Peer Review File
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Reviewer A

The authors give a retrospective one-center study with RWD on aNSCLC. From 13,340 patients with the NSCLC diagnosis, only 1,539 could be included in this analysis. In addition, data were from patients treated before 2015 and thereafter.

The authors aimed to compare patients treated with CTx 1st line in the era before ICI with patients thereafter. They concluded that patients treated by ICI are in favor in terms of OS, regardless of line of therapy.

Major points

After having RCT phase III studies with ICI monotherapy (e.g. KN24, KN42, Impower 110, EmPower01 study) as well as ICI+CTx combination (e.g. KN189, KN407, ImPower 130, 133, 150, 9LA, Poseidon) it is clear, that patients receiving ICI+/-CTx 1st line have significant survival benefit in terms of PFS and OS in comparison to patients treated with CTx alone.
All associations worldwide (e.g. ASCO, ESMO, NCCN) recommend ICI +/- CTx (depending on PD-L1 TPS) as 1st line therapy. The data presented by Liu et al. in the first part of this manuscript do not add any news to this.

Comment 1: "The data presented by Liu et al. in the first part of this manuscript do not add any news to this."
Reply 1: We thank the reviewer for the comment. We agree that this part where we show overall survival benefit of receiving ICI+/-CTx did not add to what is known from phase 3 RCTs. The reason we presented this result in the 1st part of the manuscript is to suggest that despite of its heterogeneous nature, our data is consistent with RCTs with respect to survival analysis.
Changes in the text: We added a sentence (page 9, line 9-10): "This result suggests overall survival in our data are consistent with what have been reported in randomized phase 3 trials."

The authors argue in their second part, that patients receiving ICI 2nd line after 1st line Ctx (which is not anymore state of the art) have at least the same or even better outcome in terms of OS. However, it is not possible to conclude this from the data as:
Data are retrospective
Data are collected from different eras of therapy
Data are not made comparable in terms of demographics
Only 37 patients out of 324 patients received 2nd line Ctx after 1st line ICI based therapy (in the control arm only 167 patients received 2nd line ICI out of 1215 patients receiving CTx 1st line.

In addition, this data was not compared to data from the literature. There are data from RCT

trials comparing ICI+/- CTx 1st line in comparison to CTx 1st line. These data are prospective, well balanced and with exact data on RR (no RECIST criteria in the data of this manuscript) and PFS. Furthermore, there are data of patients receiving 2nd line ICI after progressing to ICI+/- CTx as a cross over. Data from KN 24 (Pembro mono vs CTX 1st line) , KN 189 (ICI+CTx vs CTx 1st line in non squamous NSCLC) and KN 407 (ICI+CTx vs CTx 1st line in SCC NSCLC) are shown here:

KN024 – (5J Update: Reck M et al. J Clin Oncol. 2021;39(21):2339-2349.)
"Among patients initially assigned to chemotherapy, 99 received anti–PD-1 or PD-L1 (PD-[L]1) therapy (83 crossed over to pembrolizumab on-study, and 16 additional patients received anti–PD-(L)1 therapy outside the study), representing a 66.0% effective crossover rate. In the pembrolizumab group, 80 of 154 (52.9%) received additional anticancer therapy, including 12 patients who received a second course of pembrolizumab on study."
mPFS2: 24.1 months (Pembrolizumab) vs 8.5 months (Chemotherapy) HR: 0.51

KN189 – (Gadgeel S et al. J Clin Oncol. 2020;38(14):1505-1517)
"As of the data cutoff of March 8, 2022, the effective crossover rate in ITT population was 57.4%. 116/202 patients crossed over from placebo + chemotherapy to anti-PD-(L)1 therapy on (n=84) or off (n=34) study."
mPFS2 (ITT): 17.0 months (Pembrolizumab+Chemotherapy) vs 9.0 months (Placebo+Chemotherapy) HR: 0.52 (4J Update: Gray et al, WCLC 2020)
mPFS2 (PD-L1 ≥50%): 22.5 months (Pembrolizumab+Chemotherapy) vs 9.9 months (Placebo+Chemotherapy) HR: 0.47 (Gadgeel et al, ASCO 2019)
mPFS2 (PD-L1 1-49%): 16.9 months (Pembrolizumab+Chemotherapy) vs 9.1 months (Placebo+Chemotherapy) HR: 0.59 (Gadgeel et al, ASCO 2019)
mPFS2 (PD-L1 <1%): 12.6 months (Pembrolizumab+Chemotherapy) vs 8.9 months (Placebo+Chemotherapy) HR: 0.46 (Gadgeel et al, ASCO 2019)

