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Reviewer A

Major Comments

Materials/Methods -

Comment 1: The authors mention that KRAS mutations were identified using either tissue genomics or blood analysis – can the authors comment on the assays and methodology utilized for tissue vs blood based testing.

Reply 1: Thank you for this comment. This was an error in the manuscript, blood-based testing was not conducted. At our institution, *KRAS* gene testing is done in one of two ways. One way is as a single gene test using single base extension followed by mass spectrometry (OncoCarta panel and Sequenom MassARRAY instrument, Agena Biosciences) or as part of a solid organ tumor hotspot panel by next generation sequencing (Ion AmpliSeq Cancer Hotspot v2 and Ion Proton instrument, Life Technologies) on formalin fixed paraffin embedded (FFPE) tumor tissue in the Molecular Diagnostic Pathology Laboratory at Houston Methodist Hospital.

Changes in the text: We removed blood as the diagnostic confirmatory test and added the above information into the manuscript (Page 6, ln 121-129).

Comment 2: Results – Patient Outcomes: At 12 months of follow up, 28 patients had died (Table 3) – what proportion of these patients were early vs late stage?

Reply 2: Out of the 28 deaths, 4 (14.2%) were early stage disease.

Changes in the text: The authors have added the distribution of patients based on 12 month overall survival for early and late stage disease, dichotomized for *KRAS* p.G12C compared to all others mutations (Page 10, ln 215-220).

Comment 3: Results – Patient Outcomes: The probability of survival at 1yr for G12C vs other mutations was reported (Figure 4). Was this analysis stratified by late vs early stage? Early and late stage patients should not be grouped together for this analysis given the difference in prognosis between these groups.

Reply 3: Thank you for this important suggestion. An updated analysis has been done, and it was found that while advanced stage is a significant prognostic factor (p = 0.0102) of overall survival. In this model adjusted for the effect of stage, *KRAS* p.G12C mutation had a higher hazard ratio (HR=1.54), but it is not a significant prognostic factor (p = 0.2287). Upon dichotomizing for stage and *KRAS* mutations, 12 month survival was numerically lower for both early and advanced stage *KRAS* p.G12C mutations compared to all other mutations (56.3% vs 90.9% for early stage and 25.0% vs 47.6%, respectively.

Changes in text: An updated survival figure (Figure 4a) (Page 34, ln 646) has been added, Table 3 (Page 37, ln 665) has been updated, and results have been updated in the manuscript (page 10, ln 215-220).

Comment 4: Results (Figure 4): Only Figure 4A and 4B were included in the text. However, the figure legend mentions Figure A-C. Figure 4A in the legend is described as "patient survival comparison between KRAS G12C, G12D and other mutations" – this data is not shown in the figure or described in the text.

Reply 4: Thank you detecting this issue. In this initial draft, we had initially planned to compare KRAS p.G12C, p.G12D, and all others based off the proportions of mutations found. Due to small sample sizes and the scope of the manuscript, the decision was made to compare *KRAS* p.G12C to all other mutations. The primary area of interest of this manuscript was to see how patients in the community that harbored the G12C mutation did compared to other *KRAS* mutations and whether any pre-existing risk factors further influenced survival, given that there is now a targeted treatment.

Changes in the Text: Figure 4 legend has been updated. A now has survival based on stage and *KRAS* p.G12C and other mutations; B has survival based on smoking status; C has survival based on age.

Comment 5: Results – Mutation Analysis: The authors report 44.8% KRAS G12C in the clinical analysis and 8.8% in the pathologically assessed NSCLC. Can the authors clarify how the 8.8% was calculated in the pathologically assessed group (i.e. is this in the total population including KRAS mutated and wild-type patients)?

Reply 5: The authors concur that this statement may be confusing for readers. The 44.8% comes from the proportion of all *KRAS* mutations included in the clinical analysis (n=58). The 8.8% is a proportion of all diagnosed lung cancers during the time frame, including wild type.

Changes in the text: The proportion of *KRAS p.G12C* compared to all lung cancers (8.8%) was removed from the manuscript (Page 11, 233-234).

Comment 6: Results – Mutation Analysis: 16 KRAS G12C mutated patients had died and mOS of 5.6mo was reported. Were these all late-stage patients? As mentioned above, early and late stage patients should not be grouped together for this analysis given the difference in prognosis between these groups.

Reply 6: A total of 11 patients with late stage and 5 patients with early stage died.

