Peer Review File

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Reviewer A

It is a good article, patient with CCA we all know that the recurrence rate was high and patient received standard adjuvant treatment.
Monitor with periodic CA19-9 and imaging are standard
ctDNA made from patient's tumor cell is new way to detect residual disease and become standard in many solid tumors but still in the beginning era in CCA
This case report encourages further study of ctDNA as a monitoring tool for recurrence
This patient is very lucky to have MSI-H because it is infrequent finding especially in extrahepatic CCA anyway we know that pembrolizumab is standard in MSI-H in metastatic setting but this patient is consider using pembrolizumab as adjuvant setting which is not standard, I suggest author to mention this in your article
> We truly appreciate the input and comments from reviewer 1. We emphasized and mentioned that the use of pembrolizumab in the adjuvant setting is not standard (Please see page9 line 155-163).

Reviewer B

This is a fascinating case, authors present a unique situation where ctDNA detected MRD during adjuvant therapy for iCCA. the utility of ctDNA here is not standard of care but it demonstrated early rise months ahead of rise in CA-19.9. The case is equally fascinating as it detected MSI-H and TMB-H status for which relatively low-risk intervention (pembrolizumab) helped clear the ctDNA and patient remained NED.

Some minor comments to authors:
1) Consider highlighting the relative mismatch repair protein(s) loss leading to MSI-H status. Since authors indicate there was no actionable mutation - this raises the possibility of Lynch syndrome if BRAF V600E was negative and authors should highlight germline testing result as appropriate.
> We sincerely appreciate the input and comments from reviewer 2. We agree that, given the patient's BRAF V600E wild-type status, Lynch syndrome is a possibility. However, germline testing was not conducted due to the patient's age of 81, and cholangiocarcinoma was his initial malignancy. Additionally, there is no family history of malignancy. Therefore, Lynch syndrome is considered highly unlikely in this case.

2) Since Natera uses somatic mutations in their ctDNA calling, authors should attempt to obtain this information from Natera, it would add value to know which mutations were tracked and cleared with immunotherapy.
> We sincerely value the input provided by reviewer 2. Regrettably, obtaining the specified data from Natera poses a significant challenge.

3) Authors should highlight the remarkable rarity of this case, MSI-H iCCA is quite rare and while identification led to improved outcome, authors should help readers understand their rationale (risk vs. benefit) if this was a non-MSI-H setting, what would be the pros/ cons of considering alternative adjuvant regimens - and while none of the other regimens have Category 1 evidence, would it be reasonable to consider addition of additional therapy on capecitabine. Authors highlight in the conclusion that other actionable mutations could lead to similar outcomes in the appropriate setting but should also present their rationale for non-actionable mutation setting.

> We sincerely appreciate the input and comments from reviewer 2. We have added a paragraph (Please see Pages 9-10, Lines 155-168) where we addressed issues related to the potential risks and benefits of the ctDNA-guided escalation adjuvant strategy in non-MSI-H and non-actionable mutation settings. Additionally, we highlighted the rarity of MSI-H in Cholangiocarcinoma on Page 8, Line 142.

4) Building on the last point, the clinical dilemma associated with ctDNA detection in adjuvant setting should be highlighted. While ctDNA helped in this case, it should be highlighted that "this is not standard of care" and there can be risks associated with this approach. The authors appropriately highlight that this finding supports pursuing future clinical trials in this setting.

> We sincerely appreciate the input and comments from Reviewer 2. We have added a paragraph (Please see Pages 9-10, Lines 155-168) that addressed the clinical dilemma associated with ctDNA detection in the adjuvant setting. We also emphasized that the suggested ctDNA-guided escalation adjuvant approach is not standard of care (Please see Pages 9-10, Lines 155-168).