Peer Review File

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Reviewer A

I appreciate the authors to present such an interesting study.

Comment 1: The authors should avoid any "bold" statements and keep a scientific writing style.

Reply 1:

Thanks to the reviewer for the comment. We apologize for the ambiguous and inaccuracy wording in our previous manuscript. Our study has limitations. This is a retrospective study with small sample size and the selective bias was inevitable. The results in this article are far from conclusive. We aim to explore a new strategy for unresectable GBC.

We have revised our papers according to the reviewer's comments. The manuscript has been checked by a native English-speaking editor, Mitchell Arico from Liwen Bianji (Edanz) (https://www.liwenbianji.cn), for editing the language of a draft of this manuscript.

Comment 2: In our country, according to Topaz-study, GC plus Durvalumab is the first line regimen. GC plus Dur is more beneficial or equal to your study. To perform HAIC, it is necessary to place IA-port, generally GDA coiling method. This might be over invasive for some cases. Any opinion?

Reply 2:

Thanks to the reviewer for the comment. And as reviewer said, GC plus Durvalumab is the first line regimen according to the recent NCCN guideline, with an ORR of 26.7% and median overall survival (OS) of 12.8 months. Topaz-study included all kinds of advanced BTC, not just GBC. In the subgroup of GBC, the hazard ratio was 0.94 (0.65-1.37) in overall survival analysis **(1)**. This result implied that GC plus Durvalumab may not achieve survival

improvement in GBC cohort when compared with GC regimen. Hence, it is reasonable to explore new strategy for GBC.

In this exploratory study, we included a small part of GBCs: GBC with hepatic oligometastasis. These cases have no hilar invasion and distant lymphatic metastasis, and usually have adequate liver function and limited tumor involvement. Theoretically, they have adequate tolerance to be treated. We believe that these patients are very promising to get breakthrough in the setting of multidisciplinary treatment. In this pilot study, the ORR was 55.6% and median OS time was 15.0 months.

The agents with high first-pass hepatic extraction are suitable for infusion by HAIC. The "first-pass" effect results in high local drug concentrations in liver with minimal systemic distributions, which leads to significant reduction of systemic adverse events. At present, there are few reports of HAIC therapy using GC regimen. On the other hand, FOLFOX is recommended for neoadjuvant therapy and is one of second line regimens for unresectable and metastatic BTC **(2)**. FOLFOX protocol has been used for HAIC treatment for a long time, and its safety and efficacy have been confirmed in hepatocellular carcinoma and unresectable colorectal liver metastasis **(3-4)**. Therefore, we used FOLFOX for HAIC in this study. According to the reviewer's comments, we add the corresponding content in the discussion section. (see Page 10, line 27-29, Page 11, line 1-5)

HAIC was performed according to previously reported protocol **(3)**. IA-port was not used for HAIC in this study. Femoral artery puncture and catheterization were performed on day 1 in every cycle of treatment. After HAIC was completed, the catheter and sheath were removed. Repetitive catheterization was performed in the next HAIC cycle.

1.Oh DY, He AR, Qin S, Chen LT, Okusaka T, Vogel A, et al. Durvalumab plus gemcitabine and cisplatin in advanced biliary tract cancer. NEJM Evid 2022; 1: 1–11

2.Lamarca A, et al. Second-line FOLFOX chemotherapy versus active symptom control for advanced biliary tract cancer (ABC-06): a phase 3, open-label, randomised, controlled trial. Lancet Oncol 2021 May;22(5):690-701.

3.Li QJ, et al. Hepatic Arterial Infusion of Oxaliplatin, Fluorouracil, and Leucovorin Versus Transarterial Chemoembolization for Large Hepatocellular Carcinoma: A Randomized

Phase III Trial. J Clin Oncol 2022; 40: 150-160.

4.Datta J, et al. Role of Hepatic Artery Infusion Chemotherapy in Treatment of Initially Unresectable Colorectal Liver Metastasis: A Review. JAMA Surg 2019; 154: 768-776.

Comment 3: Basically, we do not usually use Bev and Sintilimab, so please show the concrete data/literature where Bev or Sintilimab is beneficial for GB cancer.

