Peer Review File

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Reviewer A: Pan and colleagues report a retrospective study of the correlation between abundance of EGFR T790M mutation and Osimertinib response in advanced non-small-cell lung cancer. The main message is that Osimertinib was equally effective for NSCLC patients with various abundance of T790M mutation. I have few comments.

Comment 1: The authors described that "The T790M abundance was calculated as mutant allele frequency (MAF), which indicated the fraction of mutated alleles relative to the corresponding WT allele to analyze the allele fractions of T790M". How did the authors distinguish tumor-derived EGFR WT from normal cell-derived EGFR WT?

Reply 1: Actually, the definition of abundance used in the present study is relatively common in the clinic. To further explain, abundance of EGFR T790M was defined as follows: mutation abundance % = copies of T790M mutants/copies of EGFR locus (total copy number)* 100%. From a technical point of view, it is very difficult to distinguish tumor-derived WT-EGFR from normal cell-derived WT-EGFR, because in both PCR and NGS tests, DNA comes from tumor cells is determined by mutation status. And combined with methylation detection or third-generation sequencing might solve above problem. Special thanks to you for your good comments.

Changes in the text: None.

Comment 2: This study evaluated PFS. Please add an explanation of the timing of imaging studies.

Reply 2: Imaging assessments for tumor lesions were performed every 6 weeks until disease progression or loss of follow-up. Special thanks to you for your good comments.

Changes in the text: We have added an explanation as advised (see Page 6, line 111-113).

Comment 3: It has been reported that the predictive efficacy of Osimertinib may be different between serum-based T790M test and tissue-based T790M test. However, the two are mixed in this analysis. If the authors want to compare the test methods, the specimens used should be matched and analyzed.

Reply 3: We have analyzed the efficacy of Osimertinib in the two cohorts of serum-based T790M test and tissue-based T790M test. But the ORR, PFS, OS indicated no

significant difference among serum-based and tissue-based specimens (Figure a). In addition, the baseline characteristics of the three test methods (including brain metastases) were added and described. Our results may only suggest that ARMS, ddPCR, and NGS were effective approaches for T790M mutation detection if the samples were selected appropriately. Special thanks to you for your good comments.

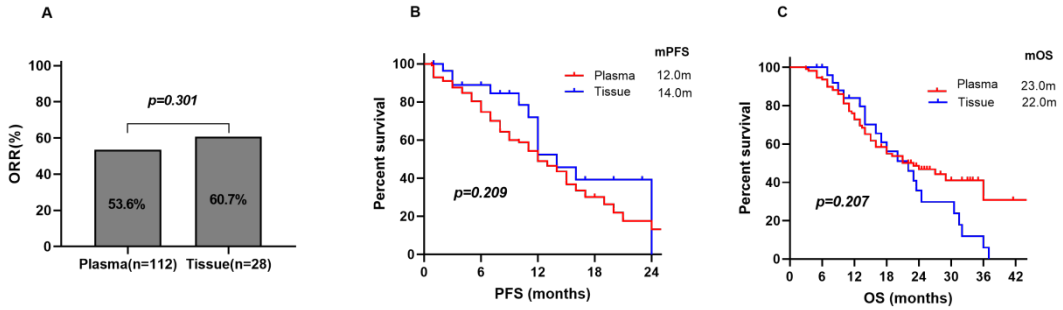

Figure a. Comparison of osimertinib efficacy between plasma detection and tissue biopsy.

Changes in the text:

1. We have analyzed the efficacy of Osimertinib in the two cohorts of serum-based T790M test and tissue-based T790M test (see Page 7-8, Line149-155; Page23, Line417-420; Fig 2).
2. We have modified the table1 and indicated the patient characteristics (see Page 18-20, table1).

Comment 4: It seems that brain metastasis may affect the efficacy of osimertinib, but was there any difference in the rate of brain metastasis between the different T790M tests (ARMS, ddPCR, and NGS)?

Reply 4: We found no difference in the rate of brain metastasis between the different T790M tests. Special thanks to you for your good comments.

Changes in the text: We have modified the table1 and indicated the patient characteristics (see Page 18-20, table1).

Comment 5: Relationship between baseline abundance of T790M mutation and the efficacy of Osimertinib. How did the authors separate the groups with high and low abundance?