KN407 – (Paz-Ares L et al. J Thorac Oncol. 2020;15(10):1657-1669)
"Of the 273 patients who discontinued study treatment in the placebo plus chemotherapy group, 114 who had progressive disease by blinded independent central review crossed over to pembrolizumab monotherapy on-study. A total of 29 patients from the placebo plus chemotherapy group received an anti–PD1/PD-L1 therapy (pembrolizumab, atezolizumab, or nivolumab) as subsequent therapy outside of the study, five of whom crossed over to pembrolizumab monotherapy within the study before receiving atezolizumab, pembrolizumab, or nivolumab outside of the study; thus, a total of 50.5% (138 of 273) patients in the placebo plus chemotherapy group who discontinued study treatment received subsequent therapy with a checkpoint inhibitor. "
mPFS2 (ITT): 13.8 months (Pembrolizumab+Chemotherapy) vs 9.1 months (Placebo+Chemotherapy) HR: 0.59 (3J Update: Robinson et al, ELCC 2021)
mPFS2 (PD-L1 ≥1%): 13.8 months (Pembrolizumab+Chemotherapy) vs 9.1 months (Placebo+Chemotherapy) HR: 0.58 (Paz-Ares et al, ESMO 2019)
mPFS2 (PD-L1 <1%): 14.1 months (Pembrolizumab+Chemotherapy) vs 9.1 months (Placebo+Chemotherapy) HR: 0.61 (Paz-Ares et al, ESMO 2019)

In all those trials patients receiving ICI 2nd line did not have the benefit in contrast to patients receiving ICI 1st line. Therefore, all these data are in clear contrast to the data presented in this small retrospective record-based study by Liu et al. in this manuscript. In addition, nothing of all the above-mentioned data is discussed in their paper draft.

Comment 2: "Therefore, all this data are in clear contrast to the data presented in this small retrospective record-based study by Liu et al. in this manuscript. In addition, nothing of all the above-mentioned data is discussed in their paper draft"

Reply 2: We very much appreciate the thorough and rigorous review. We agree that our results are in contrast with the overall conclusions from KN024, KN189 and KN407. We also recognize the small sample size in our analysis (167 CTx followed by ICI vs. 37 ICI followed by CTx), therefore our results should be interested with caution. We pointed out the limitations of our study in the original manuscript, and we further emphasized this point in the revision as listed below in "Changes in the text". We also added the references as the reviewer suggested. Nevertheless, our results that there were no statistically significant differences of overall survival and TTNT in patients received ICI as the 1st line vs. 2nd or later line are consistent with another RWD study (Oncologist. 2019;24(5):648-56). Our intention was merely to show RWD may not always be consistent with RCT results, possibly due to the difference of patient populations in RCT (selected based on rigorous inclusion/exclusion criteria) vs. real world (much more diverse).

Changes in the text:

1. We further emphasized the limitation of our RWD study, pointed out the contrast between our results and RCTs and therefore our results should be interpreted with caution (page 12, line 16-20): "We should point out that our results are in contrast with randomized phase 3 studies where patients receiving 1st line ICI had longer overall survival than those receiving chemotherapy 1st line and later crossing over to ICI treatment (38-40). This could be due to the heterogeneous nature of RWD and more diverse patient population in the real-world clinical setting; therefore, our results should be interpreted in caution.". In the original manuscript, we also acknowledge the limitation of RWD in the discussion section (page 13, line 20-21): "The retrospective nature of the study is fundamentally different than prospective randomized trials in a well-controlled target population.".
2. Additional references are added (page 12, line 18): reference 38, 39, 40.

Minor comments

It should have been discussed why only 11.4% and 13.7% of patients received 2nd line treatment with ICI and CTx, respectively. This percentage is not acceptable in a lung tumor center.

Comment 3: "It should have been discussed why only 11.4% and 13.7% of patients received 2nd line treatment with ICI and CTx, respectively"

Reply 3: We thank the reviewer for pointing this out. The percentage of patients received 2nd line treatment could be higher; however, if some of patients chose to continue treatment in a different clinic, the data will not be available in our database. We added discussion as below.

