# Peer Review File

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**Round 1:**

**Reviewer A**

*Overall, the authors have made a good attempt at prognostic impact of BRAF RNA expression.*
*However, since some uncertainty remains regarding the certainty of the methodology, the following improvements are proposed.*
*1. To reinforce the RNAscope results, qPCR should also be performed.*
*2. I understand that RNA expression patterns of wild-type BRAF gene expression in NSCLC have not been investigated, but I would like the authors to verify whether a similar trend to the present study is observed from RNAseq information on public databases.*
*Altogether I think the paper should be published but only after some revision.*

*To be honest, I did not find this paper very interesting.*
*It seemed to me that we need to carefully assess whether the paper is at the appropriate level for this journal.*

**Response:**
We thank the Reviewer for taking the time and raising important points. Accordingly, we used the Human Protein Atlas (HPA) and the TCGA database to validate our data with independent datasets. Please see the new panels in Fig 1 and 5 and the description in the Results section (page 6, line 211-219, and page 8, line 281-288). BRAF expression was prognostic in early stage (I-II) lung adenocarcinoma cases (p=0.034, n=318) according to TCGA data. We also included the section “Datasets” in the Methods (page 5, line 177-186) for the description of analyses based on publicly available data.
We agree with reviewers point to perform further validations. Unfortunately, there were no additional tumor tissue available for further studies, because of the retrospective cross-sectional nature of our study. Accordingly, we did not have frozen tumor tissue samples for every patient, therefore we couldn't perform a qPCR at this point. Furthermore tissue samples

were available in the form of TMAs, from which, even FFPE RNA-isolation would not have been feasible.

We still believe there is future potential in detecting BRAF expression in devastating cancers such as lung cancer. The low number of targetable driver alterations, especially BRAF mutation, is present in 4.29% (559/24) of LUAD cases according to the TCGA database and scientific literature (Planchard et al., 2016; Leonetti et al., 2018; Negrao et al, 2020). Therefore, we believe that wild-type BRAF RNA expression seems a promising aspect for further research to identify the exact clinical role to improve the survival of this aggressive malignancy. Also, we added the points the Reviewer raised into limitations and conclusions to highlight the need for further research to define the exact role of BRAF expression in NSCLC.

**Reviewer B**

*In this manuscript, the author investigated the expression of BRAF RNA of the wild-type (WT) BRAF gene expression in LADC. The data are novel and informative. The manuscript should be of great to the readership of the journal. I have a few major and minor comments, explained below.*

*Major comments*

*• 1. Does patients have driver alterations (EGFR, ALK, ROS1, RET, KRAS…)? If some patients have driver alterations, it would be informative to see if driver alterations were correlated with BRAF expression and associated with OS, in which driver alterations might be a cofounder for OS.*

**Response:**

We thank the Reviewer for pointing this out. Our predominantly early-stage cohort has a limited number of cases with drivers analysed in the routine clinical setting. Cases with recurrence/advanced stage were only sequenced for drivers. Also, this cohort is from 2006-2013. Therefore, gene sequencing was not widely performed at this period, and tumor tissue is not available for further studies. We included this into the limitations.

Among the total of n=64 cases, EGFR and KRAS status was available for n=12 patients, respectively, and we identified EGFR (n=1) and KRAS (n=7) mutant patients totally. Thus,

these low case numbers did not make it possible to perform statistical analyses regarding BRAF expression. There were/no co-occurrence with EGFR/KRAS status. When we excluded EGFR and KRAS mutant patients, BRAF expression was still a prognostic in our cohort (p=0.0112).

・*2. Page 3, line 109-111: Are there any patients who received ICI or targeted therapy? If they are patients who received ICI or targeted therapy, the authors should describe the detail of the types of oncotherapy into table 1.*

**Response:**

We thank the Reviewer for raising this point. ICI was not administered to our cases. However, targeted therapy was administered for n=1 case with EGFR mutation detected that had progressed/recurred into advanced stage. Only n=1 patients received Neoadjuvant chemotherapy, whereas n=26 patients received Adjuvant chemotherapy and n=24 patients received IIB-IV chemotherapy. We added the types of oncotherapies to Table 1.

