Peer Review File

Article Information: https://dx.doi.org/10.21037/tlcr-21-460

Comment 1: *All consecutive patients diagnosed with NSCLC that were candidates for immunotherapy were included in this study?*

Reply 1: All consecutive patients with metastatic NSCLC that were discussed in our multidisciplinary conference and they were candidates for receiving immunotherapy in our center were screened for this study. Patients with *EGFR* mutations or *ALK* genomic alterations were excluded before the initial screening. 93 patients in total had received PD-1/PD-L1 inhibitors as treatment for lung cancer within the time period of 2017-2020 in our center. 83 patients had sufficient clinical (BMI, body weight fluctuations) and/or radiological data in order to be classified as being cachectic or not, according to criteria set by Fearon et al. These 83 individuals were included in the final analysis.

Changes in the text 1: We have modified our text in the manuscript in section 2.1 (Patient selection), 1st paragraph, lines 76-80 and in section 2.2 (Cachexia assessment), 2nd paragraph, lines 93-97 in order to be more specific about patient selection.

Comment 2: *The authors state that they employed Fearon's criteria to define cancer cachexia syndrome, however in the flow chart of the study it looks like some patients were considered as having cachexia based on LSMI. The authors should rewrite the manuscript and the flow chart to make this more clear. I suppose 94 patients were eligible, but only 83 met Fearon's criteria and then from these patients only 54 had LSMI measurement.*

Reply 2: Thank you for this important comment. As it was stated in the reply in comment 1, only the patients that were able to be classified as cachectic or not based on the available data were included in the final analysis. 94 patients with EGFR wild type and ALK wild type metastatic NSCLC that received immunotherapy as treatment were initially screened. For all the patients that had available baseline LSMI values we also had sufficient data about their weight fluctuations, thus they were all been able to be classified for cachexia according to criteria by Fearon et al. 29 patients that did not have available baseline LSMI values were able to be classified for cachexia according to the definition by Fearon et al based only on their body weight changes and their BMI values within the last 6 months before immunotherapy initiation. The rest 11 individuals that we did not have sufficient clinical and/or radiological data in order for them to be classified for cancer cachexia they were excluded from the final analysis. We have also changed the flow chart of our study (Suppl. Figure 1) in order for the patient selection process to be clearer.

Change in the text 2: We have modified our text in the manuscript in section 2.2 (Cachexia assessment), 2nd paragraph, lines 93-97 and we also changed suppl. Figure 1 (Flow chart of our study).

Comment 3: *Which genomic tests were performed to rule out an actionable genomic alteration? Which genes were investigated?*

Reply 3: Thank you very much for your important comment. Individuals with *EGFR* mutations or *ALK* translocations were excluded before the initial screening. EGFR mutational status was examined by PCR and ALK translocations by ICH or FISH, respectively. We have included this information in our manuscript.

Change in the text 3: We have modified our text in the manuscript in section 2.1 (Patient selection), 1st paragraph, lines 77-80.

Comment 4: *At what time was PD-L1 expression evaluated? At diagnosis? Before treatment initiation? And which test was used?*

Reply 4: Thank you for this comment. PD-L1 expression levels were evaluated before the initiation of systemic treatment (Immunotherapy or platinum doublet). 36 patients (26 individuals who received pembrolizumab and 10 that received nivolumab) had their samples evaluated using staining with 22C3 monoclonal antibody pharm Dx. The remaining 12 patients with available PD-L1 status had their samples evaluated using Ventana SP142 assay.

Change in the text 4: We have modified our text in the manuscript in section 2.3 (Data collection), 2nd paragraph, lines 112-115.

Comment 5: *Regarding response rate determination, the authors informed they used RECIST 1.1. Is it common practice to use RECIST in the institution or were the images reviewed specifically for this study?*

Reply 5: Thank you for this comment. The images were reviewed specifically for this study in order to determine response assessment according to RECIST 1.1 criteria.

Change in the text 5: We have modified our text in the manuscript in section 2.4 (Outcome assessment), 1st paragraph, lines 122-123.

Comment 6: *The authors lumped up together patients treated with ICI in first and second line, nevertheless there is a high probability that patients treated in second line have worse performance status, poor fitness and higher tumor burden that could lead to a higher rate of CCS. I would recommend the authors to list and compare the clinical and demographic characteristics of the patients treated in first and second lines. If some difference arises, the authors should test the impact of CCS in each population separately.*

Reply 6: Thank you very much for this important comment. As seen in the diagram below patients that received immunotherapy as 1st line treatment experienced improved survival in comparison to individuals that received immunotherapy as 2nd line treatment.

