Peer Review File

Article information: https://dx.doi.org/10.21037/tlcr-23-449

Reviewer A

Comment 1: The author did a nice descriptive review of the real-word findings in their institution. However, they did not elaborate clearly what makes their study impactful in term of new knowledge to the field and where the field is heading.

Reply to comment 1: Thank you for your constructive suggestions to our study. Just as your concern, we have revised our manuscript after additional attentions to the aim of this study by all authors. Correspondingly, aims and underlying impacts of this study were highlighted on the abstract, introduction, discussion sections, and highlight box, respectively. Here, we introduce the problem the reviewer concerned briefly. KRAS-mutant NSCLC used to be in a dilemma that although there is a latent capacity to be targeted by small molecular inhibitors, consecutive attempts failed to develop proper targeted drugs in the past, followed by restricted efficacy of existing systematic treatment schemes in a mono or combined manner. However, there was little studies focused on the horizontal comparisons between divergent therapeutic regimes and different intervention times before the emergency of KRAS-targeted agents in NSCLC. Thus, we conducted a single-center retrospective analysis among 66 patients diagnosed with KRAS-mutant advanced NSCLC in nearly 3 months, trying to explicit the alternatives to extend their life expectancy in the absence of targeted options. Up to now, this study seemed to take the lead in this field, which made it impactful that a large group of people with KRAS-mutant NSCLC benefit from accurate genetic identification and organized treatment strategies, which underscores the need for KRAS-targeted medications. We sincerely hope that this answer can explain your confusions to the subjects to this work.

Changes in the text: Indicated words were revised on the corresponding paragraphs in Abstract (Page 1, Line 26-65), Introduction (Page 3, Line 99-121), Discussion (Page 9, Line 293-422), and Highlight box (Page 13, 427-446) with red marks.

Comment 2: KRAS co-mutation was not discussed neither brought up as having potential impact in outcomes. No referral to ongoing trials.

Reply to comment 2: We also appreciated your meticulous vision to KRAS co-mutation within NSCLC. Indeed, KRAS isotypes or coupled with other somatic mutations may confer diverse genetic characteristics and relevant biological behaviors. However, the presence of genetic heterogeneity probably dimmed genuine mutant state in light of internal medicine-based small specimen biopsy. Thus, it is extremely difficult to explore whether the KRAS con-mutations have potential impact in long-term outcomes in a restricted study cohort retrospectively, which deserves extended

population at multiple centers. Certainly, up to now, there were no relevant clinical trials on this topic, indicating an enormous exploration space in basic research field and that in practice. In the text, we detected the co-mutations of tumor samples in NSCLC by routine genetic examinations as objective descriptions, which could not be verified further in this study. We sincerely hope that this answer can explain your concerns.

Changes in the text: Further illustration was listed on Page 10, Line 314-317.

Reviewer B

Comment: Unfortunately given the heterogeneity of the pt population evaluated, both in terms of treatment received and specific KRAS mutations present, combined with the limited number of patients evaluated, I do not discern any meaningful conclusions that can be drawn from the data presented here and do not think that this study, in its current form, supports publication in a prestigious peer-reviewed journal. That said, I do think the authors have done a nice job of expanding on the many thought provoking hypothesis throughout the manuscript, especially in the discussion, and there may be a place for a review type article in TLCR, but not for this study to be presented as primary research as it does not add significantly to our knowledge of KRAS mt NSCLC given the limitation noted above.

Reply to comment: We thank for your rigorous but sincere comments on the topic and constructive advices for our manuscript. We admitted that there were several obvious shortcomings undeniably which were liable to weaken the clinical significances according to our findings in a restricted study cohort. But, sincerely speaking, our study analyzed the clinical outcomes of NSCLC patients with KRAS mutation under untargeted therapeutic regimes in the real world, indicating that there was no significant difference in the long-term survival of them, no matter which treatment schemes were performed. Additionally, a large group of people with KRAS-mutant NSCLC benefit from accurate genetic identification and organized treatment strategies, which underscores the need for KRAS-targeted medications.

As for conclusions drawn up from this study, we apologized that we failed to explicit that clearly in the primary version of this manuscript. There existed an urgent necessity for us to illustrate the results and potential outcomes in this study. First, G12C performed as the primary isotype of KRAS mutation in NSCLC. Additionally, ICIs performed seemingly beneficial to Chemotherapy and anti-angiogenesis as the first-line intervention in this restricted population. At last, therapeutic drugs and lines barely correlated to long-term outcomes in KRAS-mutant isotypes of NSCLC patients.

Although the reasons to explain your concerns seemed not sufficient, our work were still inspiring for the treatments of KRAS-mutant NSCLC to some extent in clinic.

