

Efficacy and safety of PD-1 inhibitor plus antiangiogenic treatment in patients with unresectable biliary tract cancer: A multicenter retrospective study

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Abstract. Immunotherapy and antiangiogenic therapy have shown promising clinical activity in patients with advanced biliary tract cancer (BTC) in clinical trials. As the combination of these two treatments for BTC is not well studied in the real world, the present study retrospectively analyzed the clinical outcomes of patients with unresectable BTC who received immunotherapy-antiangiogenesis combination therapy in a real-world setting. A three-center, retrospective study was performed on patients with unresectable BTC who received a combination of programmed death 1 inhibitor and antiangiogenic agent between March 26, 2019 and November 1, 2021 in China. In total, 68 patients were enrolled in the cohort. The objective response rate and disease control rate were 13.2 and 75.0%, respectively. The median time to progression, progression-free survival and overall survival were 8.2, 5.5 and 10.7 months, respectively. Adverse events of all grades occurred in 58 patients (85.3%). In conclusion, the present study demonstrated that immunotherapy-antiangiogenesis combination therapy may be considered a therapeutic option for patients with unresectable BTC. Further prospective investigations are needed.

Introduction

Biliary tract cancer (BTC) is a sporadic but highly aggressive disease that consists of intrahepatic cholangiocarcinoma (ICC), extrahepatic cholangiocarcinoma (ECC) and gallbladder cancer (GBC). In most countries, many patients suffer from locally advanced or metastatic disease at the time of diagnosis due to a lack of early recognizable symptoms, and therefore eventually receive palliative treatments with a dismal prognosis.

Cisplatin plus gemcitabine (GC) is widely used as the standard first-line treatment in patients with unresectable BTC (1,2), and there is a shortage of available antitumor regimens as second-line/late treatment. In the era of individualized cancer therapy, clinical trials of antiangiogenic therapies and immune checkpoint inhibitor (ICI) treatments have achieved impressive results in solid malignancies including lung cancer and HCC (3,4).

Antiangiogenic therapies target vascular endothelial growth factor receptor (VEGFR), which could normalize tumor vasculature and improve treatment outcome. Based on data from phase 2 non-first line studies, VEGFR-targeted tyrosine kinase inhibitors (TKIs) have shown potential efficacy and manageable safety in ICC (Apatinib, NCT03251443) and BTC (Lenvatinib, NCT02579616; Regorafenib, NCT02053376) (5-8). However, the benefits obtained from antiangiogenic therapy in this setting are limited, and predictive biomarkers for this class of agents remain elusive.

Checkpoint inhibition, represented by PD-1 blockade, also entered into clinical studies in advanced BTC. Pembrolizumab exhibited ORR in only 6-13% of patients in clinical trials KEYNOTE-028 and KEYNOTE-158 (9), and nivolumab showed similar efficacy, with an ORR of 11% in patients with refractory BTC (10).

VEGFR-targeted TKIs combined with PD-1 inhibitors have been approved to effectively target various solid tumors. Lenvatinib has shown effective anti-tumor activity in combination with pembrolizumab (LEP) in HCC, and LEP was granted as a 'breakthrough designation' in HCC, endometrial

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carcinoma and renal cell cancer by the FDA (11). Encouraged by the success of the combined therapy in the above-mentioned solid malignancies, the LEP combination showed promising efficacy and manageable toxicity in previously treated advanced BTC in phase II LEAP-005 study. For these patients, the DCR was 21.0% with duration of response (DOR) ranging from 2.1 to 6.2 months (up to April 10, 2020) (12). So far, the most favorable result was observed in regorafenib plus avelumab in a phase II trial, the median time to PFS and OS were 2.5 (95% CI, 1.9-5.5) months and 11.9 (95% CI, 6.2-NA) months, respectively (13). Moreover, the combination enabled a longer OS in BTC patients compared with regorafenib monotherapy. The conjunction of pembrolizumab and ramucirumab, however, did not perform well in JVDF study with a poor ORR of only 4%, a median PFS (mPFS) of 1.6 months, and a median OS (mOS) of 6.4 months (14).

So far, data on PD-1 inhibitor plus antiangiogenic treatment published for treating advanced BTC are still limited. This retrospective study was performed to evaluate the efficacy and safety of PD-1 inhibitor plus antiangiogenic treatment in 68 patients with unresectable BTC in the real-world setting.

Materials and methods

Patients. This was a retrospective analysis of data from patients with unresectable BTC who were treated with PD-1 inhibitor plus antiangiogenic agent from March 26th, 2019 to November 1st, 2021 across the centers in China: i) Sir Run Run Shaw Hospital, Zhejiang University; ii) The First Affiliated Hospital, Zhejiang University; iii) The Second Affiliated Hospital, Zhejiang University. A total of 68 patients with advanced BTC who received PD-1 inhibitor plus antiangiogenic agent were eligible based on the following criteria: i) BTC diagnosis based on histology; ii) patients not available for radical operation; iii) at least one measurable lesion as conformed by Response Evaluation Criteria in Solid Tumors, version 1.1 (RECIST v1.1). Exclusion criteria were: i) combined therapy for only one cycle; ii) no available follow-up data; iii) no available data for baseline assessment and response assessment; iv) combination with chemotherapy, peptide vaccines, or bi-specific/tri-specific antibodies.

