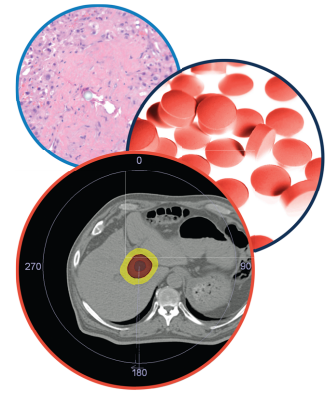
Research Article

For reprint orders, please contact: reprints@futuremedicine.com



Hepatic Oncology

Hepatic arterial infusion chemotherapy with or without lenvatinib for unresectable cholangiocarcinoma: a single-center retrospective study

Yajing Wang[‡,1,2], Zhanqi Wei[‡,2,3], Zheng Zhang[‡,1,4], Jingyi Xu[1,2], Yaqin Wang[1,2], Qian Chen[5] & Yuewei Zhang*[,1,2]

[1]School of Clinical Medicine, Tsinghua University, Beijing, 100084, China

[2]Hepatobiliary Pancreatic Center, Beijing Tsinghua Changgung Hospital, School of Clinical Medicine, Tsinghua University, Beijing, 102218, China

[3]School of Medicine, Tsinghua University, Beijing, 100084, China

[4]Department of Anesthesiology, Beijing Tsinghua Changgung Hospital, School of Clinical Medicine, Tsinghua University, Beijing, 102218, China

[5]Thorgene Co., Ltd., Beijing, 100176, China

*Author for correspondence: zhangyuewei1121@sina.com

‡Authors contributed equally

Aim: The purpose of this study is to compare the efficacy and safety of hepatic arterial infusion chemotherapy (HAIC) of oxaliplatin, fluorouracil and leucovorin (FOLFOX) plus lenvatinib and FOLFOX-HAIC alone in patients with unresectable cholangiocarcinoma. **Patients & methods:** Retrospective analysis of patients receiving FOLFOX-HAIC with or without lenvatinib. **Results:** Forty-one patients were included, with 22 patients receiving HAIC alone and 19 patients receiving HAIC plus lenvatinib. Combination treatment significantly prolonged overall survival and progression-free survival compared with HAIC alone. Grade 1–2 adverse events were more frequent in the combination group but manageable. No severe AEs or treatment-related deaths were reported. **Conclusion:** FOLFOX-HAIC plus lenvatinib has the potential to be a treatment option for unresectable cholangiocarcinoma.

Plain language summary: This study compared the effectiveness and safety of two treatments for unresectable cholangiocarcinoma (CCA), a type of liver cancer. The first treatment involved a combination of hepatic arterial infusion chemotherapy (FOLFOX-HAIC) and lenvatinib, a targeted therapy drug. The second treatment was FOLFOX-HAIC alone. The study included 41 patients with CCA, and the results showed that the group receiving FOLFOX-HAIC plus lenvatinib had significantly longer overall survival (32.0 months) and progression-free survival (20.0 months) compared with the group receiving FOLFOX-HAIC alone. The combination treatment had manageable side effects, although some mild adverse events were more common in the combination group. The study suggests that FOLFOX-HAIC plus lenvatinib could be a potential treatment option for unresectable CCA.

Tweetable abstract: Combining FOLFOX-HAIC with lenvatinib improved the survival of patients with unresectable cholangiocarcinoma (CCA), and had manageable side effects. Therefore, the combined treatment shows promise as a treatment option for unresectable CCA.

First draft submitted: 1 August 2023; Accepted for publication: 15 September 2023; Published online: 29 September 2023

Keywords: hepatic arterial infusion chemotherapy of oxaliplatin, fluorouracil, and leucovorin • lenvatinib • unresectable cholangiocarcinoma

The median survival time of unresectable cholangiocarcinoma (CCA) is only 6–10 months due to multiple focus, local tumor progression, lymph node metastasis, and other factors [1–3]. For advanced or unresectable CCA, local therapies and systemic therapies are the preferred treatment modes [4,5]. According to the ABC-02 clinical

Future Medicine

trial, cisplatin and gemcitabine were proposed as the first-line standard chemotherapy regimens for advanced or metastatic biliary tract cancer, with the overall survival (OS) of 11.7 months, and the efficacy was relatively limited [6]. According to the latest TOPAZ-1 trial, the addition of durvalumab significantly improved OS; however, the median OS with durvalumab plus chemotherapy was only 12.8 months [7,8].

