Peer Review File

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Reviewer A

Very nice paper looking at TP53 mutations as a predictive marker for inferior survival outcomes in patients on osimertinib for the T790M mutation in EGFRm NSCLC. Few minor suggestions

Comment 1: Did burden of disease correlate with TP53 mutation? Specifically liver mets? Can this be added in the table please.

Reply 1: We added the burden of disease i.e the sites of metastasis with specific emphasis on the liver metastases in correlation with TP53 status in table 1. There was no difference in the metastatic sites relative to TP53 status.
Changes in the text: see page 12, line 254

Comment 2: Please add a discussion and recommendation if TP53 should be tested? I'd say the result should not sway using osimertinib but these patients should be closely monitored.

Reply 2: We included this remark as point 3 in the discussion and the conclusion and thank the reviewer for this very important suggestion.
Discussion: Third, in routine practice we would advocate for testing patients for *TP53* mt+ and for monitoring these patients more closely when treated with TKI therapy then patients with *TP53*WT status.
Conclusion: Therefore, *TP53* status should be tested before start of therapy and strategies should be developed to monitor *TP53* mt+ patients on *EGFR* TKI more closely than *TP53*WT patients.
Changes in the text: see page 22, line 434-436; see page 23, line 449-451

Comment 3:

a) Did patients have TP53 at diagnosis tested and did this change at progression when T790M was found? This wasn't clear to me.
b) It would be also nice to know if the PFS on 1st gen TKI was shorter than the TP53 negative patients.

Reply 3: In the Results part we included:

a. All patients were studied for *TP53* mt+ at first diagnosis and 42% (n=32/77) showed a *TP53* mt+. On TKI resistance (1st or 2nd generation TKI), all patients were rebiopsied (all with tissue). All patients had a T790M. In the Oldenburg cohort, all 11 patients with

a *TP53* mt+ were restested for *TP53* status. In 2/11 (18%) there were missing data for the *TP53* status. In 3/9 (33%) patients, the *TP53* reanalysis was not successfull. In 6/6 (100%) successfully retested patients, *TP53* configuration was stable in comparison to the test before start of 1st line TKI. In the Heidelberg cohort, the retest strategy for *TP53* was different with comparison to the Oldenburg cohort. In Heidelberg, only patients with *TP53*WT were reevaluated at acquired resistance for *TP53* status. Of the 38 *TP53*WT patients, 34 (89%) were reevaluated at progression for *TP53*. 28/34 (82%) patients were successfully retested for *TP53*. In 5/28 patients *TP53* status changed at progression from WT to mutation.

This is in contrast to our 1st line cohort that was published in Oncotarget where we found that in 9/9 successfully studied patients the TP53 status remained stable.

Changes in the text: see page 10-11, line 236-246

a) The PFS of 1st line therapy with 1st gen or 2nd generation TKI was 13 months (n=31) for patients with *TP53* mt+ and patients with *TP53*WT had an PFS of 17 months (n=39) (P=0.249). In a homogenously TKI treated patient population in our center published in Oncotarget, there was a significant difference in ORR, PFS and OS in the *EGFR* mt+ group dependent on *TP53* status.

Changes in the text: see page 13, line 276-279

Reviewer B

This is an interesting paper on the effect of TP53 on efficacy data with Osimertinib. The cohort has a reasonable size, the analysis is well done, the methods are clear. The introduction and discussion sections are complete.

Comment 1: The English could be reviewed, for example in the statistical analysis section, the verb tenses are switching from past to present from one sentence to another.

Reply 1: We reviewed the English language throughout the paper, particulary in the methods section and switched all tenses to the past tense.

Changes in the text: page 6, line 137-150; page 8-9, line 200-216

Comment 2: In table 1, in the 2nd and 3rd columns, when calculating %, the denominators should be 32 and 45 respectively, it would make more sense than using a denominator from the first column. What we want to see is if there are differences between the two groups.