Changes in the text: Indicated words were revised on the corresponding paragraphs in Abstract (Page 1, Line 26-65), Introduction (Page 3, Line 114-152), Discussion (Page 9, Line 296-422), and Highlight box (Page 13, 427-446) with red marks.

Reviewer C

Comment 1: First, in the title the clinical research design should be specific to a retrospective cohort study and please clearly indicate the focuses of this study such as treatment and prognosis.

Reply to comment 1: Thank you for your kind advice. We admitted that the title failed to focus on the key points of this study. Accordingly, we transformed the title of this research into a revised one that "A retrospective observational study: clinical outcomes of KRAS-mutant non-small cell lung cancer under untargeted therapeutic regimes in the real world", hoping that the new one will match the scope of this study.

Changes in the text: Revised title was displayed on the Page 1, Line 3-4.

Comment 2: Second, the abstract needs to be revised. The background did not indicate the clinical questions to be answered by the current data. The methods did not describe the inclusion criteria, assessment of clinical variables including treatment strategies and prognosis outcomes, and how these patients were followed up. The authors need to tone down the current conclusion on the efficacy data since this is a retrospective cohort study and the sample is small.

Reply to comment 2: Your kind advices are taken into our account by adequate discussion among all authors. In practice, we revised the abstract with definite aims, detailed methods and inclusion criterion, and prognostic outcomes in a long term. In addition, we tried to explicit the data based om current conclusions as you advised, minimizing abstract as much as possible without affecting results. Corresponding revisions are marked at the proper sites.

Changes in the text: As you recommended, we revised the abstract at Page 1, Line 25-68, together with the key words as follows.

Comment 3: Third, in the introduction of the main text, the authors need to review what has been known on the treatments of KRAS mutation positive non-small cell lung cancer and their long-term prognosis outcomes, and analyze the knowledge gaps and limitations of prior studies. The authors need to explain why the current retrospective cohort data can address the limitations of prior studies.

Reply to comment 3: Thank you for your detailed advices, which indeed benefit for

our manuscript to improve readability and logicality. As mentioned in the revised version, personal financial burden and lack of sufficient evidences in KRAS isotypes weakened their clinical utilization, which still needed further attention to alternatives for KRAS-mutant advanced NSCLC besides targeted drugs instead. Other optimal strategies for KRAS mutant NSCLC seemingly failed to reach a consensus before the emergency of specific targeted therapy. It has been highly appreciated that chemotherapy performs as a fundamental strategy in combination with other interventional approaches or just as a single agent. Considering these confusions, we enrolled KRAS-mutant advanced NSCLC patients in our own center, and retrospectively analyzed their corresponding therapeutic regimes and long-term outcomes, in order to explicit the alternatives to extend their life expectancy in the absence of targeted options. Although the limitations from a restricted retrospective cohort, it was reasonable to be appreciated that this study illustrated no advantages in existing therapeutic regimes and an urgency to call for KRAS-targeted therapy. Details are mentioned on the corresponding paragraphs in introduction section as follows.

Changes in the text: Revised words were marked on the Page 4, Line 114-121. Additional illustrations were also added to the adequate locations.

Comment 4: Fourth, in the methodology of the main text, the authors need to accurately describe the clinical research design, a retrospective cohort study? But the authors described "prospectively enrolled". Please provide the sample size estimation and details of the follow up and loss to follow up. In statistics, please ensure P<0.05 is two-sided.

Reply to comment 4: We needed to apologize for the descriptive mistakes of "prospectively enrolled" in methods section, which should be revised into "retrospectively enrolled". After deliberate discussion between all authors, we rewrite this section with precise descriptions as you recommended. The sample size estimation and details of the follow-ups are mentioned in Results section in a figure format (Figure 1). Correspondingly, we ensured that two-sided P values <0.05 were regarded as significant statistically which was verified in the Statistical analyses section.

Changes in the text: Methods section was revised as you recommended on Page 4, Line 127-180.

Comment 5: Finally, please consider to review and cite several related papers as below: 1. Santarpia M, Ciappina G, Spagnolo CC, Squeri A, Passalacqua MI, Aguilar A, Gonzalez-Cao M, Giovannetti E, Silvestris N, Rosell R. Targeted therapies for KRAS-mutant non-small cell lung cancer: from preclinical studies to clinical

