Peer Review File

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Reviewer A

Comment 1: The CEA trend and imaging findings are described at various points along the patient's treatment course. Was cell-free DNA obtained in this case? This might be another data point of interest if available. Similar question for PET-CT - it would be interesting to know whether these sarcoid lesions were FDG-avid, and whether that changed throughout the treatment course.

Reply 1: Thank you. In this case, cell-free DNA was not obtained, nor was a PET-CT, but we agree with the reviewer that these investigations would be of interest.

Changes in the text: None

Comment 2. The full extent of liver disease is unclear from the case presentation - was liver resection and/or hepatic arterial infusion therapy or other locoregional strategies considered? If not, it is perhaps notable to highlight the apparent long-term disease control of colorectal liver metastasis after 6 months of ICB in this case.

Reply 2: On the basis of bilobar liver metastases as well as extra-hepatic disease (retroperitoneal lymphadenopathy), upfront systemic therapy was considered appropriate rather than hepatic arterial infusion therapy or other locoregional strategies at that time, and these approaches have not been required to date in view of the excellent ongoing response in the liver.

Changes in the text: We have added the following sentence at line 137 and 138; “we continue to observe a sustained clinical, radiological and biochemical response, including in the liver, despite no additional locoregional or systemic therapy”. We have also added detail of the burden of metastatic disease as the rationale for systemic therapy upfront in Line 97 and 98.

Comment 3. The liver lesions described around line 117 - these were felt to be sarcoid as well rather than progressive disease? One of the references (#11) reports liver sarcoid in this setting, but it does not seem definite that sarcoid in the inguinal node and mediastinum of this patient is the same process as the liver lesions. I assume if the "progressing" liver lesions were biopsied this would have been presented in the manuscript, but would suggest clarifying this point. Also in line 68, "hepatic and intra-abdominal lymphadenopathy" - new hepatic parenchymal lesions? Or new perihepatic (?portal) lymphadenopathy? Additional clarity around intraparenchymal hepatic lesions versus intra-abdominal lymphadenopathy would be useful.

Reply 3: Thank you. You are correct in suggesting that the enlarging liver lesions were not biopsied, and the decision was made that the left inguinal adenopathy was representative of

the overall disease process at our institutional multi-disciplinary meeting, and it was the most easily accessible site for biopsy. This has been clarified in the text now.
Regarding the "hepatic and intra-abdominal lymphadenopathy" mentioned in line 68 was in reference to parenchymal liver lesions, and right para-aortic nodes. This detail has been added to the text now for clarity.

Changes in the text: Addition of this sentence to the text "Given its accessibility and safe access, a left inguinal lymph node was biopsied as a representative lesion, in place of pursuing a biopsy of the liver or other sites" in Lines 126 – 128.
Addition of the details of "parenchymal liver lesions, and para-aortic adenopathy" in line 71.

Comment 4. Given the high TMB and germline MSH mutation, ICB was of course an appropriate treatment in this case. It would also be interesting to know any other mutations identified on the MSK-IMPACT panel (ie BRAF, KRAS, etc) (line 86), or if none were found state that the tumor was wild type.

Reply 4: Thank you. Neither KRAS nor BRAF mutations were identified in this case. Alterations of note including TP53 and PIK3CA alterations were identified.

Changes in the text: KRAS and BRAF alterations were not identified to line 91.

Comment 5: Figure 5 - would be ideal to show additional slices of the CT and/or point out the presumed intrahepatic sarcoid nodules for illustrative purposes. Any comment from the radiology point of view on any ways to distinguish these lesions from true hepatic progression of disease? Perhaps they are indistinguishable by imaging - in which case it would be valuable to point that out.

Reply 5: Thank you. With the help of our radiologist, we have provided an updated image for Figure 5 demonstrating liver metastasis with an arrow, and sarcoid nodules in the liver with small circles, as requested. Our radiologist who is an author on the paper, has advised that it is difficult to confidently decipher hepatic metastases from sarcoid nodules radiologically, but that the latter is favored on the basis of the fact that the subcentimeter nodules are all approximately the same size, and distributed evenly throughout the liver.

Changes in the text: Updated image provided (Figure 5), and we have added the Line 128, 129 and 130 to clarify the difficulty in distinguishing progression of disease from an alternative cause in this case.

Comment 6: Line #66 - should clarify that this biopsy was by EBUS, to distinguish from the subsequent inguinal biopsy. Currently the phrasing is slightly confusing as to the sequence of events (although clarified later in the detailed case report write-up).

Reply 6: Thank you, we appreciate this was confusing and have edited same.

Changes in the text: Line 68 clarifies that the initial lymph node sampling was by EBUS.

Comment 7: #88 - Paternal family history was unknown at all? or there was no paternal family history of malignancy? Should clarify the phrasing.

Reply 7: Paternal family history was unknown.

Changes in the text: Lines 92 and 93 have been edited to “The patient was unaware of any details of his paternal family history, including any history of malignancy”.

Comment 8: #151 - I believe an unnecessary *change* in systemic therapy is being described, but perhaps there is an editing error with a missing word.

Reply 8: Thank you for noting this omission.

Changes in the text: Line 164 has been edited to reflect “changes in systemic therapy”.

Comment 9: #85 - the figure reference is incorrect - the IHC figure is Figure 3 not Figure 2

Reply 9: Agreed, thank you. In the most recent draft we have changed the figures slightly from the original manuscript and Figure 2 now represents the IHC.

Changes in the text: None

Comment 10: Figure 3 - should confirm the labeling of the panels - A and D appear to show absent staining while B and C show present staining, but the labels indicate the opposite

Reply 10: Thank you for pointing this out. The correct labels have been edited in the figure caption.

Changes in the text: Labels corrected.

Reviewer B

Comment 1: Why did the author not perform a tissue biopsy from the liver? It is mentioned that the liver lesion was clinically diagnosed as SLR without undergoing a tissue biopsy. Could you please explain whether the new hepatic lesion could be diagnosed as SLR without liver biopsy?"

Reply 1: Thank you for this query. The case was discussed at our institutional multidisciplinary team meeting, where the consensus recommendation was that tissue sampling of one site of concern on imaging would be representative of the overall disease process, and due to ease of access and safety, the inguinal lymph node was chosen as the site for biopsy. In addition, on discussion with our radiologist, who is an author, while it is difficult to fully decipher hepatic metastases from sarcoid nodules radiologically, the latter

is favored in this case on the basis of the fact that the subcentimeter nodules are all approximately the same size, and distributed evenly throughout the liver. We appreciate your comment however, and have aimed to clarify this in the text.

Changes in the text: Edits made to Lines 128 to 134 to discuss the MDT discussion surrounding this decision.

Comment 2: Isn't the word "institution" in lines 129 and 141 a mistake for "initiation"? Please confirm it.

Reply 2: Thank you – the word institution has been changed to initiation in the text.

Changes in the text: The word "institution" has been replaced by "initiation" in the text (Line 141 and Line 153 of the updated manuscript).

Comment 3: It is believed that the "I" before "Intra-thoracic" in line 143 was accidentally left undeleted." Please confirm it.

Reply 3: Thank you for pointing this out.

Changes in the text: The I before "Intra-thoracic" has now been removed (Line 155 of updated manuscript).

Reviewer C:

Comment. 1
The clinical course of pulmonary lesions is difficult to understand because the CT scan is only presented in Figure 7 (8 months off pembrolizumab). Can you add any Figures that makes it easy to understand?

Reply 1: Additional images and updated timeline of pulmonary findings provided for Figure 7.

Changes in the text: Updated Figure 7

Comment. 2
How did the pulmonary and extra-pulmonary lesions change now (24 months since his last dose of ICB)? I am very wondering that these lesions are disappeared, reduced or remained. Especially, I think it is important to show the clinical course of pulmonary and liver SLR lesions in details because these lesions have not been pathologically proven to be SLR.

Reply 2: Additional images and updated timeline provided for Figure 1,5,6 and 7 to better describe the clinical course of the pulmonary, liver and inguinal node findings.

Changes in the text: Edited Figures 1,5, 6, 7 and captions for each.

Comment. 3
Was TBLB for lung lesions not considered?

Reply 3: Thank you. An endoscopic bronchial ultrasound and lymph node sampling was performed, with no trans-bronchial lung biopsy in this patient. The rationale behind the decision to not proceed with a TBLB was that the pulmonary findings and the lymph node changes were identified to have arisen at the same time, and therefore thought to represent the same process.

Changes in the text: None

Comment. 4
I agree that ICB is the cause of SLR, but why not mention other causes in Discussion?

Reply 4: Thank you, we have added in other possible differential diagnoses including infection, other auto-immune processes, and the possibility of "nodal flare" which has been described in the literature in the neoadjuvant setting.

Changes in the text: Addition of Line 165 – 168 to address this query.

Comment. 5
I think that the number of Figures in the main text (line 119, Figure 4,5,6) is wrong.

Reply 5: Thank you. These have been edited to correct the correct Figure numbers.

Changes in the text: Figure numbers have been corrected (Line 125, Line 126).

Comment. 6
I found a few misspellings in the main text. For example, "irEAs" in line 134, "I" Intra-thoracic involvement in line 143, "Non-nocrotizing granuloma" in Figure 4A.

Reply 6: Thank you, these have been corrected.

Changes in the text: Line 146, Line 155, Figure 4A, all edited.