Reply 2: We have adjusted table 1 as suggested by Reviewer B.
Changes in the text: see page 12, line 245

Comment 3: There are typos at lines 259 and 273: you should use its and not it's.

Reply 3: We revised it, as suggested by Reviewer B.
Changes in the text: see page 17-18, line 343 and 366

Comment 4: There are typos in figure 3 and 4 legends: further line (instead of further lin)

Reply 4: We revised it, as suggested by Reviewer B.
Changes in the text: see page 14,16 line 298 and 326

Reviewer C

Comment 1: I think it is necessary to specify the race information in Table 1.

Reply 1: Because of our past, race information is normally not captured outside of clinical trials. We do know however for this cohort that only 1 patient of 77 patients was of asian ethnicity, all other patients were of caucasian origin. We would suggest not to adapt the Table 1 to include this one asian patient, however we included the Methods section in the text.
Changes in the text: see page 6, line 144

Comment 2: The low response rate of 45% for Osimertinb and the significant difference in OS between Del and L858R (Supp Table 3) are different from the results of the AURA3 trial. The possibility of group selection bias cannot be ruled out due to the small sample size. This may affect the analysis of the association between TP53 mutations and the effect of TKIs. It should be noted to Discussion as Limitation.

https://www.nejm.org/doi/full/10.1056/NEJMoa1612674

Papadimitrakopoulou et al. 2020
doi: 10.1016/j.annonc.2020.08.2100.
https://pubmed.ncbi.nlm.nih.gov/32861806/

Reply 2: We have added this comment in the discussion and thank the reviewer for bringing this up.

Changes in the text: page 21, line 394-399

Comment 3: The authors state that the definition of TP53 mutations was taken from a previous study, Ref (5). However, this paper and the Ref 3 paper define the type of TP53 mutation used in the analysis. Are the TP53 mutations employed in this study disruptive or non-disruptive? Which mutation or type of mutation had a stronger impact on the effect of TKIs? Suppl. Table1-4 shows the analysis data by mutation type. However, the authors only briefly mention in the text that the data should be referred to the Suppl. However, in the text, the authors simply state, "See Suppl". It is necessary to clearly state whether the results of this study were reproduced as in previous studies or not.

Reply 3: For PFS, the classification of TP53 mutations were reproduced in this cohort. There is one exception which is the Exon 8 vs. non exon 8 TP53 mutations. However the group of exon 8 mt+ NSCLC was only 4 patients.
For OS, we could not reproduce the predictive significance of the different classification of TP53. However, TP53 mutations irrespective of the type of the mutation were predictive of OS. We added a sentence in the result section to make this fact clear.
Changes is in the text: see page 14 and 15, line 290-291; line 318-321

Comment 4: In this study, there was no correlation between smoking and the presence of TP53 mutations. Does this mean that the type of TP53 mutation that is less associated with smoking was more important in the effect of EGFR-TKIs on EGFR-mutated lung cancer?

Reply 4: We could not find any differences in the type of TP53 mutation based on smoking status.
Changes in the text: We have not included this statement in the text.

Comment 5: Quite a few papers have been reported on the relationship between EGFR-TKI and TP53. This study is an analysis focused on the relationship between the effect of osimertinib and TP53 in T790M-positive EGFR lung cancer. However, it seems that the relationship between the effect of

osimertinib and TP53 has already been reported. It needs to be clarified what is the new finding compared to the existing studies.

Chen L 2019

https://doi.org/10.1093/annonc/mdz243.015

https://www.annalsofoncology.org/article/S0923-7534(19)58627-6/fulltext

Fu Y. 2021

https://doi.org/10.3389/fonc.2021.621992

https://www.frontiersin.org/articles/10.3389/fonc.2021.621992/full

Reply 5: We would like to point out that the two studies refer to asian patients with one specific TP53 mutation (Fu et al.) and that the other data set has only been published in abstract form also only referring to asian patients (Chen et al.). Therefore, we feel that our data in predominantly caucasian patients adds in a relevant form to the literature.

Changes in the text: We haved not included this piece of information in the text.

