Peer Review File

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Reviewer A

Ma J. et al. conducted this study to investigate the efficacy of osimertinib in the second/subsequent line after resistance to first- and second- generation (1/2G) EGFR-TKI. In addition, they evaluated differential outcomes between plasma vs tumor T790M+ patients treated with osimertinib. They found that the efficacy of osimertinib in second line/beyond in T790M+ patients was comparable to previous reports, but plasma T790M+ patients had inferior outcomes compared to tumor T790M+ patients.

- - In AURA3, patients with a plasma T790M+ status show comparable responses to osimertinib with patients who are tissue T790M+ even though tissue test is required if plasma test for T790M is negative because of lower sensitivity of plasma test, which contrasts the results of this study. Authors explained that the lower response in only plasma T790M+ patients may be attributed to higher level of ctDNA shed by the tumor in plasma T790M+ patients suggesting that plasma ctDNA is better able to reflect total tumor burden compared to tissue biopsy. However, it is unlikely that the tumors with higher burden leading to lower response to osimertinib exhibit tissue T790M- and plasma T790M+ results.

- Even if we accept this theory, when tumor T790M is positive, the response is very good regardless of plasma T790M positivity. On the contrary, when only plasma T790M is positive, the response is not so good. Then, is there any necessity to do pared plasma-tissue test for T790M although authors highlighted the advantage of this test?

Reply to Reviewer A:

We thank Reviewer A for the very insightful comments, and we have divided and labelled the comments as parts (1.1), (1.2) and (2) (in blue) accordingly for ease of reference for the Reviewer.

Comment (1.1):

In AURA3, patients with a plasma T790M+ status show comparable responses to osimertinib with patients who are tissue T790M+ even though tissue test is required if plasma test for T790M is negative because of lower sensitivity of plasma test, which contrasts the results of this study.

Reply to (1.1):

In the AURA3 study, all patients had confirmed tissue T790M-positive NSCLC. As what Reviewer A has highlighted, in their retrospective analysis of ctDNA plasma samples (Papadimtrakopoulou et al. Cancer 2020), patients who were cobas plasma T790M-positive had similar ORR as those who were cobas plasma T790M-negative (76% and 71% respectively). In our study, we also demonstrated the same: ORR was 63% for patients who were plasma T790M-positive/tumour T790M-positive, and 67% for those plasma

T790M-negative/tumour T790M-positive (please refer to table 6). However, we were not able to evaluate PFS for these 2 groups due to small patient numbers.

Changes to text:

We have made reference to AURA3 (Papadimitrakopoulou et al. Cancer 2020) and added the following text regarding limited test sensitivity of cobas plasma test (see page 9 lines 283-286).

Comment (1.2):

Authors explained that the lower response in only plasma T790M+ patients may be attributed to higher level of ctDNA shed by the tumor in plasma T790M+ patients suggesting that plasma ctDNA is better able to reflect total tumor burden compared to tissue biopsy. However, it is unlikely that the tumors with higher burden leading to lower response to osimertinib exhibit tissue T790M- and plasma T790M+ results.

Reply to (1.2):

We agree with the Reviewer's point and would like to clarify that in our study, the group of patients who were tissue T790M-positive had a numerically longer median PFS and OS than those who were plasma T790M-positive, however we were unable to verify the statistical significance due to overlap between these 2 subgroups and a significant proportion of patients did not have known plasma T790M mutation status. Nevertheless, we found that T790M positivity on tumour was more predictive for treatment response compared to plasma T790M positivity. We postulated that our findings might suggest higher level of ctDNA shed by the tumour in plasma T790M-positive tumours. Consistent with this, Papadimtrakopoulou and colleagues also showed from AURA 3 that plasma T790M detection was associated with larger baseline tumour size and presence of extrathoracic disease, similar to what other studies including Thress et al. Lung Cancer 2015 and Sueoka-Aragane et al Cancer Science 2016 have reported. We also acknowledge that our study was a retrospective observational one, with a small number of patients with paired tissue and plasma T790M testing. To our knowledge, currently there is still limited data on outcomes of patients with plasma T790M-positive/tissue T790M-negative status treated with osimertinib. Our findings for this subgroup therefore remains hypothesis-generating, and will require a larger future prospective study to validate.

Changes to text:

We have clarified our results regarding mPFS/mOS of tumour T790M+ and plasma T790M+ patients (see page 7 line 226-227) and have moderated our conclusion to more accurately reflect our findings (see Conclusion section in abstract). We have also modified our text under "Discussion" (see page 9 lines 294-299 and lines 303-305) accordingly, taking into account the Reviewer's comments.

Comment (2):

Even if we accept this theory, when tumor T790M is positive, the response is very good regardless of plasma T790M positivity. On the contrary, when only plasma T790M is positive,

the response is not so good. Then, is there any necessity to do pared plasma-tissue test for T790M although authors highlighted the advantage of this test?

Reply to (2):
Yes, therefore we concluded that tumour T790M positivity is more predictive of treatment response to osimertinib than plasma test. We agree with the Reviewer that in clinical practice and guidelines (ESMO expert consensus guidelines 2022/NCCN guidelines version 1.2022), it is an accepted approach for physicians to base decision for treatment with second-line osimertinib after progression on prior EGFR TKI on a positive plasma T790M test alone. We have proposed performing paired plasma and tissue T790M testing wherever feasible as our study found that tissue T790M test appeared to be more predictive for response with osimertinib compared with plasma test. Aside from T790M mutation, tissue testing may also offer additional insights into resistance mechanisms including information about the transcriptomic subtype, tumour microenvironment, as well as histologic transformation (KP Chua et al. CCR 2021).

Changes in the text:
We have added in the statement under "Discussion" (see page 10, line 317-320) to support the additional value of tissue testing besides detection of T790M mutation.

Reviewer B
Activating mutations in the EGFR gene are aberrations of major clinical significance. Currently, ozymertinib is a drug that is often chosen for first-line therapy. However, sequential treatment , including 1st or 2nd generation TKIs, also has documented clinical value. The paper presented by the authors deals with this particular patient population.

The manuscript presented is carefully prepared.

I have one comment- based on their analysis, could the authors indicate the population in which the T790M mutation is more likely to be found? This could help in decision-making in daily practice and identifying patients for sequential treatment.

Reply to Reviewer B:

We thank Reviewer B for the kind comments. We have looked through our dataset and found that in the patients who were tested positive for T790M mutations, the median duration of prior TKI therapy was longer than that of patients who were tested negative for T790M mutations and there was higher proportion with presence of exon 19 deletion as the primary EGFR mutation compared to those who tested negative for T790M mutations. However, we acknowledge that our study would have an inherent selection bias, as our study had included patients who received osimertinib in the second line or later setting. Majority of these patients had received osimertinib based on detection of T790M mutation as per current standard of

care and numbers of patients with T790M negative disease was very low, as such we did not include these findings in our manuscript.