Reply 3:

Thanks to the reviewer for this comment. At present, the treatment effect of unresectable GBC is still unsatisfactory. This is an exploratory study to investigated a new strategy for unresectable GBC. The attempts to treat GBC with targeted-immune and FOLFOX-HAIC mainly stem from their success in hepatocellular carcinoma (HCC).

Vascular endothelial growth factor (VEGF) is over-expressed in 45%–75% of BTCs and has been implicated in the control of lymphangiogenesis and lymphatic metastasis in GBC **(1)**, which have made VEGF-driven angiogenesis a logical target in BTC, especially in the setting of targeted-immune therapy. TKIs (e.g. lenvatinib) and monoclonal antibody (e.g. bevacizumab) are the main choice of antiangiogenic drugs.

In recent years, targeted-immune therapy, single or combined with chemotherapy, has been investigated in BTC **(2)**. Several studies had reported the efficacy and safety of PD-1 inhibitor plus lenvatinib for advanced GBC, with an ORR of 32.3% **(3,4)**. In fact, bevacizumab is more widely used. Bevacizumab combined PD-1/PD-L1 have achieved encouraging results in HCC, nasopharyngeal carcinoma and colon cancer **(5-8)**. It is worth noting that, bevacizumab combined with PD-1/PD-L1 achieved anti-tumor efficacy in hepatocellular-cholangiocarcinoma (cHCC-CCA) **(9)**, which imply the effectiveness of targeted-immune therapy containing bevacizumab against BTC. There were also case reports showing their potential value on GBC **(10,11)**.

Sintilimab (China, Innovent Biologics Suzhou Co. Ltd.) is a PD-1 inhibitor. Given the stratospheric price and health insurance policies, PD-L1 inhibitors is far out of the reach of the average GBC patients at present. Sintilimab is often used instead of PD-L1 inhibitors in China. Bevacizumab plus sintilimab have achieved encouraging results in the treatment of

HCC, metastatic nasopharyngeal carcinoma and metastatic colorectal cancer **(7,8,12)**. Hence, we chose bevacizumab combined with sintilimab as targeted-immune therapy in this study.

According to the reviewer's comments, we will add the corresponding content in the discussion section. (see Page 11, line 13-27)

1.Lin W, et al. Vascular endothelial growth factor-D promotes growth, lymphangiogenesis and lymphatic metastasis in gallbladder cancer. Cancer Lett 2012; 314(2): 127-136.

2. Shi GM, 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.

3. Zuo BY, et al. A real-world study of the efficacy and safety of anti-PD-1 antibodies plus lenvatinib in patients with advanced gallbladder cancer. Cancer Immunol Immunother 2022; 71: 1889-1896.

4. Wu T, et al. Comparison of Efficacy and Safety of Anti-Programmed Cell Death-1 Antibody Plus Lenvatinib and Chemotherapy as First-Line Therapy for Patients with Stage IV Gallbladder Cancer: A Real-World Study in a Chinese Population. Biomedicines 2023;11(11):2933.

5. Finn RS, et al. Atezolizumab plus Bevacizumab in Unresectable Hepatocellular Carcinoma. N Engl J Med 2020; 382: 1894-1905.

6. Zeng X, et al. A real-world analysis of survival and cost-effectiveness of sintilimab plus bevacizumab biosimilar regimen in patients with advanced hepatocellular carcinoma. J Cancer Res Clin Oncol 2023 Sep;149(11):9213-9219

7. Lu N, et al. Efficacy and safety of sintilimab plus bevacizumab in metastatic nasopharyngeal carcinoma after failure of platinum-based chemotherapy: an open-label phase 2 study. EClinicalMedicine 2023; 62:102136.

8. Fang X, et al. Sintilimab plus bevacizumab, oxaliplatin and capecitabine as first-line therapy in RAS-mutant, microsatellite stable, unresectable metastatic colorectal cancer: an open-label, single-arm, phase II trial. EClinicalMedicine 2023 Jul 27:62:102123

9. Gigante E, et al. Atezolizumab and bevacizumab for non-resectable or metastatic combined hepatocellular-cholangiocarcinoma: A multicentric retrospective study. United European Gastroenterol J 2023 Dec 7. doi: 10.1002/ueg2.12503

10. Guo LH, et al. Successful Treatment of Metastatic Gallbladder Carcinoma with PD-L1 Expression by the Combination of PD-1 Inhibitor Plus Bevacizumab with Chemotherapy: A Case Report. Onco Targets Ther 2022; 15:629-636.

