Peer Review File
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Comment 1: Authors performed a narrative review to investigate the mechanism of resistance to TKI therapies in ALK-positive NSCLC, especially focusing on the lineage transformation of cancer cells. Lineage transformation in ALK-positive NSCLC during TKI therapies is rare and the biology and clinical implications are mostly unknown. The paper is well-written and well-discussed. But I have a few comments and questions that want to be addressed in order to improve this manuscript.

Authors concluded that lineage transformation was likely to be driven by transcriptional reprogramming rather than acquired genomic mutations in ALK-positive NSCLC. Although it is sure that molecular alterations for lineage transformation remain unknown, some research had demonstrated the important role of key transcriptional factors for cell fate decision, such as ASCL1 (Cell rep. 2016;16:1259-1272) or SOX2, in small cell lung cancer (SCLC) or squamous cell lung cancer tumorigenesis, for example. If authors speculate that lineage transformation via transcriptional reprogramming is more common, they should cite these articles more and discuss in more detail.

Reply 1: We thank the reviewer for highlighting this aspect of our manuscript that requires further clarification. As the reviewer states, the definitive molecular mechanisms of lineage transformation remain unknown. In our view, the strongest evidence that lineage transformation is likely to be driven by transcriptional reprogramming is the lack of identification of recurrent acquired genomic mutations causally associated with transformed tumors, together with the available data showing evidence for evolution of gene expression and methylation changes. We have added text to **lines 358-368** in the revised manuscript to further discuss this. We agree that published literature about the role of transcription factors such as ASCL1 and SOX2 in cell fate and tumorigenesis suggest a possible role for such transcriptional programs in lineage transformation, and we have added this suggested reference to the revised manuscript **(line 367)**.

Comment 2: In this time, authors focused on the lineage plasticity in ALK-TKI resistance in ALK-positive NSCLC, but not other rare driver gene mutant NSCLC. Furthermore, histologic transformation has been reported not only during TKI therapies for driver gene mutant NSCLC but also other drug therapies such as immune checkpoint inhibitors (ref. 95-98). It seems that histologic transformation is rare but could happen anytime in any patients during any treatments in lung cancer. What does authors think about this? Do we need to pay special attention to ALK-positive NSCLC?

Reply 2: Thank you for this comment. Our intention in writing this review article specifically tailored towards lineage transformation in ALK-positive NSCLC was because this manuscript is part of a review series thematically focused on ALK-positive NSCLC. However, we agree with the reviewer that lineage transformation has been described in other subsets of NSCLC. In this review, we specifically highlight the published data on lineage transformation in the context of EGFR-mutant NSCLC as this is where it has been most frequently described. As the reviewer points out, we also reference rare case reports of lineage transformation following non-targeted therapies such as immune checkpoint inhibitors (**lines 259-261**). Based on the data we have at

this time, it is difficult to fully know the probability or incidence of lineage transformation in ALK-positive NSCLC compared to other subsets of NSCLC. In our view, further studies of both the clinical incidence of lineage transformation and the underlying biologic mechanism(s) are needed to more definitively answer this. We have added some text on **lines 261-262** of the revised manuscript to highlight this.

Comment 3: It has been repeatedly discussed that SCLC has features of epithelial to mesenchymal transition (EMT)-like. For example, SCLC cells usually show decreased intercellular junctions, loss of apical-basal polarity with irregular and discontinuous basal lamina, or decreased expression of E-cadherin, and so on. From the point of view of transcriptional regulation of EMT, it was shown that ASCL1, lineage specific key transcriptional factor of SCLC, acts to modulate epithelial or mesenchymal phenotypic change in SCLC cells (e.g., Transl Lung Cancer Res. 2018;7:32-49). From these points of view, it may not always accurate to separate the part of SCLC transformation and EMT completely as described in Figure 1. I suggest discussing about these in the main section of manuscript of EMT (line 508-523) or figure legends.

Reply 3: We thank the reviewer for this feedback and agree that morphologic and transcriptional features of EMT can be seen to have some overlap with SCLC transformation. To address this point, we have added some text to the EMT section of our review, as suggested by the reviewer (**lines 584-586**). From our perspective and interpretation of available data, the precise relationship between EMT and SCLC transformation (e.g. whether they are distinct entities governed by similar/overlapping biologic mechanisms or whether EMT-like changes occur as part of SCLC transformation) remains a topic of ongoing and future study.