Peer Review File

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Reviewer A

Comment 1: The case is interesting, but from a clinico-molecular standpoint, ALK as the original driver can be debated in this case for two reasons: 1) it is never found in ctDNA and 2) the benefit from alectinib is not clear.

Reply 1: The cytoblock obtained from the right pleural effusion at diagnosis was positive for *ALK* by immunohistochemistry. This was confirmed with NGS on the pleural effusion cytoblock using Oncomine Focus Assay targeted panel which was positive for *EML4-ALK*. The NGS did not show any other relevant alterations including *MET* exon 14 skipping mutation. The patient achieved a durable response with stable disease radiographically and the patient had improvement in clinical symptoms of shortness of breath and chest tightness on alectinib. The *ALK* rearrangement may not have been detected by ctDNA as the patient was actively on alectinib at the time of plasma NGS collection. We agree with the reviewer that we do not have a tissue biopsy at the time of recurrence/progression, which is a limitation of the case report.

Changes in the text: We have modified our text as advised (see Page 4, lines 120-129 and Page 6, lines 339-346).

Comment 2: Authors are invited to provide more details on the baseline molecular analyses, ideally ALK IHC and/or FISH on the cytoblock or the tissue biopsy. Did the original NGS cover MET exon 14 skipping mutations?

Reply 2: The cytoblock obtained from the right pleural effusion at diagnosis was positive for *ALK* by immunohistochemistry. This was confirmed with NGS on the pleural effusion cytoblock using Oncomine Focus Assay targeted panel, which was positive for *EML4-ALK*. The NGS covered MET exon 14 skipping mutations which was not detected. A liquid biopsy using the FoundationOne Liquid CDx assay was also performed which did not identify the *ALK* rearrangement or MET mutations.

Changes in the text: We have modified our text as advised (see Page 4, lines 120-126).

Comment 3: Methodological details on molecular analyses should be provided (I guess that ctDNA was analyzed through Foundation One).

Reply 3: The right pleural effusion biopsy at diagnosis was analyzed using Oncomine Focus Assay targeted panel. The liquid biopsy performed at time of diagnosis and recurrence/progression were analyzed using FoundationOne Liquid CDx assay. Patient declined a tissue biopsy sample at time of recurrence/progression.

Changes in the text: We have modified our text as advised (see Page 4, lines 120-126 and lines 131-133).

Minor
Comment 4: The right column of table 1 (change from previous) could be avoided.
Reply 4: I revised the table and removed the change from previous column in table 1.
Changes in the text: We have modified our text as advised (see Table 1).

Reviewer B
I particularly enjoyed reading this case report. The article is well written, and the topic, exploring the mechanism of resistance to TKIs and strategies to overcome it, is particularly of interest and relevant. Here are some suggestions you may want to consider:

Introduction
Comment 1: Line 73: "Identifying these resistance mechanisms are important" I think there is a typo, it should be "is important".
Reply 1: I corrected the sentence as recommended.
Changes in the text: We have modified our text as advised (see Page 3, line 105).

Case presentation
Comment 2: Line 88-91: please clarify the diagnostic process, was a biopsy performed or just a cytology? Was the NGS performed on tissue or blood? The NGS showed other relevant alterations?
Reply 2: Cytology was performed on the right pleural effusion at diagnosis. The pleural fluid cytoblock was positive for *ALK* by immunohistochemistry. This was confirmed with NGS using Oncomine Focus Assay targeted panel which was positive for *EML4-ALK*. The NGS did not show any other relevant alterations including *MET* exon 14 skipping mutation. A liquid biopsy using the FoundationOne Liquid CDx assay was also performed at time of diagnosis which did not identify the *ALK* rearrangement or *MET* mutations (see table 1).
Changes in the text: We have modified our text as advised (see Page 4, lines 120-129).

Comment 3: Line 95: If the first NGS was performed on blood, were the two liquid biopsy both done with Foundation OneCDx?
Reply 3: The NGS at diagnosis was performed on the cytoblock obtained from the right pleural effusion using the Oncomine Focus Assay targeted panel. The two liquid biopsies at the time of diagnosis and recurrence/progression were performed using the FoundationOne Liquid CDx assay. The patient declined a tissue biopsy at the time of recurrence/progression.
Changes in the text: We have modified our text as advised (see Page 4, lines 120-129 and lines 131-133).

Comment 4: Line 102: Why was it decided to continue also with alectinib despite

the absence of ALK fusion in the liquid biopsy? Delve deeper into the reasons.
Reply 4: The *ALK* inhibitor was continued as this was believed to be the clonal driver mutation and the MET exon 14 skipping mutation was believed to be the subclonal resistance mutation. The *ALK* rearrangement may not have been detected by ctDNA at the time of recurrence/progression as the patient was actively on alectinib at the time of plasma NGS collection. The lack of tissue biopsy and NGS at the time of recurrence/progression is a clinical limitation.
Changes in the text: We have modified our text as advised (see Page 5, lines 215-218 and page 6, lines 339-346).

Comment 5: Line 102: For completeness, a small paragraph could be added that introduces another treatment alternative in patients with ALK fusion, the chemotherapy. The authors could briefly mention the chemosensitivity of patients affected by NSCLC with ALK fusion and the reasons that led to a different choice (e.g. ESRD).
Reply 5: We added additional text to discuss pemetrexed chemotherapy as an alternative option and the chemosensitivity observed with *ALK* fusions. We also added additional text discussing the reasons for not pursuing chemotherapy in our patient.
Changes in the text: We have modified our text as advised (see Page 4, lines 135-148).

Discussion
Comment 6: Line 118: "Initially significant response to alectnib" Please clarify if this means that the patient achieved a reduction in lung lesions (although not enough to reach a partial response), did the patient present an improvement of the symptoms? It would be important to add it.
Reply 6: The patient achieved a durable response with stable disease radiographically and had improvement in clinical symptoms of shortness of breath and chest tightness on alectinib.
Changes in the text: We have modified our text as advised (see Page 4, lines 126-129 and Page 5, lines 194-196).

Comment 7: Line 132: typo capmatinib
Reply 7: We have corrected the misspelling.
Changes in the text: We have modified our text as advised (see Page 5, line 209).

Comment 8: Please add this reference to the discussion section "Efficacy and Tolerability of ALK/MET Combinations in Patients With ALK-Rearranged Lung Cancer With Acquired MET Amplification: A Retrospective Analysis", PMID: 37533439. There are 3 cases reported in the literature about the combination of alectinib and capmatinib.
Reply 8: Additional text and the reference above were added to the discussion.
Changes in the text: We have modified our text as advised (see Page 5, line 224-

233).

Comment 9: Furthermore, to publish the case report the patient should give his informed consent. The checklist reports that the patient has not given informed consent.
Reply 9: Informed consent was obtained.
Changes in the text: We have modified our text as advised (see CARE Checklist).

Reviewer C

General comments:
This is a relevant and interesting case report on combination therapy with alectinib and capmatinib in a patient with NSCLC who developed resistance to alectinib. However, a few aspects must be clarified before the manuscript is ready for publication.

Specific comments:
Comment 1: Line 30-177: More consistency is required in relation to the use of italics in relation to genes as well as the use of abbreviations. Please review the manuscript carefully with respect to these issues.
Reply 1: All genes were italicized and abbreviations were corrected and clarified.
Changes in the text: We have modified our text as advised (see throughout manuscript text).

Comment 2: Line 95: I assume that the FoundationOne Liquid CDx assay was used. If so, please correct.
Reply 2: Yes, the FoundationOne Liquid CDx assay was used for both the plasma NGS at the time of diagnosis and recurrence/progression.
Changes in the text: We have modified our text as advised (see Page 4, lines 124-125 and lines 131-133).

Comment 3: Line 96: MET exon 14 skip alteration = MET exon 14 skipping mutation?
Reply 3: Yes, we used *MET* exon 14 skip alteration interchangeably with *MET* exon 14 skipping mutation. We have changed all *MET* exon 14 skip alteration to *MET* exon 14 skipping mutation for clarity.
Changes in the text: We have modified our text as advised (see throughout manuscript text).

Comment 4: Line 128-131: Coexistence of MET exon 14 skipping mutations and MET amplification has been observed. Please comment on this and whether the patient was tested for MET amplification. This may be significant, as both capmatinib and tepotinib have been shown to be effective in patients with MET amplification.

Reply 4: Yes, the patient was tested for *MET* amplification at diagnosis using Oncomine Focus Assay targeted panel, which was performed on the cytoblock obtained from the right pleural effusion. The patient was also tested for *MET* amplification at time of diagnosis and recurrence/progression with plasma NGS using the FoundationOne Liquid CDx assay. Results from both did not show MET amplification.
Changes in the text: We have modified our text as advised (see Page 5, lines 211-215).

Comment 5: Line 159-164: The low sensitivity and risk of false-negative test results of liquid biopsy assays must be briefly discussed. Owing to this issue, the US FDA has included a statement in the labeling for this type of companion diagnostic assay, indicating that patients with negative test results should undergo routine biopsy and their tumor mutation status should be verified using an FDA-approved tumor tissue test. This is also the situation for the FoundationOne Liquid CDx (https://www.accessdata.fda.gov/cdrh_docs/pdf19/P190032S005C.pdf)
Reply 5: Yes, unfortunately this is a limitation of our case report as the patient declined a tissue biopsy at time of recurrence/progression. The *ALK* rearrangement may not have been detected by ctDNA as the patient was actively on alectinib at the time of plasma NGS collection.
Changes in the text: We have modified our text as advised (see Page 6, lines 339-346).

Reviewer D
Interesting article.

Comment 1: I would like to know a little more details about the hemodialysis, and a consideration of doing serum concentration measurements of alectinib/capmatinib.
Reply 1: Additional text was added to discuss alectinib/capmatinib use and serum concentration measurements of alectinib/capmatinib in patients on hemodialysis.
Changes in the text: We have modified our text as advised (see Page 6, lines 331-337).

Comment 2: Furthermore, the case description could include tox details of the combo, which now partly are described in discussion.
Reply 2: Additional text and reference was added to discuss toxicities observed in patients treated with a combination of alectinib and capmatinib.
Changes in the text: We have modified our text as advised (see Page 5, lines 220-233).

Comment 3: Also - would it be feasible to do a deeper sequencing of the primary tumour, to look for pre-existing METex14?

Reply 3: The cytoblock obtained from the right pleural effusion at diagnosis was positive for *ALK* by immunohistochemistry. This was confirmed with NGS on the pleural effusion cytoblock using Oncomine Focus Assay targeted panel which was positive for *EML4-ALK*. The NGS did not show any other relevant alterations including *MET* exon 14 skipping mutation. A liquid biopsy using the FoundationOne Liquid CDx assay was also performed at time of diagnosis, which did not identify the *ALK* rearrangement or *MET* mutations.
Changes in the text: We have modified our text as advised (see Page 4, lines 120-129).

Comment 4: In line 132 there is a misspelling of capmatinib.
Reply 4: We have corrected the misspelling.
Changes in the text: We have modified our text as advised (see Page 5, line 209).