In total, data of 11 patients were excluded: 4 because they underwent additional chemotherapy, peptide vaccine, or bi-specific antibody; 1 because he discontinued for economic reasons after the first medication; 6 because they had no available follow-up data. Data from the remaining 68 patients were analyzed. Data including clinical information and follow-up data were gathered from patients' electronic health records. This study was carried out in accordance with the ethical guidelines of the Declaration of Helsinki and was approved by the ethics committee of the three participating hospitals (approval no. 2021332 for Sir Run Run Shaw Hospital, Zhejiang University; approval no. 2021734 for The First Affiliated Hospital, Zhejiang University; approval no. I20211065 for The Second Affiliated Hospital, Zhejiang University).

Treatment procedure. PD-1 inhibitors plus antiangiogenic agents were applied as off-label therapies for BTC in our cohort. The treatment strategy was designed based on previous treatment strategies, individual characteristics,

Table I. Baseline characteristics.

Variable	Value
Median age, years (range)	65 (33-82)
Patients aged ≥ 60 years, n (%)	40 (58.8)
Sex, n (%)	
Male	36 (52.9)
Female	32 (47.1)
ECOG PS, n (%)	
0-1	21 (30.9)
2-3	47 (69.1)
Alcohol status, n (%)	
Current or ex-drinker	8 (11.8)
Never-drinker	60 (88.2)
Histology, n (%)	
ICC	51 (75.0)
ECC	7 (10.3)
GBC	10 (14.7)
Tumor stage, n (%)	
Stage III	14 (20.6)
Stage IV	54 (79.4)
Metastasis present, n (%)	
Intrahepatic metastasis	52 (76.5)
Lymph node metastasis	39 (57.4)
Lung metastasis	13 (19.1)
Bone metastasis	5 (7.4)
Peritoneum metastasis	18 (26.5)
Intra-abdominal implantation	13 (19.1)
Previous treatment lines, n (%)	
0	27 (30.9)
≥ 1	41 (69.1)
Previous therapy, n (%)	
Immunotherapy	16 (23.5)
Antiangiogenic therapy	9 (13.2)
Local treatment (non-surgical)	23 (33.8)
Surgery	34 (50.0)

PS, performance status; ICC, intrahepatic cholangiocarcinoma; ECC, extrahepatic cholangiocarcinoma; GBC, gallbladder cancer.

patient willingness and economic condition. PD-1 inhibitors included camrelizumab, sintilimab, toripalimab, tislelizumab, pembrolizumab and nivolumab, which were administrated intravenously according to the following doses: camrelizumab 200 mg, sintilimab 200 mg, toripalimab 240 mg, tislelizumab 200 mg, pembrolizumab 200 mg every 3 weeks, or nivolumab 3 mg/kg every 2 weeks. Antiangiogenic agents included lenvatinib, apatinib, anlotinib, sorafenib, bevacizumab and fruquintinib, which were administered orally except bevacizumab. Lenvatinib was given 8 mg/day (body weight <60 kg) or 12 mg/day (body weight ≥ 60 kg). The initial dose of sorafenib was 400 mg/day and increased to 400 mg/12 h if tolerable. The patients received apatinib at a dosage of 250 mg daily,

