Lenvatinib plus toripalimab with local-regional therapy in patients with advanced biliary tract cancer: a retrospective study

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Research Article

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Abstract

Background: Lenvatinib plus anti-PD-1 antibodies with local-regional therapy which was called stereotactic therapy have demonstrated potent antitumor activity in solid tumors. However, the efficacy and safety of stereotactic therapy in patients with advanced biliary tract cancer (BTC) remains unclear.

Methods: This was a retrospective study including patients with advanced BTC who received a combination of Lenvatinib plus toripalimab or Lenvatinib plus toripalimab with local-regional therapy (stereotactic therapy). This study evaluated the efficacy and safety of Lenvatinib plus toripalimab and stereotactic therapy in advanced BTC patients. Tumor tissues were collected to retrospectively evaluate the expression status of PDL1.

Results: This study included 55 patients: 24 in Lenvatinib plus toripalimab therapy; 31 in stereotactic therapy. All patients had undergone at least 1 line of antitumor treatment. The stereotactic therapy group showed longer median progression-free survival (mPFS) (9.6 versus 4.6 months, p=0.035), longer overall survival (mOS) (13.7 versus 9.2 months, p=0.023) than the Lenvatinib plus toripalimab group. The ORR was 35.5% [95% confidence interval (CI): 17.6-53.3] with stereotactic therapy versus 25% (95% CI: 6.3-43.7) with toripalimab plus Lenvatinib. Four patients received surgery after the Lenvatinib plus toripalimab therapy or after stereotactic therapy. All patients experienced any-grade adverse events (AEs) without grade 5 AEs. PDL1 expression was associated with improved clinical benefits.

Conclusions: Stereotactic therapy had acceptable toxic effects and might improve survival compared with Lenvatinib plus toripalimab for advanced BTC.

Introduction

Biliary tract cancers (BTCs), which mainly include intrahepatic cholangiocarcinoma (ICC), extrahepatic cholangiocarcinoma (ECC), and gallbladder cancer (GBC), are aggressive malignancies [1]. Most patients have an advanced-stage disease at presentation, and the prognosis is poor, with a 5-year survival of less than 5–15%[2, 3]. Presently, the first-line treatment for patients with advanced disease is gemcitabine and cisplatin (GC)[4], and FOLFOX is recommended as a second-line treatment. Unfortunately, the objective response rate (ORR) of FOLFOX was only 5% and the disease control rate (DCR) was 33%. The subsequent treatment options are limited for patients who experienced disease progression despite chemotherapy. Therefore, new effective therapeutic approaches are needed to improve the clinical outcomes of patients with advanced BTC.

Accumulating evidence has demonstrated that programmed cell death ligand 1 (PD-L1) or programmed cell death protein 1 (PD-1) expression has been observed and that suppressed immune microenvironments contribute to the pathogenesis of BTC, which suggests that there is a potential role for immunotherapy that is directed against the PD-1 pathway in BTC patients[5, 6]. However, the KEYNOTE-158 and KEYNOTE-028 studies showed that the treatment efficacy of using immunotherapy alone is far from satisfactory[7]. A previous study showed that the combination of anti-PD-1 therapy with

Lenvatinib could increase the ORR to 21.4% in patients with previously treated BTC [8]. The combination of toripalimab, Lenvatinib, and gemox chemotherapy could increase the ORR to 80% (24/30) in the phase 2 clinical trial[9].

In addition, a real-world practice found that advanced hepatocellular carcinoma (HCC) patients received the combination of Lenvatinib plus anti-PD-1 antibodies with local-regional therapy which was called stereotactic therapy had a satisfactory efficacy [10]. Stereotactic therapy is defined by receiving Lenvatinib plus anti-PD-1 antibodies with local regional therapy, including radiotherapy, hepatic arterial infusion chemotherapy (HAIC), transcatheter arterial chemoembolization (TACE), and radiofrequency ablation (RFA). Anti-PD-1 therapy in combination with antiangiogenic and local regional therapy may enhance the endogenous immune response.

Toripalimab, a humanized programmed death-1 (PD-1) antibody, has shown a manageable safety profile and has promising antitumor activity in patients with advanced gastric cancer and metastatic mucosal melanoma[11, 12]. Based on these results, we investigated the efficacy and safety of the combination of Lenvatinib plus toripalimab therapy or stereotactic therapy in patients with advanced BTC.

Materials And Methods

Study design and participants

We performed a retrospective study to assess the efficacy and safety of Lenvatinib plus toripalimab or stereotactic therapy in patients with advanced biliary tract cancer from March 2019 until December 2021. Eligible patients were aged 20 years or older with histologically confirmed ECC, ICC, and GBC. All patients had been diagnosed with advanced BTC and had progressed despite having at least 1 line of systemic therapy previously. The other eligibility criteria included patients who had at least one measurable or evaluable tumor lesion according to the Response Evaluation Criteria in Solid Tumors version 1.1 (RECIST 1.1) and who had an Eastern Cooperative Oncology Group (ECOG) performance status of 0-2. The patients received Lenvatinib and toripalimab with or without local-regional therapy. The eligible participants were also required to have received >3 months of treatment. The demographic information and the surgical, pathological, regional, and systemic treatment information were recorded. A study flow diagram is shown in ***Figure 1***.

The study protocol was compliant with the Declaration of Helsinki and was approved by the Institutional Review Board and Ethics Committee at Peking Union Medical College Hospital. All patients provided written informed consent before enrollment (PUMCH-JS-1391). Once the patients with advanced BTC had progressed on at least 1 line of systemic therapy, treatment with Lenvatinib plus toripalimab or stereotactic therapy was recommended. The final treatment plan was principally made based on the condition of the patient and the patient's disease status, including the ECOG score of the patient and the number and location of tumors. If the patients with stable disease (SD) or progressive disease (PD) received toripalimab plus Lenvatinib therapy, local regional therapies, such as radiotherapy, hepatic

arterial infusion chemotherapy (HAIC), transcatheter arterial chemoembolization (TACE), and radiofrequency ablation (RFA), were also recommended.

Treatment

In the Lenvatinib plus toripalimab group, patients received toripalimab at a fixed dosage of 240 mg or 3 mg/kg every 3 weeks and a Lenvatinib dosage of 12 mg (for patients with a bodyweight ≥60 kg) or 8 mg (for patients with a bodyweight <60 kg) orally once a day.

In the stereotactic therapy group, the patients not only received toripalimab with a fixed dosage of 240 mg or 3 mg/kg every 3 weeks and a dosage Lenvatinib of 12 mg (for patients with a bodyweight ≥60 kg) or 8 mg (for patients with a bodyweight <60 kg) orally once a day, the patients also received radiotherapy, HAIC, TACE, and RFA. Lenvatinib plus toripalimab was administered 2-3 weeks earlier than the local regional therapies.

