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Original Article

Local-regional therapy combined with toripalimab and lenvatinib in patients with advanced biliary tract cancer

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Abstract: Local-regional therapy combined with PD-1 inhibitors and lenvatinib (triple combination therapy) has demonstrated potent antitumor activity in solid tumors. However, the efficacy and safety of the triple combination therapy in patients with advanced biliary tract cancer (BTC) remain unclear. This retrospective study evaluated the efficacy and safety of the triple combination therapy in advanced BTC. Tumor tissues were collected to assess the expression status of PDL1 to identify efficacy biomarkers. Forty-nine patients were included: 24 in lenvatinib plus toripalimab therapy; 25 in the triple combination therapy. The triple combination therapy group showed longer median progression-free survival (mPFS) (7.9 versus 5.6 months, P=0.015) and longer median overall survival (mOS) (13.7 versus 11.1 months, P=0.145) than the lenvatinib plus toripalimab group. The overall response rate (ORR) was 32% [95% confidence interval (CI): 12.3-51.7] with the triple combination therapy versus 25% (95% CI: 6.3-43.7) with toripalimab plus lenvatinib. Three patients received surgery after the triple combination therapy. All patients experienced any-grade adverse events (AEs) without any specific toxicities. PDL1 expression was associated with improved clinical benefits. Local-regional therapy combined with PD-1 inhibitors and lenvatinib may be an encouraging treatment choice for advanced BTC without an increase in specific toxicities.

Keywords: Advanced biliary tract cancer, toripalimab, lenvatinib, local-regional therapy, synergic effect

Introduction

Biliary tract cancers (BTCs), including intrahepatic cholangiocarcinoma (ICC), extrahepatic cholangiocarcinoma (ECC), and gallbladder cancer (GBC), are aggressive malignancies [1]. Most patients have advanced-stage disease at presentation, and the prognosis is poor [2, 3]. Although gemcitabine, cisplatin (GC), and FOLFOX are recommended as first-line and second-line therapy for advanced BTC [4, 5], the overall survival is still dismal.

Strategies based on chemotherapy have also attempted to improve the low survival rate of BTC patients. 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) [6]. Recently, the TOPAZ-1 trial testing immunotherapy plus chemotherapy as first-line treatment for advanced BTC showed that mOS was 12.8 months with durvalumab plus gemcitabine and cisplatin vs. 11.6 months with gemcitabine and cisplatin alone (HR: 0.80; 95% CI [0.66, 0.97]; P=0.021), and mPFS was significantly prolonged with chemoimmunotherapy vs. chemotherapy alone (HR: 0.75; 95% CI [0.64, 0.89]; P=0.001) [7]. The ORR was also significantly improved (26.7% vs. 18.7%). Growing evidence indicates that chemotherapy can promote immune response by reducing

T-regulatory cell activity [8] and increasing the immunogenicity of malignant cells [9].

Accumulating evidence has demonstrated that immunotherapy has antitumor activity in different tumors [10, 11]. The FDA has approved Pembrolizumab for tumors with high microsatellite instability (MSI-H) or mismatch repair deficiency (dMMR). Immunotherapy [12] alone is not satisfactory in patients with advanced BTC. A previous study showed that the combination of PD-1 inhibitor with Lenvatinib could increase the ORR to 21.4% in patients with advanced BTC [13]. Dual anti-PD-1 and VEGF/VEGFR blockade can reprogram the immunosuppressive tumor microenvironment into an immunostimulatory environment and enhance the antitumor efficacy of immunotherapy [14, 15]. BTC's complex and heterogeneous tumor immune microenvironment makes the ORR of lenvatinib combined with anti-PD-1 inhibitor ambiguous.

Previous studies showed that locoregional therapies, which refer to radiotherapy, hepatic arterial infusion chemotherapy (HAIC), and transcatheter arterial chemoembolization (TACE), enhance the endogenous immune response. PD-1 inhibitors combined with radiotherapy, HAIC, and TACE have been shown to have survival benefits for cholangiocarcinoma [16-18]. A previous study showed that Lenvatinib plus toripalimab with local-regional therapy prolonged survival and converted hepatocellular carcinoma (HCC) to be surgically resectable in patients with extrahepatic metastasis [19].

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 [20, 21]. Based on the above views, we investigated the efficacy and safety of triple combination therapy in patients with advanced BTC.