development—a narrative review. Transl Lung Cancer Res 2023;12(2):346-368. doi: 10.21037/tlcr-22-639. 2. Spagnuolo A, Maione P, Gridelli C. The treatment of advanced non-small cell lung cancer harboring KRAS mutation: a new class of drugs for an old target—a narrative review. Transl Lung Cancer Res 2022;11(6):1199-1216. doi: 10.21037/tlcr-21-948. 3. Fung AS, Karimi M, Michiels S, Seymour L, Brambilla E, Le-Chevalier T, Soria JC, Kratzke R, Graziano SL, Devarakonda S, Govindan R, Tsao MS, Shepherd FA; on behalf of the LACE-Bio Collaborative Group. Prognostic and predictive effect of KRAS gene copy number and mutation status in early stage non-small cell lung cancer patients. Transl Lung Cancer Res 2021;10(2):826-838. doi: 10.21037/tlcr-20-927. 4. Yang R, Wang D, Li X, Mao K, Wang J, Li P, Shi X, Zhang S, Wang Y. An advanced non-small cell lung cancer patient with EGFR and KRAS mutations, and PD-L1 positive, benefited from immunotherapy: a case report. Ann Transl Med 2022;10(6):381. doi: 10.21037/atm-22-403.

Reply to comment 5: Thank you for your kind advices according to several related articles to our study. These recommended articles are related to therapeutic strategies and sophisticated mutation status of KRAS-mutant NSCLC, which may further enrich the clinical meanings of our research. And these references are cited at the corresponding sites.

Reviewer D

1. **Please confirm if the exclusive criterion is missing. It seems that the following sentences are not the exclusive criterions.**

159 genetic examinations to confirm the presence of KRAS mutation after the suspected
160 malignant lesions according to computer tomographic scanning. Exclusive criterion
161 was listed below. The clinical data of the biological features of participants were
162 collected, including age, sex, smoking status, clinical stage, Eastern Cooperative
163 Oncology Group (ECOG) performance status at diagnosis, histology and molecular
164 status, programmed death ligand 1 (PD-L1) TPS score (<1%, 1–49%, ≥50%), presence
165 of brain metastases at diagnosis and at any time following initial diagnosis, systemic
166 therapy including start and stop dates, date of diagnosis of metastatic or recurrent
167 disease, date of death, and date of last follow-up. The first line of therapy was defined
168 as the initiation of systemic therapy drug. Several treatment-related characteristics were
169 also collected, including the starting time of each first-line treatment, the time of each
170 systemic evaluation of progress, the specific organ of progress, and the drug
171 combination of different treatment regimens. End points were confirmed at the time of
172 death. This study was approved by the Medical Ethics Committee of the First Affiliated

Reply: We have revised the sentence: **Exclusive criterion was shown in figure 1.**

2. **Title**

It is suggested to revise the title as follows:

Clinical outcomes of KRAS-mutant non-small cell lung cancer under untargeted therapeutic regimes in the real world: A retrospective observational study

3 **A retrospective observational study: clinical outcomes of KRAS-mutant non-small**
4 **cell lung cancer under untargeted therapeutic regimes in the real world**

Reply: Thanks for your suggestion. We have revised the title as you suggested.

3. Abstract (word limit: 200~350)

a. We helped to make some minor revisions in below sentences. Please confirm whether you are ok with this.

32 cancer (NSCLC). However, whether these non-selective therapy schedules for KRAS
33 mutation matters is still under debate.

72 to benefit KRAS mutant sufferers regardless of isotypes, making the KRAS-targeted
73 drugs urgent.

Reply: We agree to these revisions.

b. Please extend the content of the Background. This paragraph should contain 'study background' and 'study objective'.

Background: Kirsten rat sarcoma viral oncogene homolog (KRAS) mutation seemingly suffered less effective therapeutic regimens in the absence of widely-accepted targeted drugs compared with other mutation types in non-small cell lung cancer (NSCLC). However, whether these non-selective therapy schedules for KRAS mutation matters is still under debate.

Reply: We have extended the content of the Background.

4. The main text should be structured as #Introduction, #Methods, #Results, #Discussion and #Conclusions; please add "#Conclusions" accordingly.

Reply: We have added "#Conclusions" accordingly in the text.

5. Please check if citations are missing in below sentences, since "previous studies" is mentioned.

*Please note that the references should be cited in order of their appearance in the text.

a. Previous studies reported that diverse isotypes of KRAS-mutation exist, which may contribute to various molecular biological characteristics, and in turn, distinctive clinical outcomes to the same treatment.

Reply: We have added the reference in the text.

b. Similar to the results of previous studies, the incidence of KRAS gene mutation in NSCLC patients in this study was 10.8%, with a median age of 62 years, which

mainly occurred in lung adenocarcinoma patients.

Reply: The previous studies were mentioned above, we have revised the sentence

6. **Some references are duplicated: (6 and 19), (13 and 16), (8 and 11). Please revise and update the citation in both the main text and reference list.**

Reply: We have revised and updated the citation in both the main text and reference list.

7. **Figure 3 and Figure 4**

There are no symbols (*, ns) in figures 3 and 4, but you indicated them in the figure legend. Please check and revise.