The clinical target volume covered an extended involved-site field that covered the primary tumor, adjacent organs, and lymph node areas. The radiation doses that were given to the clinical target volume were usually 30-60 Gy in 8 to 28 fractions by a 6 MV X-ray linear accelerator and with intensity modulated radiotherapy (IMRT)[13].

TACE was performed according to the patient's condition and decision. Using the Seldinger technique, the patients were catheterized via the femoral artery, and hepatic arteriography was performed. Agents, including iodized oil (3–5 ml), fluorouracil (5-FU) (750 mg/m^2), and oxaliplatin (60 mg/m^2), were injected through the appropriate hepatic artery. The length of time that was used to infuse the chemotherapy drugs was generally not less than 15 minutes. According to the type of tumor, the alpha-fetoprotein (AFP) level, and the physical recovery, the decision of whether to perform another TACE was made.

The RFA was performed as follows: The overall duration of the radiofrequency varied according to the size and number of the lesions (range 10–90 min), and the radiofrequency current was emitted by a 200 W generator, which was set to deliver the maximum power with the automatic impedance control method. HAIC was performed as follows: A catheter/microcatheter was placed in the main feeding hepatic artery, and then the following regimen was administered via the hepatic artery: oxaliplatin 85 mg/m^2 from hour 0 to 2 on day 1; leucovorin 400 mg/m^2 from hour 2 to 3 on day 1; and a 5-fluorouracil 400 mg/m^2 bolus at hour 3 and then 2400 mg/m2 over 46 h on days 1 and 2. The treatments may have been interrupted and even discontinued when unacceptable or serious adverse events (AEs) occurred or when there was disease progression.

Assessments

The clinical objective response used enhanced computed tomography (CT) and magnetic resonance imaging (MRI) according to RECIST 1.1 at 6–8 weeks after the patient's treatment. The imaging examinations were evaluated by professional radiologists. The therapeutic efficacy included the objective

response rate (ORR) (the proportion of patients with a confirmed complete and partial response), the progression-free survival (PFS) (the time from the initial treatment to disease progression or death from any cause), the overall survival (OS) (the time from the initial treatment to death from any cause), the disease control rate (DCR) (the proportion of patients who achieved an objective response or SD), and the safety. The AEs were categorized and graded according to the National Cancer Institute Common Terminology Criteria for Adverse Events, version 4.0 (CTCAE 4.0). The worst grade for each AE that was experienced by each patient during the observation period was recorded.

Assessment of PD-L1 expression

Formalin-fixed, paraffin-embedded (FFPE) tumor specimens from 28 patients were used to perform immunohistochemistry (IHC). IHC staining was performed according to a standard protocol. The tumor tissues were fixed, embedded, and cut into 3 μm thick sections. Anti-PDL1 antibody (IHC PD-L1 (E1L3N) XP rabbit mAb, Cell Signaling Technology) was used. The percentages of PD-L1-expressing tumor cells were assessed by independent pathologists who were blinded to the clinicopathologic data, including the therapeutic response and survival time. PDL1 positivity was defined as a CPS ≥ 1 in the tumor cells.

Statistical analysis

The data were last updated on December 31, 2021. The PFS and OS were calculated from the date of the initiation of treatment until the date of disease progression or death. The baseline characteristics and efficacy data of the two treatment groups were compared using the chi-square test or Fisher's exact test. The Kaplan–Meier method and bilateral log-rank test were used to generate PFS and OS curves. The hazard ratios of each clinicopathological feature for the PFS and OS were estimated by Cox proportional hazard modeling. All statistical analyses were undertaken using SPSS 22 (vision 22.0, SPSS, Inc., Chicago, IL) and R (version 4.0.3).

Results

The patient demographics and baseline characteristics

From March 2019 until June 2021, 55 patients with advanced BTC met the eligibility criteria: 31 patients received stereotactic therapy, and 24 patients received Lenvatinib plus toripalimab. The median age of the patients was 60 (IQR, 57-65) years, and 67.3% of the patients were male. Of this total number, 37 patients (67.3%) had ICC, 8 (14.5%) had ECC and 10 (18.2%) had GBC. Forty-three (78.2%) of the participants had an ECOG PS of 0-1. In total, the pathological differentiation types of 24 (43.6%) patients were unknown due to a lack of further pathological tissue analyses. All patients had undergone ≥1 line of antitumor treatment. Twenty-four (43.6%) patients underwent radical surgical resection. Nineteen (34.5%) patients had received systemic chemotherapy. Due to concerns about the side effects of chemotherapy, 17 (30.9%) patients chose targeted therapy, including afatinib (n=1) and Lenvatinib (n=16). During the initial diagnosis, 32 (58.2%) patients presented with lymph node metastasis. The baseline characteristics of two cohorts are summarized in ***Table 1***, and no difference was observed. In both the stereotactic

therapy group and the Lenvatinib plus toripalimab group, lymph node metastasis was the most common metastasis. In the stereotactic therapy group, 16 (51.6%) patients had radiotherapy. One patient underwent TACE, HACI, RFA, and radiotherapy due to having multiple metastatic sites. The treatments are listed in ***Table 1***.

Efficacy

The data were last updated on 31 December 2021. In this study, the median duration of follow-up was 21.1 (IQR, 14.5–26.4) months. As of December 31, 2021, 35 (63.6%) deaths had occurred: 17 (70.8%) deaths in the Lenvatinib plus toripalimab therapy group and 18 (58.1%) deaths in the stereotactic therapy group. The median PFS in the stereotactic therapy group was 9.6 (95% CI: 4.7-14.5), compared with 4.6 (95% CI: 2.5-6.6) in the toripalimab plus Lenvatinib therapy group (HR 0.50 [95% CI: 0.26-0.97]; p=0.035, ***Figure 2A***). The median OS was 13.7 months (95% CI: 9.4-17.9) in the stereotactic therapy group versus 9.2 months (95% CI: 7.9-10.4) in the toripalimab plus Lenvatinib therapy group (HR 0.45 [95% CI: 0.22-0.91]; p=0.023, ***Figure 2B***). The ORR was 25% (6/24; 95% CI: 6.3-43.7), and the DCR was 75% (18/24; 95% CI: 56.3-93.7) in the Lenvatinib plus toripalimab therapy group. However, in the stereotactic group, the ORR was 35.5% (11/31; 95% CI: 17.6-53.3), and the DCR was 87.1% (27/30; 95% CI: 74.6-99.6) (***Table 2***). Among the two cohorts, 4 patients achieved PR and had surgery after the above treatment: 3 patients received stereotactic therapy, and 1 patient received therapy with toripalimab plus Lenvatinib (***Figure 3***). Evidence shows that patients in the stereotactic therapy group have better survival than those in the Lenvatinib plus toripalimab therapy group.