Materials and methods

Patients

This retrospective study aimed to assess the efficacy and safety of the triple combination therapy in patients with advanced BTC from March 2019 until June 2021 at Peking Union Medical College Hospital. Patients with advanced BTC who received the triple combination therapy and those with advanced BTC who received lenvatinib and toripalimab were enrolled. The inclusion criteria included: histologically confirmed ECC, ICC, and GBC; at least one line of systemic therapy previously; 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 exclusion criteria included patients with diverse pathological tumor types and those who had received additional antitumor therapy, including chemotherapy while receiving triple combination therapy, lenvatinib, and toripalimab during this study. The demographic, surgical, pathological, regional, and systemic treatment information was recorded. Patients were followed-up by outpatient clinic visits every 6-8 weeks after the patient's treatment. 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 (approval number: JS-1391).

Treatment

In the lenvatinib plus toripalimab group, patients received toripalimab at a fixed dosage of 240 mg or 3 mg/kg every three weeks. Lenvatinib was 12 mg (for patients with a body weight ≥60 kg) or 8 mg (for patients with a body weight <60 kg) orally once a day. In the triple combination 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 of 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, and TACE. Lenvatinib plus toripalimab was administered 2-3 weeks earlier than the local regional therapies and was not discontinued before or after the local regional treatments.

Radiotherapy is given to metastatic lesions. The radiation doses 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) [22]. For lesions with rich intrahepatic blood

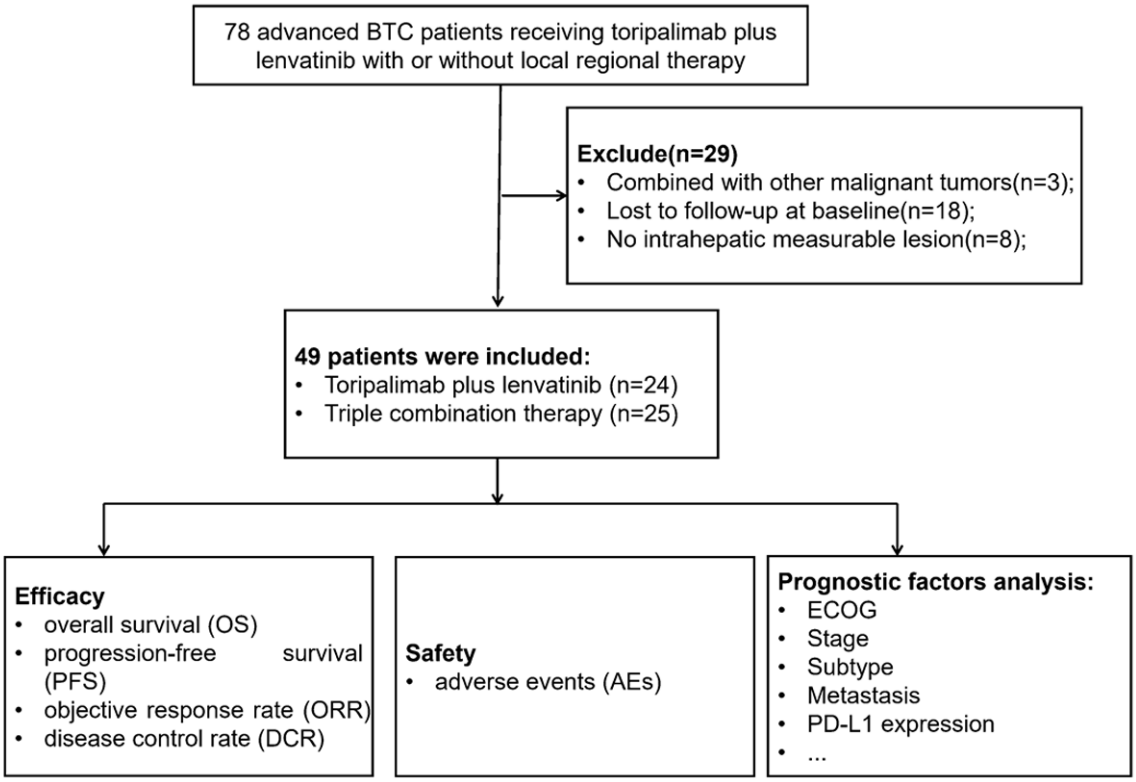

Figure 1. Study flow diagram. BTC, biliary tract cancer; LRT, local-regional therapy; Triple combination therapy: Lenvatinib plus toripalimab with LRT.