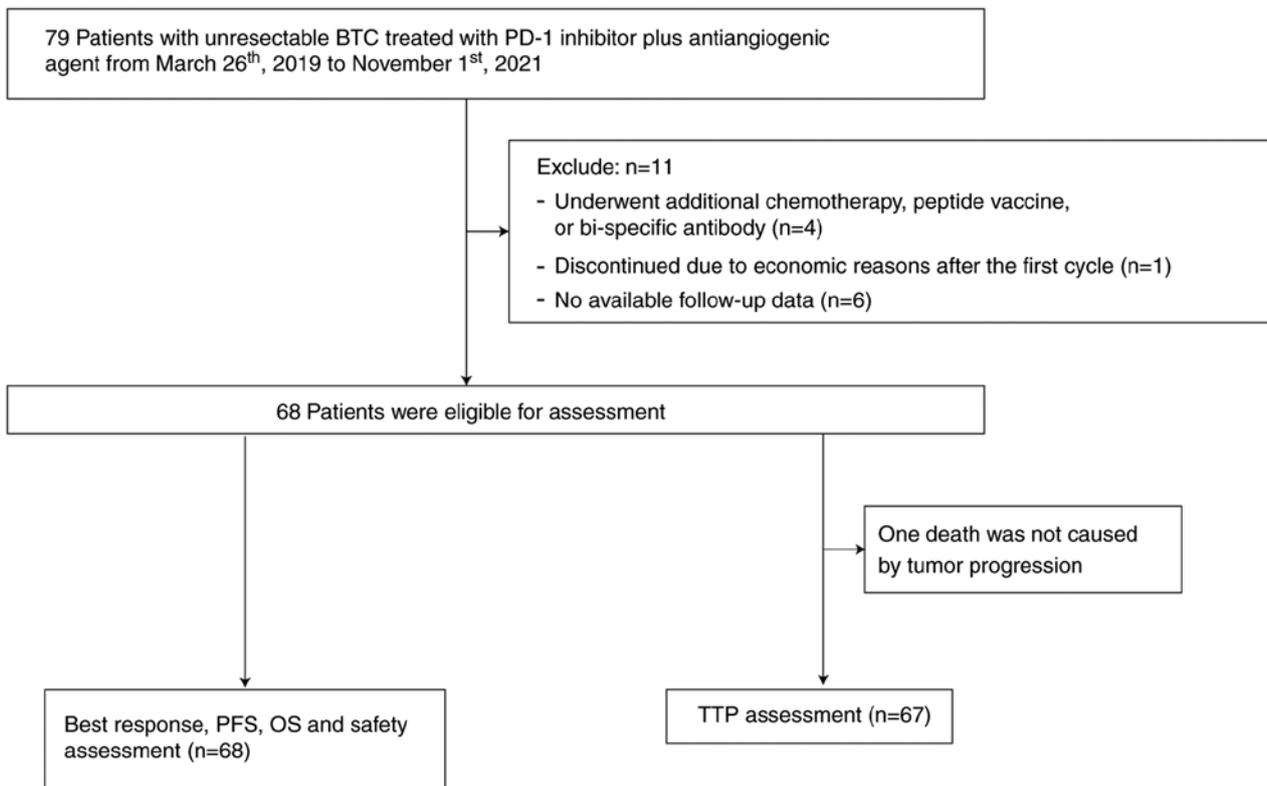


Figure 1. Patient selection flowchart. BTC, biliary tract cancer; OS, overall survival; PFS, progression-free survival; TTP, time to progression.

anlotinib at a dosage of 8 mg (2 weeks on/1 week off), or bevacizumab at a dosage of 7.5 mg/kg every 3 weeks intravenously. Fruquintinib was administered at 5 mg/day, day1-14/21 days in this cohort. All patients continued combination treatment until disease progression or unacceptable toxicity.

Assessments. Tumors were assessed using dynamic computed tomography (CT) and/or magnetic resonance imaging (MRI) at baseline and every 8 to 12 weeks until disease progression or treatment discontinuation. Tumor responses were evaluated according to RECIST v1.1: (1) complete response (CR) as the complete disappearance of all target lesions; (2) partial response (PR) as a $\geq 30\%$ decrease of the diameter of the target lesions; (3) stable disease (SD) as insufficient shrinkage to qualify as PR but insufficient increase to qualify as PD; (4) progressive disease (PD) as $\geq 20\%$ increase of the diameter of the target lesions, or new lesions development.

The therapeutic efficacy assessment included the ORR and DCR, and the survival analysis included TTP, PFS and OS. ORR was defined as the sum of CR and PR, and DCR was defined as the sum of CR, PR and SD. TTP was calculated from admission to progression confirmed by radiology. PFS was calculated from initial dose to clinical or radiographic progression or death. OS was calculated from initial dose to the date of death of any cause. Treatment-related adverse event (TRAE) data were collected and evaluated according to the Common Terminology Criteria for Adverse Events, version 5.0.

Statistical analysis. Clinical characteristic categorical variables were analyzed by Pearson's χ^2 test or Fisher's exact test.

Treatment strategy categorical variables were analyzed using the logistic regression model. Survival data were estimated using the Kaplan-Meier method, univariate analysis and multi-univariate analysis were performed using Log-rank test and Cox regression model, respectively. Statistical analysis was performed by IBM SPSS version 23.

Results

Baseline characteristics and therapeutic strategies. In this retrospective cohort, sixty-eight unresectable BTC patients who had received PD-1 inhibitor plus antiangiogenic therapy (Fig. 1), with a median follow-up of 7.9 (95% CI, 6.9-9.0) months by the time of data lock (November 1st, 2021). Baseline patient disposition is summarized in Table I. Of the 68 patients, thirty-six patients are male (52.9%) and thirty-two are female (47.1%) with a median age of 65 (range 33-82 years). Forty-seven patients (69.1%) had an Eastern Cooperative Oncology Group performance status (PS) of 2 or 3. Fifty-one patients (75.0%) had ICC, seven (10.3%) had ECC, and ten (14.7%) had GBC. According to TNM staging, fourteen patients were in stage III, and fifty-four patients in stage IV. Half of patients experienced post-operative recurrence. Fifty-two patients (76.5%) had intrahepatic metastasis, and lymph node (57.4%) was the most frequent site for extrahepatic disease followed by peritoneum (26.5%), lung (19.1%) and intra-abdominal implantation (19.1%). More than half of the patients (69.1%) in the study had undergone prior systemic therapies. In addition, twenty-three patients (33.8%) received non-surgical local treatment previously, including locoregional therapy and radiotherapy.