Safety

All patients experienced ≥1 adverse event (AE), and no treatment-related deaths occurred in this study (***Table S1***). The most common AEs were an ALT or AST elevation (74.5%, n = 41) followed by fatigue (72.7%, n = 40). The most frequent grade 3-4 AE was an elevation in the bilirubin levels (7/55, 12.7%). The stereotactic therapy group had a higher incidence of AEs than the Lenvatinib plus toripalimab therapy group. The most common AEs with the stereotactic therapy were Fatigue (25/31, 80.6%), AST or ALT increased (25/31, 80.6%), and abdominal pain (12/31, 38.7%). A detailed list of the AEs and their associated frequencies are shown in ***Table 3***. No drug-related deaths occurred in any group.

PD-1 expression and the subgroup analysis

A total of 28 samples were used for the assessment of PD-L1 expression by two pathologists (***Table S2***). Overall, 29.1% of the samples were PD-L1 positive (≥1%). The subgroup with positive PDL1 expression showed a higher PFS than the subgroup without PDL1 expression (14.7 vs. 5.9, P=0.007) (***Figure 4***). For the OS, ORR, and DCR, the subgroup with positive PDL1 expression was not significantly different compared with the PD-1-negative subgroup (***Table S3***).

Discussion

Patients with advanced BTC who were pretreated with systemic antitumor treatments have limited treatment options and an overall poor prognosis[14], so new effective treatment strategies are needed. In this retrospective study, although all of the patients with advanced BTC received one or more systemic antitumor treatments, our study showed that stereotactic therapy may lead to longer OS and PFS. The analyses of predefined subgroups revealed that the patients in the stereotactic therapy group achieved a significantly better mPFS (9.6 vs. 4.6 months, p = 0.035). Compared with the Lenvatinib plus toripalimab therapy group, the patients in the stereotactic therapy group had a longer mOS (13.7 vs. 9.2 months, p = 0.023), indicating that stereotactic therapy has the potential to improve the long-term survival. The ORR and DCR were also better in the stereotactic therapy group (ORR: 35.5% vs. 25%, DCR: 87.1% vs. 75%). In addition, 3 patients received surgery after the combination of Lenvatinib plus toripalimab with local regional therapy which is called the stereotactic therapy. The results indicate that the combination of Lenvatinib plus anti-PD-1 antibodies with local-regional therapy is a possible conversion therapy that can successfully convert unresectable BTC to a resectable status, and it has the potential to improve long-term survival.

For patients who experienced disease progression despite chemotherapy, the subsequent treatment options are limited. Accumulating preclinical studies have demonstrated that immunotherapy[7] or Lenvatinib[15] alone is not satisfactory in patients with advanced BTC. A phase 2 study showed that the ORR of Lenvatinib monotherapy as a second-line treatment in unresectable biliary tract cancer was 11.5% [15]. The results from the KEYNOTE-028 (KN028) and KEYNOTE-158 (KN158) basket studies showed that the ORRs were 13.0% and 5.8%, respectively, in patients who had PD-L1–positive tumors[16]. A single-arm study revealed that pembrolizumab combined with Lenvatinib could enhance the antitumor efficacy of immunotherapy, with an ORR of 25% and a DCR of 75% for patients with refractory BTC[17]. A recent conference abstract that evaluated Lenvatinib and toripalimab in 31 patients with advanced ICC showed a high response rate with an ORR of 32.3% (10/31; 95% CI: 16.7%-51.4%) and a DCR of 74.2% (23/31; 95% CI: 55.4%-88.1%) [18]. This promising efficacy indicates that anti-PD-1 plus TKI therapy may exert activity in BTC patients, especially in patients who have biliary tract cancer with positive PD-1 expression[17, 19]. As we observed in this study, the subgroup with positive PDL1 expression had a higher PFS than the subgroup with negative PDL1 expression (14.7 vs. 5.9, P = 0.009), suggesting that patients with positive PDL1 expression may have a survival benefit. Increasing evidence suggests that the inhibition of angiogenesis by targeting TKIs can reprogram the immunosuppressive tumor microenvironment into an immunostimulatory environment and can enhance the antitumor efficacy of immunotherapy that targets PD1/PDL1 [20, 21]. Dual anti-PD-1 and VEGF/VEGFR blockade has shown some benefits in patients with previously treated advanced non-small-cell lung cancer, gastro-esophageal cancer, hepatocellular carcinoma, or urothelial carcinoma [21–23]. This combination may be a promising alternative for patients with previously treated advanced BTC. Therefore, Lenvatinib combined with pembrolizumab is recommended as a second-line treatment option for biliary tract cancer in CSCO guidelines.

Continuous improvements have been made for patients with advanced BTC who have limited treatment options, and multimodality therapies, including anti-PD-1 therapy combined with chemotherapy, and

locoregional treatment approaches, are now often used following cancer progression because these therapies may be efficacious[24]. A phase 2 clinical trial reported toripalimab, Lenvatinib and gemox chemotherapy as first-line treatment for an advanced and unresectable ICC showed an ORR of 80% (95% CI, 61.4–92.3) and a DCR of 93.3% (95% CI, 77.9–99.2)[9]. Growing evidence indicates that chemotherapy may affect the immune system and increase the ratio of cytotoxic lymphocytes to regulatory T cells[25]. Toripalimab, Lenvatinib, and gemox chemotherapy are recommended as first-line therapy in advanced biliary tract malignancies in CSCO guidelines. All of the above studies indicate that new combination therapy approaches that induce the release of antigens and seek to increase response rates are the current hotspots. Anti-PD-1 antibodies combined with traditional treatment regimens, such as radiotherapy[26], HAIC[27], and TACE[28], attracted more attention. A previous study showed that stereotactic therapy not only prolonged survival but also converted the hepatocellular carcinoma (HCC) to be surgically resectable in patients with extrahepatic metastasis[10]. In this study, we also observed that Lenvatinib plus toripalimab with local-regional therapy benefits of patient survival presents “cooperative binding”.