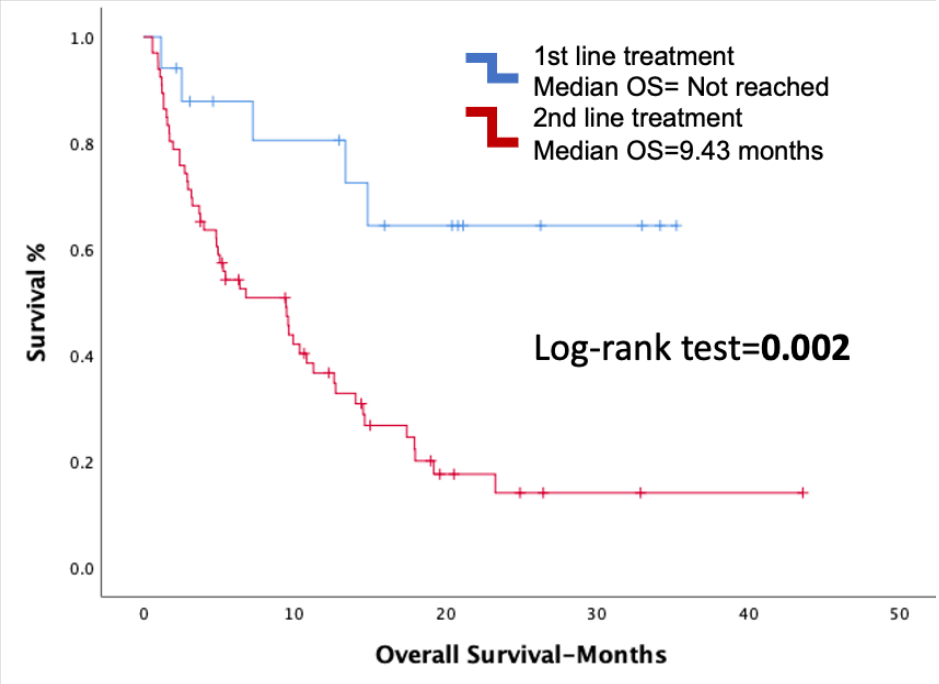
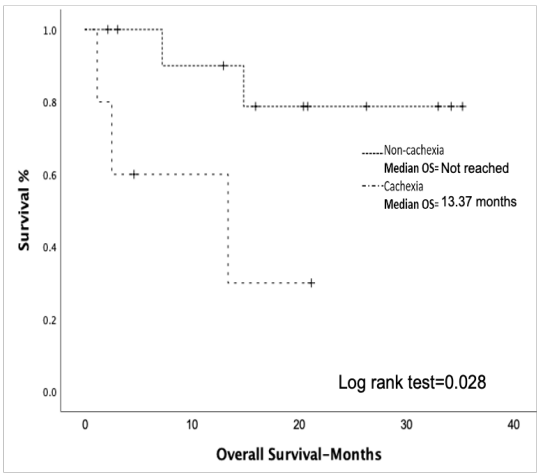


Figure 1: Log-rank test demonstrating the difference in survival curves amongst the patients that received immunotherapy as 1st line treatment vs those that received immunotherapy as 2nd line treatment.

However, this is an expected result, thus we have not included it in our manuscript. Following your comment, we performed additional subgroup analysis on the effect of cachexia syndrome on overall survival amongst the patients that received immunotherapy as 1st line therapy and those who received immunotherapy as 2nd line, respectively by applying log-rank test. Cachexia was significantly associated with reduced survival in both subgroups of patients as demonstrated in the figures below. Due to the low number of patients in these subgroups we did not perform any additional multivariate analyses.

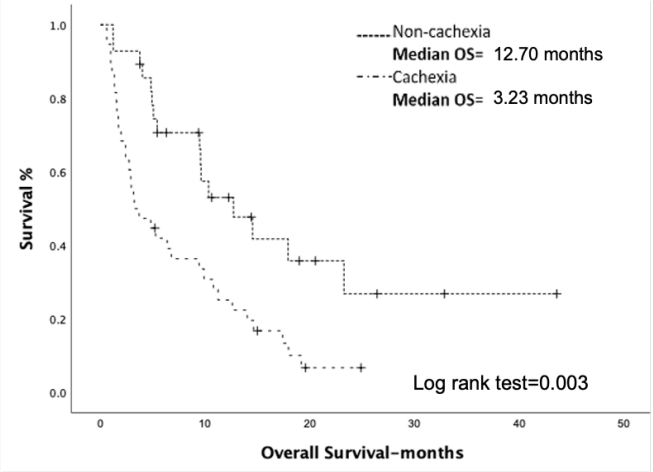
A.



Number at risk:

Non-cachexia	12	9	6	3	0
Cachexia	5	2	1	0	0

B.



Number at risk:

Non-cachexia	28	13	5	2	1	0
Cachexia	38	11	1	0	0	0

Figure 2: Log rank test demonstrating the effect of cancer cachexia syndrome amongst the patients subgroups that received PD-1/PD-L1 inhibitors as **A.** First line treatment and **B.** Second line treatment.

We have added these results in our manuscript as response to your comments.