Reply 5: From the ROC curve analysis, we did not find the best cutoff value to separate the groups. From the scatter plot, we found the abundance of T790M showed a skewed distribution, so the median of T790M abundance was adopted to distinguish the high and low abundance values. Special thanks to you for your

good comments.

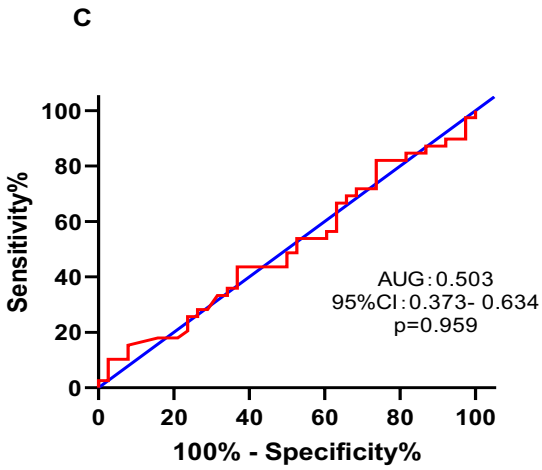

Figure b Receiver Operating Characteristic (ROC) Curve for T790M abundance predicting objective response.

Changes in the text: We have indicated in txet (see Page 8-9, Line 175-178) and modified the Figure 4.

Comment 6:

Discussion

- Line 195: "…is" is a misnomer?

Reply 6: We have modified our text as advised. Special thanks to you for your Correction.

Changes in the text: We have modified our text as advised (see Page 10, Line217-220)

Comment 7:

Tables

- The authors should also indicate the patient characteristics by measurement methods (ARMS, ddPCR, and NGS). The type of specimen used in the analysis should also be indicated there.

Reply 7: We have modified the table1. Special thanks to you for your good comments.

Changes in the text: We have modified the table1 and indicated the patient characteristics (see Page 18-20, table1).

Reviewer B: This is the retrospective analysis about the correlation between the

abundance of EGFR T790M mutation and osimertinib response in advanced non-small-cell lung cancer.

Comment 1: Since the response of osimertinib differs depending on the mutation status, it is better to add the mutation status to the patient background. It is also better to consider whether the mutation status affects this analysis.
Reply 1: A total of 88 (61.1%) patients originally harbored EGFR exon 19 deletion and 52 (36.1%) carried L858R mutation, while 3 patients had G719X mutation and 1 patient showed co-mutation of 19 deletion and L858R. Moreover, the baseline of EGFR mutation status in two groups with high and low abundance was comparable. Special thanks to you for your good comments.
Changes in the text: We have added an explanation as advised (see Page 6, line 128-129); we have modified the table2 and indicated the patient characteristics (see Page 21-22, table2).

Comment 2: It is not good to classify by treatment line of osimertinib in the patient background. Since the number of regimens is important for EGFR-TKI, the treatment line as EGFR-TKI should be described. And, based on this date, you should analyze the result and discussion.
Reply 2: Prior EGFR-TKIs were gefitinib used in 52 (36.1%) patients, icotinib in 85 (59.0%) patients, erlotinib in 2 (1.4%) patients and afatinib in 1 patient (0.7%). Most of the patients received osimertinib directly or after chemotherapy when resistance to prior EGFR-TKIs. The baseline of prior EGFR-TKIs in two groups with high and low abundance was comparable. Special thanks to you for your good comments.
Changes in the text: We have added an explanation as advised (see Page 6-7, line 131-136); we have modified the table2 and indicated the patient characteristics (see Page 21-22, table2).

Comment 3: Since there is no information on the detection threshold of ddPCR and the depth of the sequence of NGS, the accuracy is unknown. You should put this information in Met hods, without which you will not be able to discuss the Results and Discussions of subsequent papers.
Reply 3: Generally, the minimum detection limit of ddPCR was 0.01% if providing sufficient sample and operating according to the standard procedures. The high-throughput sequencing of NGS contained 168 genes related to the pathogenesis and targeted therapy with ≥500 average sequencing depth, and the detection for alterations covered single-nucleotide variant (SNV), short fragment insertions or deletions (INDEL), copy number variation (CNV) and rearrangements within the

range of +/-20bp of target gene exon. Special thanks to you for your good comments.

Changes in the text: We have added an explanation as advised (see Page 5, line 94-101).