supply, TACE treatment is recommended. 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 [23-25]. The length of time that was used to infuse the chemotherapy drugs was generally not less than 15 minutes. The HAIC regimen was composed of oxaliplatin (40 mg/m^2 for 2 h) and 5-fluorouracil (800 mg/m^2 for 22 h) on days 1-3 every 3 or 4 weeks. Leucovorin calcium was dripped intravenously at 200 mg/m^2 for 2 h during a 5-fluorouracil infusion. The treatments may have been interrupted and even discontinued when unacceptable or severe adverse events (AEs) occurred or when there was disease progression.

Definition of outcomes

The patient outcomes were the efficacy and safety of the triple combination therapy in patients with advanced BTC. 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 each patient experienced during the observation period was recorded. The objective clinical response used enhanced computed tomography (CT) or magnetic resonance imaging (MRI) according to RECIST 1.1 at 6-8 weeks after the patient's treatment. Professional radiologists evaluated the imaging examinations.

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 stan-

dard protocol. The tumor tissues were fixed, embedded, and cut into three 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 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 June 30, 2022. The PFS and OS were calculated from the date of the initiation of treatment until the date of disease progression or death. The two treatment groups' baseline characteristics and efficacy data were compared using the chi-square test or Fisher's exact test. The Kaplan-Meier and bilateral log-rank tests 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

A total of 49 patients with advanced BTC met the eligibility criteria: 25 received triple combination therapy, and 24 received lenvatinib plus toripalimab. The population was predominantly male (65.3%). The median age of the patients was 62 years. The majority (67.3%) was ICC. In total, 38 (77.6%) patients had an ECOG performance status of 0-1. Most patients had metastatic cancer in the liver (28/49, 57.1%) and lymph nodes (35/49, 71.4%). Although Toripalimab plus lenvatinib group showed higher frequencies of ECOG performance status of 1, the difference did not reach a statistically significant level (P=0.09). Most patients had TNM stage III, 17 in toripalimab plus lenvatinib, and 15 in triple combination therapy. The baseline characteristics of the two cohorts are summarized in **Table 1**, and no difference was observed. In the triple combination therapy group and the lenvatinib plus toripalimab group, lymph node metastasis was the most common. In the triple combination therapy group, 16 (51.6%) patients had radiotherapy, 3 (9.7%) had received HAIC, and 6 (19.4%) had received TACE.

Efficacy

The data were last updated on June 30, 2022. In this study, the median duration of follow-up was 27.5 (IQR, 18.8-36.2) months. As of June 30, 2022, 39 patients dead: 21 patients dead in the lenvatinib plus toripalimab therapy group and 18 dead in the triple combination therapy group. The median PFS in the triple combination therapy group was 7.9 (95% CI: 2.8-13.1), compared with 5.6 (95% CI: 3.8-7.5) in the toripalimab plus lenvatinib therapy group (HR: 0.48; 95% CI: [0.0.26-0.88]; P=0.015, **Figure 2A**). The median OS was 13.7 months (95% CI: 10.4-17.0) in the triple combination therapy group versus 11.1 months (95% CI: 7.9-14.3) in the toripalimab plus Lenvatinib therapy group (HR: 0.62; 95% CI: [0.32-1.19]; P=0.145, **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 triple combination therapy group, the ORR was 32% (8/25; 95% CI: 12.3-51.7), and the DCR was 88% (22/25; 95% CI: 74.3-101.7) (**Table 2**). Among the two cohorts, four patients achieved PR and had surgery after the above treatment: 3 received triple combination therapy and one received therapy with toripalimab plus lenvatinib (**Figure 3**). Evidence shows that patients in the triple combination therapy group have better survival than those in the Lenvatinib plus toripalimab therapy group.

Multivariate analyses were performed to identify independent prognostic factors associated with OS and PFS. Potential predictors include age, sex, subtype, TNM stage, ECOG, therapies, PD-L1 expression, and metastasis. The results indicate that therapies and PD-L1 expression were significant prognostic associations with OS and PFS (**Figure 4**).