Change in the text 6: We have added these results in the text of our manuscript in section 2.5 (Statistical analysis), paragraph 3, lines 145-147 and on section 3.3 (Effect of the studied variables on survival outcomes), paragraph 4, lines 206-208.

Comment 7: *The median follow-up time is quite short and is quite superimposed to the median survival time. Besides, the observed median overall survival is shorter than it would be expected even when ICI is used as second line treatment (median 12 months for non squamous NSCLC). Additionally, it is said the study started collecting data from 2017 till 2020, this would give us 3 years at least of follow up for the entire population. Were patients preferentially included in the more recent years? The authors should provide a landmark survival analysis at 6 months or show actuarial survival curves to account for the impact this short follow up period could have on overall survival analysis.*

Reply 7: Thank you very much for this insightful and important comment. Because treatment with PD-1/PD-L1 inhibitors became common practice in our center after 2018 there was a gradual increase in accrual after the second half of 2018. In order to avoid this potential selection bias we performed a landmark survival analysis at 6 months using 6 months survival as a cut-off value. Individuals (6 patients in total) that were alive at the time of data analysis but had a follow-up time shorter than 6 months were censored at the time of their last follow-up. In addition, we performed a univariate Cox regression analysis to estimate the hazard ratio of cancer cachexia on 6 months survival time.
As seen in the diagram below the presence of baseline cancer cachexia was significantly associated with inferior 6 months survival rate. In addition, individuals with baseline cancer cachexia had almost 4 times higher probability of death within the first 6 months since the initiation of immunotherapy.

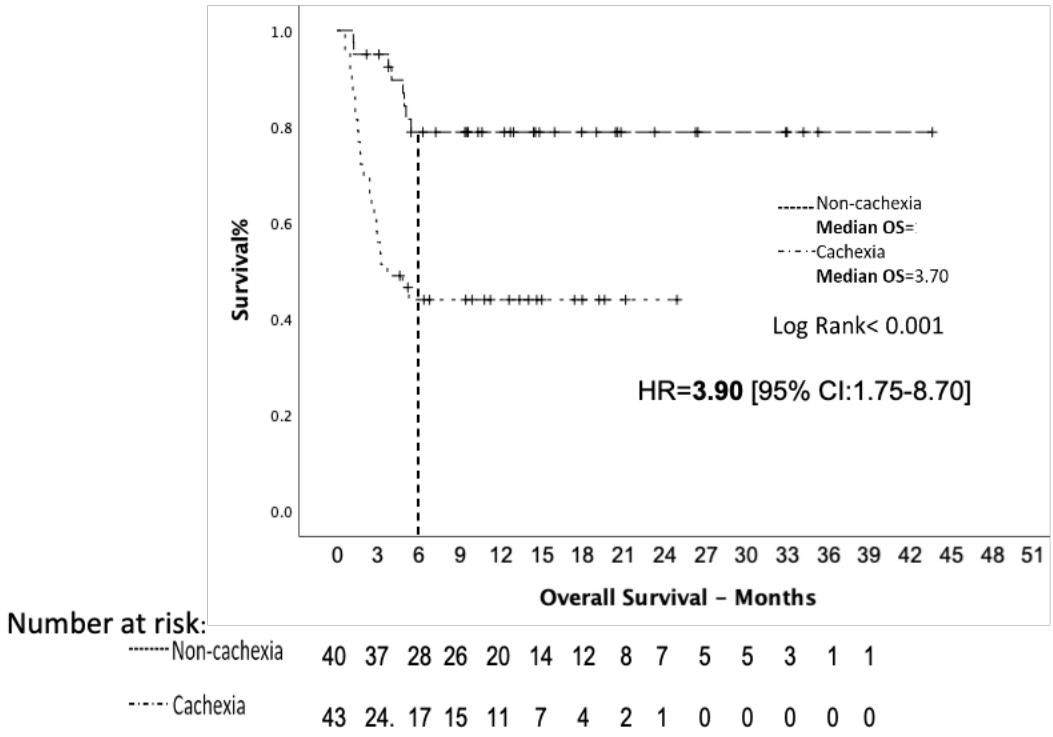


Figure 3: Log-rank test demonstrating the effect of cancer cachexia syndrome on 6 months survival.

Change in the text 7: We have added this information in our manuscript in section 2.5 (Statistical analysis), paragraph 4, lines 152-155, section 3.3 (Survival outcomes), paragraph 4, lines 208-210 and section 3.4 (Univariate and multivariate analysis), paragraph 3, lines 222-224. In addition, we have added the above figure as supplementary figure 4.

Comment 8: *Images and legends are too small in Figure2. The legends are also too small in figures 3 and 4.*
In figures 2, 3 and 4, write down what the abbreviations ICI, CCS and LSMI mean.

Reply 8: Thank you very much for this comment. We have changed figures 2,3 and 4 in order to improve their visibility and we have added the requested abbreviations.

Change in the text 8: We have increased the size of figures 2, 3 and 4 and we have added the abbreviations ICI, CCS and LSMI.