

Dear Editors,

On behalf of my co-authors, we thank you very much for giving us an opportunity to revise our manuscript, we appreciate editor and reviewers very much for their positive and constructive comments and suggestions on our manuscript entitled “Response to dacomitinib in advanced NSCLC harboring the rare delE709_T710insD mutation: A case report and literature review” (Manuscript NO: 75261).

Those comments are all valuable and very helpful for revising and improving our paper, as well as the important guiding significance to our researches. We have studied comments carefully and have tried our best to revise our manuscript according to the comments which we hope meet with approval. Revised portion are highlighting marked in the paper. The main corrections in the paper and the responds to the reviewer’s comments are as following:

Reviewer #1:

Thank you for the possibility to review the manuscript titled “Response to dacomitinib in advanced NSCLC harboring the rare delE709_T710insD mutation: A case report and literature review”. The study is interesting and easy to read. Moreover, the study underlines new and important data for clinical practice. DelE709_T710insD is present in only 0.16% of cases, which means that the drug can be used in a limited number of cases. Nevertheless, the case report is important to consider for publication. There are no major or minor recommendations.

Response: We really appreciate your acknowledgment of the article.

Reviewer #2:

The authors come up with a very interesting case study reporting a patient with EGFR delE709_T710insD after the initiation of dacomitinib. The case study is very well interpreted and the efficacy of dacomitinib on rare mutations needs was interpreted using the genetic analysis albeit to be validated using in vitro and in vivo I only wish the authors delve upon the cell block samples and PCR as the authors were not clear

on the results interpreting the PCR. There could be a table mentioning the same. Am I missing anything? Otherwise a very much needed case for clinical community.

Response: We are very sorry for our negligence of unclear indication which caused a misunderstanding.

Malignant pleural effusion (MPE) is a common complication of advanced lung cancer, occurring in 15% of lung cancer patients(1). To make cell blocks, the pleural effusion specimens were centrifuged and smeared routinely, the cell sediments were embedded in paraffin. Cell distribution in hematoxylin-eosin staining of the cell block retains a similar arrangement to that of solid tumor tissue, which can be used for immunohistochemical diagnosis. As for molecular research, the tumor cell quantity was initially evaluated to ensure the quality control. Köksal D et.al(2) reported cell block method increases the diagnostic yield in exudative pleural effusions accompanying lung cancer compared with conventional smear and further confirmed the subtyping of lung cancer as adenocarcinoma. Liu X et.al(3) reported the sensitivity of MPE cell blocks for EGFR mutation detection was 81.8%, which will be valid surrogates for NSCLC tumour EGFR mutation detection when tissue is not available.

Polymerase chain reaction (PCR) is a targeted approach designed to detect prespecified mutations in vitro with the advantages of high sensitivity, speed, and accuracy. Compared with next-generation sequencing(NGS), the method only can detect most common mutations. In our study, we initially performed routine molecular genetic testing of cell block by PCR diagnostic kits, including mutation of *EGFR*, *KRAS*, *NRAS*, *BRAF*, *HER2*, *MET*, and *PIK3CA*, and fusion of *ALK*, *RET*, and *ROS1* as shown definitely in the table below. For *EGFR* exon 18, the PCR kits could only detect G719X mutation, but failed to identify delE709_T710insD, as it is a rare complex in-frame deletion mutation in exon 18. Consequently, it is not certain whether the mutation was present at the time of initial diagnosis.

In conclusion, results of common genetic test for cell block and tumor tissue were in good agreement. Due to the limitations of PCR assays, uncommon mutations or less

frequent alterations could not be identified.

Considering the reviewer's suggestion, we have corrected the description of genetic testing of cell block by PCR assays in the section of further diagnostic work-up and added a supplementary table listing all gene and mutation sites of the PCR kits. Because there are no major changes to the content of the manuscript, no further language polishing performed.

We would like to express our great appreciation to you and reviewers for comments on our paper. Looking forward to hearing from you.

Thank you and best regards.

Yours sincerely,

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3. Wang S, Chen H, Zhong J, Qin H, Bai H, Zhao J, et al. Comparative study of EGFR mutations detected in malignant pleural effusion, plasma and tumor tissue in patients with adenocarcinoma of the lung. *Lung cancer (Amsterdam, Netherlands)*.

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