In terms of therapeutic safety, systemic therapies based on toripalimab have been clinically feasible and safe. Although most patients experienced AEs, no grade 5 AEs were reported. Approximately 45.5% (25/55) of the patients experienced grade 3–4 AEs, but they were generally manageable. The adverse events that were more frequently noted in this study were elevations in the ALT and ALP. The most common treatment-related grade 3 or higher adverse event was elevations in the bilirubin level. Many AEs were more frequent in the stereotactic therapy group than in the Lenvatinib plus toripalimab therapy group, as demonstrated in a systematic review [29]. The possible reason might be as follows. First, the proportion of patients in the stereotactic therapy group who had local treatment after therapy with Lenvatinib plus toripalimab had failed. Second, local regional therapy, such as radiotherapy, HAIC, and TACE, may cause more side effects. However, none of the AEs in either group were unexpected, which was due to the regular outpatient care.

However, as a retrospective study, there are some limitations. First, part of the clinical data was obtained while the patients were hospitalized in the local hospital, and this data was recorded by the patients or their families at the follow-up or during outpatient visits. Second, the follow-up time was relatively short. Third, although Lenvatinib plus toripalimab therapy and stereotactic therapy were more commonly used for advanced patients, we need to assess the precise efficacy and safety in well-designed clinical trials. Although these factors somewhat weaken the validity and reliability of the conclusions, these ‘real-world’ data are still helpful for a subsequent prospective study.

Conclusion

Our data indicated that stereotactic therapy and Lenvatinib plus toripalimab have antitumor activity. Stereotactic therapy is a promising alternative for patients with previously treated advanced BTC, and this treatment may provide a significantly and clinically relevant improvement in antitumor activity and has the potential to improve the long-term survival for patients with previously treated advanced BTC.

Meanwhile, patients with extrahepatic metastasis may obtain clinical benefits and have a chance to convert to surgery, which can allow them to achieve tumor-free survival through Lenvatinib plus toripalimab therapy or stereotactic therapy. Retrospective analysis of PDL1 expression showed that PDL1 expression was associated with improved clinical benefits.

Abbreviations

BTCs, biliary tract cancers; ICC, intrahepatic cholangiocarcinoma; ECC, extrahepatic cholangiocarcinoma; GBC, gallbladder cancer; GC, gemcitabine and cisplatin; FOLFOX, fluorouracil, folinic acid, and oxaliplatin; PD-L1, programmed cell death ligand 1; PD-1, programmed cell death protein 1; Lenvatinib, tyrosine kinase inhibitors; ORR, objective response rate; PFS, progression-free survival; OS, overall survival; DCR, disease control rate; AEs, adverse events; SD, stable disease; PD, progressive disease; CR, complete response; PR, partial response; HR, hazard rate; AST, aspartate transaminase; ALT, aminoleucine transferase; HAIC, hepatic arterial infusion chemotherapy; TACE, transcatheter arterial chemoembolization; RECIST, response evaluation criteria in solid tumors; CTCAE, Common Terminology Criteria Coastocellular Group; FPFE, formal HCC, fixed paraffocellular carcinoma; ECE, Eastern Coastern Group.

Declarations

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Not applicable.

Statement of Ethics

All patients were fully informed about the objectives of this study and provided formal written consent *a priori*. The protocols of this study were compliant with the principles of the Declaration of Helsinki and were also approved by the institutional review board and ethics committee at Peking Union Medical College Hospital (PUMCH-JS-1391).

Conflict of Interest Statement

The authors have no conflicts of interest to declare.

Author Contributions

YCW and XY collected the data and wrote the manuscript. KZ, XTS and HTZ designed and examined the study. YYW, BYZ, JYL, DXW, and JNX helped to collect the literature and participated in discussions. ZHL, JXZ, ZYX, HSS, YRL, XBYand NZ performed the statistical analyses. All authors read and approved the final manuscript.

Data Availability Statement

All data generated or analyzed during this study are included in this published article and its supplementary information files.

References

1. Bridgewater JA, Goodman KA, Kalyan A, Mulcahy MF: **Biliary Tract Cancer: Epidemiology, Radiotherapy, and Molecular Profiling**. *American Society of Clinical Oncology educational book American Society of Clinical Oncology Annual Meeting* 2016, **35**:e194-203.
2. Tella SH, Kommalapati A, Borad MJ, Mahipal A: **Second-line therapies in advanced biliary tract cancers**. *Lancet Oncol* 2020, **21**(1):e29-e41.
3. Kim ST, Kang JH, Lee J, Lee HW, Oh SY, Jang JS, Lee MA, Sohn BS, Yoon SY, Choi HJ *et al*: **Capecitabine plus oxaliplatin versus gemcitabine plus oxaliplatin as first-line therapy for advanced biliary tract cancers: a multicenter, open-label, randomized, phase III, noninferiority trial**. *Annals of oncology : official journal of the European Society for Medical Oncology* 2019, **30**(5):788-795.
4. Valle J, Wasan H, Palmer DH, Cunningham D, Anthoney A, Maraveyas A, Madhusudan S, Iveson T, Hughes S, Pereira SP *et al*: **Cisplatin plus gemcitabine versus gemcitabine for biliary tract cancer**. *N Engl J Med* 2010, **362**(14):1273-1281.
5. Weinberg BA, Xiu J, Lindberg MR, Shields AF, Hwang JJ, Poorman K, Salem ME, Pishvaian MJ, Holcombe RF, Marshall JL *et al*: **Molecular profiling of biliary cancers reveals distinct molecular alterations and potential therapeutic targets**. *J Gastrointest Oncol* 2019, **10**(4):652-662.
6. Loeuillard E, Yang J, Buckarma E, Wang J, Liu Y, Conboy C, Pavelko KD, Li Y, O'Brien D, Wang C *et al*: **Targeting tumor-associated macrophages and granulocytic myeloid-derived suppressor cells augments PD-1 blockade in cholangiocarcinoma**. *J Clin Invest* 2020, **130**(10):5380-5396.
7. Bang YJ, Ueno M, Malka D, Chung HC, Doi TJJoCO: **Pembrolizumab (pembro) for advanced biliary adenocarcinoma: Results from the KEYNOTE-028 (KN028) and KEYNOTE-158 (KN158) basket studies**. 2019, **37**(15_suppl):4079-4079.
8. Zhao JLWSSZJHH: **Lenvatinib plus checkpoint inhibitors in patients (pts) with advanced intrahepatic cholangiocarcinoma (ICC): Preliminary data and correlation with next-generation sequencing %J Journal of Clinical Oncology**. 2018, **36**(4_suppl):500-500.
9. Jian Z, Fan J, Shi G-M, Huang X-Y, Wu D, Yang G-H, Ji Y, Chen Y, Liang F, Lu J-C *et al*: **Gemox chemotherapy in combination with anti-PD1 antibody toripalimab and lenvatinib as first-line**

treatment for advanced intrahepatic cholangiocarcinoma: A phase 2 clinical trial. 2021, **39**(15_suppl):4094-4094.

