Author response 1

We greatly appreciate your support in the smooth review process of our manuscript and providing the opportunity to revise our manuscript. We also thank the reviewer for their time in evaluating our work and providing helpful suggestions to improve our manuscript. We have updated the case report and thoroughly revised the manuscript and figures to incorporate their suggestions wherever possible. We have also added a figure on blood tumor markers as Figure 1G and also replaced Figure 2 to clarify the representation of the two red columns as the specific base change. We hope you find the revised manuscript suitable for publication.

Reviewer 1 Comments: The case report by Jia Hu et al. describe the response of an EML4-ALK rearranged malignant pleural mesothelioma (MPM) patient treated with two different next generation ALK TKI. The case is interesting.

1) The main comment is about the nomenclature of mutations that needs to be adjusted to avoid confusion for the readers:

The term “compound mutation” has to be restricted to mutations detected on the same allele, i.e. in cis.

This seems to be the case for the L1196M/G1202R mutation at lorlatinib relapse. However this has not be investigated for the I1171N mutation so the term “compound” cannot be used here. The reviewer suggest using the term “multiple” mutations when several mutations are detected in one sample but the cis/trans status cannot be confirmed.

For instance, the sentences below (including the title and abstract) are wrong or imprecise and have to be reformulated:

“Acquired compound mutations ALK I1171N, L1196M and G1202R”

“Meanwhile, compound ALK I1171N, L1196M and G1202R mutations”

“speculate that these compound mutations synergistically”

“compound mutations involving ALK I1171N, L1196M and G1202R”

“potentially mediated by compound mutations ALK I1171N/L1196M and ALK I1171N/L1196M/G1202R”

“compound missense mutations in ALK kinase domain I1171N, L1196M and G1202R”

These are examples but in all the manuscript this has to be carefully corrected.

Author response: We greatly appreciate your time in reviewing our work and providing helpful suggestions to further improve our manuscript. We have revised our manuscript to correct the terminology and replace all use of “compound” to “multiple”. Thank you for this comment.

2) Precision about figure 2 would be necessary. The two red bars would suggest that both point mutations are present in 100% of the reads? Why did the authors add these red rectangular that seem to hide what is behind?

Author response: The figure was a screenshot from the integrated genome viewer (IGV) and the red rectangular boxes were not drawn/added to replace/highlight/hide the base change. In the IGV, the red color represents the base thymine (T) and so the two red vertical columns actually represent the base change to T. We have replaced Figure 2 with two figures, the left panel which shows the zoomed out version to illustrate the sequencing reads so as not to make the mutations appear to be 100% of the total reads, and the right panel which shows the zoomed in to base level version to illustrate the specific base change. As indicated by the table in Figure 1, the allelic fraction/abundance of L1196M and G1202R detected at lorlatinib PD were only 2.90% and 3.07%, respectively. Targeted sequencing was performed at a target sequencing depth of 10,000X for liquid biopsy (both pleural effusion supernatant and plasma sample).

3) the authors should define what they used as “blood tumor markers”

Author response: We have included the specific blood tumor markers in the revised manuscript. A plot to summarize the blood tumor marker levels during the treatment course was also added as Figure 1G.

Reviewer 2 Comments:

The article titled “Acquired compound mutations ALK I1171N,

L1196M and G1202R mediate lorlatinib resistance in EML4-ALK rearranged malignant pleural mesothelioma: a case report” is an interesting and novel case of MPM where ALK fusion has been detected and third generation ALK inhibitor, lorlatinib, has been used for its treatment for the first time. Given the rarity of ALCL of the oral cavity cases, the author’s report adds to the current knowledge. The report is well written and well cited. I only have a few minor suggestions/comments to the authors.

1. Line 48, ‘report described’ should be ‘report describes’

2. Line 49, ‘response from’ should be ‘response with’.

3. Line 65, ‘MPM cases is diagnosed’ should be ‘MPM cases has been observed’.

4. Line 134, ‘with overall survival’ should be ‘with an overall survival’.

5. Line 136, ‘our study was’ should be ‘our study is’.

6. Line 137, ‘and described’ should be ‘ and describes’.

7. Line 148-149, ‘alectinib inhibition’ should be ‘alectinib mediated inhibition’.

8. Line 251, ‘B-D’ should be ‘B-E’ as the description is for figures B-E and not just B-D.

9. In Figure-2 legend, the authors have mentioned tumor size at all the stages except for (C) PR, I think they should include it so that the readers can see the reduction in tumor size.

10. In Figure-2, the authors have highlighted the 3604G and 3586C base positions with a complete vertical red column. Perhaps, it would be clearer to the readers if they could show the base ‘A’ in the 3604 and 3586 base positions to make it clear.

Author response for comments 1-10: We greatly appreciate your time in thoroughly reviewing our work and providing clear instructions and helpful suggestions to further improve our manuscript. We have revised our manuscript to include all the grammatical corrections you have provided. We have also indicated the specific tumor size in the figure legends for Figure 1C. In the integrated genome viewer, the red color represents the base thymine (T) and so the two red vertical columns actually represent the base change to T. We have replaced Figure 2 with two figures, the left panel which shows the zoomed out version to illustrate the sequencing reads so as not to make the mutations appear to be 100% of the total reads, and the right panel which shows the zoomed in base-level version to illustrate the specific base change. Thank you very much for these suggestions.

11. The patient was detected to have EML4-ALK fusion by targeted NGS and the authors suggest that the patient's tumor was majorly driven by the oncogenic EML4-ALK fusion. Expression of ALK protein is generally confirmed in lung cancer patients by Ventana ALK (D5F3) CDx Assay, FoundationOne CDx or Vysis ALK Break Apart FISH Probe Kit. I think it would add more value to the report if the authors also had the ALK confirmation in the diagnosis tissue biopsy samples.

Author response: Thank you for this suggestion. We also agree that confirmatory tests with FISH or IHC could add another data for the presence of ALK fusion or overexpression in the MPM tumor of our patient. Unfortunately, tumor samples obtained thru biopsy were not adequate for further testing. The limited tumor sample was also the primary reason why pleural effusion samples were submitted for the initial targeted NGS genotyping (indicated in Figure 1 table of mutations). Actually, the targeted NGS panels that we have used for detecting the somatic mutations in several time-points during the treatment course of our patient are commercially-available from Burning Rock Biotech (a CLIA-certified, CAP-accredited clinical laboratory in China), which offers similar service as FoundationOne CDx. The gene panels have already been cited in numerous publications for the detection of various somatic mutations including EML4-ALK in various solid cancers including lung cancer (For specific methods, please refer to Li et al. 2018 Ann Oncol PMID:29346604; for a recent publication on EML4-ALK detection, please refer to Zhang et al. 2020 J Thorac Oncol PMID:32112982). We also believe that the clinical response of our patient to both alectinib and lorlatinib are evidences that EML4-ALK is one of the major clones driving the MPM tumorigenesis in our patient.