Peer Review File

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Reviewer A:

Comment 1: Among the initial 11 patients, 5 exhibited stable disease (SD) or progressive disease (PD). In contrast, in the entire study cohort of 32 patients, only 5 had SD or PD. This suggests that all of the subsequent 21 patients achieved either complete response (CR) or partial response (PR), which is somewhat hard to believe. It is particularly surprising that, among the 18 patients with extrahepatic metastasis, most did not show progression of their extrahepatic metastatic lesions. How do the authors explain this?

Reply 1: Thank you for your comments. In fact, with the proceeding of study treatment, two of the five participants who previously achieved SD achieved CR or PR among the first 11 enrolled participants, while two exhibited SD or PD in the subsequent 21 participants. Therefore, only five participants finally exhibited SD or PD in this trial. The relevant sentences have been modified in the section of **Results-Tumor Response** (See Page 9, line 197).

The tumor response was evaluated synthetically based on the changes of the target lesions both in the liver and outside of the liver. Participants might be considered to achieve PR when the target lesions in the liver had shrunk in size significantly, while the target lesions outside of the liver remained stable or progressed slightly. However, as described in the section of **Discussion**, the TOPAZ-1 and KEYNOTE-966 trials showed a synergistic effect between chemotherapy and immunotherapy in patients with advanced biliary tract cancers (1,2). Previous studies have shown promising results in patients with advanced biliary tract cancers treated with chemotherapy, anti-VEGF therapy, and immunotherapy (3,4). Thus, the combination of HAIC, bevacizumab, and immunotherapy in this trial presented a synergistic anti-tumor effect, suggesting that the systemic anti-tumor effect of immunotherapy may be enhanced by chemotherapeutic agents and bevacizumab administered via HAIC. Therefore, most patients with extrahepatic metastases did not show progression of the extrahepatic metastatic lesions at the first and second evaluations. The following sentence has been included in the section of **Discussion**: “The phenomenon that most participants with extrahepatic metastatic disease did not show progression of their extrahepatic metastatic lesions at the time of first and second evaluation in this trial also suggested that HAIC may enhance the systemic anti-tumor effect of immunotherapy.” (See Page 12, line 292).

Change in the text:

Page 9, line 197: With the proceeding of study treatment, two of the five participants who previously achieved stable disease (SD) achieved CR or PR among the first 11 enrolled participants. Finally, CR, PR, SD, and PD per imRECIST criteria were achieved in 3 (9.38%), 24 (75.0%), 4 (12.50%), and 1 (3.13%) participant, respectively, in all enrolled participants.

Page 12, line 292: The phenomenon that most participants with extrahepatic metastatic disease did not show progression of the extrahepatic metastatic lesions at the first and second evaluations in this trial also suggests that HAIC may enhance the systemic anti-tumor effect of immunotherapy.

References:

1. Oh D-Y, He AR, Bouattour M, et al. Durvalumab or placebo plus gemcitabine and cisplatin in participants with advanced biliary tract cancer (TOPAZ-1): updated overall survival from a randomised phase 3 study. The Lancet

Gastroenterology & Hepatology 2024;9:694-704.
2. Kelley RK, Ueno M, Yoo C, et al. Pembrolizumab in combination with gemcitabine and cisplatin compared with gemcitabine and cisplatin alone for patients with advanced biliary tract cancer (KEYNOTE-966): a randomised, double-blind, placebo-controlled, phase 3 trial. The Lancet 2023;401:1853-65.
3. El-Khoueiry AB, Ren Z, Chon H, et al. IMbrave151: A phase 2, randomized, double-blind, placebo-controlled study of atezolizumab with or without bevacizumab in combination with cisplatin plus gemcitabine in patients with untreated, advanced biliary tract cancer. Journal of Clinical Oncology 2023;41:491-.
4. Shi GM, Huang XY, Wu D, et al. Toripalimab combined with lenvatinib and GEMOX is a promising regimen as first-line treatment for advanced intrahepatic cholangiocarcinoma: a single-center, single-arm, phase 2 study. Signal Transduct Target Ther 2023;8:106.

Comment 2: A major concern with intensive local chemotherapy for biliary tract cancer is the risk of cholangitis. It is noted that 20 out of 32 patients had biliary drainage in place prior to chemotherapy. However, what was the incidence of cholangitis during treatment? Additionally, how frequently was biliary drainage replacement or intervention required?