Safety

All patients experienced ≥1 adverse event (AE), and no treatment-related deaths occurred in this study. The most common AEs were an ALT or AST elevation (73.4%, n=36) and fatigue (73.4%, n=36). The triple combination therapy group had a higher incidence of AEs than the

Table 1. Baseline characteristics

Characteristics	Toripalimab plus lenvatinib (n=24)	Triple combination therapy (n=25)	*P* value
Median Age (range), y	62 (58-65)	64.5 (58.8-65)	0.18
Gender, n (%)			0.48
Male	14 (58.3)	18 (72)	
Female	10 (41.7)	7 (28)	
Tumor subtype, n (%)			0.66
ICC	15 (62.5)	18 (72)	
ECC	5 (20.8)	1 (4)	
GBC	4 (16.7)	6 (24)	
ECOG performance, n (%)			0.09
0	3 (12.5)	9 (36)	
1	16 (66.7)	10 (40)	
2	5 (20.8)	6 (24)	
Differentiated histology, n (%)			0.25
Well	2 (8.3)	0	
Moderate	6 (25)	3 (12)	
Poor	4 (16.7)	7 (28)	
Moderate-poor	1 (4.2)	4 (16)	
Well-moderate	1 (4.2)	0	
Unsure	10 (41.6)	11 (44)	
Previous therapy, n (%)			
Radical surgery	10 (41.7)	10 (40)	0.91
Systemic chemotherapy	7 (29.2)	9 (36)	0.62
Targeted therapy	10 (41.7)	11 (44)	0.87
Site of metastases, n (%)			
Intrahepatic	14 (58.3)	14 (56)	0.87
Lymph nodes	17 (70.8)	18 (32)	0.93
Lung	2 (8.3)	2 (8)	0.97
Bone	2 (8.3)	2 (8)	0.97
Tumor diameter, mean ± SD (cm)	6.5±7.1	5.1±3.2	0.85
TNM stage, n (%)			0.62
III	17 (70.8)	15 (60)	
IV	7 (29.2)	10 (40)	
Local regional therapy			-
RT	-	16 (51.6)	
HAIC	-	3 (9.7)	
TACE	-	6 (19.4)	

ICC, intrahepatic cholangiocarcinoma; ECC, extrahepatic cholangiocarcinoma; GBC, gallbladder cancers; Triple combination therapy: Toripalimab + lenvatinb + LRT; LRT, local-regional therapy; HAIC, Hepatic artery infusion chemotherapy; TACE, Transarterial chemoembolization; RT, radiotherapy.

Lenvatinib plus toripalimab therapy group. The most common AEs with the triple combination therapy were Fatigue (21/25, 84%) and AST or ALT increase (21/25, 84%). 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 24 samples were used for the assessment of PD-L1 expression by two pathologists (Table S1). Overall, 58.3% (14/24) of the samples were PD-L1 positive (≥1%). The subgroup with positive PDL1 expression showed a longer

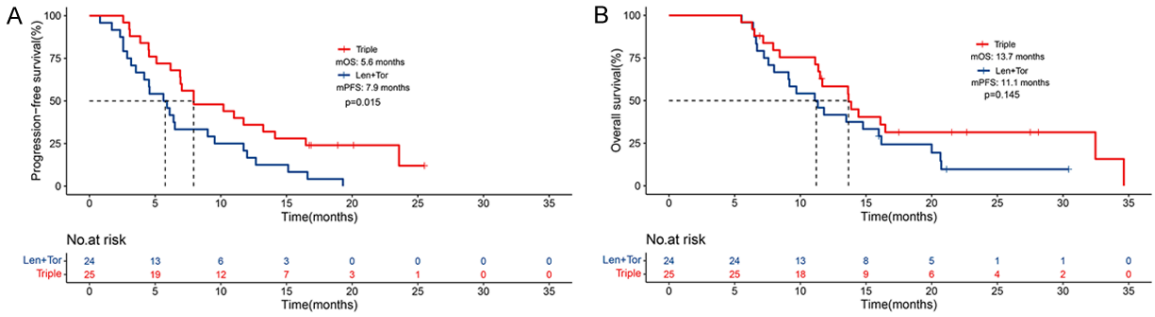

Figure 2. Kaplan-Meier estimates progression-free survival (A) and overall survival (B) in the Lenvatinib plus toripalimab group and the triple combination therapy group.

