Peer Review File

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Reviewer A:

The original article entitled, "KRASG12C/TP53 co-mutations identify long-term responders to first line palliative treatment with pembrolizumab monotherapy in PD-L1 high (≥50%) lung adenocarcinoma" by Frost N, et al. reported the impact of mutated KRAS subtypes with TP53 mutations as concomitant mutations on the efficacy of pembrolizumab monotherapy in high PD-L1 expressed lung adenocarcinoma in the real world setting. These results revealed the clinical novel impact of TP53 mutations in NSCLC with KRASG12C mutations. The reviewer respectfully provides the following comments:

1. *It was curious data to cause significant differences in clinical outcomes with ICI treatment between Kras-G12C and others among KRAS mutated patients. The authors have to discuss these points, based on biological or clinical aspects. In addition, patients with KRAS other plus TP53 mutations showed poor outcomes, such as PFS and OS. While it might be influenced by the small samples, the authors should discuss them.*

   Reply: We thank the reviewer for acknowledging the novelty of our study providing a rationale for the integration of KRAS and TP53 mutations into therapeutic concepts. The reviewer made an important remark questioning the underlying potentially causative mechanism for the differences observed in our investigation. As we pointed out in the discussion, extensive data linking clinical characteristics and outcome with KRAS subgroups and/or TP53 mutations have not been reported from clinical trials yet. We observed no clinical differences between KRAS/TP53-defined molecular subgroups. Additionally, the distribution of KRAS mutations in the $KRAS^{other}$ subgroups was also coincident.

   However, KRAS subgroups might display significant differences concerning key issues for response to immunooncologic (IO) approaches like smoking history, tumor mutational burden (TMB) and PD-L1 expression level. Several investigations demonstrated a different spectrum of KRAS mutations according to smoking habits. $KRAS^{G12D}$ mutations are more frequently observed in non-smokers, whereas $KRAS^{G12C}$ mutations predominantly occur in smokers (*Riely GJ et al., Clin. Cancer Res. 14 (18) (2008) 5731–5734*, *Ruppert AM et al., JTO Clinical and Research Reports. 2020; 1(3):100052*). Biologically, these clinical differences might translate into different levels of TMB. Indeed, $KRAS^{G12D}$ mutations less likely exhibit high TMB (defined as >10 mut./Mb), thus providing a potential biological rationale for a different response and outcome to checkpoint inhibition. In contrast, $KRAS^{G12C}$ mutations display higher shares of PD-L1 positivity (≥50%) as well as high TMB (*Liu SV et al., Journal Clin Oncol. 2020; 38(15_suppl):9544-9544; DOI: 10.1200/JCO.2020.38.15_suppl.9544*). Already just analyzing patients with a PD-L1 expression ≥50%, we did not observe any differences in PD-L1 expression among KRAS subgroups. However, $KRAS^{G12C}$/$TP53^{mut}$ tumors more frequently had a PD-L1 expression within the highest percentile (≥90%: 41.7 vs. 20.0%, p=0.14), which has been demonstrated as a useful predictive and prognostic marker in patients treated with pembrolizumab as first-line palliative therapy (*Aguilar EJ et al., Ann Oncol. 2019 Oct 1;30(10):1653-1659. doi: 10.1093/annonc/mdz288*; see also response to question #3 for a more detailed discussion).

Furthermore, different expression profiles of additional co-occurring mutations like STK11 and/or KEAP1, known to be negatively predictive to IO, might also have contributed to the survival differences observed in our study. The reasons, why TP53 co-mutations were either associated with the best (in combination with $KRAS^{G12C)}$ or worst survival (in combination with $KRAS^{other)}$, respectively, remain speculative. Whether TP53 subgroups themselves (disruptive vs. non-disruptive mutations and nonsense/missense/frameshift mutations, respectively) may be also linked to a distinct outcome to IO clearly warrants further investigation.