Changes in text: We discussed the possible reason that only 11.4% and 13.7% of patients received 2nd line treatment with ICI and CTx, respectively (page 13, line 27-31): "In our analysis of treatment sequencing (Fig. 3), only 13.7% (167/1214) and 11.4% (37/324) of patients received 2nd line treatment with ICI (after 1st line chemotherapy) and chemotherapy (after 1st line ICI), respectively. This is most likely due to the incompleteness of the data, because if patients were treated in a different clinic following the 1st line therapy at Mount Sinai Health System, the 2nd line treatment information are not available in our database."

In the Kaplan-Meier curve (Fig 5) only 12 and 25 patients received different lines of 1st- 2nd line treatments. No realistic statistical comparison can be made with this numbers. In addition, there can no conclusions be drawn.

Comment 4: "In the Kaplan-Meier curve (Fig 5) only 12 and 25 patients received different lines of 1st- 2nd line treatments."
Reply 4: We thank the reviewer for the comment. In Fig. 5, there are 3 group of patients received different lines of 1st and 2nd line treatment: 1st chemo, 2nd chemo (n=158), 1st ICI single agent, 2nd chemo (n=12), and 1st ICI-chemo, 2nd chemo (n=25). Although we showed survival curves of 3 groups, we only discussed the difference between group 1 (n=158) and group 3 (n=25). We agree the sample size is still small; in addition to the limitations that we discussed in our original manuscript, we specifically emphasized again as below.
Changes in text: We discussed the small sample size of this analysis in the revision (page 14, line 12-15): "In our analysis of 2nd line chemotherapy (Fig. 4 and 5), we are comparing 2 groups of patients (chemo followed by chemo, ICI-chemo followed by chemo) with only 158 and 25 patients in each group respectively. The small sample size underscores the necessity for replicating our findings in additional cohorts."

The scenario discussed with patients receiving 1st line CTx because analysis of driver mutations cannot be done before 4 weeks is implausible in industrialised areas of the world and against all international recommendations. In addition, ICI should be given with CTx for the 2nd or 3rd cycle of therapy when a treatable mutation could be ruled out or large irradiation of metastases is finished.

Comment 5: "The scenario discussed with patients receiving 1st line CTx because analysis of driver mutations cannot be done before 4 weeks is implausible in industrialised areas of the world and against all international recommendations. In addition, … "
Reply 5: We agree the turnaround time for genetic testing should be within 14 days as specified in guidelines. However, in routine clinical practice, the recommended turnaround time may not be always achieved. In a recent publication (Implementing Genomic Testing for Lung Cancer Into Routine Clinical Practice - The Welsh Experience; Clinical Oncology Volume 34, Issue 11, November 2022), it was reported that median turnaround time of DNA testing was 26 days in UK Welsh Thoracic Oncology Group. Regarding ICI treatment, there are still challenges in low-and middle-income countries to access the contemporary treatment options (Priorities for cancer research in low- and middle-income countries: a global perspective; Nature Medicine, VOL 28, April 2022, 649–657).

Changes in text: We added discussions of genetic testing turnaround time and access to treatment options (page 12, line 2-5): "In a recent publication (34), it was reported that median turnaround time of DNA testing was 26 days in UK Welsh Thoracic Oncology Group. Furthermore, there are still challenges in low-and middle-income countries to access the contemporary treatment options including ICI (35)."

Reviewer B

The authors explored prognostic impact of treatment sequences among patients with advanced non-small cell lung cancer applicable to immune checkpoint inhibitors. The theme itself is quite interesting and can provide significant clinical implications. However, in my opinion, substantial revisions are essential before considering this manuscript for publication. Please see my comments below.

Major comments:
1. Page 8, line 29, "~p-value=0.007; Fig. 2)": The authors need to provide p-value corresponding to K-M curve (P= 0.075), obtained from univariate analysis. I could not understand why the factor (1st Chemo or 1st ICI) could have significance (P =0.007) in multivariate analysis, not in univariate analysis.

Comment 1: as above
Reply 1: We thank the reviewer's comment. We added the p-value of univariate analysis in addition to multivariate analysis in the text. Regarding the difference between univariate and multivariate analysis, we noticed that Fig. 2A and Fig. 2B in the original manuscript were based on 2 different patient populations. We apologize for the error. In the revision, univariate analysis (Fig. 2A, p-value=0.075, HR=0.83) and multivariate analysis (Fig. 2B, p-value=0.066, HR=0.82) have similar results.
Changes in text: We provided p-values of both univariate analyses corresponding to the K-M curves and multivariate analysis (page 9, line 8-9), and updated Fig. 2.