11. Ning C, et al. Conversion therapy of stage IVb unresectable gallbladder carcinoma. Hepatobiliary Surg Nutr 2022; 11: 335-337.

12. Zeng X, et al. A real-world analysis of survival and cost-effectiveness of sintilimab plus bevacizumab biosimilar regimen in patients with advanced hepatocellular carcinoma. J Cancer Res Clin Oncol 2023; 149(11): 9213-9219.

Comment 4. In case of advanced GB cancer, lymph node involvements are frequent. Is HAIC-FOLFOX and Bev/anti PD-L1 also beneficial for lymph node involvements? The size of LN metastases of advanced GB cancer is not always big so FDG-PET sometimes shows false-negative. What I mean, it is quite difficult to choose appropriate patient for HAIC. Any opinion?

Reply 4:

Thanks to the reviewer for the constructive comment. We agree with reviewer that lymphatic metastasis is frequent in GBC patients. The overall rate of lymph node metastasis in GBC ranges from 54% to 64% **(1)**. In this study, 33.3% of the cases had radiographically detectable lymph metastasis in hilar region of liver. According to reviewer's comment, we revised the inclusion criteria: detectable lymph node metastasis was confined to the hilar region of liver (see Page 4, line 16-20); we supplemented the radiographic data of detectable lymph node metastases before treatment in Table.1 and 5, due to the small number of cases, we only listed the data without further analysis; we added the data of pathological reports about metastatic lymph nodes in Figure 6.

For potentially regional lymph metastasis, we believe that HAIC can play a direct therapeutic role. In theory, the blood supply of hilar lymph nodes come from capillary branch

of proper hepatic artery. During HAIC procedure, we did not embolize gastroduodenal artery (GDA) and right gastric artery (RGA) which are routinely performed at other centers **(2)**. This was designed to make the chemotherapeutics cover the area around the portal of the liver. We have added this in the manuscript (see Page 5, line 22-24; Page 11, line 5-9).

For distant lymph metastasis, it is difficult to play a direct therapeutic role by HAIC. Frankly, this is the limitation of locoregional chemotherapy. This really limits the scope of HAIC applications. Hence, patients with detectable distant lymphatic metastasis were actually excluded. But we believe that HAIC may have therapeutic effect by some indirect mechanism. Tumor cells are killed by locoregional chemotherapy and release more specific antigens which were captured by antigen-presenting cells which enhance the effect of PD-1 inhibitor. Chemotherapeutic agents also have been shown to induce immunomodulatory effects **(3)**. These phenomena improve the efficacy of systematic targeted-immune therapy for both tumor and potentially metastatic lymph nodes. According to the reviewer's opinion, this part is also added to the discussion (see Page 12, line 6-11).

We agree with reviewer that PET-CT can't accurately detect lymph node metastasis. Therefore, as reviewer said, a small part of unresectable GBC (GBC with liver oligometastasis) may benefit from this combination therapy. We emphasize this limitation in our paper (see Page 13, line 16-17). Even so, we believed that it is reasonable because there are only limited treatment options for unresectable GBC at the present stage. These cases had no hilar invasion and distant lymphatic metastasis, and usually have adequate liver function and limited tumor involvement. Theoretically, they have adequate tolerance to receive combination treatment. There is still a chance that these patients will be converted to surgical resection. The lesions on gallbladder and liver can be covered simultaneously and take full advantage of HAIC.

1.Lin W, et al. Vascular endothelial growth factor-D promotes growth, lymphangiogenesis and lymphatic metastasis in gallbladder cancer. Cancer Lett 2012; 314(2): 127-136.

2.Zheng K, et al. Hepatic Arterial Infusion Chemotherapy with Oxaliplatin and 5-Fluorouracil for Advanced Gallbladder Cancer. Cardiovasc Intervent Radiol 2021; 44: 271-280.