Table 2. Tumor response to treatment in each treatment group

	Toripalimab plus lenvatinib (n=24)	Triple combination therapy (n=25)	*P*-value	Effect size (95% CI)
ORR (n, %), 95% CI	25 (6.3-43.7)	32 (12.3-51.7)	0.75	-
CR (n, %)	0	0		-
PR (n, %)	6 (24)	8 (32)		-
SD (n, %)	12 (50)	14 (56)		-
PD (n, %)	6 (25)	3 (12)		-
DCR (95% CI)	75 (56.3-93.7)	88 (74.3-101.7)	0.29	-
mOS, months (95% CI)	11.1 (7.9-14.3)	13.7 (10.4-17.0)	0.145	0.62 (0.32-1.19)
mPFS, months (95% CI)	5.6 (3.8-7.5)	7.9 (2.8-13.1)	0.015	0.48 (0.26-0.88)
Down staging and resection	1 (4.2)	3 (12)	0.61	-

ORR, objective response rate; CR, complete response; PR, partial response; SD, stable disease; PD, progressive disease; mOS, median overall survival; mPFS, median progression-free survival.

mPFS and mOS than the subgroup without PDL1 expression (mPFS: 12.7 vs. 5.9 months, P=0.013; mOS: 16.2 vs. 8.4 months, P=0.005) (**Figure 5**). For the OS, ORR, and DCR, the subgroup with positive PDL1 expression was not significantly different compared with the PD-1-negative subgroup (Table S2).

Discussion

Compared to the published literature, this is the first study to evaluate the efficacy and safety of the local-regional therapy combined with toripalimab and lenvatinib in advanced BTC patients. Our study revealed that triple combination therapy could improve the overall survival of patients with advanced BTC without an increase in specific toxicities. In addition, three patients received surgery after the triple combination therapy. The results indicate that triple combination therapy may be an encouraging treatment choice for advanced BTC.

Previous reports were mainly focused on PD-1 inhibitors plus lenvatinib use in patients with advanced BTC, yielding an undesirable ORR of 10-25% [26-28]. In this study, adding local-regional therapy to toripalimab and lenvatinb yielded confirmed response rates (32%). Preclinical and clinical evidence suggested that PD-1 inhibitors combined with radiotherapy, TACE, or HAIC could induce additive antitumor effects. Local-regional therapy caused cell death and released tumor-associated antigens [29]. A combination of radiotherapy and immunotherapy led to a complete response for patients with advanced cholangiocarcinoma [30, 31]. The combination of HAIC and PD-1 inhibitors proved safe and effective [17]. TACE plus immune checkpoint inhibitors improved survival and were well-tolerated in patients with unresectable cholangiocarcinoma [32]. The results of this study were consistent with the antitumor activity observed in HCC [19]. Local-regional therapy combined with toripalimab