Changes in the text: Median overall survival was updated according to all patients with early stage and *KRAS* p.G12C mutation, and all patients with late stage and *KRAS* p.G12C mutation (Page 11, ln 238-240).

Comment 7: Results & Discussion – The authors reported a numerically (non-significant) lower 12-month survival of KRAS G12C patients (vs non-G12C patients). In TableS1, 7 of the 16 stage IV KRAS G12C did not receive any systemic therapy, and an additional 3 patients only received 1-2 cycles of treatment. In comparison, 4 out of 14 stage IV KRAS non-G12C patients did not receive treatment. Were the G12C patients sicker and unable to tolerate/receive treatment (i.e. was performance status evaluated)? If so, this would impact the 12-month survival of this subgroup, and it would be difficult to attribute the worse 12-month survival purely to the presence of the G12C mutation. Were survival outcomes in KRAS mutated vs wild type patients assessed based on treatment received? It would be nice to have some discussion of the treatments received in KRAS mutated patients and the impact on survival outcomes.

Reply 7: Thank you for pointing this out. It is true that 7/16 G12C and 5/21 non-G12C patients with advanced disease were not able to receive chemotherapy or immunotherapy following diagnosis, either due to critical illness at the time of diagnosis or refusal to receive treatment. Patients with p.G12C at diagnosis and advanced disease had a median ECOG performance status of 2, compared to a median performance status of 1 for all other *KRAS* mutations, indicating these patients were more ill at the time of diagnosis.

Changes in the text: Median ECOG performance status was added to Table 2, dichotomized according to early and advanced stage, as well as *KRAS* p.G12C vs all other *KRAS* mutations (Page 35, ln 670, Table 2). In addition, the results *Page 9-10, ln 201-215) section and discussion (Page 12, ln 265-269, page 13-14, ln 290-294) were both updated reflecting this point.

Comment 8: Discussion – Was a multivariate analysis not feasible due to small patients numbers? If so, this should be included as a limitation of the study, as confounding factors were not adjusted for when evaluating the effect of KRAS mutations on outcomes

Reply 8: A multivariate analysis was not feasible for all outcomes due to the sample size limitations. However, we did run a multivariate model of sorts; with this updated draft, we ran both *KRAS* mutation and stage.

Changes in text: This point was added to the limitations section (Page 17, ln 383-384).

Minor comments

Comment 9: KRAS is not consistently italicized in the manuscript

Reply: Thank you for finding this detail.

Changes in the text: All *KRAS* is now italicized

Comment 10: Discussion (page 8, line 220-223) – please include reference for this statement.

Reply: The authors agree a clinical study citation should be added.

Change in text: A reference for the phase 1 trial by Hong et al. and the clinical trial number for the CodeBreak 101 trial has been appropriately inputted (Page 12, ln 253-254).

Reviewer B

Comment 1: I understand the importance of KRAS mutations, smoking status and stage in survival and I think all three can act as confounding factors in the analysis. Therefore, I recommend performing the survival analysis and Figure 4 controlling for stage.

Reply 1: Thank you for this suggestion. The authors agree that these three can act as confounding factors in the analysis, and that a survival analysis controlling for stage should be conducted.

Changes in text: A survival analysis controlling for stage (early vs advanced) has been amended in the text, also controlling for *KRAS* p.G12C vs all other *KRAS*

mutations (Figure 4A, (Page 34, ln 646).

Comment 2: I recommend the use of roman numbers instead of Arabic, when referring to stages.

Reply 2: The authors agree with this suggestion.

Changes in the text: Roman numerals were used when referring to disease stage (Page 7, ln 146).

Comment 3: Some references are duplicated (for example, 23 and 30), please, review them and amend the text accordingly.

Reply 3: Thank you for finding this error. This was a duplicate reference.

Changes in the text: All references have been reviewed and no additional duplicate references have been found. The referencing numbers have been adjusted in the manuscript accordingly.

Comment 4: The authors mention actionable mutations in the text, what about NTRK? What is detected within the study patients?

Reply 4: NTRK gene-rearrangements are uncommon and occur in <1% of lung cancer diagnoses. While rare, the authors agree that it should be included in the introduction of the manuscript since there are targeted treatments available. At our hospital network, we routinely test for *NTRK* 1 and 3 as part of the lung mutational panel. However, as stated in the results, all *KRAS* mutations were mutually exclusive and no other driver mutations were found.

Changes in the text: NRTK gene rearrangement has been added to the introduction (page 4, ln 88).