Reply 2: Thank you for your comment. The reason for biliary drainage in 20 patients prior to HAIC was obstructive jaundice. Treatment was only initiated after the total bilirubin level decreased to less than five times the upper limit of normal after biliary drainage. Among the 20 patients, 13 had the drainage catheter removed after receiving the study treatment for a few cycles due to recanalization of the biliary tract, one patient received biliary stent placement, and two patients replaced the drainage catheter every 6 months. However, symptomatic cholangitis did not occur during or HAIC. The relevant sentence has been modified in the section of **Results-Participants Characteristics** (See Page 8, line 183-184), and the sentence "The study treatment did not result in any case of symptomatic cholangitis." has been added to the section of **Results-Safety** (See Page 10, line 226).

Change in the text:

Page 8, line 183-184: Twenty participants underwent biliary drainage before the study treatment for obstructive jaundice, and the study treatment was initiated until the total bilirubin level decreased to less than five times the upper limit of normal after biliary drainage.

Page 10, line 226: The study did not report any cases of symptomatic cholangitis during or after HAIC.

Comment 3: Details regarding second-line therapies after disease progression should be provided.

Reply 3: Thank you for your comment. Details regarding second-line therapies after disease progression have been provided in the section of **Results-Participants Characteristics** (See Page 9, line 194).

Change in the text:

Page 9, line 194: As second-line treatment, one participant received HAIC using other regimens; four received HAIC using other regimens plus immunotherapy; two received HAIC using other regimens plus oral tyrosine kinase inhibitors (TKI), such as regorafenib and lenvatinib, and immunotherapy; three received oral tyrosine kinase inhibitors plus immunotherapy; three received systemic chemotherapy; one received systemic chemotherapy plus immunotherapy; and two received oral tyrosine kinase inhibitor.

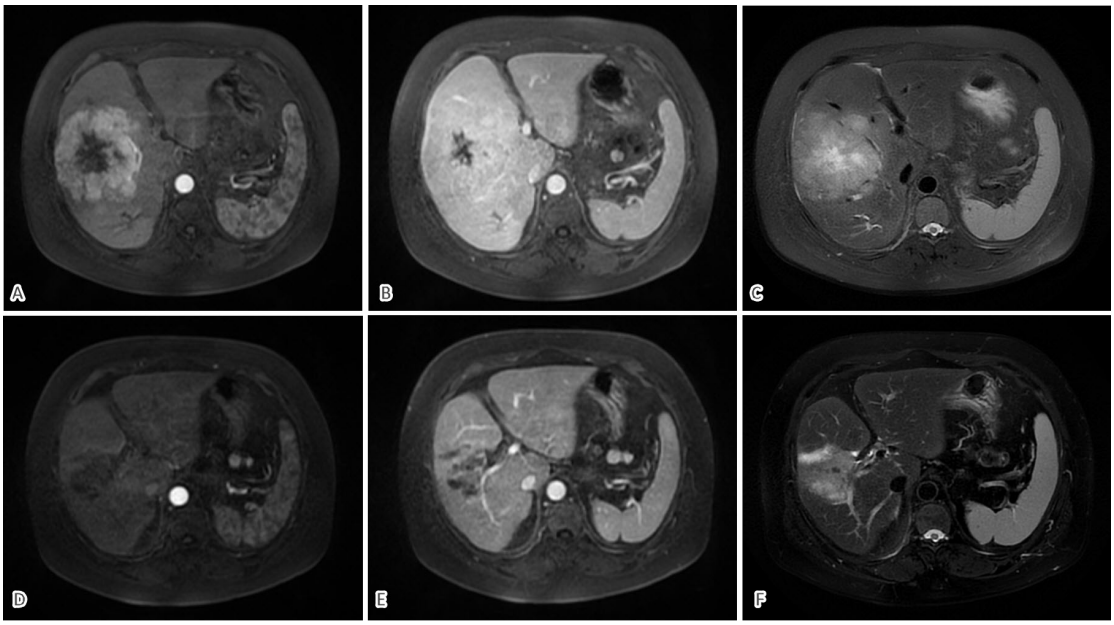
Comment 4: In Figure 1, panels A–C and D–F show images of different slices. These should be presented using the same slices for consistency.

Reply 4: Thank you for your comment. Figure 1 has been modified accordingly (See Page 24, line

504).

Change in the text:

Page 24, line 504:



Reviewer B:

Comment 1: To better contextualize their findings with this single-arm phase II trial, it may be interesting for the authors to share outcomes of a similar patient population treated at their institution with standard of care systemic therapy. While this would not be a head-to-head comparison, it would provide a better "historical" set of results against which to frame their results than other trials that may deal with very different patient populations, diseases, etc. Instead, they may be able to look back at a contemporary cohort of patients who meet the same inclusion/exclusion criteria treated with standard of care systemic therapy.