10. Yang X, Xu H, Zuo B, Yang X, Bian J, Long J, Wang D, Zhang J, Ning C, Wang Y *et al*: **Downstaging and resection of hepatocellular carcinoma in patients with extrahepatic metastases after stereotactic therapy**. *Hepatobiliary Surg Nutr* 2021, **10**(4):434-442.
11. Sheng X, Yan X, Chi Z, Si L, Cui C, Tang B, Li S, Mao L, Lian B, Wang XJSSEP: **Axitinib in Combination with Toripalimab, a Humanized IgG4 mAb Against Programmed Death-1 (PD-1) in Patients with Metastatic Mucosal Melanoma: A Non-Randomized, Open-Label, Dose-Finding, and Cohort-Expansion Phase 1b Trial**.
12. Wang F, Wei XL, Wang FH, Xu N, Shen L, Dai GH, Yuan XL, Chen Y, Yang SJ, Shi JHJAooojotESfMO: **Safety, efficacy and tumor mutational burden as a biomarker of overall survival benefit in chemo-refractory gastric cancer treated with toripalimab, a PD1 antibody in phase Ib/II clinical trial NCT02915432**. 2019.
13. Brunner TB, Seufferlein T: **Radiation therapy in cholangiocellular carcinomas**. *Best practice & research Clinical gastroenterology* 2016, **30**(4):593-602.
14. Marin JJG, Prete MG, Lamarca A, Tavolari S, Landa-Magdalena A, Brandi G, Segatto O, Vogel A, Macias RIR, Rodrigues PM *et al*: **Current and novel therapeutic opportunities for systemic therapy in biliary cancer**. *British journal of cancer* 2020, **123**(7):1047-1059.
15. Ueno M, Ikeda M, Sasaki T, Nagashima F, Mizuno N, Shimizu S, Ikezawa H, Hayata N, Nakajima R, Morizane C: **Phase 2 study of lenvatinib monotherapy as second-line treatment in unresectable biliary tract cancer: primary analysis results**. *BMC cancer* 2020, **20**(1):1105.
16. Bang Y-J, Ueno M, Malka D, Chung HC, Nagrial A, Kelley RK, Piha-Paul SA, Ros W, Italiano A, Nakagawa K *et al*: **Pembrolizumab (pembro) for advanced biliary adenocarcinoma: Results from the KEYNOTE-028 (KN028) and KEYNOTE-158 (KN158) basket studies**. 2019, **37**(15_suppl):4079-4079.
17. Lin J, Yang X, Long J, Zhao S, Mao J, Wang D, Bai Y, Bian J, Zhang L, Yang X *et al*: **Pembrolizumab combined with lenvatinib as non-first-line therapy in patients with refractory biliary tract carcinoma**. *Hepatobiliary surgery and nutrition* 2020, **9**(4):414-424.
18. Jian Z, Fan J, Shi G-M, Huang X-Y, Wu D, Liang F, Yang G-H, Lu J-C, Chen Y, Ge N-L *et al*: **Lenvatinib plus toripalimab as first-line treatment for advanced intrahepatic cholangiocarcinoma: A single-arm, phase 2 trial**. 2021, **39**(15_suppl):4099-4099.
19. Mody K, Starr J, Saul M, Poorman K, Weinberg BA, Salem ME, VanderWalde A, Shields AF: **Patterns and genomic correlates of PD-L1 expression in patients with biliary tract cancers**. *Journal of gastrointestinal oncology* 2019, **10**(6):1099-1109.
20. Hegde PS, Wallin JJ, Mancao C: **Predictive markers of anti-VEGF and emerging role of angiogenesis inhibitors as immunotherapeutics**. *Semin Cancer Biol* 2018, **52**(Pt 2):117-124.
21. Shigeta K, Datta M, Tai H, Kitahara S, Chen IX, Matsui A, ikuchi HK, Mamessier E, Aoki S, Ramjiawan RRJH: **Dual Programmed Death Receptor-1 and Vascular Endothelial Growth Factor Receptor-2**

Blockade Promotes Vascular Normalization and Enhances Antitumor Immune Responses in Hepatocellular Carcinoma. 2019, **71**(4).

22. Herbst RS, Arkenau H-T, Santana-Davila R, Calvo E, Paz-Ares L, Cassier PA, Bendell J, Penel N, Krebs MG, Martin-Liberal J *et al*: **Ramucirumab plus pembrolizumab in patients with previously treated advanced non-small-cell lung cancer, gastro-oesophageal cancer, or urothelial carcinomas (JVDF): a multicohort, non-randomised, open-label, phase 1a/b trial**. *Lancet Oncol* 2019, **20**(8):1109-1123.
23. Chae YK, Arya A, Iams W, Cruz MR, Chandra S, Choi J, Giles F: **Current landscape and future of dual anti-CTLA4 and PD-1/PD-L1 blockade immunotherapy in cancer; lessons learned from clinical trials with melanoma and non-small cell lung cancer (NSCLC)**. *J Immunother Cancer* 2018, **6**(1):39.
24. Thiruthaneeswaran N, Bibby BAS, Yang L, Hoskin PJ, Bristow RG, Choudhury A, West C: **Lost in application: Measuring hypoxia for radiotherapy optimisation**. *Eur J Cancer* 2021, **148**:260-276.
25. Roselli M, Cereda V, di Bari MG, Formica V, Spila A, Jochems C, Farsaci B, Donahue R, Gulley JL, Schlom J *et al*: **Effects of conventional therapeutic interventions on the number and function of regulatory T cells**. *Oncoimmunology* 2013, **2**(10):e27025.
26. Ozpiskin OM, Zhang L, Li JJ: **Immune targets in the tumor microenvironment treated by radiotherapy**. *Theranostics* 2019, **9**(5):1215-1231.
27. He MK, Liang RB, Zhao Y, Xu YJ, Chen HW, Zhou YM, Lai ZC, Xu L, Wei W, Zhang YJ *et al*: **Lenvatinib, toripalimab, plus hepatic arterial infusion chemotherapy versus lenvatinib alone for advanced hepatocellular carcinoma**. *Therapeutic advances in medical oncology* 2021, **13**:17588359211002720.
28. Zhu XD, Li KS, Sun HC: **Adjuvant therapies after curative treatments for hepatocellular carcinoma: Current status and prospects**. *Genes & diseases* 2020, **7**(3):359-369.
29. Kroeze SGC, Fritz C, Hoyer M, Lo SS, Ricardi U, Sahgal A, Stahel R, Stupp R, Guckenberger M: **Toxicity of concurrent stereotactic radiotherapy and targeted therapy or immunotherapy: A systematic review**. *Cancer treatment reviews* 2017, **53**:25-37.