Changes in the text: Smoking behavior is correlated to a distinct spectrum of KRAS mutations with $KRAS^{G12D}$ more frequently observed in never smokers and $KRAS^{G12C}$ being the predominant mutation in smokers. The lower probability for a high TMB in $KRAS^{G12D}$ mutations might provide a molecular rationale for different responses to IO, whereas $KRAS^{G12C}$ mutations display higher shares of PD-L1 positivity (≥50%) as well as high TMB. (*Discussion, page 14, line 23 – page 15, line 3*)

2. *In table2B and Figure 2,3, the authors mentioned the Kras mutation types with or without TP53 mutations for clinical outcomes. How about TP53 mutation status (mutant or wild) for clinical impacts in NSCLC patients with Kras G12C mutations?*

   Reply: We now have updated table 2B as well as figures 2 and 3 with the requested information. Therefore, follow-up time and availability of RECIST evaluation were comparable among KRAS/TP53-defined subsets. However, substantial differences were observed for the number of cycles administered, response rate, duration of treatment and number of patients still on therapy (see table 2B). PFS for $KRAS^{G12C}/TP53^{mut}$, $KRAS^{G12C}/TP53^{wt}$, $KRAS^{other}/TP53^{mut}$ and $KRAS^{other}/TP53^{wt}$ was 33.3, 15.6, 2.8 and 13.1 months, respectively. OS was not estimable (NE), 17.9, 9.7 and 22.0 months, respectively.

   Changes in the text: However, $KRAS^{G12C}/TP53^{mut}$ patients experienced the by far longest PFS (33.3 months; 95% CI, not estimable (NE), 1- and 2-year PFS 83 and 67%) as compared to $KRAS^{G12C}/TP53^{wt}$ (15.6 months; 95% CI, 10.8 – 20.4, HR, 0.48, 95% CI, 0.17 – 1.35, p=0.16), $KRAS^{other}/TP53^{wt}$ (13.1 months; 95% CI, 10.3 – 15.9; HR 0.23, 95% CI, 0.08 – 0.72, p=0.01) and $KRAS^{other}/TP53^{mut}$, the latter group displaying the worst PFS (2.8 months; 95% CI, 0.0 – 6.2; HR, 0.18, 95% CI, 0.06 – 0.53, p=0.002, Figure 2D). (*Results, page 11, line 23 – page 12, line 3*)

   Again, survival was strongly influenced by $KRAS^{G12C}/TP53^{mut}$ (median OS not yet reached; 1 and 2-year OS 92 and 79%), as compared to $KRAS^{G12C}/TP53^{wt}$ (17.9 months; 95% CI, 12.0 – 23.8; 1 and 2-year OS 79 and 41%, HR, 0.24, 95% CI, 0.05 – 1.07, p=0.06) and $KRAS^{other}/TP53^{wt}$ (22.0 months; 95% CI, 13.6 – 30.6, 1 and 2-year OS 81 and 44%, HR, 0.23, 95% CI, 0.05 – 1.05, p=0.06). (*Results, page 12, line 17-22*)

   Table 2B and figures 2/3 have been updated likewise.

3. *In table 3, how about clinical impacts on PD-L1 expression levels between (≥90%) vs (<90%)?*

   Reply: This is an important remark. By trend, PD-L1 expression was higher in $KRAS^{mut}$ tumors (75 vs. 65%, p=0.13). Whereas no differences were observed among KRAS subgroups, $KRAS^{G12C}/TP53^{mut}$ tumors more frequently had a PD-L1 expression within the highest percentile (≥90%: 41.7 vs. 20.0%, p=0.14). According to a multicenter retrospective study from Aguilar and colleagues, a PD-L1 expression within the highest percentile might be a useful predictive and prognostic marker in patients treated with pembrolizumab as first-line palliative therapy (*Aguilar EJ et al., Ann Oncol. 2019 Oct 1;30(10):1653-1659. doi: 10.1093/annonc/mdz288*). ORR, PFS and OS according to

PD-L1 expression level (50-89% vs. ≥90%) were 32.7%, 4.1 and 15.9 months vs. 60.0%, 14.5 months and NE. In our investigation, the overall percentage of patients with a PD-L1 expression level ≥90% was 21% (25/119) and therefore only half the number of patients as compared to the study from Aguilar (42%; 80/187). However, response and outcome were comparable. Patients with a PD-L1 expression of 50-89% had an ORR, PFS and OS of 42.5%, 6.2 and 18.9 months vs. 68.0%, 13.1 months and NE with PD-L1 ≥90%.

