Peer Review File

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Reviewer A

This is a well written and timely case report, describing MYC amplification-conferred primary resistance to capmatinib in a MET amplified NSCLC patient. As the authors highlight nicely in the manuscript, responses to MET tyrosine kinase inhibitors have been lower than other oncogene driven subsets of NSCLC, and there are clearly underlying molecular mechanisms that are potentially driving primary or acquired resistance to MET inhibitors. Given the rarity of MET amplification, publication is of value to the thoracic oncology community at large.
Confirmation of resistance using patient-derived cells (PDCs) including sensitivity to MYC inhibitor ICX-101 is nice proof-of-concept translational work and the authors should be commended on the translational component of the paper which elevates the manuscript.

Thank you for the encouraging and favorable comments.

Some questions / comments:
[Comment 1]
- Was NGS sequencing used prior to treatment with capmatinib? Was it the same NGS platform used? This needs to be clarified. If not performed, it is of course possible that the MYC amplification was not present in the tumor prior to capmatinib therapy and would then be an acquired mechanism of resistance to MET inhibition. If not performed, it is not possible to know. It would be important to comment re acquired vs primary resistance to capmatinib.

Reply 1:
Thank you for this constructive comment. After the patient progressed with first-line capmatinib, a re-biopsy was performed from the liver, and NGS sequencing was performed with the re-sampled tumor tissue. We have rephrased the sentence in the manuscript to clarify this point. Since pre-treatment NGS was not performed, we agree with the reviewer that it is not possible to know whether MYC amplification was a co-existing alteration or an acquired change. However, the duration of capmatinib was only 6 weeks, which is a relatively short time for an acquired resistance mechanism to arise, and the tumor did not respond at all. Therefore, we believe that MYC amplification conferred primary resistance to capmatinib in this case patient.

Changes in the text: (Page 5 – 6, lines 97 – 102)
We performed fluorescent in situ hybridization (FISH) for *MET*, and an amplicon-based next-generation sequencing (NGS, Illumina), with a panel consisting of 91 genes using the re-biopsied tumor samples from the liver. The NGS analysis revealed gene amplification of *MYC* (estimated copy number 5) and *CEBPA* without other nucleotide variants or fusion mutations, as well as *MET* amplification on FISH.

[Comment 2]
- Mentioning the type of NGS platform used and no of genes tested on this platform should also be included.

Reply 2:
Along with the response to the previous comment, we have added the information of the NGS platform in the revised manuscript.

Changes in the text: (Page 5 – 6, lines 97 – 102)
We performed fluorescent in situ hybridization (FISH) for *MET*, and an amplicon-based next-generation sequencing (NGS, Illumina), with a panel consisting of 91 genes using the re-biopsied tumor samples from the liver. The NGS analysis revealed gene amplification of *MYC* (estimated copy number 5) and *CEBPA* without other nucleotide variants or fusion mutations, as well as *MET* amplification on FISH.

[Comment 3]
- Please include clinical trials.gov identifier for the phase 2 trial of capmatinib

Reply 3:
In response to this suggestion, we added the ID of the trial for clinicaltrials.gov.

Changes in the text: (Page 5, lines 86 – 87)
As the initial tumor tissue was intensely stained for MET, he was screened for a phase 2 trial of capmatinib (GEOMETRY mono-1, NCT02414139) as first-line treatment.

[Comment 4]
- MYC Amplification has been previously described following crizotinib inhibition in a patient with ALK-driven NSCLC. It would be worth mentioning this and giving reference to the paper [doi: 10.1016/j.tranon.2018.09.013. Epub 2018 Oct 2.]

Reply 4:
We appreciate this comment. The paper suggested by the reviewer is mentioned and cited in the revised manuscript.

Changes in the text: (Page 8, lines 155 – 157)
In accordance with this notion, *MYC* amplification was suggested as a potential cause of primary resistance to crizotinib in a case of advanced NSCLC with ALK-rearrangement

[Comment 5]
- the prevalence of MYC alteration in MET-driven lung cancer is largely unknown, and so this case adds to the literature. It would be worth including brief discussion re the placement of MYC amplification as a cancer target. For example, that the MYC gene which consists of 3 paralogs, C-MYC, N-MYC and L-MYC, and is one of the most frequently deregulated driver

genes in human cancer – this is not mentioned in the text. Also, the point that, as a potential anticancer target, MYC has been traditionally regarded as undruggable because of the lack of a suitable pocket for high-affinity binding by low molecular weight inhibitors – this would be worth mentioning.

