Peer Review File

The reviewer points out that the novelty of the work is a weakness of the manuscripts, since previous studies *(Jakobsen et al. 2017 and Xu S., et al. 2017)* have already demonstrated EMT as a resistance mechanism in HCC827 cells. We acknowledge this concern of EMT already having been demonstrated as a resistance mechanism in HCC827 NSCLC cells. The two papers referred to address resistance mechanisms to EGFR-TKIs and found EMT as an acquired resistance. However, we address the clinically relevant condition of acquired resistance to sequential MET-TKI treatment in EGFR-TKI resistant cells that have acquired resistance based on *MET* amplification. This sequential TKI resistance has only been investigated in two previous studies (Yamaoka et al. 2016)(A. Li et al. 2017), with no evidence of EMT as a resistance mechanism. Additionally, our results are the first evidence of EMT as a resistance mechanism to capmatinib and crizotinib, at least to our knowledge. Since *MET* amplification drives resistance to EGFR-TKIs in up to 20% of EGFR-TKI resistant patients, we believe knowledge on resistance to the next line of TKI treatment is important in the clinical management of resistance. Our findings contribute to validation of EMT as a resistance mechanism in TKI resistance.

Major concerns:

1. The author should derive cell lines from more than one cell line model (HCC827) to ensure these are not clonal effects.

We understand this concern, but with the aim of investigating sequential MET-TKI resistance in *MET*-amplification-mediated EGFR-TKI resistant cells, HCC827 was the only cell model available to us. We have previously established EGFR-TKI resistance in PC9 NSCLC cells in addition to HCC827 but were only able to detect acquired *MET* gene amplification in HCC827 (personal communication). This is in accordance with previous studies, where HCC827 is prone to gain *MET*-amplification at EGFR-TKI resistance (Jakobsen et al. 2017)(Engelman et al. 2007)(Turke et al. 2010), while PC9 more often acquires EGFR T790M mutations and not *MET*-amplification (Ogino et al. 2007)(Ware et al. 2013)(Bean et al. 2007). Furthermore, we did not find introduction of *MET* gene amplification by experimental genetic approaches biological relevant, since it is difficult to control the copy number inserted. EMT is not a phenomenon restricted to HCC827 cells as it has

been reported as a resistance mechanism in other NSCLC cell lines than HCC827 and to several TKIs (Ware et al. 2013)(L. Li et al. 2017)(Fukuda et al. 2019)(Rastogi et al. 2016). Additionally, we established MET TKI resistance in three distinct *MET*-amplified erlotinib-resistant cell clones to investigate biological variation in resistance development and used two distinct MET-TKIs to evaluate the effect of both a specific and a broad TKI.

2. The author should demonstrate that genetic or pharmacological alteration of EMT-associated genes can render resistant cells sensitive to MET TKI – rescue experiments.

We agree that such studies could validate EMT as a resistance mechanism to MET-TKI in our sequential resistant *in vitro* model. However, EMT is established as a mechanism of TKI resistance in several published studies. Furthermore, genetic and pharmacological reversion of EMT have been demonstrated in different cancer cell lines resistant to different TKIs to overcome resistance and to render resistant cells sensitive to the TKI to which resistance was acquired (Fukuda et al. 2019)(Witta et al. 2006)(Suda et al. 2011). The aim of our study was to investigate if EMT was associated with resistance in our cell model of sequential resistance, and to identify drivers of survival of the resistant cells. We demonstrated that EMT is a common phenotype of the resistant cells and that FGFR inhibition inhibits the resistant cells.

3. Immunoblot showing upregulation of FGFR1 should be reorganized from Figure 2 to 3 in the FGFR1 section.

Thank you for pointing that out, this is corrected in the revised figures.

4. The authors should use more than one FGFR inhibitor (AZD4547) to ensure on-target effects given the promiscuity of kinase inhibitors.

AZD4547 is a potent inhibitor of FGFR1-3. AZD4547 was the inhibitor of choice as it has previously successfully been used to inhibit FGFR signaling and decrease viability of FGFR1 overexpressing cells (Ware et al. 2013)(Jakobsen et al. 2017). Based on these previous studies we used AZD4547 in our inhibitor experiments.

5. The authors should perform dose curves to determine the IC50s of each line rather than using only one time-point that should be specified in the Figure legends and text. Pharmacodynamic analysis should also be performed to verify target engagement of compounds

We agree with the reviewer that the IC50 value of each cell line contributes with superior information and have included these in Figure 1B in the revised manuscript. We have verified the engagement of MET-TKI in inhibition of MET, which is illustrated in the WB in Figure 2A. Here, MET phosphorylation is absent in the resistant cells, which are cultured in medium supplemented with MET-TKIs. In contrast MET is phosphorylated in the parental cells cultured in medium without MET-TKIs. Removal of the MET-TKI from the culture medium resulted in phosphorylation of MET in the resistant cells (personal communication).

6. *Quantification and statistical analyses should be done on IF data in Figure 2C.*
The IF data was added to the figure to support and illustrate the WB data and the mRNA expression analyses of vimentin and e-cadherin. Accurate quantification of IF data has not been possible in our hands in a manner that we find reproducible to support publishing and we therefore have based our conclusions on the qualitative data retrieved from the IF images.

7. *No data is shown for this statement on line 245, "Additionally, sequencing with the Oncomine Focus panel, covering SNVs, indels, CNVs, and fusions in 52 cancer-associated genes revealed no de novo genetic changes in the MET-TKI resistant cells, hereby supporting FGFR1-driven EMT as the central resistance mechanism.". It is also unclear how such a conclusion can be drawn from that data.*
We acknowledge that the text in the manuscript was unclear and has accordingly corrected this section in the revised manuscript (page 11, line 253). The conclusion is based on our targeted NGS data, which we now have included in the supplementary data of the revised manuscript (Supp. Table S3). This data showed no genetic changes between resistant and parental cell lines in genes previously described to confer TKI resistance. That no previously described genetic resistance mechanism was present in the resistant cells is in alignment with our findings of EMT and associated FGFR1 overexpression being the central resistance mechanism. That this is the conclusion, and not as previously written *"FGFR1-driven EMT as the central resistance mechanism"*, have been corrected and clarified in the revised Results and Discussion sections (page 11, line 253) (page 13, line 304).

Minor concerns:
1. *There are only 3 Figures, but the authors have labeled Figure 3 as Figure 4.*

Thank you for pointing this out. This error is now corrected in the revised figure legends.

2. *Missing p-values (statistical analyses) in Figure 1B, Figure 2B-D or Figure 3A or 3B*

We agree statistical analyses will strengthen our findings. This is now corrected in the revised figures and described in the statistics section under Methods (page 8, line 183).

3. *The authors should state where HCC827 were obtained and whether STR fingerprint profiling was used for cell line authentication, especially since the crux of this paper is based solely on this one model.*

HCC827 was obtained from ATCC and STR fingerprint analysis was performed on the erlotinib resistant cell line, HCC827ER, compared to parental HCC827 and demonstrated that HCC827ER is genetically equivalent to HCC827. This verified the authenticity of the *MET*-amplified clones which were derived from HCC827ER by single cell dilution. Our sequencing results of the MET-TKI resistant and parental cell lines revealed similar gene mutational signature in the 52 genes analyzed indicating genetically equivalent background. A statement of where HCC827 was obtained and if fingerprint analyses were performed is included in the Methods section in the revised manuscript (page 5, line 97).

4. *Figure legends would benefit with more details for graph axis.*

We have considered this concern in the revision of figures and figure legends.

5. *Scale bars should be included in the IF-data.*

We have corrected the missing scale bars in the revised figure.