Peer Review File

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**Reviewer A**

I read with interest your manuscript that tries to shed light on clinicopathological characterization of NGS detected mutations in lung cancers. I have really appreciated the intent of the manuscript to underline the essential importance of NGS correlating clinicopathological features to specific pattern of mutations. In this way the authors try to find a sort of scheme which can lead to associate mutations with specific characteristics, trying to improve the use of these panel testing among patients who could benefit more, even in view of a cost-sparing health policy.

However, there are some major concerns that need to be solved:

Comment 1: Not all patients are tested for all mutations and no explanation is given as to why.

Reply 1: Thank you for this remark. During the observation period due to technological progress different gene panels, with an increasing number of genes, were used to test patients in clinical routine. While analyzing the data for our study we thoroughly discussed how to best handle this issue, and not introduce bias. Finally, we decided to analyze genes that were present in all panels in all patients, and use subgroups for some genes that were not included in all testing panels. We have amended the method section to better reflect this issue.

Changes in the text 1: As mentioned above, during the observation period due to technological progress the panels used for testing included an increasing number of genes. To reflect this issue when calculating the prevalence of genes not included in all panels we analyzed. Not all panels used for testing included all genes. Therefore, the following mutations were analyzed in subgroups of patients: RET (n = 133 patients), PTEN and TP53 (n = 102 patients), STK11 (n = 86 patients), KIT and PDGFRA (n = 59 patients), and AR, CCND1, ERG, MYC and MYCN (n = 58 patients). See page 5 line 110.

Comment 2: Study population is categorized into patients with metastases (stage IV) and without metastases (stages I-III). However, stage III (particularly stages IIIB and IIIC) represents a locally-advanced disease, more similar to stage IV than to early stage (I and II). So, a more precise categorization of patient's population should be done.

Reply 2: We have changed the categorization of stage in to the proposed categories and have adjusted all tables and figures, as well as the text in the manuscript accordingly. The change did not result in major changes of our findings and their interpretation.

Changes in the text 2: E.g., please see page 7 line 150, and page 8 line 163.

Comment 3: Even if the topic is very interesting, many findings couldn't have a tangible application. For example, while the overexpression of PD-L1 can lead to suppose the presence of some mutations, I can't image how we can decide to choose a testing panel by only considering the age, gender or the smoking attitude of patients.

Reply 3: Thank you for this comment, it is true than some findings may not have a direct influence on treatment decisions. However, we aimed to give a full picture of genetic profiles in lung cancer patients, therefore we included this information. We added a comment in the limitations in the discussion section regarding this issue.

Changes in text 3: Additionally, we are aware that not all results in our analysis are clinically relevant, and can help making treatment decisions. However, the aim in this study was to give a full overview of gene mutations in NSCLC patients with as many gene alterations as possible. See page 11 line 271.

Comment 4: Discussion should be implemented with the results obtained by other authors who performed NGS testing on liquid biopsy of lung cancer patients (e.g. doi: 10.3390/biomedicines11010153).

Reply 4: Thank you for this recommendation. We have added this publication to our discussion section.

Changes in text 4: A mutation in BRAF was also found using liquid biopsy in one patient with early stage NSCLC in cohort of 20 patients, where a mutation would not have been expected due to the sample size. See page 11 line 237.

Comment 5: Some typos need to be corrected, e.g. line 62 clinicopathologcigal, line 243 sex stands for female???

Reply 5: We have thoroughly edited the manuscript for typos.

Changes in text 5: See e.g. page 3 line 48,54, and 55. Page 10 line 218. Page 12 line 281.

**Reviewer B**

Authors conducted the retrospective study about NGS detected mutations in lung cancers. They showed the prevalence of various mutations in lung cancer and the related clinicopathological characterization.

Comment 1: As they mentioned, the study cohort is too small to detect any confirmed conclusion. Furthermore, because they used the relatively small gene panel, they might fail to detect the important gene alteration. To analyze more precise gene alteration in lung cancer, I

recommend use large data set available in public.

Reply 1: We are aware that data from a single-center cannot cover a large enough patient cohort and give a full picture of the whole population of NSCLC patients. However, we believe that a center-specific cohort has the advantage of allowing a more comprehensive analysis of clinical features of patients. This information is often not or not completely available in public data bases. This is true for example in terms of follow-up, verification of tumor stage, and the NGS panel that was used for diagnostics. We have amended the discussion section with some sentences regarding this issue.

Changes in text 1: Additionally, a small sample size might not have enough power to detect all relevant differences, and a larger (public) dataset might be needed to analyze more precise gene alteration in lung cancer. However, we also believe that although using data from a single center might lead to a smaller dataset, a big advantage is that this data allows a comprehensive analysis of clinical features not available in public datasets. Page 11 line 261.