Reply 5:
Thank you for the constructive comment. This is a valid point to be discussed in the paper. We have now added additional descriptions about the importance of MYC as a potential anticancer target.

Changes in the text: (Page 8, lines 163 – 168)
The MYC family has three paralogs: C-MYC (encoded by *MYC*), N-MYC (encoded by *MYCN*), and L-MYC (encoded by *MYCL*). MYC is enhanced and deregulated in many types of malignancies, which is one of the most frequently deregulated driver genes in human cancer. However, despite its critical role, MYC has been traditionally regarded as an undruggable target because it lacks a preferred pocket to allow high-affinity binding by small molecule drugs (14).

Reviewer B

Choi and colleagues present interesting case report of a patient with MET amplified NSCLC with resistance to capmatinib found to have MYC amplification. The authors nicely describe patient-derived tumor cell culture experiments for drug response. This is an excellent approach to advancing our understanding of targeted therapies and contributes to our understanding of mechanisms of resistance to capmatinib. The pursuit of PDC experiments provides preclinical evidence for the investigational agent ICX-101. The conclusion presents a nice review of what is known about myc amplification in MET altered NSCLC, and describes important future directions. A few comments and suggestions:

We deeply appreciate these encouraging and favorable comments.

[Comment 1]
1. Did molecular analysis of the initial diagnostic biopsy demonstrate MYC amplification (lines 78-79)? It is possible the MYC amp developed during the capmatinib therapy as it is only noted on the post-treatment tissue. Agree the clinical picture suggests it was there primarily (2 months on therapy with progressive disease), but the mutational analysis are not able to determine this as currently described. Furthermore, the evidence described in lines 133-136 suggests MYC amp has been implicated in both primary and acquired resistance. This should be clarified and discussed further. Similarly, line 153, consider 'primary or acquired resistance'

Reply 1:
This is a valid point to be addressed. Tests for MYC amplification were not tested with the initial diagnostic sample, and we agree that without this data there is a possibility that MYC amplification could have developed during capmatinib treatment. However, as mentioned in

the comment, the duration of capmatinib treatment was only about 6 weeks, which is a relatively short time for an acquired resistance mechanism to arise.

The paper cited in the manuscript (Shen et al., Cancer Res 2015) demonstrates that MYC activation is a critical mechanism in conferring resistance to a MET-inhibitor in initially MET-dependent cancer cells. In their experiments, EBC-1, a lung cancer cell line with MET amplification, shows high sensitivity to SGX-523 which is an investigational MET inhibitor. After generating acquired resistance to SGX-523 (termed EBC-1/SR), these cells still show a similar level of MET amplification, but an enhanced expression of MYC. They also show that overexpression of MYC in parental EBC-1 cell line results in diminished drug sensitivity to SGX-523. Together these results suggest that MYC confers resistance to both primary and acquired resistance to MET inhibitors. These data stand in line with the role of MYC as an important mediator of therapy resistance to MET inhibitors.

We have added these points to clarify the cited reference, and added descriptions in the revised manuscript.

Changes in the text: (Pages 7 – 8, lines 144 – 153)
Notably, another preclinical study suggested the role of MYC as a downstream effector to dictate the therapeutic response of MET inhibitors, and also a driver that leads to both primary and acquired resistance in *MET*-amplified cancer cells (10). In this study, a lung cancer cell line with *MET* amplification showed high sensitivity to an investigational MET inhibitor. After generating acquired resistance to this drug, these cells still showed a similar level of *MET* amplification but an enhanced expression of MYC. They also demonstrated that overexpression of MYC in the parental cell line resulted in diminished drug sensitivity to the MET inhibitor. Together these results suggest that MYC confers both primary and acquired resistance to MET inhibitors, suggesting its role as an essential mediator of therapy resistance (10).