3.Fournel L, et al. Cisplatin increases PD-L1 expression and optimizes immune check-point blockade in nonsmall cell lung cancer. Cancer Lett 2019; 464: 5–14

Reviewer B

Comment 1: We believe this article is suitable for publication in the journal although some revisions are needed. The main strengths of this paper are that it addresses an interesting and very timely question and provides a clear answer, with some limitations. Certainly, a linguistic revision is necessary, since there are some grammar mistakes and the manuscript needs corrections.

Reply 1:

Thanks to the reviewer for the comment. Our study does have limitations. We aim to explore a new strategy for unresectable GBC. We have revised our papers according to the reviewer's comments. The manuscript has been checked by a native English-speaking editor, Mitchell Arico from Liwen Bianji (Edanz) (https://www.liwenbianji.cn), for editing the language of a draft of this manuscript (please see the tracked copy of the manuscript).

Comment 2: The background of the changing scenario of medical treatment for biliary tract cancer should be better discussed, and some recent papers regarding this topic should be included (PMID: 37535194; PMID: 33756174; PMID: 35031442; PMID: 33571059), only for a matter of consistency.

Reply 2:

Thanks for the valuable refences provided by reviewer. Conversion therapy has been discussed in the treatment of hepatocellular carcinoma. However, limited study focused on the conversion therapy of GBC. With the help of these references, we furtherly discussed the conversion therapy of GBC (see Page 12, line 12-16). In the meantime, we cited one of the references above (PMID 3375617, Reference 21). In addition, we explored the synergies between different treatment modalities (see Page 12, line 6-12).

Comment 3: The abstract should be modified. Some changes are necessary.

Reply 3:

Thanks for reviewer' s advice. The abstract has been modified in terms of syntax and logic. We invited a native English-speaking editor to revised the abstract (please see the tracked copy of the manuscript).

Reviewer C

Comment 1: A very interesting study discussing a timely topic in HCC patients. Some changes are needed. A linguistic revision is required since there are some grammar mistakes and oversights to be corrected. A professional service is recommended.

Reply 1:

Thanks for reviewer' s advice. The attempts to treat GBC with targeted-immune and FOLFOX-HAIC mainly stem from their success in HCC. Therefore, we cite some relevant results in the field of HCC in manuscript. This study preliminarily investigated the value of hepatic artery infusion chemotherapy (HAIC) combined bevacizumab plus PD-1 inhibitor in the treatment for gallbladder cancer (GBC) with hepatic oligometastasis.

The manuscript has been checked by a native English-speaking editor, Mitchell Arico from Liwen Bianji (Edanz) (https://www.liwenbianji.cn), for editing the language of a draft of this manuscript (please see the tracked copy of the manuscript).

Comment 2: The introduction should be expanded and the authors should further discuss the evolving systemic treatment scenario for HCC, by also adding some recently published papers regarding this topic (PMID: 32772560; PMID: 35031442; PMID: 33820447; PMID: 37535194).

Reply 2:

Thanks for the valuable refences provided by reviewer. The introduction has been modified in terms of syntax and logic (please see the tracked copy of the manuscript). With the help of these references, we furtherly discussed the conversion therapy (neoadjuvant therapy) of GBC (see Page 12, line 12-16). In addition, we explored the synergies between different treatment modalities (see Page 12, line 6-12).

Comment 3: A more personal perspective should be included in the Discussion. Major changes needed.

Reply 3:

Thanks for reviewer' s advice.

According to reviewer's comment, we added some personal perspective in discussion:

⑴We added our views and reasons for the use of FOLFOX-HAIC in the treatment of unresectable GBC (see Page 10, line 27-29, Page 11, line 1-5);

⑵We also added our review on the use of bevacizumab plus sintilimab as targeted-immune therapy for unresectable GBC in this study (see Page 11, line 13-27).

⑶ In fact, patients with detectable distant lymphatic metastasis were actually excluded. Therefore, a small part of unresectable GBC (GBC with liver oligometastasis) may benefit from this combination therapy. Even so, we believed that it is reasonable because we have only limited treatment options for unresectable GBC at the present stage. We state our view that this part of patients should be treated actively (see Page 10, line 1-11).