Peer Review File

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Reviewer A

This case report, which provides a detailed explanation of a new treatment for the progression of ALK+ NSCLC, was indeed very interesting.

Reply to the reviewer's general comment: *We thank very much the reviewer for the positive comment.*

However, I would like to offer some critical issues from various perspectives:

Comment 1. In the case report, MET amplification was not detected by DNA and RNA-NGS testing, but was confirmed by IHC and FISH testing. This suggests a lack of consistency between the testing methods used. Further explanation and consideration are needed for these differing results.

Reply to comment 1: *We thank the reviewer for the constructive comment. We completely agree, the diagnostics of MET-amplification in NSCLC may be challenging, given the frequent heterogenous and subclonal nature of this alteration. This heterogeneity may indeed cause NGS (where bulky DNA of a tissue sample is analyzed) to miss MET-amplification, which otherwise may be detected by FISH (where MET-amplified cells can be seen and quantified by microscopy).*
Changes in the text: *Thus, we have further specified and explained this issue in the Discussion by modifying the text as follows:* "This reflects the possible discordance between NGS and FISH as diagnostic methods, given the frequent heterogenous and subclonal nature of *MET*-amplification in NSCLC (5,9,28-31). This heterogeneity may indeed cause NGS, where bulky DNA of a tissue sample is analyzed, to miss *MET*-amplification, which otherwise may be detected by FISH, in which *MET*-amplified cells can be identified and quantified by microscopy" (*page 14-15, line 233-238*).

Accordingly, we have slightly modified the legend to figure 4, by adding "(but not by NGS)" *(page 13, line 206 for legend inserted in the text; page 25, line 472 for separately written legend) so that the new sentence becomes* "De novo MET-amplification was detected in the initial diagnostic biopsy from the primary lung tumor by retrospective IHC and FISH analysis (but not by NGS), after identifying *MET*-amplification in the hepatic metastasis at progression on Brigatinib".

Moreover, we have added a reference related to the issue (*new nr. 31*: Xiang C, Lv X, Chen K, et al. Unraveling the Significance of MET Focal Amplification in Lung Cancer: Integrative NGS, FISH, and IHC Investigation. Mod Pathol 2024; 37:100451), *which is cited on page 14,*

line 236 and page 15, line 247 of the revised manuscript. The reference list and citations have been adjusted accordingly.

Comment 2. The conclusion states that the combination of Alectinib and Crizotinib is an effective treatment for ALK+ NSCLC. However, this conclusion is based on a single case, and it may not apply to all ALK+ NSCLC patients. Furthermore, this conclusion is based on six months of treatment results, and the long-term effects and side effects are unknown. Authors should be described it in discussion.

Reply to comment 2: *We thank the reviewer for the relevant comment. We agree that we cannot make firm conclusions regarding the treatment of ALK+ NSCLC with de novo MET-amplification only based on a single case.*
Changes in the text: *Thus, we have slightly modified the conclusion in the Abstract from the original "*The combination of Alectinib and Crizotinib is a feasible and effective treatment for *ALK*+ NSCLC with *de novo MET*-amplification*" to the new version "*The combination of Alectinib and Crizotinib may be a feasible and effective treatment for *ALK*+ NSCLC with *de novo MET*-amplification*." (page 2, line 55).*
Furthermore, we have also modified the paragraph number 4, "Conclusions", as follows: "The combination of Alectinib and Crizotinib has provided significant clinical benefit to our patient, and no adverse events have been observed so far. The treatment is ongoing, and we are awaiting the results of the long-term outcome." (*page 17, line 299-300*).

Comment 3. This case report is based on a single case, and further clinical trial is needed to determine how applicable its results are to the general patient population. Authors should be discussed in detail.

Reply to comment 3: *We thank the reviewer for this comment, which is in line with the previous one.*
Changes in the text: *We added the word "clinical" to the last sentence of the paragraph number 4, "Conclusions", so that the sentence now is:* "Further clinical studies of combining ALK-TKI with a more potent MET-TKI are needed for optimizing this approach." (*page 17, line 300).*

Reviewer B

This is a really impressive case, which will also give guidance to oncolgists who face the choice of either moving to the next treatment line or combining molecularly targeted therapy against the driver with a second TKI to target the resistance mechanism. This is a clincial scenario relevant to clincial practice.

Interestingly, the MET amplification as targetable resistance mechanism is described in an ALK-fusion case, though it would have been more likely expected in a EGFR mutated case.
The case is clearly described, and the manuscript is well written. The discussion is to the point

and highlights the potential of combining two TKIs.

REPLY to REVIEWER B
We thank very much the reviewer for the very positive comments and nice words regarding our manuscript.
Changes in the text: *No changes were required by reviewer B.*

Reviewer C

The article named: ALK-TKI intrinsic resistance due to de novo MET-amplification in metastatic ALK-rearranged NSCLC effectively treated by Alectinib-Crizotinib combination is an interesting case report on the role of MET amplification as resistance to ALK TKIs treated with combination of crizotinib and Alectinib.
First of all, I would like to congratulate with the authors for the quality of this paper and for the original idea. I think that resistance in ALK population is an important theme that should be more investigated.

Reply to the reviewer's general comment: *We thank very much the reviewer for such positive comments.*

Here you find some little suggestion that I hope could help you to increase the quality of your paper:
Comment 1. The introduction is well written; I would cite more about the context of on-target and off-target mutations in ALK population (there are many papers on this theme doi: 10.7573/dic.2022-3-1.)

Reply to comment 1: *We thank the reviewer for the suggestion. Due to the restricted number of references (max. 35 for case reports), we preferred not to cite the suggested reference, as the subject of on-target and off-target resistance mechanisms in ALK+ NSCLC patients is comprehensively presented in the more recent references that we have already cited (nr. 2-4). No changes in text seem necessary.*

Comment 2. In the discussion, it could be interesting introduce the role of liquid biopsy on monitoring mutations and heterogenous disease doi: 10.1016/j.jtho.2021.06.017

Reply to comment 2: *Thank you for suggesting this generally relevant issue. Yet, the suggested reference seems to be out of scope with respect to our manuscript, which is focused on de novo MET-amplification as mechanisms of intrinsic TKI-resistance in ALK+ NSCLC. However, the idea of mentioning liquid biopsies in the Discussion is useful and well appreciated.*
Changes in text: *Thus, we added a sentence to the Discussion: "*Furthermore, the possibility of detecting *MET*-amplification and other co-alterations in plasma cfDNA from *ALK*+ NSCLC patients may help monitor their response to therapy", *and we have also added a related reference (nr. 35) (page 17, line 284-286).*

Comment 3. I did not understand if ALK fusion was revealed by NGS RNA; any way MET amplification was identified only by FISH and IHC. Do you believe it could be useful to test MET amplification? Please explain it in discussion section.

Reply to comment 3: *We thank the reviewer for this comment. First, we would like to confirm that the EML4-ALK-fusion v.3 was detected by RNA-NGS as described in the text (Section 2., Case Description, page 4, line 102-105).*
As to whether "it could be useful to test MET amplification" upfront (i.e., at baseline), we would like to be cautious before being conclusive, as it is only one case report. We have addressed this issue already in the Discussion and we believe that MET-FISH increases the chance of detecting MET-amplification, especially in NGS-negative cases. To underline more clearly this issue, as suggested by the reviewer, we have rephrased a sentence in the Discussion.
Changes in the tex*t:* *the rephrased sentence is as follows: "A*dditional real-world data and future clinical studies may help elucidated whether it is of cost-benefit to test upfront *ALK*+ NSCLC patients for potential mechanisms of intrinsic resistance such as de novo *MET*-amplification as this may concern only a minority of these patients*." (page 17, line 282-284).*

Comment 4. I think it would be more complete if the authors could introduce a table including similar with studies reporting these cooccurring mutations and clinical factors that could influence the prognosis of ALK population (doi: 10.1007/s12094-024-03481; doi: 10.1016/j.jtho.2023.08.007; doi: 10.1002/cam4.4663; https://doi.org/10.1016/j.cpccr.2024.100291; doi: 10.1016/j.tranon.2022.101471; doi: 10.3390/cancers15133422)

Reply to comment 4: *We thank the reviewer for the valuable suggestions. To keep the focus of our case report (i.e., de novo MET-amplification as intrinsic TKI-resistance mechanism in ALK+ NSCLC), we prefer to avoid introducing a new table with all the prognostic co-mutations and clinical factors. Moreover, we extensively discuss the reported resistance mechanisms in the text.*
Considering TLCR's restricted number of citations, we cannot add all the six suggested references. Thus, we cite only three of them, which are the most relevant for our Discussion.
Changes in the text: *The three citations are the new references nr. 25, 26 and 34. The sentence added in connection with the citations 25 and 26 is:* "Baseline *KRAS* co-mutations or *CDKN2A*-deletion were also reported as potential mechanisms of intrinsic resistance to ALK-TKIs (25, 26)" *on page 14, line 228-230. The sentence related to reference 34 is:* "Shorter *EML4-ALK* fusion variants (v.3, v.5) and baseline *TP53* co-mutations were also linked to shorter OS in a Chinese population (34)", *on page 16, line 280-281.*

Comment 5. are there any clinical trials investigating the role of combination of alectinib and crizotinib? please cite

Reply to comment 5: *Regrettably there are no clinical trials currently available to address the role of combined Alectinib and Crizotinib, as shown in reference nr. 10 and stated in the Introduction, line 86, i.e., "*Currently no clinical trials exploring combination of ALK-TKI and MET-TKI are registered (10)*".*
Changes in the next: *Thus, we do not feel that we need to make changes.*

Reviewer D

Well done on this superb precision diagnostic work and on the successful treatment.
Excellent

REPLY to REVIEWER D
We thank very much the reviewer for the very positive comments and nice words regarding our manuscript.
Changes in the text: *No changes were required by reviewer D.*

Reviewer E

The manuscript is quite well written and organized. English could be improved.
Figures and tables are comprehensive and clear.
The introduction explains in a clear and coherent manner the background of this study.

Reply to the reviewer's general comment: We *thank the reviewer for the very positive comments regarding our manuscript. Minor linguistic adjustments have been made.*

Comment 1: We suggest the following modifications:
Introduction section: although the authors correctly included important papers in this setting, we believe the evolving systemic treatment scenario for lung cancer should be further discussed and some recent papers added within the introduction (PMID: 36533070; PMID: 36695827; PMID: 38791914; PMID: 33225800), only for a matter of consistency. We think it might be useful to introduce the topic of this interesting case report.

Reply to comment 1: *We thank reviewer E for suggesting the inclusion of the 4 indicated references in the Introduction's section. However, they seem to be out of context, especially considering TLCR's restrictions regarding text and references. Indeed, three of these 4 references deal with possible prognostic biomarkers in advanced cancer patients treated with immunotherapy and the last one is related to a prognostic tool across diverse cancer types.*
Changes in the text: *Accordingly, we do not see the necessity to include these references in the Introduction, as they would take the focus of the manuscript away from its topic, which is ALK-TKI intrinsic resistance by de novo MET-amplification.*

Comment 2: The timeline in Figure 4 should be re-organized in order to help readability of the

manuscript.

Reply to comment 2: *Thank you for the constructive comment.*

Changes in the text: *We have re-organized and simplified the figure to make it comprehensible.*