Tables

Table 1 Baseline characteristics

Characteristics	Toripalimab plus lenvatinib (n=24)	Stereotactic therapy (n=31)	P value
Median Age（range）, y	62(58-65)	64.5(59-65)	0.08
Gender, n (%)			0.255
Male	14(58.3)	23(74.2)	
Female	10(41.7)	8(25.8)	
Tumor subtype, n (%)			0.563
Intrahepatic cholangiocarcinoma	15(62.5)	22(71)	
Extrahepatic cholangiocarcinoma	5(20.8)	3(9.6)	
Gallbladder cancer	4(16.7)	6(19.4)	
ECOG performance status, n (%)			0.064
0	3(12.5)	12(38.7)	
1	16(66.7)	12(38.7)	
2	5(20.8)	7(22.6)	
Differentiated histology, n (%)			<0.01
Well	2(8.3)	0	
Moderately	6(25)	5(16.1)	
Poorly	4(16.7)	8(25.8)	
Moderately-poorly	1(4.2)	4(12.9)	
Well-moderately	1(4.2)	14(45.2)	
Unsure	10(41.6)	0	
Previous antitumor therapy, n (%)			0.587
Radical surgery resection	10(41.7)	14(45.2)	
Systemic chemotherapy	7(29.2)	12(38.7)	
Targeted therapy	10(41.7)	7(22.6)	
Interventional therapy	5(20.8)	6(19.4)	
Site of metastases, n (%)			0.903
Intrahepatic	10(41.7)	11(35.5)	
Lymph nodes	14(58.3)	18(58.1)	

Lung	1(4.2)	3(9.7)	
Bone	1(4.2)	2(6.5)	
Local regional therapy			-
Radiotherapy	-	16(51.6)	
HAIC	-	3(9.7)	
TACE	-	6(19.4)	
TACE+ Radiotherapy	-	2(6.5)	
HAIC+ Radiotherapy	-	3(9.7)	
TACE+HACI+RFA+ Radiotherapy	-	1(3.2)	

Stereotactic therapy: Toripalimab +lenvatinb+LRT; LRT, local-regional therapy; HAIC, Hepatic artery infusion chemotherapy, TACE, Transarterial chemoembolization; RFA, Radiofrequency ablation.

Table 2 Tumor response to treatment in each treatment group

	Toripalimab plus lenvatinib (n=24)	Stereotactic therapy (n=31)	P value	Effect size (95% CI)
Objective response rate (n, %), 95% CI	25(6.3-43.7)	35.5(17.6-53.3)	0.558	OR 1.65
Complete response (n, %)	1(4.2)	1(3.2)	-	-
Partial response (n, %)	5(20.8)	10(32.3)	-	-
Stable disease (n, %)	12(50)	16(51.6)	-	-
Progressive disease (n, %)	6(25)	4(12.9)	-	-
DCR(95% CI)	75(56.3-93.7)	87.1(74.6-99.6)	0.304	OR 2.25
Median overall survival, months (95% CI)	9.2(7.9-10.4)	13.7(9.4-17.9)	0.023	HR 0.45 (0.22-0.91)
Median progression-free survival, months (95% CI)	4.6(2.5-6.6)	9.6(4.7-14.5)	0.035	HR 0.50 (0.26-0.97)
Downstaging and resection	1(4.2)	3(9.7)	-	OR 2.46

Table 3 Safety summary of toripalimab plus lenvatinib and stereotactic therapy

Adverse events	Toripalimab plus lenvatinib n (%) (n=24)			Stereotactic therapy n (%) (n=31)		
	Any grade	Grade 1–2	Grade 3-4	Any grade	Grade 1–2	Grade 3-4
Fatigue	15(62.5)	14(58.3)	1(4.2)	25(80.6)	23(74.2)	2(6.5)
Nausea	4(16.7)	4(16.7)	0	9(29)	8(25.6)	1(3.2)
Vomitting	3(12.5)	3(12.5)	0	6(19.4)	5(16.1)	1(3.2)
Proteinuria	7(29.2)	6(25)	1(4.2)	10(32.3)	8(25.6)	2(6.5)
Stomatitis	2(8.3)	2(8.3)	0	8(25.6)	7(22.6)	1(3.2)
Arthralgia	2(8.3)	2(8.3)	0	2(6.5)	2(6.5)	0
Rash	6(25)	5(20.8)	1(4.2)	8(25.6)	5(16.1)	3(9.7)
Abdominal pain	9(37.5)	9(37.5)	0	12(38.7)	11(35.5)	1(3.2)
Diarrhea	6(25)	6(25)	0	10(32.3)	8(25.6)	2(6.5)
Fever	1(4.2)	1(4.2)	0	2(6.5)	2(6.5)	0
Anorexia	3(9.7)	3(9.7)	0	9(29)	8(25.6)	1(3.2)
Gastrointestinal haemorrhage	2(8.3)	2(8.3)	0	4(12.9)	4(12.9)	0
Epistaxis	1(4.2)	1(4.2)	0	1(3.2)	1(3.2)	0
Hypertension	9(37.5)	8(33.3)	1(4.2)	11(35.5)	10(32.3)	1(3.2)
Headache	1(4.2)	1(4.2)	0	1(3.2)	0	1(3.2)
Myocarditis	4(16.7)	4(16.7)	0	3(9.7)	2(6.5)	1(3.2)
AST or ALT increased	16(66.7)	15(62.5)	1(4.2)	25(80.6)	23(74.2)	2(6.5)
Anemia	4(16.7)	4(16.7)	0	6(19.4)	6(19.4)	0
Bilirubin elevation	7(29.2)	5(20.8)	2(8.3)	15(48.4)	10(32.3)	5(16.1)
Hypothyroidism	3(9.7)	3(9.7)	0	9(29)	9(29)	0
Hypoproteinemia	7(29.2)	6(25)	1(4.2)	11(35.5)	10(32.3)	1(3.2)
Thrombocytopenia	5(20.8)	5(20.8)	0	3(9.7)	3(9.7)	0
Leukopenia	5(20.8)	5(20.8)	0	10(32.3)	10(32.3)	0