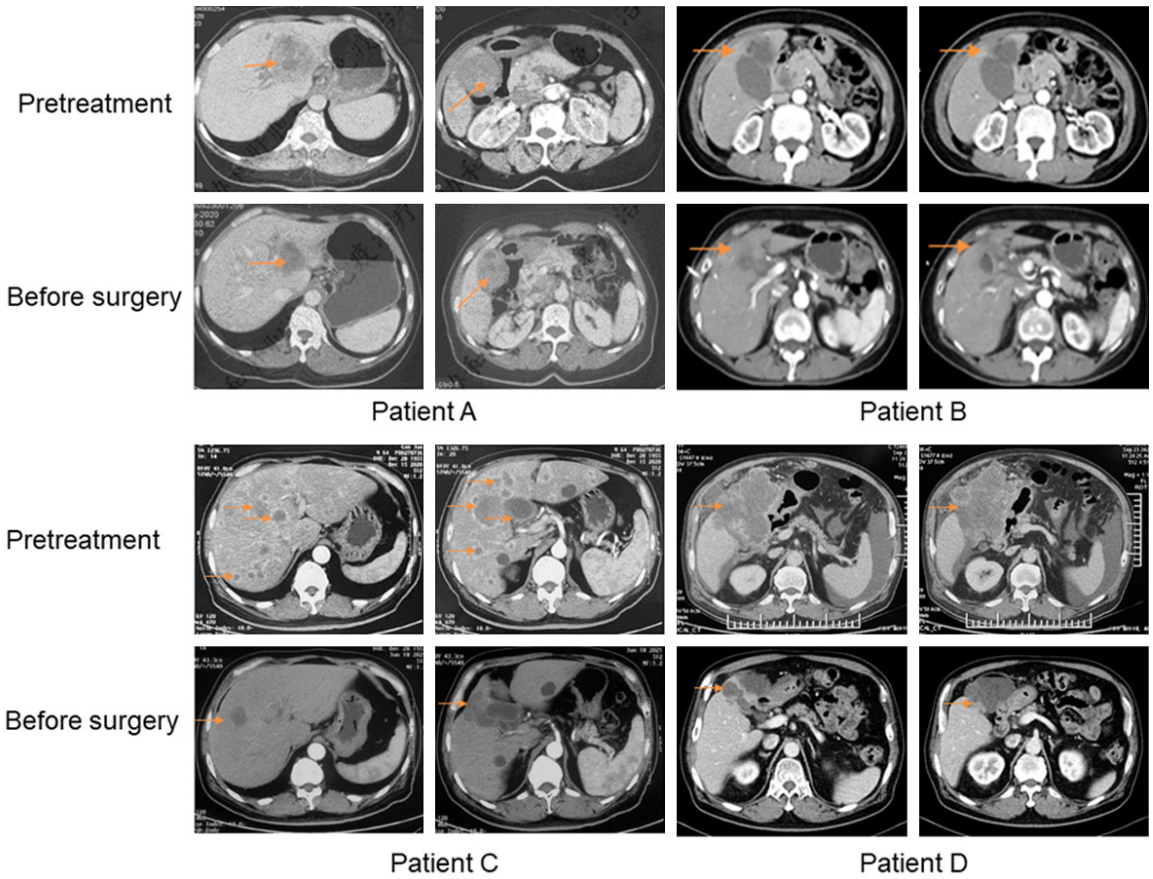

Figure 3. The pretreatment and preoperative CT scans in 4 patients. Patients A, B, and C received triple combination therapy, while patient D received Lenvatinib plus toripalimab.

and lenvatinib presents "synergic effect". Adding local-regional therapy to immunotherapy and lenvatinb improves the overall survival of patients with advanced BTC.

Predictive biomarkers for combination immunotherapy have been performed. In our study, we found that positive PD-L1 expression in tumor tissues was significantly associated with a longer PFS and OS. Given the limited sample size, the current correlation between PDL1 expression status and treatment outcome should be understood with caution. Further research is needed to demonstrate the role of PD-L1 expression.

In terms of therapeutic safety, systemic therapies based on toripalimab have been clinically feasible and safe. Although most patients experienced AEs, no grades 5 AEs were reported. Approximately 40.8% (20/49) 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 triple combination therapy group than in the Lenvatinib plus toripalimab therapy group, as demonstrated in a systematic review [33]. The possible reason might be as follows. First, the proportion of patients in the triple combination therapy group who had local treatment after therapy with Lenvatinib plus toripalimab had failed. Second, local, and regional therapy, such as radiotherapy, HAIC, and TACE, may cause more side effects. However, none of the AEs in either group were unexpected due to the regular outpatient care.

However, there are some limitations. First, this study lacks a control group (such as chemotherapy drugs or best supportive care). Second,