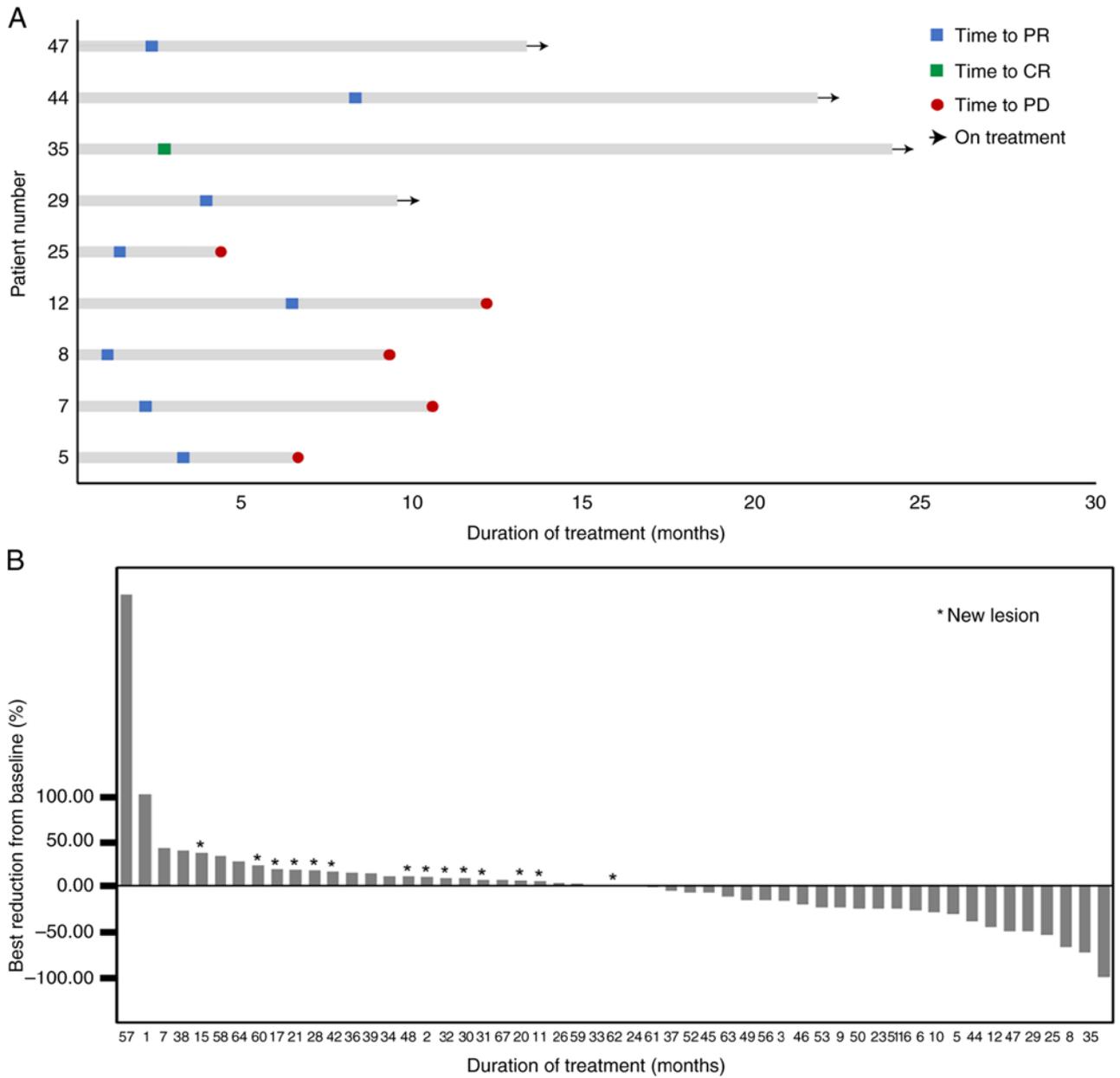


Figure 2. (A) Swimmer plots showed time to first response and duration of response. (B) Waterfall plot of the best reduction from baseline at response evaluation (* indicates new lesions). CR, complete response; PD, progressive disease; PR, partial response.

In the study, patients were given different combination strategies of six types of PD-1 inhibitors plus six types of antiangiogenic agents (Table II). The three most frequently used PD-1 inhibitors were camrelizumab (39.7%), sintilimab (22.1%) and toripalimab (19.1%). Synchronously, the majority of patients were treated with lenvatinib (51.5%), apatinib (26.5%) and anlotinib (14.7%). These angiogenetic drugs consisted of anti-VEGFR2 antibody (3.0%) and multitargeted TKIs (97.1%).

Treatment outcomes

Tumor response. In our cohort, the ORR was 13.2% (n=9) with 1 patient achieving CR, and the swimmer plot showed the median DOR (mDOR) was 8.4 (95% CI, 4.9-11.9) months in these patients (Fig. 2A). Besides, forty-two participants (61.8%) had SD and the DCR was 75.0% (Table III). Waterfall

plot presented the best percentage change from baseline in tumor measurement (Fig. 2B). Twenty-four (35.3%) patients exhibited a decrease in tumor size from baseline. None of the evaluated baseline characteristics was significantly associated with objective response (Table IV).

Survival and disease progression. In the cohort, one death was not caused by tumor progression, and median TTP (mTTP) was 8.2 (95% CI, 4.9-11.6) months for the remaining 67 patients; mPFS and mOS were 5.5 (95% CI, 3.3-7.8) months and 10.7 (95% CI, 2.3-19.0) months, respectively, for all patients (Table III). Up to the end of follow-up, 54 patients (79.4%) had PD and 36 deaths had occurred (52.9%). Univariate analyses showed that only tumor stage was significantly associated with TTP and PFS. The mTTP of patients in stage III and stage IV were 24.0 and 6.3 month, respectively (P=0.042). The mPFS of patients in stage III and stage IV were 7.83 and 4.37 month,

Table II. The combination immune checkpoint inhibitor and antiangiogenic therapy strategies.