[Comment 2]
2. Describe the MYC amplification in more detail (copy number gain), line 97

Reply 2:
The estimated copy number of MYC on the NGS report was 5. We have added this information in the revised manuscript.

Changes in the text: (Page 6, lines 100 –101)
NGS analysis revealed gene amplification of MYC (estimated copy number 5)

[Comment 3]
3. Clarify whether the IC50 for ICX-101 is described in MYC amplified NSCLC cells or all NSCLC cells, this is relevant when describing the comparison to the IC50 in PDCs. Include in lines 115-117 and Fig 2C caption.

Reply 3:

The IC50 value for ICX-101 was calculated by testing all NSCLC PDCs. We have added these points for clarification in the revised manuscript and the caption for Fig 2C.

Changes in the text: (Page 6 – 7, lines 120 – 122)
So far, we tested the sensitivity of ICX-101 using multiple NSCLC PDC samples developed in our institute (n=100) and the IC50 value was 0.91 μM (interquartile range 0.54 – 1.54 μM) for all NSCLC PDCs.

(Caption of Fig 2C)
(C) IC50 values of ICX-101 among PDCs of all NSCLC (n = 100).

[Comment 4]
4. Lines 119-120 this conclusion is overstated. The MYC amplification may be the primary resistance mechanism to capmatinib, but with RR of only 40%, it is not definitive that MYC amp is the cause. A more appropriate conclusion is that the concurrent MYC amplification may have conferred primary resistance to capmatinib (or acquired resistance, see comment 1). Additional PDC data showing a synergistic response to the combination of capmatinib and ICX-101, or that ICX-101 could restore sensitivity to capmatinib would be important to support this claim.

Reply 4:
We appreciate this constructive comment. As suggested, we have toned down the conclusion in the manuscript and revised it as below. We have also performed in vitro experiments suggested by the reviewer. In brief, patient-derived cancer cells were seeded in 96-well plates at passage 3, and after 24 hours of incubation, each concentration of ICX-101 or capmatinib was treated. After 48 hours, the other drug was treated in a dose-matrix manner. After further incubation for 72 hours, cell viability was measured by ATP assay. In these PDC cells, when ICX-101 was treated first, an additive effect, not a synergistic effect, was shown, whereas when capmatinib was treated first, a synergistic effect was shown. Presumably, a more substantial synergistic effect is expected if capmatinib is treated at an appropriate dose for a more extended period and MYC inhibitor is treated sequentially. We have added these additional experimental data in the supplementary data section, and also described these points in the revised manuscript.

Changes in the text: (Page 7, lines 123 – 131)
In order to see if there is a synergistic effect between ICX-101 and capmatinib, we treated the PDCs of the case patient with both drugs either in combination or as sequential treatments. Co-treatment of ICX-101 and capmatinib was not synergistic, and ICX-101 followed by capmatinib showed only an additive antitumor effect (Supplementary Figures 3, and 4A). However, a synergistic effect was shown when capmatinib was treated with subsequent ICX-101 (Supplementary Figure 4B). These clinical and in vitro data imply that the primary resistance to capmatinib in this patient even with MET amplification may be conferred by the concurrent *MYC* amplification, and a MYC inhibitor could be utilized to inhibit resistance to capmatinib.

[Comment 5]

5. In the concluding paragraph, would posit that MYC could be used as an alternative therapy, but also in sequence or potentially in combination w MET-targeting agents if MYC amp is truly a mechanism of resistance.

Reply 5:
Thank you for this insightful comment. As selective MYC inhibitors suitable for in vivo treatment are not available yet, the statement in the conclusion is based on our speculations. The decision to use MYC inhibiting strategy as either an alternative or combination therapy should be validated through clinical studies.

Minor comments:
[Minor comment 1]
1. Recommend "selective agents that can match a tumor's driver oncogenic alteration" in line 52
Reply 1:
Thank you for this comment. We have rephrased the sentence as suggested.

Changes in the text: (Page 4, lines 51 – 53)
The treatment of advanced non-small cell lung cancer (NSCLC) has been revolutionized by selectively targeted agents that match driver oncogenic alteration.