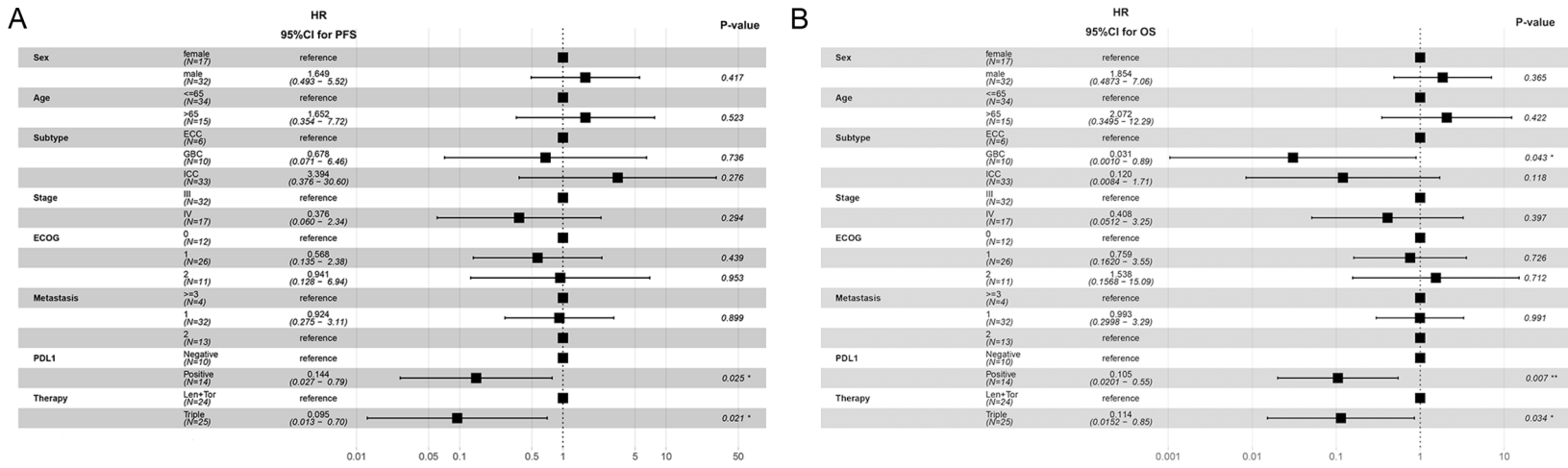

Figure 4. Multivariate analyses based on the Cox regression model were performed to identify independent prognostic factors associated with OS and PFS.

Table 3. Safety summary of toripalimab plus lenvatinib and Triple combination therapy

Adverse events	Toripalimab plus lenvatinib n (%) (n=24)			Triple combination therapy n (%) (n=25)		
	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)	21 (84)	20 (80)	1 (4)
Nausea	4 (16.7)	4 (16.7)	0	9 (36)	8 (32)	1 (4)
Vomitting	3 (12.5)	3 (12.5)	0	6 (24)	5 (20)	1 (4)
Proteinuria	7 (29.2)	6 (25)	1 (4.2)	9 (36)	9 (36)	0
Stomatitis	2 (8.3)	2 (8.3)	0	3 (12)	2 (8)	1 (4)
Arthralgia	2 (8.3)	2 (8.3)	0	2 (8)	2 (8)	0
Rash	6 (25)	5 (20.8)	1 (4.2)	8 (32)	5 (20)	3 (12)
Abdominal pain	9 (37.5)	9 (37.5)	0	12 (48)	11 (44)	1 (4)
Diarrhea	6 (25)	6 (25)	0	7 (28)	5 (20)	2 (8)
Fever	1 (4.2)	1 (4.2)	0	2 (8)	2 (8)	0
Anorexia	3 (9.7)	3 (9.7)	0	8 (32)	8 (32)	0
Gastrointestinal haemorrhage	2 (8.3)	2 (8.3)	0	4 (16)	4 (16)	0
Epistaxis	1 (4.2)	1 (4.2)	0	1 (4)	1 (4)	0
Hypertension	9 (37.5)	8 (33.3)	1 (4.2)	9 (36)	8 (32)	1 (4)
Headache	1 (4.2)	1 (4.2)	0	1 (4)	0	1 (4)
Myocarditis	4 (16.7)	4 (16.7)	0	3 (12)	2 (8)	1 (4)
AST or ALT increased	16 (66.7)	15 (62.5)	1 (4.2)	21 (84)	20 (80)	1 (4)
Bilirubin elevation	7 (29.2)	5 (20.8)	2 (8.3)	13 (52)	10 (40)	3 (12)
Hypothyroidism	3 (9.7)	3 (9.7)	0	5 (20)	5 (20)	0
Hypoproteinemia	7 (29.2)	6 (25)	1 (4.2)	11 (44)	10 (40)	1 (4)
Thrombocytopenia	5 (20.8)	5 (20.8)	0	3 (12)	3 (12)	0
Leukopenia	5 (20.8)	5 (20.8)	0	10 (40)	10 (40)	0