Drug	Value, n (%)
Immune checkpoint inhibitor	
Camrelizumab	27 (39.7)
Sintilimab	15 (22.1)
Toripalimab	13 (19.1)
Tislelizumab	10 (14.7)
Pembrolizumab	2 (2.9)
Nivolumab	1 (1.5)
Antiangiogenic drug	
Lenvatinib	35 (51.5)
Apatinib	18 (26.5)
Anlotinib	10 (14.7)
Sorafenib	2 (2.9)
Bevacizumab	2 (2.9)
Fruquintinib	1 (1.5)

Table III. Results of tumor response and prognosis.

Variable	Value
CR, n (%)	1 (1.5)
PR, n (%)	8 (11.8)
SD, n (%)	12 (17.6)
ORR, n (%)	9 (13.2)
DCR, n (%)	51 (75.0)
mTTP, months (95% CI)	8.2 (4.9-11.6)
mPFS, months (95% CI)	5.5 (3.3-7.8)
mOS, months (95% CI)	10.7 (2.3-19.0)

CR, complete response; PR, partial response; SD, stable disease; ORR, objective response rate; DCR, disease control rate; mTTP, median time to progression; mPFS, median progression-free survival; mOS, median overall survival. Survival data were estimated using the Kaplan-Meier method.

respectively ($P=0.035$). Therefore, patients in stage III had longer TTP and PFS compared to patients in stage IV. None of the evaluated baseline characteristics were independent prognostic factors for OS (Table V).

Relation of treatment strategy and efficacy and prognosis. In this study, 39.7% of patients used Camrelizumab as the PD-1 inhibitor and 51.5% of patients used Lenvatinib as the antiangiogenic drug. Since this is a small cohort of only 68 patients, it was divided into Camrelizumab-based group and the other PD-1 inhibitors group in the subgroup analysis of PD-1 inhibitors, and it was divided into Lenvatinib-based group and the other antiangiogenic drugs group in the subgroup analysis of antiangiogenic drugs. Univariate and multivariate analyses were conducted to identify the relationship between drug types and treatment responses, TTP, PFS and OS. Univariate analyses showed that types of antiangiogenic drugs was

Table IV. Univariate analyses of the effects of baseline characteristics on tumor response.

Baseline characteristics	P-value	ORR	
		OR	95% CI
Age	0.109	3.4	0.7-14.8
Sex	0.585	0.7	0.2-2.8
PS	0.098	3.4	0.8-14.1
Alcohol	0.309	0.4	0.1-2.4
Histology	0.995		
ICC	0.999	3.0x10 ⁸	NA
ECC	0.999	2.7x10 ⁸	NA
GBC	1 (Ref)		
Tumor stage	0.319	2.2	0.5-10.1
Extrahepatic metastasis	0.800	1.2	0.2-6.8
Lymph node metastasis	0.907	1.1	0.3-4.5
Lung metastasis	0.520	2.0	0.2-18.0
Bone metastasis	0.999	2.7x10 ⁸	NA
Peritoneum metastasis	0.757	1.3	0.2-6.9
Intra-abdominal implantation	0.520	2.0	0.2-17.9
Macrovascular invasion	0.931	0.9	0.1-8.5
Previous immunotherapy	0.921	1.1	0.2-5.9
Previous antiangiogenic therapy	0.840	1.3	0.1-11.4
Previous treatment lines	0.304	2.1	0.5-8.7
Previous non-surgical local therapy	0.473	1.7	0.4-7.0
Previous surgery	0.721	0.8	0.2-3.2

ORR, objective response rate; PS, performance status; ICC, intrahepatic cholangiocarcinoma; ECC, extrahepatic cholangiocarcinoma; GBC, gallbladder cancer. P-values were determined by Pearson's χ^2 test or Fisher's exact test. Classification for categorical variables: Age, <60 or ≥ 60 ; sex, male or female; PS, 0-1 or 2-3; alcohol status, current drinker/ex-drinker or never drinker; histology, ICC or ECC or GBC; tumor stage, stage III or stage IV; extrahepatic metastasis, yes or no; lymph node metastasis, yes or no; lung metastasis, yes or no; bone metastasis, yes or no; peritoneum metastasis, yes or no; intra-abdominal implantation, yes or no; macrovascular invasion, yes or no; previous immunotherapy, yes or no; previous antiangiogenic therapy, yes or no; previous treatment lines, 0 or ≥ 1 ; previous non-surgical local therapy, yes or no; previous surgery, yes or no.

significantly associated with PFS ($P=0.037$), but multivariate analyses did not show significance. No significant difference was found in ORR, TTP, or OS among different PD-1 inhibitors and antiangiogenic drugs (Table VI).