Changes in the text:

By trend, PD-L1 expression was higher in $KRAS^{mut}$ tumors (75 vs. 65%, p=0.13). Whereas no differences were observed among KRAS subgroups, $KRAS^{G12C}$/$TP53^{mut}$ tumors more frequently had a PD-L1 expression within the highest percentile (≥90%: 41.7 vs. 20.0%, p=0.14). (*Results, page 10, line 24 – page 11, line 1*)

We identified a PD-L1 expression ≥70% as threshold for an improved survival, but observed an even more pronounced benefit in patients with a PD-L1 expression ≥90% (ORR, PFS and OS 68.0%, 13.1 months and NE vs. 42.5%, 6.2 and 18.9 months in PD-L1 <90%), thereby confirming recently published findings. (*Discussion, page 15, lines 18-22*)

Reviewer B

This is a well written manuscript presenting interesting new information on KRAS mutated lung cancer. Understanding the efficacy of immune therapy in PD-L1 high KRAS lung cancer is a significant contribution to the lung cancer literature.

Reply: We thank the reviewer for the positive assessment and for acknowledging the importance of our study.

Comments:

1. *It would be appropriate to state that the antibodies used to test PD-L1 are different from the antibody used in Keynote 24.*

   Reply: We thank the reviewer for this important remark and added the following statement to the discussion section:

   Changes in the text: The use of different diagnostic antibodies (22C3 in the KEYNOTE trials, E1L3N and QR1 in our investigation) as well as the examination by different pathologists might have biased results for PD-L1 staining. However, a growing body of evidence supports the comparability of different standardized assays and laboratory-developed tests (e. g.: *Koomen BM et al., Histopathology 2020; 76(6): 793-802; Scheel AH et al., Pathologe 2016; 37(6): 557-64*). All participating centers were certified by the quality management initiative of the German Society of Pathology (QuIP®) after having successfully passed round-robin tests for PD-L1 testing, therefore results can be regarded as comparable. (*Discussion, page 16, line 25 – page 17, line 6*)

2. *It is stated in the discussion that STK11 mutations are prognostic for response to immune therapy for lung cancer. However the current literature is also consistent with STK11 mutations being negatively predictive for response to immune therapy. It would be important to note that STK11 may be negatively prognostic or predictive.*

   Reply: We thank the reviewer for bringing up this important issue and stated more precisely as following:

   Changes in the text: We did not account for additional, presumably negative predictive and prognostic KRAS-associated co-mutations like STK11 or KEAP1, as

they were not included into the routine NGS assay (*Skoulidis F et al., Cancer Discov 2018, 8(7):822-835*). (*Discussion, page 17, line 8*)

3. *The authors appropriately note that the lack of STK11 testing is a weakness of this study. It would also be appropriate to note that this group of PD-L1 high cancers may be enriched for STK11 wild type cancers, since STK11 mutated cancers may be more likely to be PD-L1 low, and immunologically cold. The high response rate reported in this study may be related to a low rate of STK11 mutations.*
   Reply: We thank the reviewer for this valuable remark. The described molecular constellation might indeed have leaded to improved responses to pembrolizumab in our investigation. However, the available data are not conclusive in this setting. The LC-SCRUM-Japan study included 791 $KRAS^{mut}$ patients. Rates for STK11 comutations were observed in 5-9% and were comparable among KRAS subgroups (*Tamiya Y et al., Journal Clin Oncol. 2020; 38(15_suppl):9589-9589; DOI: 10.1200/JCO.2020.38.15_suppl.9589)*. Rates were substantially higher in 4.706 $KRAS^{mut}$ patients from the US with differences observed among KRAS subgroups. However, whereas STK mutations were less frequently observed in patients with $KRAS^{G12D}$ (14.2%), they were even more common in $KRAS^{G12C}$ (23.0%). $KRAS^{G12A,V,Q61X}$ exhibited comparable higher rates of STK11 comutations (*Liu SV et al., Journal Clin Oncol. 2020; 38(15_suppl):9544-9544; DOI: 10.1200/JCO.2020.38.15_suppl.9544*). Both studies included patients irrespective from PD-L1 expression. Therefore, a different behavior in the PD-L1 high subgroups (≥50%) cannot be excluded and should be addressed in further investigations.
   Changes in the text: Lower frequencies of e. g. STK11 mutations leading to immunologically cold cancers might have contributed to the improved outcome in $KRAS^{G12C}$ patients. However, recently published data in this setting are inconclusive. Whereas no differences among KRAS subgroups were observed in the LC-SCRUM-Japan study, STK11 co-mutations occurred less frequently in $KRAS^{G12D}$ but were equally present in $KRAS^{G12A,\ C,\ V\ or\ Q61X}$ in a large US cohort. (*Discussion, page 17, lines 11-16*)