[Minor comment 2]
2. Refer to the cancer/tumor/NSCLC or rather than the "patient" when describing mutations, responses etc, per the IASLC Language Guide, lines 52, 55, 57, 124, 125, 128, 154, Fig 2C caption

Reply 2:
We appreciate this comment. Along with the first minor comment, we have rephrased the term 'patient' into 'tumor, 'NSCLC, or 'lung cancer' in the revised manuscript.

[Minor comment 3]
3. Eliminate 'However' line 85 and line 92

Reply 3:
We rephrased the sentence and eliminated 'however' as suggested.

Changes in the text:
(Page 5, lines 87 – 90)
Real-time polymerase chain reaction (RT-PCR) analysis for METex14 was negative, but MET GCN as determined by fluorescence in situ hybridization (FISH) was 13.5 indicating gene amplification.

(Page 6, lines 103 – 104)
Subsequently, he received paclitaxel and carboplatin as second-line therapy and irinotecan and

cisplatin as third-line therapy, without tumor response to either regimen.

[Minor comment 4]
4. Consider "…third-line therapy, without tumor response to either regimen." in line 99
Reply 4:
Along with the third minor comment, we have rephrased the sentence as suggested.

[Minor comment 5]
5. Consider including OS in line 101-2, eg "but succumbed to death due to disease progression XX months after initial diagnosis"

Reply 5:
Thank you for this constructive comment. The patient died 6 months after the initial diagnosis of metastatic NSCLC. This point is added in the revised manuscript.

Changes in the text: (Page 6, lines 106 – 107)
…eventually succumbed to death due to disease progression 6 months after the initial diagnosis of metastatic NSCLC.

[Minor comment 6]
6. Would describe the frequency of MYC amplification in NSCLC after sentence lines 137-9.

Reply 6:
Unfortunately, there is no reliable data reporting the frequency of MYC amplification in a large cohort of NSCLC, especially confined to advanced stages. Thus, we find it inappropriate to state the frequency of MYC amplification.

[Minor comment 7]
7. Add NSCLC line 149 "KRAS mutated NSCLC and small cell…"

Reply 7:
Thank you for the comment. We have rephrased the sentence as suggested.

[Minor comment 8]
8. Line 154, omit "even"

Reply 8:
We have omitted 'even' as suggested.

[Minor comment 9]
9. Its worth nothing that capmatinib is not yet approved for MET amplified tumors (in the US), though the authors describe the promising data, particularly for GCN >10.

Reply 9:

We have added this point in the revised manuscript.

Changes in the text: (Page 4, lines 60 – 62)
Based on these trial data, capmatinib was granted accelerated approval by the FDA for NSCLC with *MET*ex14.

Reviewer C

[Comment 1]
This is a well-written and very compelling case report demonstrating the role of c-MYC inhibitors to overcome c-MET resistance.
Albeit the role of c-MYC for the c-MET pathway is not fully understood, this case report for the first time could show the inhibition of c-MYC is important to restore capmatinib resistance.
Several mechanisms have been shown to be implication in resistance to highly selected c-MET inhibitors (e.g., capmatinib, tepotinib). This. I would recommend to briefly list these mechanisms in a table to provide more information for the readers.
In addition, the authors just excluded a c-MET exon 14 skipping mutation, however, other mutations can also confer resistance to capmatinib (see Dempke et al. TLCR, 2021).
I would suggest to discuss this briefly in the discussion section.

Reply:
Thank you for this comment. As mentioned, several mechanisms that confer resistance to capmatinib or tepotinib have been reported and were described briefly in the original manuscript. In the revised manuscript, we added an additional reference study (PMID 33545388) and also created a table to list these alterations to provide more information for the readers.

Changes in the text: (Page 7, lines 137 – 141)
Studies that investigated the resistance mechanisms against various MET inhibitors in NSCLC patients with METex14 have shown that alterations in mitogen-activated protein kinase (MAPK) pathway genes (EGFR, KRAS, HER3, and BRAF), de novo mutations of MET, and phosphoinositide-3 kinase (PI3K) signaling alterations were highly implicated (5-8) (Table 1).

Table 1. Summary of genetic alterations that cause resistance to MET inhibitors