite different, and in some studies the difference may appear significant. We performed another survival analysis between the two groups and still obtained a Logrank $p > 0.05$. This could be because the difference in the number of patients who are at risk is not sufficient to find a statistical significance. We understand that this study has a small population size, and therefore the verification of survival curves from TCGA cohort is critical. Both cohorts did not find significant survival differences in EGFR-mutant NSCLC with varying levels of PD-L1 expression. We also agree that exon21L858R with low PD-L1 expression had the most favorable survival outcomes. This observation was not mentioned in the manuscript and we agree for an additional discussion on this topic. We thank the reviewer for emphasizing this.
Changes in the text: We provided explanation that justifies the good prognostic value of exon21L858R with low PD-L1 expression. Please see Discussion (Page 10, Lines 354-360). We also appended the bibliography for the additional references (45-46).

Comment 5: Could you discuss the status of the engagement of the PD-1/PD-L1 pathway in the Filipino cohort? Could the expression level of PD-L1 and PD-1 be insufficiently significant to predict the real impact of the checkpoint engagement on EGFR mutant NSCLC?
Reply 5: We agree that discussing the status of PD-1/PD-L1 expression patterns and signaling pathway in Filipino cohort is important to provide sufficient explanation about the findings of our study.
Changes in the text: We added a section that discusses and compare the expression pattern of PD-L1 in Filipino and other Asian cohort. We also providing reasoning about the potential insufficiency of PD-L1/PD-1 pathway in predicting treatment outcomes of EGFR-mutant NSCLC and provided other checkpoints that may also be valuable marker for prognostic studies. Please see Discussion (Page 12, Lines 414-425). We also appended the bibliography for the additional references (66-69).

Comment 6: Could you think of other confounding factor that could influence the results of the PD-L1 status as prognostic of the Filipino whose gender bias might be affecting?
Reply 6: This is an interesting discussion to be included as sex is a known factor for differential prognostic outcomes in EGFR-mutant NSCLC. We agree that discussing potential deviations and biases in the observed outcomes will clarify issues that the readers may have in the future.
Changes in the text: We added a section that discusses certain confounding factors and sex-specific correlation of different PD-L1 expression in tumor biopsies. Please see Discussion (Page 12, Lines 427-433). We also appended the bibliography for the additional reference (70).

Comment 7: Discussion part line 368: I am not sure that the patient with exon 19 outperform the PSF of patients with exon21. It does not seem to me that the difference is significant (53% vs 58% 1-year rate)

Reply 7: We previously meant to compare the survival, relatively. However, we agree that the phrasing of the sentence may convey statistically inaccurate comparison. We thank the reviewer for pointing this out.

Changes in the text: We decided to omit this sentence to prevent confusing the readers. Please see Discussion (Page 10, Line 323; second paragraph, deletion has been marked red through "Track Changes").

Comment 8: Minor point : Some of the graphs' legends are not easily readable (fig3f) even with high magnification.

Reply 8: We agree that texts in some figures, especially in Fig. 3F, are not readable. We thank the reviewer for pointing this out.

Changes in the text: We provided better figures with enlarged texts. We also deactivated image compression in MS Word and increased the DPI of other images.

Reviewer C

Comment 9: The authors claim that this is the first study to examine the prognostic value of PD-L1 in EGFR-mutant NSCLC in the Filipino population. While all of the endpoints are important, similar studies have been widely reported in other Asian countries. Moreover, the case numbers in this study are so small that it is extremely difficult to evaluate any endpoint. This is the greatest weakness of this study. Although it is favorable that the study was conducted prospectively, the first priority should be to increase the number of patients, even retrospectively. Another disadvantage of the present study is the short observation period, even when the prognosis of EGFR-mutant NSCLC is taken into consideration. The novelty of this study is not apparent except that it is limited to Filipinos, and even that novelty is of little significance with this number of cases. In light of the above, this study does not meet the criteria for publication in this journal, and must be considered a rejection.

Reply 9: We thank the reviewer for giving us pointers in improving our study. We are also aware of the limitations of our research, which are mentioned in the limitation section of the paper. However, we believe that the novelty of our study emanates from its timeliness and originality. No other literatures have described a prospective study that assessed the prognostic value of PD-L1 expression in EGFR-mutant NSCLC among Filipinos. To this date, the prognostic value of PD-L1 is fundamentally variable in different populations of EGFR-mutant NSCLC, and our study provides a population-specific findings that are distinct to Filipinos. We believe that our results could serve as a foundation for future researches that involves EGFR mutations and PD-L1/PD-1 expression in NSCLC. Our findings will be critical to curb the growing cases of lung cancer in the recent years.