Figures

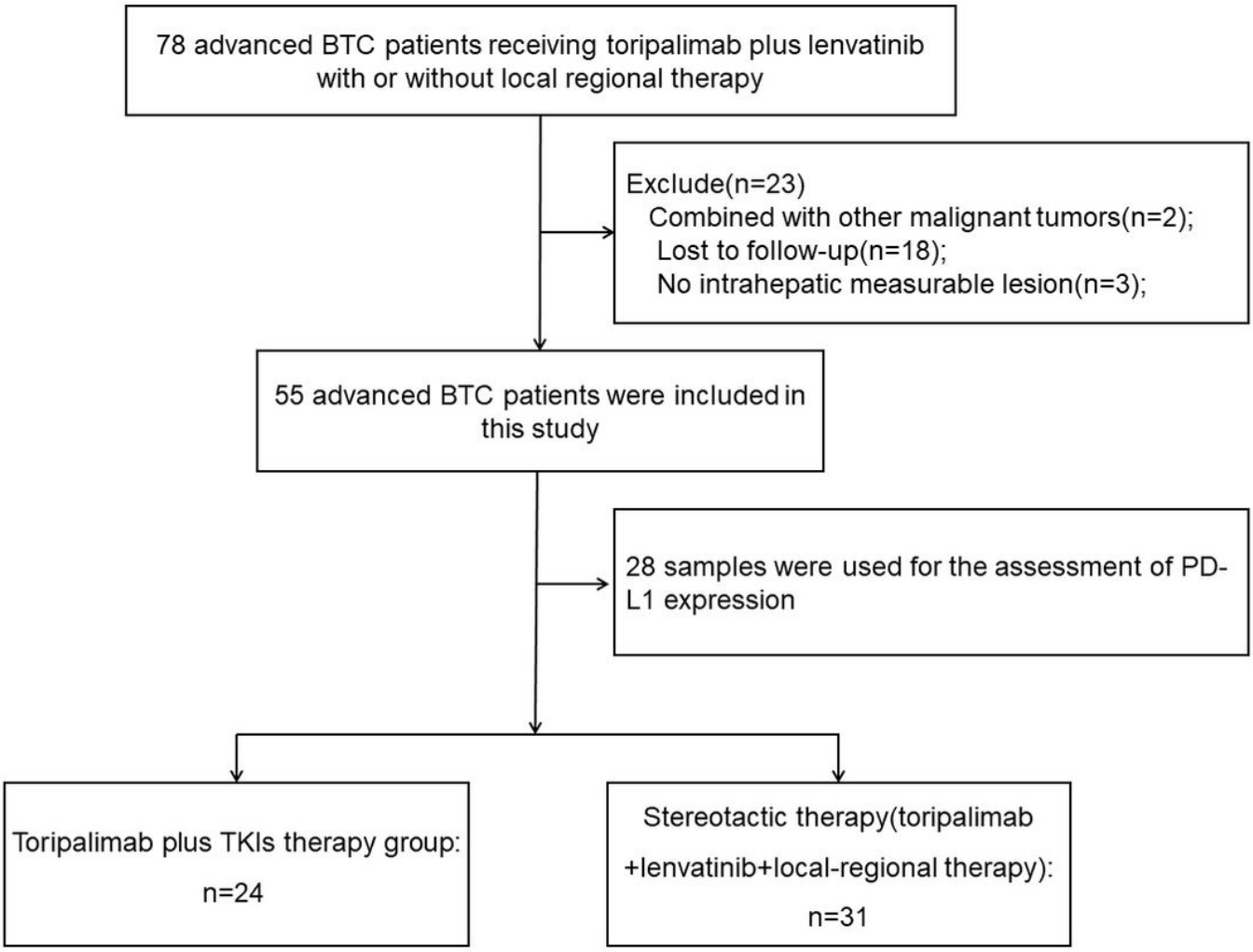

Figure 1

Study flow diagram. BTC, biliary tract cancer; LRT, local-regional therapy; Stereotactic therapy: Lenvatinib plus toripalimab with LRT.

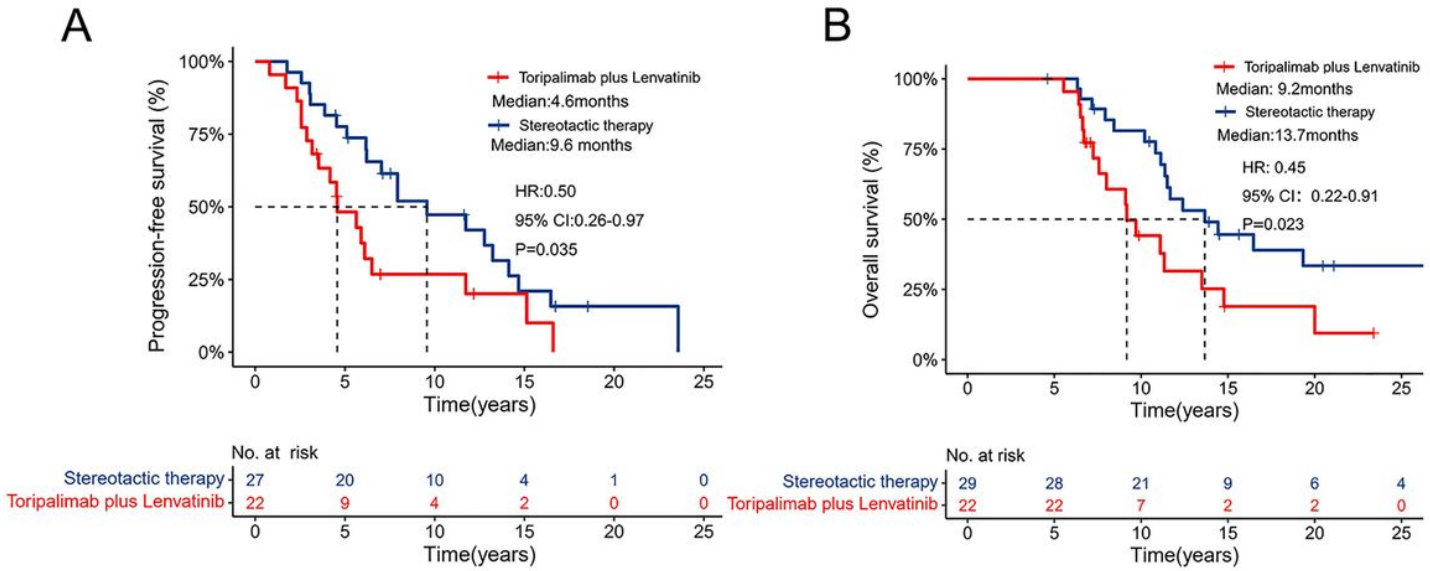

Figure 2

Kaplan–Meier estimates of the progression-free survival (A) and overall survival (B) in the Lenvatinib plus toripalimab group and the stereotactic therapy group.