・*3. Table 1: In some rows such as smoking and tumor grade, the total number of each row is not equal to n=64. I guess it included some missing values or patients in the other category. I would suggest the total number of each row should be matched to n=64.*

**Response:**

We thank the Reviewer. In our retrospective study, there were no available/enough tumor tissue to evaluate all the markers (SFig1). Accordingly, we included the number of missing values for each related category in Table 1.

・*4. Table 1, 2, 3: The authors did not mention Performance Status, which would be an important confounder for OS. If the authors make univariate or multivariate analyses, the authors should include it.*

**Response:**

We thank the Reviewer for raising this point. Our predominantly early-stage cohort cases underwent lung resection surgery with overall good performance status and appropriate lung function parameters, which is required for the procedure. Accordingly, this is a homogeneous

population. Since this is a retrospective study, we tried but could not gather more specific information from the electronic records. Also, we added the points raised by the Reviewer into limitations and conclusions to highlight the need for further research to define the exact role of BRAF expression in NSCLC.

・*5. There is no patient flow diagram, which makes the selection bias. The authors should present the patient flow diagram as a supplementary figure or describe it in the main manuscript.*

**Response:**

Accordingly, a flow chart was included into our manuscript (Supplementary Figure 1). Of note, this is a retrospective study on a predominantly early stage cases with tumor tissue available. According to the Reviewer's request, we conclude that further prospective studies are needed to indentify the exact role of BRAF expression in NSCLC.

Minor comments

・*1. The authors need to provide a clear explanation for the limitation section. For example, if there is no data with driver alterations or TMB, the authors should describe it.*

**Response:**

Accordingly, we included the limitations. “Since our cohort is predominantly early stage cases, we had no data on tumor mutation burden. Additionally, we identified only a few cases with driver alterations (EGFR (n=1) or KRAS (n=7)) and targeted therapies were a standard of care only later periods (after the period from 2006 to 2013, from which the retrospective cohort dates).

・*2. Page 8, line 369-371: It would be appropriate to discuss the possible mechanism more why wild-type BRAF can still be overexpressed in the absence of BRAF gene mutation.*

**Response:**

We thank the Reviewer for pointing this out. Accordingly, we further discussed this important issue in the Discussion section (page 10, line 365-371)

・*3. Table 1: The authors should provide the number of lung biopsy vs. surgical resection.*

**Response:**

We now included this in Table 1 and the flow chart.

・*4. Figure 5: The authors should describe the median follow-up.*

**Response:**

We now included the last follow-up and the median follow up time regarding our patient cohort in the legends of Figure 5.

*While it is an interesting topic, certain areas need minor improvements.*

**REFERENCES**

Planchard D, Besse B, Groen HJ, et al. Dabrafenib plus trametinib in patients with previously treated BRAFV600E-mutant metastatic non-small cell lung cancer: an open-label, multicentre phase 2 trial. *Lancet Oncol* 2016;17:984-93. 10.1016/S1470-2045(16)30146-2

Leonetti A, Facchinetti F, Rossi G, et al. BRAF in non-small cell lung cancer (NSCLC): Pickaxing another brick in the wall. Cancer Treat Rev. 2018 May;66:82-94. doi: 10.1016/j.ctrv.2018.04.006. Epub 2018 Apr 24. PMID: 29729495.

Negrao MV, Raymond VM, Lanman RB, et al. Molecular Landscape of BRAF-Mutant NSCLC Reveals an Association Between Clonality and Driver Mutations and Identifies Targetable Non-V600 Driver Mutations. J Thorac Oncol. 2020 Oct;15(10):1611-1623. doi: 10.1016/j.jtho.2020.05.021. Epub 2020 Jun 13. PMID: 32540409; PMCID: PMC7529990.

**Round 2:**

**Review Comments**

**Comment:** Although the presented data is a little old and thus has some limitations, I am satisfied with the revised paper. It is well written and an interesting study. I do not have any substantial amendments to suggest.

We thank the reviewer for their positive comments and the constructive criticism that helped improving our manuscript.