Peer Review File
Article Information: https://dx.doi.org/10.21037/tlcr-23-643

Reviewer A

I enclose my comments regarding the TLCR submission titled "Effectiveness and Safety of Amivantamab in EGFR exon 20 insertion 3 (E20I) mutations in Non-small cell lung cancer (NSCLC)" authored by Dae-Ho Choi, Hyun Ae Jung, Sehhoon Park, Jong-Mu Sun, Jin Seok Ahn, Myung-Ju 6 Ahn, Keunchil Park, and Se-Hoon Lee provides a review of the efficacy of amivantamab in the real-world population for EGFR E20I-59 mutated NSCLC. The submission is well-written and provides a good overview of the methods, interpretations, and conclusions. I have several suggestions regarding this submission:

1. In page 3, line 93-94, there is an analysis to explore the synergistic effects of amivantamab and immune checkpoint inhibitors. Please explain if this is a phenomenological exploration or if there is an underlying mechanism linking the two mechanisms of action together.

Answer) We appreciate your positive feedback. In the process of analyzing real-world data and performing subgroup analysis, we identified adverse outcomes within the PD-L1 positive group. To elucidate this observation, we delved into the underlying mechanisms, framing the investigation as a phenomenological exploration in the paper.

2. In page 4, line 148, it would be helpful to understand the prior treatments of the patients and their respective responses to better understand the scale of amivantamab responses. There are indications of prior treatments in Table 1 and Supplementary Table 1. Can more information about the patient be provided to better understand the profile of the results? Likewise, commentary would be critical to better understand the overall outcomes listed from line 177.

Answer) Thank you for your comment. I fully acknowledge the significance of evaluating potential variations in effectiveness based on prior treatments. To address this concern, we have incorporated an analysis of the Objective Response Rate (ORR) in Table 3, stratified by whether patients had previously undergone treatment with Immune Checkpoint Inhibitors (ICI) or Tyrosine Kinase Inhibitors (TKI). Regarding patients who had previously received platinum-based treatment, we opted to exclude them from the analysis due to their considerable number (40 out of 42), causing a notable imbalance between the groups and compromising the statistical reliability of the analysis. Consequently, this specific subgroup was not further investigated.

I have added the following results to Table 3

Kinds of Previous therapy		
Previous use TKI	2/9 (22%)	0.36
No TKI	12/33 (36%)	
Previous use ICI	5/16 (31%)	0.55

No ICI	9/26 (35%)

3. Page 4, line 164-166, it is not clear how the different methods can be compared. Please provide some more information about the sensitivity of the different methods for EGFR analyses. This interpretation can be critical to determine if there are changes due to classification in the patient responses.

Answer) Thank you for your input. I resonate deeply with your perspective. I acknowledge that variations in sensitivity for detecting EGFR mutations can potentially introduce distortions in the results. Exon 20 insertion mutations, known for their heterogeneity compared to well-established mutations like del(19) or L858R, pose a practical challenge in detecting all exon 20 insertions through PCR. According to the literature, the sensitivity for detecting exon 20 insertion mutations is reported to be around 15-50%, and PCR testing using Cobas version 2 is occasionally associated with false positives. Therefore, all PCR tests using Cobas version 2 were excluded in this study. On the other hand, NGS, leveraging sequencing, holds a significant advantage in detecting almost all exon 20 insertion mutations. I appreciate your valuable insights.

4. This submission does not demonstrate a clear correlation between PD-L1 expression and amivantamab response. perhaps diving more into patient profiling or earlier treatment profiles can provide better understanding of responses.

Answer) Thank you for your comment. I deeply appreciate the acknowledgment that clear statistical significance may not be evident. To delve further into this, we explored the Objective Response Rate (ORR) based on patients' previous treatment history. Out of the 16 patients who underwent prior treatment with Immune Checkpoint Inhibitors (ICI), 13 had available PD-L1 status information. Among these, 10 were PD-L1 positive, and 3 were PD-L1 negative. In terms of ORR, among the PD-L1 positive patients who had received prior ICI treatment, it was observed to be 20% (2/10), while for PD-L1 negative patients, it was 67% (2/3). However, due to the limited sample size, especially with only 3 PD-L1 negative patients who received ICI treatment, establishing statistical significance proves to be challenging. Therefore, additional investigations are deemed necessary.

5. Pages 10-12, 16 , please highlight the number of patients that describe the curve

Answer) Thank you for your valuable input. I have added numbers of patients all of KM plot.

6. Page 13, Table 1, please provide more information about the definition of site of EGFR mutation. The initial description focuses on genotype. Please align the nomenclature for EGFR mutations. Please do the same on p 17, supplementary table 1.

Answer) Thank you for your valuable input. I have added information regarding the mutation

sites to Table 1 and Supplementary Table 1.

Reviewer B

①Did you investigate whether there were differences in the efficacy of amivantamab based on a more detailed classification of PD-L1 expression levels (not just categorizing as less than 1% or 1% and above, but specifically into less than 1%, 1-49%, and 50% or more) ?

Answer) Thank you for your valuable input. I have added additional analysis based on a more detailed classification of PD-L1 expression levels (less than 1%, 1-49%, and 50% or more). I provided these analyses in Supplementary figure 2.

②Was there a difference in efficacy based on the number of treatment lines in chemotherapy?

Answer) Thank you for your valuable input. I have added additional analysis based on the number of treatment lines in chemotherapy (1st group: 1st and 2nd line of therapy, 2nd group: 3rd line of therapy and more). I provided these analyses in Supplementary figure 3.