2. Results: The median survival time (OS and TTNT) for every analysis should be provided along with hazard ratio and p-value.

Comment 2: as above
Reply 2: We thank the reviewer's advice and added the median survival time accordingly.
Changes in text: We added median survival time with 95% confidence internal in the figure legends of Fig 2, 3, 4, 5 (page 20) and Supplementary Fig 2, 3 (page 22).

3. Figure 4 & 5: Why the number at risk for Chemo-Chemo at baseline is 114, not 158? Please explain the discordance with the figure for study flow.

Comment 3: as above
Reply 3: Upon further investigation, we confirm the number of patients for the chemo-chemo

group in the study flow (n=158) is correct. However, some of the patients were inadvertently omitted in Fig. 4 and 5 during data processing. We fixed the errors and updated Fig. 4 and 5. The result section has also been updated accordingly. We very much appreciate the reviewer's question that helped us to identify the error. We also repeated all the analyses to ensure there are no other errors.
Changes in text: We updated the Fig. 4 and 5, and the result section (page 10, line 15-16).

4. Table 1: This table seems to be almost useless to interpret the data on main theme of the study. The authors should alternatively provide baseline characteristics for the groups of main interest (Chemo->ICI, Chemo->Chemo, vs. ICI -> Chemo), clearly differentiating ICI-Chemo and ICI monotherapy. Also, the authors are encouraged to provide sufficient information on treatment regimen either for chemotherapy and ICI therapy.

Comment 4: as above
Reply 4: We appreciate the reviewer's advice. We moved Table 1 from the main text into supplementary information as Supplementary Table 1. We also added Supplementary Table 2 to provide baseline characteristics for the groups of main interest in Fig. 4 and 5. The suggestion to provide more details on treatment regimen for chemotherapy or ICI is similar to reviewer C's 1st comment (see below), and we updated the result section.
Changes in text: We changed Table 1 to Supplementary Table 1. We added Supplementary Table 2. We added more details on treatment regimen (page 8, line 26 to page 9, line 3): "We further examined more details on treatment regimens for ICI-based therapy and chemotherapy. In 1,214 patients treated with 1st line chemotherapy, 874 (72%) received platinum-based therapy. In 324 patients treated with 1st line ICI-based therapy, 155 (48%) received ICI agents without chemotherapy (145 ICI single agent, 10 nivolumab/ipilimumab); 169 (52%) patients were treated with ICI-chemotherapy combination, and majority (150 of 169) were pembrolizumab-containing regimens. In 167 patients received 2nd line ICI after progressing on 1st line chemotherapy, 143 (86%) were treated with a single ICI agent; 22 (13%) were treated with ICI-chemotherapy combination (pembrolizumab-based or atezolizumab-based); 2 (1%) patients received nivolumab-ipilimumab combination."

5. Supplementary figure 1: The authors are encouraged to provide study flow with precise numbers of subjects for the subgroup of interest corresponding to K-M curves. Without sufficient explanation in the main text. It might be hard for readers to understand the selection process for each analysis.

Comment 5: as above
Reply 5: We thank the reviewer's suggestion and updated the consort diagram with the number of subjects corresponding to K-M curves in Fig. 2-5.
Changes in text: Supplementary Fig. 1 is updated.

Minor comments:
1. Abstract: The numbers of subjects for each subgroup of interest should be provided along with results in the abstract. As sample size for each group is relatively small, the description,

"A retrospective cohort study of 13,340 lung cancer patients~", without these numbers seems to be misleading and not fair.

Comment 6: as above
Reply 6: We agree with the reviewer the number 13,340 is mis-leading and unfair.
Changes in text: Abstract is updated. We explicitly point out the 2,106 patients with systemic therapy data is the start point of data analysis (page 2, line 7-8): "Systemic therapy data of aNSCLC in 2,106 patients was the start point in our analysis to investigate … ". Details of sample size in each specific analysis are described in the main text and labelled in figures.

2. Abstract, Results, "reflecting the advancement in developing novel immunotherapy and new generations of targeted agents": This part should be removed because it seems subjective with authors' interpretation.

Comment 7: as above
Reply 7: We appreciate the reviewer's advice and removed "reflecting the advancement in developing novel immunotherapy and new generations of targeted agents" in the abstract.
Changes in text: Abstract is updated (page 2, line 13-14).