Reply 1: Thank you for your comment. We had designed this trial as a prospective single-arm phase II trial before initiation, and the comparison between the results of this trial and the contemporary cohort of patients in our institution may violate the design principle of the phase II trial. Nevertheless, we consider that it would be reasonable to compare the results of this trial with those of phase III trials using the standard first-line systemic treatment for advanced biliary tract cancers with almost the same inclusion criteria. Therefore, we have modified this sentence in the section of **Discussion** (See Page 12, line 294-295).

Change in the text:

Page 12, line 294-295: The triple combination therapy showed superior outcomes compared to the recommended first-line treatment for advanced BTCs in prior published phase III trials, with a higher ORR (84.38% vs. 26.1% [GemCis] vs. 26.7% [durvalumab plus GemCis] vs. 29% [pembrolizumab plus GemCis]), longer PFS (13.20 vs. 8.0 [GemCis] vs. 7.2 [durvalumab plus GemCis] vs. 6.5 months [pembrolizumab plus GemCis]), and longer OS (19.0 vs. 11.7 [GemCis] vs. 12.9 [durvalumab plus GemCis] vs. 12.7 months [pembrolizumab plus GemCis]) (1-4).

References:

1. Oh D-Y, He AR, Bouattour M, et al. Durvalumab or placebo plus gemcitabine and cisplatin in participants with advanced biliary tract cancer (TOPAZ-1): updated overall survival from a randomised phase 3 study. The Lancet Gastroenterology & Hepatology 2024;9:694-704.
2. Kelley RK, Ueno M, Yoo C, et al. Pembrolizumab in combination with gemcitabine and cisplatin compared with gemcitabine and cisplatin alone for patients with advanced biliary tract cancer (KEYNOTE-966): a randomised, double-blind, placebo-controlled, phase 3 trial. The Lancet 2023;401:1853-65.
3. Valle J, Wasan H Fau - Palmer DH, Palmer Dh Fau - Cunningham D, et al. Cisplatin plus gemcitabine versus gemcitabine for biliary tract cancer. N Engl J Med 2010;362:1273-81.
4. Oh D-Y, Ruth He A, Qin S, et al. Durvalumab plus Gemcitabine and Cisplatin in Advanced Biliary Tract Cancer. NEJM Evidence 2022;1:EVIDoa2200015.

Comment 2: Given the importance of molecular profiling nowadays, especially in biliary tract cancers, can the authors provide an account of the mutations present in the tumors included in the study?

Reply 2: Thank you for your comment. Most participants with perihilar cholangiocarcinoma were enrolled in this trial, and they were diagnosed based on cytological pathology due to the absence of tumor tissue. Next-generation sequencing was only performed on a fraction of the participants with intrahepatic cholangiocarcinoma and gallbladder cancer. Considering the possibility of misleading results due to the limited results of next-generation sequencing technology in this trial, we did not provide an account of the mutations present in the included tumors. Instead, Olink proteome technology is a circulating protein biomarker test that can predict prognosis and monitor the efficacy of treatment and can be performed using the plasma of patients without the requirement of tumor tissue (1, 2). Olink proteome technology has a high accessibility and is cost-effective. Therefore, Olink proteome technology was employed for 29 participants to detect protein biomarkers associated with outcomes after treatment for advanced biliary tract cancers. Finally, 13 proteins were found to be associated with response, and high level of MMP12 was associated with worse progression-free survival. The following sentence has been added to the the section of **Discussion**: “Olink proteome technology is a circulating protein biomarker test that can predict prognosis and monitor the efficacy of treatment, and can be performed using the plasma of patients without the requirement of tumor tissue. Therefore, the Olink proteome technology was employed to detect protein biomarkers in this trial owing to both its accessibility and its cost-effectiveness.” (See Page 13, line 315).

Change in the text:

Page 13, line 315: Olink proteome technology is a circulating protein biomarker test that can predict prognosis and monitor the efficacy of treatment, and can be performed using the plasma of patients without the requirement of tumor tissue (1, 2). Therefore, the Olink proteome technology was employed to detect protein biomarkers in this trial owing to both its accessibility and its cost-effectiveness.

References:

1. Jordan HA, Thomas SN. Novel proteomic technologies to address gaps in pre-clinical ovarian cancer biomarker discovery efforts. Expert Review of Proteomics 2023;20:439-50.
2. Gao M, Wu X, Jiao X, et al. Prognostic and predictive value of angiogenesis-associated serum proteins for immunotherapy in esophageal cancer. Journal for ImmunoTherapy of Cancer 2024;12:e006616.

Comment 3: I would encourage the authors to mark within the waterfall plots which patients had a) liver-limited vs. extrahepatic disease, and b) which disease site (iCCA vs. pCCA vs. GBC).

Reply 3: Thank you for your comment. The waterfall plots were modified according to your suggestion (See Page 25, line 517).

Change in the text:

Page 25, line 517: