Peer Review File

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Reviewer A

Comment 1: Update the information with regards to non-genetic mechanisms of resistance that have not been investigated, keeping in mind cutting edge research. See: 1. Zhu et al. Targeting c-Myc to overcome acquired resistance of EGFR mutant NSCLC cells to the third-generation EGFR tyrosine kinase inhibitor, Osimertinib. Cancer Research 2021; 2. Kashima et al. Single-cell analyses reveal diverse mechanisms of resistance to EGFR tyrosine kinase inhibitors in lung cancer. Cancer Research 2021.

Reply 1: Thank you very much for your advice. We have revised the manuscript to include these references in the discussion section.

Changes in the text: Page 4, lines 93.

Comment 2: Could you re-confirm that no co-mutations were found? TP53 and others?

Reply 2: We tested for TP53 and other gene mutations by NGS, but none of them were detected. We mentioned in the discussion section that we did not find any specific gene mutations.

Changes in the text: Page 4, lines 95-96 and page 5, line 97.

Comment 3: Did you perform NGS in liquid biopsy?

Reply 3: We did not perform NGS in liquid biopsy. The blood samples had not been collected. We have revised the manuscript to mention that we did not perform liquid biopsy in this case.

Changes in the text: Page 5, lines 117-119.

Comment 4: See Robichaux et al. Structure-based classification predicts drug response in EGFR-mutant NSCLC. Nature 2021. The study clearly describes types of mutations responding to osimertinib or afatinib or vice versa.

Reply 4: Thank you for the latest information on the resistance mechanism of EGFR-TKI. We have added a description of the resistance mechanism of EGFR-TKI in the discussion section.

Changes in the text: Page 4, line 94.

Reviewer B

Comment 1: Resistance testing had been done on pleural effusion and not from the progressing tumor or as (preferred) liquid biopsy. Therefore, any new mutations/translocations might have been missed.

Reply 1: Thank you for your valuable comment. Because the patients did not agree with receiving bronchoscopy or CTNB, we could not obtain the rebiopsy specimen from increasing primary tumor. As the reviewer mentioned, maybe we should perform liquid biopsy in our case to find a specific gene mutation involved in EGFR-TKI resistance. We have added this point to our manuscript as a limitation.

Changes in the text: Page 5, lines 116-119.

Comment 2: The therapeutic effect on afatinib was moderate with 5 months and the patient passed away. Therefore, the response will not have confirmed and it is unclear what the cause of death was. This should be discussed.

Reply 2: As the reviewer mentioned, this patient did not have long duration of response to afatinib. The reason for death was carcinomatous meningitis, and the primary tumor remained shrunken at his death. We apologize for the lack of information about the cause of death and have revised the manuscript to include additional information.

Changes in the text: Page 4, line 81-83.

Comment 3: There is a recent study from the town of the authors in Niigata, Japan where patients not responding to osimertinib were treated with osimertinib and afatinib. (Miura et al., WCLC MA02.05, 2021). This study should be included and discussed.

Reply 3: Thank you for your advice. We have included the study from Niigata City in the discussion section.

Changes in the text: Page 5, line 104-108 and page 7, lines 150-151.

Comment 4: A comprehensive section of limitations is completely missing. Here, at least, should be discussed that the patient might have not taken the oral medication of osimertinib in daily manner but the afatinib (costs of drugs?) and points 1) to 3).

Reply 4: We confirmed that this patient had no financial problems and was taking osimertinib daily. According to the reviewer's comment, we have mentioned this point and added limitations of this study in the discussion section.

Changes in the text: Page 3, lines 71-72 and page 5, lines 116-119.

Minor point: Arrows should be placed on the CT scans to indicate tumor lesions.

The term primary resistance is misleading meaning molecular alterations tested before therapy. It should be renamed in early progression.

Reply minor point: We agree with the reviewer's suggestion. We have placed arrows on the CT scans to indicate tumor lesions. In addition, we have changed the title to "Effectiveness of afatinib in an NSCLC patient with *EGFR* mutation and early progression to osimertinib".

Changes in the text: Page 1, lines 2-3, page 2, line 48, page 3, lines 49, 56, 59, and page 6, line 121. Fig. 1A, 1B and 1C.