Adverse events. A total of fifty-eight patients (85.3%) experienced at least one TRAE (Table VII). The most frequent types of AEs were elevated liver enzymes (39.7%), thrombocytopenia (32.4%), hyperbilirubinemia (29.4%), rash (20.6%), anemia (19.1%) and anorexia (19.1%). Twenty-five patients reported grade 3/4 AEs, and the three most common ≥ 3 -grade AEs were hypertension (5.9%), anemia (5.9%) and thrombocytopenia (4.4%). One patient had a confirmed fatal TRAE

Table V. Univariate analyses of the effects of baseline characteristics on TTP, PFS and OS.

Baseline characteristics	TTP			PFS			OS		
	P-value	HR	95% CI	P-value	HR	95% CI	P-value	HR	95% CI
Age	0.283	0.7	0.4-1.3	0.182	0.7	0.4-1.2	0.989	1.0	0.5-1.9
Sex	0.938	1.0	0.6-1.8	0.867	1.0	0.6-1.8	0.933	1.0	0.5-1.9
PS	0.301	0.7	0.4-1.3	0.623	1.2	0.6-2.6	0.098	1.9	0.9-3.9
Alcohol	0.093	0.4	0.1-1.2	0.144	0.5	0.2-1.2	0.859	0.9	0.4-2.4
Histology	0.606			0.951			0.133		
ICC	1 (Ref)			1 (Ref)			1 (Ref)		
ECC	0.805	0.9	0.3-2.5	0.753	1.2	0.5-3.0	0.284	1.8	0.6-5.2
GBC	0.322	0.6	0.2-1.7	0.991	1.0	0.4-2.2	0.064	2.2	1.0-5.2
Tumor stage	0.042	2.5	1.0-5.9	0.035	2.3	1.1-4.9	0.244	1.8	0.7-4.5
Extrahepatic metastasis	0.143	1.9	0.9-4.5	0.206	1.6	0.8-3.3	0.417	1.5	0.6-3.8
Lymph node metastasis	0.673	1.1	0.6-2.1	0.833	0.8	0.5-1.4	0.599	1.2	0.6-2.3
Lung metastasis	0.205	1.6	0.8-3.2	0.168	1.6	0.8-3.0	0.579	0.8	0.3-1.9
Bone metastasis	0.741	0.8	0.3-2.7	0.381	1.5	0.6-3.9	0.269	1.8	0.6-5.1
Peritoneum metastasis	0.739	0.9	0.5-1.7	0.084	1.7	0.9-3.0	0.225	1.5	0.8-3.1
Intra-abdominal implantation	0.281	0.7	0.3-1.4	0.400	1.3	0.7-2.5	0.826	1.1	0.5-2.4
Macrovascular invasion	0.288	0.5	0.2-1.7	0.722	0.8	0.3-2.2	0.733	0.8	0.2-2.7
Previous immunotherapy	0.114	1.7	0.9-3.4	0.302	1.4	0.7-2.6	0.248	1.5	0.7-3.2
Previous antiangiogenic therapy	0.506	0.7	0.3-1.8	0.984	1.0	0.5-2.1	0.461	0.7	0.2-1.9
Previous treatment lines	0.422	1.3	0.7-2.4	0.067	1.7	1.0-3.1	0.239	1.5	0.8-3.1
Previous non-surgical local therapy	0.477	1.3	0.7-2.3	0.705	0.9	0.5-1.6	0.916	1.0	0.5-2.0
Previous surgery	0.377	1.3	0.7-2.4	0.836	1.1	0.6-1.8	0.749	0.9	0.5-1.7

TTP, time to progression; PFS, progression-free survival; OS, overall survival; PS, performance status; ICC, intrahepatic cholangiocarcinoma; ECC, extrahepatic cholangiocarcinoma; GBC, gallbladder cancer. P-values were determined by log-rank test.

Table VI. The influence of therapy strategies on tumor response, TTP, PFS and OS.

Treatment strategy	ORR			TTP			PFS			OS		
	P-value	HR	95% CI	P-value	HR	95% CI	P-value	HR	95% CI	P-value	HR	95% CI
PD-1 inhibitors (Camrelizumab vs. other)	0.300	2.4	0.5-12.8	0.273	1.4	0.7-2.8	0.077	1.6	0.9-2.9	0.975	1.0	0.5-2.0
Antiangiogenic drugs (Lenvatinib vs. the other)	0.394	0.5	0.1-2.3	0.374	1.3	0.7-2.5	0.265	0.7	0.4-1.3	0.146	0.6	0.3-1.2

ORR, objective response rate; TTP, time to progression; PFS, progression-free survival; OS, overall survival. P-values were calculated using the logistic regression model (ORR) and Cox regression model (TTP, PFS, OS).

which was hepatic failure caused by autoimmune hepatitis. During our observation period, 23 (33.8%) patients required dose delay, dose reduction, or discontinued treatment due to TRAE.

Discussion

With broader accesses to tumor diagnostics and a deep understanding of tumor microenvironment, the strategies using antiangiogenic therapies and ICI have been realized for

several solid malignancies, while BTC remains a cancer type with scarce therapeutic options. We conducted a retrospective study in patients with unresectable BTC, not only to study the efficacy and safety of PD-1 inhibitor plus antiangiogenic treatment but also to promote the implementation of correlative clinical trials in the real world.