3. Abstract, Conclusion, "Docetaxel, gemcitabine or pemetrexed, routinely used following platinum doublet 1st LOT, is effective": This is not the conclusion drawn from this research. As the authors did not provide details on regimens of chemotherapy, it can be overinterpretations.

Comment 8: as above
Reply 8: We agree with the reviewer the statement can be overinterpretations. We changed this sentence to be less specific regarding the drugs, but more precisely aligned with the scope of our work (see below).
Changes in text: Abstract is updated (page 2, line 25): "The chemotherapies routinely used following platinum doublet 1st LOT, is effective …".

Reviewer C

This is an interesting single-center study that evaluates the impact of ICI treatment in advanced-stage disease patients with NSCLC. Although important and necessary the data set lacks essential endpoints and insights:
Please specify all Chemo/ICi protocols in the first line.
Please specify all ICI treatment options in the second line.

Comment 1: as above, for additional details of the data.
Reply 1: We thank the reviewer's suggestions. We added details of treatment regimens in the revision; this is also to address reviewer B's major comment #4.
Changes in text: We added more details on treatment regimen (page 8, line 26 to page 9, line 3): "We further examined more details on treatment regimens for ICI-based therapy and

chemotherapy. In 1,214 patients treated with 1st line chemotherapy, 874 (72%) received platinum-based therapy. In 324 patients treated with 1st line ICI-based therapy, 155 (48%) received ICI agents without chemotherapy (145 ICI single agent, 10 nivolumab/ipilimumab); 169 (52%) patients were treated with ICI-chemotherapy combination, and majority (150 of 169) were pembrolizumab-containing regimens. In 167 patients received 2nd line ICI after progressing on 1st line chemotherapy, 143 (86%) were treated with a single ICI agent; 22 (13%) were treated with ICI-chemotherapy combination (pembrolizumab-based or atezolizumab-based); 2 (1%) patients received nivolumab-ipilimumab combination.".

Please add all PD-L1 status of all patients
Comment 2: as above.
Reply 2: We added PD-L1 status summary in the discussion section.
Changes in text: We summarized PD-L1 status in the discussion section and pointed out only a subset of patients treated with ICI had PD-L1 data (page 13, line 25-27): "Of the 553 patients (Supplementary Fig. 2) received ICI in their treatment history, only 359 (65%) had PD-L1 data (237 positive and 122 negative)."

Please show data in depth of sequence pattern of treatment after second line.
Comment 3: as above.
Reply 3: We responded to reviewer A's comment #3 regarding small percentages of patients who received 2nd line treatment, and the number of patients with 3rd treatment information is even smaller. Since the focus of this study is on the 2nd line treatment, we felt it would be inadequate to present 3rd line treatment data due to the small sample size. Nevertheless, we agree with the reviewer that the depth of sequencing pattern is important, and therefore added this point in the discussion section.
Changes in text: We discussed the importance of treatment depth and the lack of data in our study (page 14, line 15-17): "Moreover, although the depth of treatment is an important aspect of cancer care, we do not have a sufficient number of patients who received more than 2 lines of therapy to study the depth of treatment."

There is NOS pathology listed in 30% of patients - is this correct?
There is all lack of data regarding the details of the protocol.

Comment 4: as above.
Reply 4: We checked the 2,106 patients with systemic therapy as the start point of our analysis, NOS is listed in 32% patients based on our curation of the data. Regarding study protocol, since this is a retrospective analysis of the real-world data, therefore, there is no specific "protocol" of the study.

The overall impact of the manuscript could be strengthened by highlighting the failure of first line and identifying the parameters why so.

Comment 5: as above
Reply 5: We agree with the reviewer. Due to the nature of real-world data, oftentimes there is

no specific descriptions on the reason of treatment failure. This is in contrast with clinical trials where reasons for treatment discontinuation (e.g., disease progression or adverse events) are well documented. Therefore, TTNT (time-to-next treatment) is typically used as a surrogate, real-world clinical endpoint for PFS. For some of the patients, reasons for treatment failure could be captured in the medical oncologists' notes. However, if would take significant amount of work to manually curate through the free-text notes of the patient population in this study.

Changes in text: We added discussion of this topic in the section on study limitations (page 14, line 4-8): "Fourth, while reasons for treatment failure such as disease progression or adverse events would be valuable to analyze and share with scientific and clinical community, the information is not always captured in the RWD. Even for those patients with such information discussed in medical oncologists' notes, manual curation of free-text notes can be resource consuming."