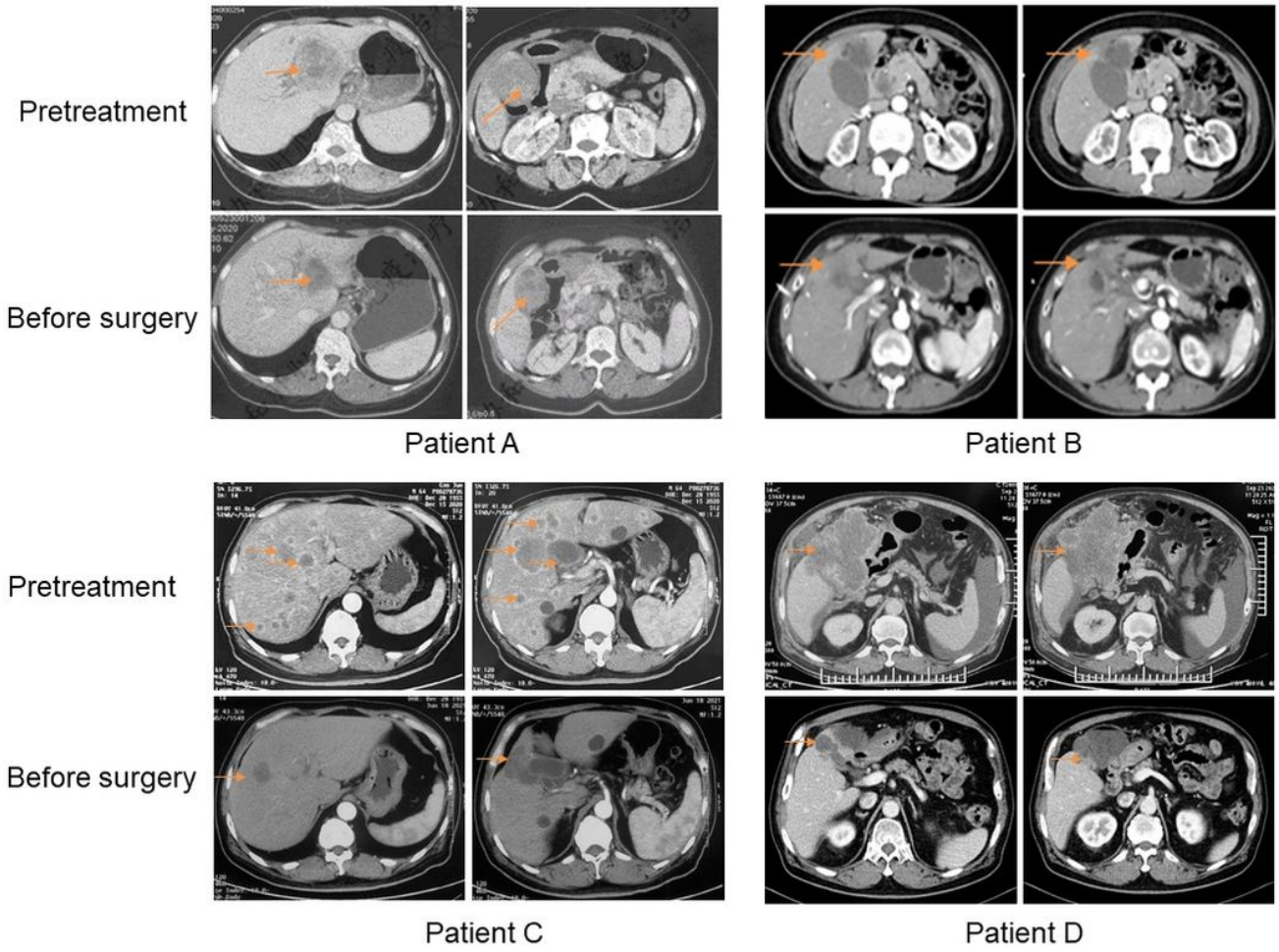

Figure 3

The pretreatment and preoperative CT or MR scans in 4 patients. Patients A, B, and C received stereotactic therapy, while patient D received Lenvatinib plus toripalimab.

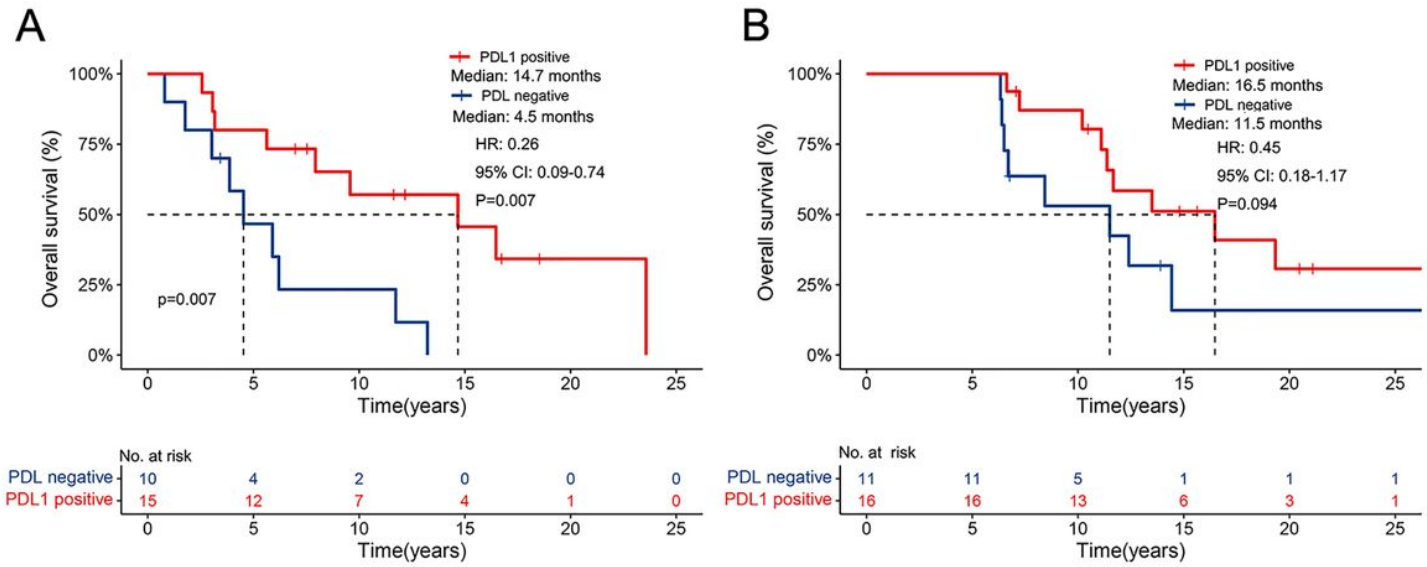

Figure 4

Kaplan–Meier estimates of the overall survival (A) and progression-free survival (B) in 28 patients with BTC whose tissues were tested for PD-L1 expression.

Supplementary Files

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