The ORR of PD-1 inhibitors plus antiangiogenic agents was 13.2% for unresectable BTC in our study, which was similar to the ORR (4.0-13.8%) of the combination regimens for unresectable BTC reported in clinical trials (12-14). Furthermore,

Table VII. Treatment-related adverse events according to category and grade.

Event	Grade, n (%)		
	Any	3/4	5
Elevated liver enzymes	27 (39.7)	2 (2.9)	0 (0.0)
Thrombocytopenia	22 (32.4)	3 (4.4)	0 (0.0)
Hyperbilirubinemia	20 (29.4)	2 (2.9)	0 (0.0)
Rash	14 (20.6)	2 (2.9)	0 (0.0)
Anemia	13 (19.1)	4 (5.9)	0 (0.0)
Anorexia	13 (19.1)	0 (0.0)	0 (0.0)
Hypertension	10 (14.7)	4 (5.9)	0 (0.0)
Leukopenia	10 (14.7)	1 (1.5)	0 (0.0)
Diarrhea	10 (14.7)	0 (0.0)	0 (0.0)
Fatigue	7 (10.3)	2 (2.9)	0 (0.0)
Hand-foot syndrome	7 (10.3)	2 (2.9)	0 (0.0)
Pruritus	6 (8.8)	1 (1.5)	0 (0.0)
Constipation	6 (8.8)	0 (0.0)	0 (0.0)
Hypothyroidism	4 (5.9)	0 (0.0)	0 (0.0)
Nausea/vomiting	3 (4.4)	1 (1.5)	0 (0.0)
Oral mucositis	3 (4.4)	0 (0.0)	0 (0.0)
Enterocolitis	2 (2.9)	2 (2.9)	0 (0.0)
Hemorrhage	2 (2.9)	0 (0.0)	0 (0.0)
Autoimmune hepatitis	1 (1.5)	0 (0.0)	1 (1.5)
Interstitial pneumonia	1 (1.5)	1 (1.5)	0 (0.0)
Myocarditis	1 (1.5)	1 (1.5)	0 (0.0)
Renal dysfunction	1 (1.5)	0 (0.0)	0 (0.0)

our cohort showed the DCR achieved 75.0%, mPFS and mOS were 5.5 months and 10.7 months, respectively. In LEAP-005, the DCR was 21.00%, and in REGOMUNE, mPFS and mOS were 2.5 months and 11.9 months, respectively (12,13). Compared with the very limited prospective studies mentioned above, the result of our study appears to be more optimistic possibly because we enrolled more patients with better outcomes who had not previously received systemic therapy as those patients reported in prospective studies. In addition, the strategy chosen for patients in the clinic not only follows the guidance but also takes cost, drug accessibility and patient willingness into consideration.

Nowadays, systemic treatment in BTC is dominated by chemotherapy with ongoing clinical trials on precision therapy and immunotherapy. The efficacy of antiangiogenic treatments with antibodies (e.g. bevacizumab and ramucirumab) or TKIs (e.g. sorafenib and regorafenib) and anti-PD-1 therapy (e.g. pembrolizumab and nivolumab) has been investigated in combination with chemotherapy. There is accumulating evidence that chemotherapy might have a synergistic effect with immunotherapy on advanced BTC (15-17). A recent phase II study of the combination of pembrolizumab with capecitabine and oxaliplatin has shown moderate efficacy with DCR of 81.8% and mPFS was 4.1 months, which is not as good as the result of another phase II study of nivolumab plus GC (DCR 92.6%, mPFS 6.1 months) (18,19). The value of immunotherapy predictive response biomarkers such as PD-L1 in

BTC has not been confirmed in clinical settings due to the high genetic heterogeneity in BTC and the lack of heterogeneous subgroups of patients in cohorts. Besides, PD-1 inhibitors combined with antiangiogenic therapies, including the regimens in our study, are not as effective as the combination with chemotherapy. Unlike immunotherapy, the combination of antiangiogenic treatment with conventional chemotherapy did not confer any advantage in phase II AIO study as there was no difference in the mPFS and mOS for gemcitabine plus sorafenib versus GC (mPFS, 3.0 vs. 4.9 months, $P=0.859$; mOS, 8.4 versus 11.2 months, $P=0.775$) (20), and the result in our study also compares favorably to which of group gemcitabine plus sorafenib. Due to limited data, more studies are needed to reveal whether the regimen of antiangiogenic agent plus chemotherapy is recommended in this setting.