Reviewer C

Comment 1: The authors describe a retrospective chart analysis including 7 hospitals and focusing on epidemiology, clinical and molecular characteristics and outcome of KRAS G12C patients. Only 58 patients were included in this analysis, only 64% of them had advanced stage disease. Within the past 3 years there has been a large number of reports describing these patients, mostly with substantially larger cohort. Here, no new information is given for age, gender-ratio, smoker status, frequency, co-occurring mutations. OS is described to be lower, however, without statistic

significance and in contrast to most other analyses with larger patient numbers.

Reply 1: The authors thank you for your assessment of this manuscript. It is true there are similar analyses in the literature on *KRAS* mutations. This manuscript aimed to highlight outcomes in *KRAS* p.G12C vs other *KRAS* mutations in the community setting with a diverse patient population. There are varying degrees of outcomes in different studies in *KRAS* mutated lung cancers which was highlighted extensively in the discussion section. While not significant due to low sample size, the *KRAS* p.G12C mutation seemed to have a poorer 12 month survival, and patients with both early and advanced stage *KRAS* p.G12C mutation had poorer initial performance status at diagnosis, which has been updated in the manuscript. In addition, other studies primarily focus on either early or advanced only, whereas our analysis incorporated all patients to report on the culmination of the patient experience. Furthermore, this is the second *KRAS* lung cancer analysis that reports upon other malignancies occurring in this patient population. While it did not impact prognosis in this particular patient population, it is a unique correlation.

Changes in the text: Survival has been controlled for stage, performance status has been added for *KRAS* mutation status and cancer stage.

Reviewer D

The paper is a well-written, methodologically well performed analysis of outcome in KRAS mutant lung cancer.

Comment 1: As a retrospective analysis from a local 7 institution network, the work mainly suffers from very small sample size. Thus, all further statistical analysis can be assumed to have insufficient power. It remains unclear why the analysis of this small cohort should be of further interest to a general audience. I also dont really see a question asked or answered, but purely descriptive statements.

Reply 1: The authors agree that the manuscript is limited by a small sample size. As mentioned in the introduction of the manuscript, the aim of this analysis was to identify and categorize potential prognostic contributors that impact survival in patients with early and advanced stage lung cancers in a “real-world,” community based hospital setting. Since our hospital reflexively tests mutational burden regardless of stage, the goal was to see if the patients with *KRAS* mutations, particularly p.G12C, had differences in outcomes. While there was a poorer overall survival found with *KRAS* p.G12C mutations, it was not significant, likely reflective

of the small sample size. There is conflicting data on the prognostic impact of *KRAS* p.G12C in the literature (as mentioned in the discussion), and recognizing potential factors that may impact outcomes in *KRAS* mutated lung cancer with targeted therapy on the horizon is important. While our results were not significant, it does further corroborate that patients with p.G12C may have poorer survival compared to other *KRAS* mutations. Furthermore, early and advanced stage p.G12C mutations had poorer performance status at diagnosis, which may have impacted the reported survival difference. In addition to this, it is interesting to note that one-quarter of the patients in this study were found to have a second cancer (history of, concurrent, or developed a second primary cancer).

Changes in the text: The authors updated 12 month survival based on stage (early, advanced), and added performance status characteristics for stag (early, advanced), and *KRAS* mutation.

Comment 2: At the very least, the authors should clearly state these limitations and suggest some added value derived from their work.

Reply 2: The authors agree that there are clear limitations to this retrospective study, and recognize that it may not have previously been clearly delineated in the last paragraph of the discussion section. As for added value derived from the work, the authors do mention the large burden of additional malignancies, as well as the non-significant but numerically lower overall survival in *KRAS* p.G12C mutated lung cancers, which adds value to conflicting literature on the prognostic impact of these specific mutations.

Changes in the text: The limitation section has been modified to more clearly state the limitations, especially with regard to sample size (Page 317, ln 365-377).

Comment 3: l159ff.: the interesting feature of the Kaplan Meier method is to calculate median survival times in cohort in which not all patients reached the endpoint. Thus, I don't so much see the value in constructing median OS for those that died - the median OS for the full cohort (or a homogenous sub-cohort, such as st. IV disease) would be of interest

Reply 3: Thank you for this suggestion. Not all subjects died in the analysis and were still included. We have censored times and thus feel that Kaplan-**Meier is both appropriate and necessary.**

Changes in the text: Survival by stage (early and advanced) while controlling for *KRAS* mutation was added, and survival analysis based on age were also added.