Peer Review File

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Reviewer A

Comment 1: For those patients who are not good candidates for repeat biopsies pre, during, and post-treatments with anti-PD-L1, this work shows a way to follow whether PD-L1 positive CTCs are being eliminated. Immunohistochemistry on repeat biopsies would not show elimination of specific cancer cells; whereas, using CTCs one would be able to show by performing immunohistochemistry on the single CTCs whether PD-L1 positive cells were being selectively reduced. The work presented is original, significant, and of good quality.

Reply1: The authors are grateful for the recommendation

Reviewer B

The authors investigated PD-L1 expression on CTCs in SCLC patients using two methods. One is the CellSearch system which isolates EpCAM positive circulating cells, and another is the Parsortix system which separates cells based on size and shape. They found the positive rate of CTCs between the two methods was similar and could assess PD-L1 expression on CTCs.

Comments

Comment 1. The correlation of PD-L1 expression between tumor tissue and CTCs needs to be evaluated.

Reply 1: We agree with the reviewer that this is an important information. Unfortunately, we were not able to compare the expression of PD-L1 on CTCs to that of the matching tumours as samples were not available for profiling for most patients. PD-L1 assessment is not routinely done for SCLC patients. We have indicated in the revised manuscript as a study limitation Page 14 line 337-340: *'Finally, PD-L1 expression assessment is not a routine practice for SCLC. Thus, we could not compare the expression of PD-L1 on CTCs to that of the matching tumours as samples were not available for evaluation.'*

Comment 2. As mentioned by the authors, previous phase III studies have not demonstrated the association between the expression of PD-L1 on tumor cells and clinical responses to ICIs in SCLC patients. The authors need to explain why they assessed CTCs in SCLC patients.

Reply 2: We agreed with the reviewer and as we already stated in the manuscript tumour PD-L1 expression has been demonstrated to be a non-discriminatory biomarker in SCLC. However, However, it is possible that retaining PD-L1 might represent one of the mechanisms

that CTCs use to survive immune system attack while in circulation and, therefore a better readout of a pre-existing anti-tumour response. Moreover, studies in NSCLC and melanoma have shown that PD-L1 expression on CTCs a promising prognostic biomarker in patients treated with immune checkpoint inhibitors, despite the lack of correlation with the expression on matching tumours. We have expanded our explanation in the discussion session. See page 14, line 313 to 318: '*However, it is possible that retaining PD-L1 might represent one of the mechanisms that CTCs use to survive immune system attacks while in circulation and, therefore a better readout of a pre-existing anti-tumour response. Previous studies in melanoma and NSCLC have shown that PD-L1 expression on CTCs a promising prognostic biomarker in patients treated with immune checkpoint inhibitors, despite the lack of correlation with the expression on matching tumours'.*

Comment 3. In this study, SCLC patients who did not receive ICIs were included. The impact of PD-L1 expression on CTCs on survival should be evaluated in SCLC patients treated with ICI.

Reply 3: The treatment plan for SCLC patients evolved during the study. Only 9 patients of the 14 patients with CTCs, were treated with chemotherapy combined with immunotherapy. The association of PD-L1^{+}CTCs with survival among patients treated with immunotherapy was impossible, given their small number in this cohort. We have indicated in the revised manuscript as a study limitation Page 14 line 330-332: '*It was not possible to assess the association of PD-L1+CTCs with survival among patients treated with immunotherapy, given*

their small number of cases (9 of 21) in this subgroup'.

Comment 4. The number of CTCs differed between the two methods (Fig. 3). The authors need to explain the reason for this.

Reply 4: The difference in CTCs counts between the two methods was primarily in two samples (1355 and 1360), with EpCAM-magnetic beads recording high numbers compared to the Parsortix system. This might be partly because SCLC CTCs are relatively small and may not be efficiently retained by the Parsortix system which isolates CTCs based on size and deformability. We have expanded our explanation in the discussion section. The section has been revised, Page 12 Line 272-276: *'On the other hand, the number of Parsortix-isolated CTCs was lower those isolated using EpCAM-beads, in particular for the two patients with the largest number of CTCs. SCLC CTCs are relatively small, compared to other carcinomas and may not be efficiently retained by the Parsortix system which isolates CTCs based on size and deformability'*

Reviewer C

Comment 1: The correct agency name is the U.S. Food and Drug Administration. Note that this accelerated approval was rescinded in 2021.

Reply 1: We have made changes to the correct agency name. Page 3 line 75: '*Combination*

with chemotherapy gained US Federal Drug Administration (FDA)'.

Comment 2: "The observed clinical outcomes from these trials have not been striking, with only a week of progression-free survival (PFS) and 2 months of overall survival (OS) benefits [8, 9, 11]." This sentence fails to recognize the key metric in immunotherapy trials is the percentage of patients with prolonged remissions, i.e., the plateau on the survival curve. Since there has never been much of one before, many would consider this outcome striking, I suggest revision.

Reply 2: We have edited the sentence in Page 3 Line 76 – 80: '*Despite immune checkpoint inhibitors becoming a primary component of SCLC treatment, their efficacy is modest, with only 2 months of overall survival (OS) benefit and limited to a small subset of patients. Hence, there is a need to identify biomarkers that will help determine a subgroup of SCLC patients most likely to benefit from these treatments.'*

Comment 3: Smoking status in table 1 should be described as current, former, and never smokers rather than yes or no. Similarly, the radiation should be described as whether it was pre or post-blood draw.

Reply 3: The data on smoking status was collected as smokers and non-smokers. The smokers included those who smoked at least 10 packs a year (i.e. one pack a day for 10

years) either former or current. There was no specific time frame defined. We have specified this in the data collection section for clarity. Page 4 Line 135 – 137: 'Text changes: *collected. Smoking status was collected as smokers and non-smokers. The smokers included those who smoked at least 10 packs a year (ie one pack a day for 10 years) either former or current.'*

Comment 4: The study population has a surprisingly low survival given that 23% of the patients had a single site of metastasis and a relatively young age and good performance status. Some thoughts on this by the authors would be helpful to know if the population is representative of the disease. Also, the better survival in patients with poor performance status is downright weird and deserves some kind of commentary or explanation.

Reply: We revisited the data and realized of our error when entering the data in Table 2. In the univariate analysis results for ECOG status we used ECOG >=2 as reference group instead of ECOG 0. We have corrected this in Table 2 in Page 23. However, the values entered for age are correct.