It is noteworthy that our study enrolled 27 patients who were not previously treated at baseline, and the efficacy of PD-1 inhibitors combined with antiangiogenic agents as first-line therapy are as follows: the ORR and DCR achieved 18.5 and 80.8%, respectively; mPFS and mOS were 9.1 (95% CI, 5.0-13.1) months and 18.3 (95% CI, 4.0-32.8) months, respectively. As the standard first-line regimen for unresectable or recurrent BTC, GC therapy had a DCR of 81.4%, a mPFS of 8.0 (95% CI, 6.6-8.6) months, and a mOS of 11.7 (95% CI, 9.5-14.3) months (1). Therefore, our results suggested that the new strategy of PD-1 inhibitor combined with antiangiogenic agent as first-line treatment might be superior to standard chemotherapy. Based on TOPAZ-1 study, durvalumab in combination with GC has been recommended as standard first-line therapy for recurrent BTC recently (16). Data showed that the ORR in TOPAZ-1 study was higher than the ORR in our study (26.7% vs. 18.5%), however, mPFS and mOS in TOPAZ-1 study were shorter than those in our study (mPFS, 7.2 vs. 9.1 months; mOS, 12.8 vs. 18.3 months). The difference of efficacy and prognosis might be attributed to the treatment strategy, enrollment criteria and later therapy. Besides, clinical trials of chemotherapy with antiangiogenic therapy as first-line therapy for BTC are under investigation. A phase II study found that mPFS in ramucirumab plus GC group and merestinib plus GC group were 6.5 (80% CI, 5.7-7.1) months and 7.0 (80% CI, 6.2-7.1) months, respectively (21), which were shorter than those in our cohort. However, the efficacy of the first-line therapy in our study and first-line therapy of camrelizumab plus oxaliplatin-based chemotherapy in a phase 2 trial are comparable based on confirmed ORR (18.5% vs. 16.3%) and DCR (80.8% vs. 75.0%) (22). Thus, as the first-line therapy, PD-1 inhibitor combined with antiangiogenic agent might provide promising efficacy in advanced BTC patients and offer an alternative for advanced BTC who cannot tolerate chemotherapy. Further prospective studies are needed to confirm this finding.

Hyperprogressive disease (HPD) is a new pattern of response consisting in a sudden acceleration of tumor growth in ICIs (23). The incidence of HPD across solid tumors is reported between 4-29% (24). The diagnostic criteria for HPD remains controversial and the most widely used criteria contain RECIST and tumor growth rate (TGR) or tumor growth kinetic of the target lesions according to RECIST 1.1 criteria per month) (25,26). So far, there is a lack of available published data on HPD in clinical studies

conducted of BTC treatment. In our retrospective analysis, 2 patients had an initial progressive disease defined by visualization of the significantly increased target lesion (100 and 320%, respectively) based on RECIST within 2 months of treatment. The TGR of these two patient after treatment is more than twice the previous rate. Thus, 2 (2.9%) patients with BTC experienced HPD in our study.

The frequency of TRAEs in our study were comparable to the known AEs of PD-1 inhibitor and antiangiogenic agent. Grade ≥ 3 TRAEs to the combined strategy of PD-1/PD-L1 inhibitor with antiangiogenic agent occurred from 17.6 to 48.4% in clinical trials of BTC (12-14), similar with which reported in our study (36.8%). Thrombocytopenia is one of known AEs to anti-PD-1 therapy, rash and hypertension are common AEs to antiangiogenic therapy. Though elevated liver enzymes and hyperbilirubinemia were the most common AEs in this study, a number of patients with BTC suffer from complications of liver insufficiency due to primary disease progression. In addition, most TRAEs observed in the study including hypertension, rash and hand-foot syndrome, were related to antiangiogenic agents. Further research is required to identify predictive biomarkers to improve assessment before enrollment to ensure safety of patients who receive the combination therapy.

There are a few limitations in our study. First, it's a retrospective study with a small sample of patients, which inevitably caused bias. Second, we observed OS from thirty-five patients (51.5%) in this cohort and insufficient follow-up information is an inevitable issue. Hopefully, prospective clinical trials including LEAP-005 study is providing novel findings. Third, all of the combined regimens were heterogeneous and off-label used in the study, though different combinations have been shown to have no significant effect on the efficacy and prognosis of BTC, there is currently a lack of independent prospective studies to provide appropriate drug strategies. Finally, biomarkers were not recorded and analyzed to select the molecular subgroups that are most likely to benefit from the combined regimens in our study.

Immunotherapy represented by PD-1 inhibitors has shown initial effect in advanced BTC, and the combination of drugs is worth further exploration. In addition, biomarkers are required to select the molecular subgroups most likely to benefit from immunotherapy and immunotherapy-based combinations. With the collective data of PD-1 inhibitor combined with antiangiogenic agent, the combination regimens might exhibit active clinical activity for BTC.

In conclusion, in this multicenter and retrospective study, we evaluated the efficacy and safety of the combination of PD-1 inhibitor and antiangiogenic therapy in patients with unresectable BTC in a real-world setting. These promising data might provide opportunities for chemo-free therapy in advanced BTC patients, and the feasibility of the combination therapy in the clinic is waiting to be validated by further prospective studies with larger-scale samples and biomarker detections.

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Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Author's contributions

ZW, XuZ, XiZ, YW, YZ, WH, HP and JY contributed to the study conception and design. Material preparation, data collection and analysis were performed by ZW, XuZ and XiZ. The first draft of the manuscript was written by ZW and JY, and all authors commented on previous versions of the manuscript. JY, XuZ and XiZ confirm the authenticity of all the raw data. All authors read and approved the final manuscript.

Ethics approval and consent to participate

This study was performed in line with the principles of the Declaration of Helsinki. Approval was granted by the Institutional Review Board of Sir Run Run Shaw Hospital (Hangzhou, China; approval no. 2021332), The First Affiliated Hospital, Zhejiang University (Hangzhou, China; approval no. 2021734) and The Second Affiliated Hospital, Zhejiang University (Hangzhou, China; approval no. I20211065).

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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