Comment 6: A basic experiment using a genetically modified mouse model is reported to explain why TP53 mutation reduces the efficacy of EGFR-TKI. I think it is worth citing in Disucussion.

Foggetti et al. 2021 Genetic Determinants of EGFR-Driven Lung Cancer Growth and Therapeutic Response *In Vivo*

DOI: 10.1158/2159-8290.CD-20-1385

https://cancerdiscovery.aacrjournals.org/content/11/7/1736.full-text.pdf

Reply 6: We did not include the paper in the text or the discussion, as in this Trp53 deficient mouse model, the authors stated an impact of KEAP1 mutations, but not of Trp53 mutation on EGFR driven tumor growth.

Changes in the text: We did not include the paper.

Reviewer D

The authors suggest that TP53 mutation is associated with worse clinical outcomes in T790M (+) patients treated with osimertinib. However, there are a lot of redundancy and incorrect citations, and several points to be discussed.

Comment 1: The authors need to describe the inclusion and exclusion criteria clearly.

Reply 1: We revised it, as suggested by Reviewer D.

Changes in the text: see page 6, line 137-142

Comment 2: What is the median follow-up period of the subjects?

Reply 2: Median follow up calculated from start of osimertinib was 21 months

Changes in the text: see page 8, line 185-187

Comment 3: In page 6 line 220, 'p<0.362' should be revised.

Reply 3: We revised it, as suggested by Reviewer D.

Changes in the text: see page 13, line 273

Comment 4: Overall survival can be affected by subsequent treatment after osimertinib failure. Please elaborate on that in the Methods.

Reply 4: In order to account for the influence of subsequent therapy after osimertinib on OS, we captured the therapy after stop of osimertinib: 4/77 patients (5%) were treated after osimertinib failure with I/O therapy (n=1), chemotherapy (n=3) or TKI therapy (n=3). Thus the influence of subsequent therapy on OS is limited in this cohort.

Changes in the text: page 8, line 187-190

Comment 5: Along with brain metastasis, hepatic metastasis is an unfavorable predictor in EGFR-mutant patients treated with EGFR-TKIs. The impact of hepatic metastasis needs to be evaluated in the PFS and OS analysis and inserted in Table.

Reply 5: We added the burden of disease (for instance: hepatic metastasis) in correlation with *TP53* status in Table 1.

We have not included liver mets in the multivariate analysis due to small number of patients and equal distribution of liver mets in the TP53 WT (n=6) and mt+ (n=6) groups.

Changes in the text: see page 12, line 254

Comment 6: 'The interested reader can find them in a supplementary appendix online.' Instead of this sentence, authors should specify which supplementary material they would like to refer.

Reply 6: We revised it, as suggested by Reviewer D.

Changes in the text: see page 7, 14, 15, line 175, 293-294, 321-322

Comment 7: In Table 2, authors provided HR of each clinical and molecular parameter. The parameter of each row should be modified as follows: 'TP53 status' to 'TP53 mutation (vs wild-type)'.

Reply 7: We revised it, as suggested by Reviewer D.

Changes in the text: see page 20, line 378

Comment 8: In the Discussion, authors stated "Our data show that the presence of TP53 mt+ impact the ORR as well as PFS and OS". However, ORR difference was not significant.

Reply 8: We have changed it in the text as follows: Our data show that the presence of *TP53* mt+ impact ORR (not significant) and PFS and OS significantly.

Changes in the text: see page 21, line 394-395

Comment 9: In the Discussion, authors stated "The data of the FLAURA trial, which could provide an answer to this question, are eagerly awaited". However, FLAURA data had already been published.

Reply 9: We changed the sentence in the text: An analysis of the TP53 mutation analysis within the FLAURA trial and their impact on PFS and OS are eagerly awaited.

Changes in the text: see page 22, line 426-427

Comment 10: What is the clinical implication of the different TP53 mutational status classified by three different algorithms?

Reply 10: see comment Reviewer C comment 3

Changes in the text: see page 14 and 15, line 290-291; line 318-321