Peer Review File

Article information: http://dx.doi.org/10.21037/ tcr-20-754.

Lung cancer is the leading cause of cancer-associated death worldwide and has more than 50 histomorphology subtypes. In the manuscript “The clinical significance of RET gene fusion among Chinese patients with lung cancer”, the authors analyzed 39 samples with RET fusions using next-generation sequencing and collected the anonymized data to establish the molecular features of RET fusion in Chinese NSCLC patients.

Comment 1: The methods section is missing from the Abstract.

Reply 1: Sorry for the mistake we made. The methods section has been added in the Abstract and carefully revised by the authors.

Changes in the text: Line 34-39. Methods: We applied formalin-fixed, paraffin-embedded (FFPE) samples of 39 patients with NSCLC using the Lung Plasma panel covering 168 cancer-associated genes and performed capture-based targeted deep sequencing to identify the RET fusion partners and concurrent gene mutation with Miseq. The log-rank test was used to compare the survival difference of patients according to treatment strategies. Statistical analyses and graphs were performed using R language and GraphPad Prism.

Comment 2: There are many major mistakes in the manuscript. The language of the

paper needs to be polished and modified by a native English speaker.

Reply 2: We feel sorry for the mistakes. The language of this manuscript has been edited by a native speaker. We have made some editorial changes, re-worded some sentences, and corrected some spelling and grammar errors. The modifications are marked in red font with yellow background. We hope the revised manuscript could be easy to follow.

Changes in the text: The modifications are marked in red font with yellow background in the revised manuscript.

Comment 3: What does "FFPE" refer to?

Reply 3: FFPE refers to Formalin-fixed, paraffin-embedded. Tissue samples are fixing in formaldehyde to preserve proteins and vital structures. In our study, we applied FFPE samples with NSCLC using the Lung Plasma panel (Burning rock biotech) and capture-based targeted deep sequencing with Miseq to find the fusion partners and concurrent gene mutations.

Changes in the text: Line 115. DNA of formalin-fixed, paraffin-embedded (FFPE) samples were extracted using QIAamp DNA FFPE tissue kit (QIAGEN, Valencia, CA) and the DNA concentration was measured by Qubit dsDNA assay.

Comment 4: The markers (A) and (B) are missing from Figure 4.

Reply 4: Sorry for the mistakes we made. The markers have been added in Figure 4.

Changes in the text: Line 217. Survival comparison between patients who received

and not received RET inhibitors indicated that there was no significant difference between the two groups in terms of PFS (Figure 4A) and OS (Figure 4B).

Comment 5: A total of 46 RET fusions were identified in the 39 patients. Please list these 46 RET fusions in a supplementary table.

Reply 5: Thanks for your suggestion. 46 RET fusions are listed in supplemental Table 1.

Changes in the text: Line 182. Several new and rare RET fusions occurred in one patient including that RET fused with ZNF438, ZNF43, STK33, REEP3, MRPS30, MIR7854, MARCH8, LOC401312, LINC01435, LINC00841, KIAA1217, ERC1, CBWD6 and ARHGAP12 (Figure 2) (Supplemental Table 1).

Comment 6: Why was a representative RET fusion not chosen for FISH testing?

Reply 6: Thank you for your suggestion. The samples with RET fusions were tested by the Lung Plasma panel (Burning Rock Biotech). This panel includes genomic regions harboring known driver mutations and exons containing recurrent SNVs in 168 cancer-associated genes including *RET*. The regions are selected according to whole-exome sequencing data from The Cancer Genome Atlas (TCGA) as more than 20 patients in TCGA lung adenocarcinomas and squamous cell carcinoma datasets harboring the mutations covered per kilobase of the exons which are adequate to identify the majority of known driver gene mutation. We then performed capture-based targeted deep sequencing on the samples with Miseq (1, 2). Comparing to reverse transcription PCR

(RT-PCR) and break-apart fluorescence in situ hybridization (FISH), next-generation sequencing (NGS) would be better in detecting the fusion partners along with the concurrent genomic alterations. FISH was a useful screening tool in previous clinical studies for *RET* rearrangements in tumor samples. In this study, we performed screening using the Lung Plasma panel and NGS trying to find some novel and unusual RET fusion partners (3, 4). We feel grateful for your suggestion and will compare different methods for detection RET fusions in the future studies.

Reference

1. Mao X, Zhang Z, Zheng X, Xie F, Duan F, Jiang L, et al. Capture-Based Targeted Ultradeep Sequencing in Paired Tissue and Plasma Samples Demonstrates Differential Subclonal ctDNA-Releasing Capability in Advanced Lung Cancer. Journal of thoracic oncology : official publication of the International Association for the Study of Lung Cancer. 2017;12(4):663-72.

2. Zhang J, Fujimoto J, Zhang J, Wedge DC, Song X, Zhang J, et al. Intratumor heterogeneity in localized lung adenocarcinomas delineated by multiregion sequencing. Science (New York, NY). 2014;346(6206):256-9.

3. Drilon A, Hu ZI, Lai GGY, Tan DSW. Targeting RET-driven cancers: lessons from evolving preclinical and clinical landscapes. Nature reviews Clinical oncology. 2018;15(3):151-67.

4. Sussman RT, Oran AR, Paolillo C, Lieberman D, Morrissette JJD, Rosenbaum JN. Validation of a Next-Generation Sequencing Assay Targeting RNA for the Multiplexed

Detection of Fusion Transcripts and Oncogenic Isoforms. Archives of pathology & laboratory medicine. 2020;144(1):90-8.