Figure 3 PFS of NSCLC patients with KRAS mutation. **A.** mPFS in different lines. **B.** mPFS in 1st-line angiogenesis inhibitors. **C.** mPFS in 1st-line ICIs. **D.** mPFS in age-dependent subgroups. P values are listed under the annotation on the right. P*<0.05; Pns>0.05. PFS, progression-free survival; NSCLC, non-small cell lung cancer; KRAS, Kirsten rat sarcoma viral oncogene homolog.

Figure 4 The survival analysis of patients with NSCLC complicated with KRAS mutation. **A.** OS in unclassified patients. **B.** OS in genders. **C.** OS in ages. **D.** OS in subtypes of NSCLC. **E.** OS in patients with or without brain metastasis. **F-H.** OS in first line schemes with chemotherapy, angiogenesis inhibitor, and ICIs, respectively. **I.** OS in isotypes of KRAS. **J.** OS in the presence of ICIs. P values are listed under the annotation on the right. P*<0.05; Pns>0.05. mOS, median overall survival; BMS, brain metastases.

Reply： Symbols (*, ns) are not required in the figure, we have deleted "P*<0.05" and "Pns>0.05" in the figure legend..

8. **Table 1**

Please check whether these data are correct, as they do not match the table 1.

histological examinations, accounting for 87.9%. The expression level of PD-L1 was detected in 22 patients, of which 8 cases (36.4%) were <1%, 6 cases (27.3%) were 1–49%, and eight cases (36.4%) were ≥50%. Brain metastases were seen in 12 cases, accounting for 18.2%

PD-L1 expression	
<1%	8 (12.1)
1–49%	6 (9.1)
≥50%	8 (12.1)
Unknown	44 (66.7)

Reply: The proportion in the table is the proportion of all enrolled patients. The proportion described in the text is the proportion of the patients detected PD-L1. We are sorry for the misunderstanding caused by the unclear description, and we have made modifications in the text on Page 7, line 206.

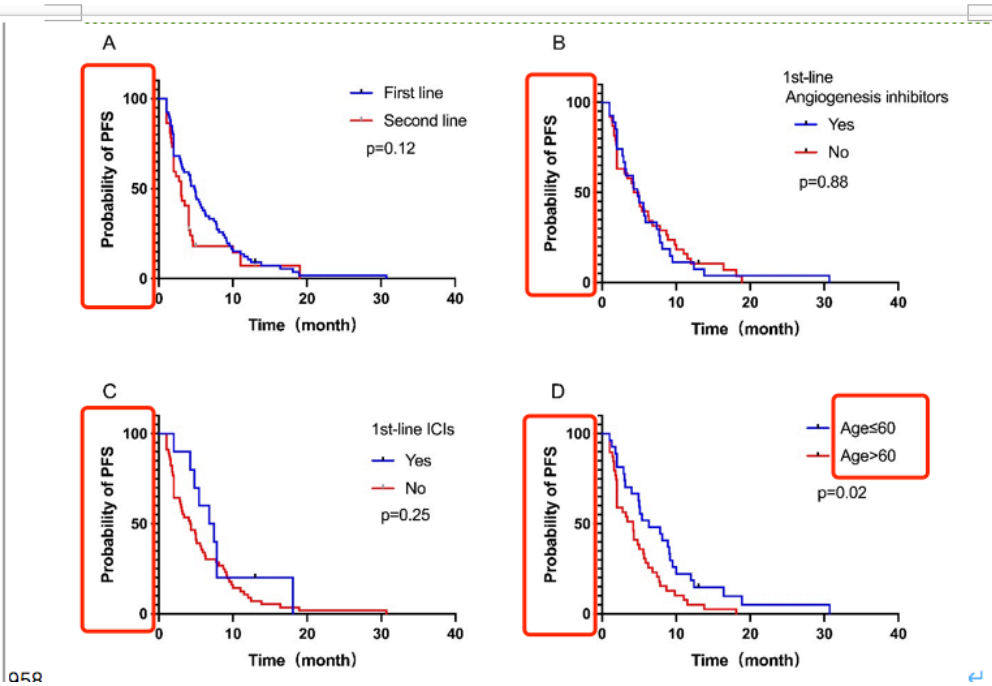
9. Figure 3 and Figure 4

The correct format for the y-axis should be one of the following, please revise.

a) If the description is "Probability of PFS, %", the numbers should be 0-100.

b) If the description is "Probability of PFS", the numbers should be 0-1.0.

Please revise.



Reply: We have revised the y-axis format in Figure 3 and Figure 4 and replaced the original figures in the main manuscript.

10. Figure 3D and Figure 4C

Please provide the unit for "age".

Reply: We have provide the unit for "age" in Figure 3D and Figure 4C and replaced the original figures in the main manuscript.