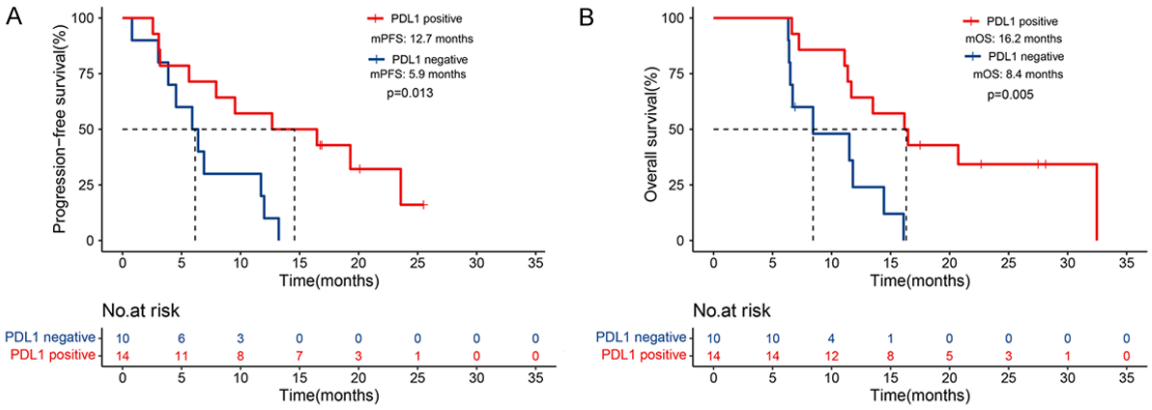

Figure 5. Kaplan-Meier estimates the overall survival (A) and progression-free survival (B) in 24 patients with BTC whose tissues were tested for PD-L1 expression.

although the two treated groups were well balanced, some selection bias arising from patients in the two groups who probably had different tumor burdens cannot be ruled out. Although these factors somewhat weaken the validity and reliability of the conclusions, these data are still helpful for a subsequent prospective study.

Conclusion

Our data indicated that the addition of local-regional therapy to toripalimab and lenvatinib might confer better efficacy, improving the overall survival of patients with advanced BTC. Local-regional therapy combined with toripalimab and lenvatinib presents “synergic effect”.

Although more side effects are caused by topical therapy, they are controllable. The triple combination therapy may be a promising alternative for patients with previously treated advanced BTC. A prospective clinical is needed to assess the precise efficacy and safety of the triple combination therapy.

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Disclosure of conflict of interest

None.

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.

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Table S1. Comparisons of major clinicopathological features between patients with PDL1 expression

Characteristics	PDL1 positive (n=14)	PDL1 negative (n=10)	*P* value
Median Age (range), y	68 (66-70)	60 (59-62)	0.51
Gender, n (%)			0.83
Male	8 (57)	7 (70)	
Female	6 (43)	3 (30)	
Tumor subtype, n (%)			0.46
Intrahepatic cholangiocarcinoma	9 (64)	7 (70)	
Extrahepatic cholangiocarcinoma	1 (7)	2 (20)	
Gallbladder cancer	4 (29)	1 (10)	
ECOG performance status, n (%)			0.71
0	4 (29)	4 (40)	
1	8 (57)	4 (40)	
2	2 (14)	2 (20)	
Differentiated histology, n (%)			0.53
Well	0	0	
Moderately	2 (14)	1 (10)	
Poorly	6 (43)	2 (20)	
Moderately-poorly	2 (14)	1 (10)	
Well-moderately	0	1 (10)	
Unsure	4 (29)	5 (50)	
Tumor diameter, mean ± SD (cm)	4.8±2.9	4.9±4.3	0.81
Site of metastases, n (%)			
Intrahepatic	10 (71)	6 (60)	0.59
Lymph nodes	8 (57)	4 (40)	0.43
Lung	0	0	-
Bone	1 (7)	0	0.34

Table S2. Tumor response between patients with PDL1 expression

	PD-L1 negative (n=10)	PD-L1 positive (n=14)	*P* value	Effect size (95% CI)
Objective response rate (n, %), 95% CI	20 (-10.2-50.2)	57.1 (27.5-86.8)	0.10	-
Complete response (n, %)	0	0	-	-
Partial response (n, %)	2 (20)	8 (58)	-	-
Stable disease (n, %)	5 (50)	3 (21)	-	-
Progressive disease (n, %)	3 (30)	3 (21)	-	-
DCR (95% CI)	70 (35.4-104.6)	78.6 (54-103.2)	0.67	-
Median overall survival, months (95% CI)	8.4 (2.0-14.9)	16.2 (10.7-21.6)	0.005	HR: 0.25 (0.09-0.71)
Median progression-free survival, months (95% CI)	5.9 (3.0-8.8)	12.7 (0-25.4)	0.013	HR: 0.30 (0.11-0.81)