Hepatic arterial infusion chemotherapy (HAIC) of oxaliplatin, fluorouracil, and leucovorin (FOLFOX) is a treatment that delivers high-dose chemotherapeutic drugs directly to the liver through the artery supplying the tumor. FOLFOX-HAIC has shown good efficacy and safety in advanced hepatocellular carcinoma. Since liver invasion is the main reason why advanced CCA cannot be resected surgically, FOLFOX-HAIC may be an attractive alternative to surgical resection in unresectable CCA [9–15]. The ABC-06 clinical trial provided reliable, high-quality evidence on the potential benefits and low risk of FOLFOX chemotherapy in the treatment of advanced biliary tract cancer [16]. FOLFOX-HAIC has shown significant tumor inhibition and survival benefit in the treatment of both intrahepatic and extrahepatic CCA [15,17].

According to previous studies, EGFR is upregulated in CCA, which also indicates stronger tumor invasion and poorer prognosis [18]. Lenvatinib, a micromolecule tyrosine kinase inhibitor, can inhibit tumor angiogenesis by inhibiting VEGF receptor (VEGFR), FGF receptor (FGFR) and other receptors, which has been widely used in the treatment of various solid tumors [19,20]. A single-center phase II clinical trial reported a median progression-free survival (PFS) of 3.19 months and a median OS of 7.35 months for unresectable CCA treated with lenvatinib [21].

This study reviewed patients with unresectable CCA who received FOLFOX-HAIC treatment in our center over the past three years and attempted to compare the clinical outcomes of FOLFOX-HAIC plus lenvatinib and FOLFOX-HAIC alone in unresectable CCA.

Patients & methods

Patients with unresectable CCA who received FOLFOX-HAIC plus lenvatinib or FOLFOX-HAIC alone at our center from November 2018 to November 2021 were included in this study. The study was conducted in accordance with the ethical guidelines of the Declaration of Helsinki. The patient data analysis underwent review and approval by the ethics committee at Beijing Tsinghua Changgung Hospital. Treatment commenced after obtaining informed consent from all enrolled individuals.

Patient selection

Inclusion criteria: Patients aged 18–80 years; Patients with CCA confirmed by histopathology in our center or other hospitals; Patients diagnosed by experienced hepatobiliary surgeons as unresectable or unable to achieve R0 resection with multiple lesions, vascular invasion, local lymph node metastasis, distant metastasis, etc.; Patients who refuse systemic chemotherapy; Patients with an Eastern Cooperative Oncology Group performance status (ECOG PS) of 0–2 before FOLFOX-HAIC treatment; Patients receiving at least a cycle of FOLFOX-HAIC; Patients with complete follow-up data.

Exclusion criteria: patients combined with other malignant tumors; patients with mixture tumor of hepatocellular carcinoma and cholangiocarcinoma confirmed by histopathology; patients with unconfirmed histopathology; patients with malignant hypertension; Patients with Child-Pugh class C; patients combined with other treatments, including systemic chemotherapy, radiotherapy, immune checkpoint inhibitors, and transarterial chemoembolization (TACE); and patients with missing clinical data or follow-up. Within the month preceding the commencement of the initial treatment, all pertinent laboratory data and imaging data, including CT or MRI images, were gathered.

Figure 1 illustrates the inclusion and exclusion procedures employed in this study. Ultimately, a total of 41 patients were enrolled for analysis.

Data collection

The clinical data utilized in this study were retrieved from the electronic medical record system at Beijing Tsinghua Changgung Hospital. The subsequent data points were compiled and subjected to analysis: Age, sex, HBV, ECOG-PS score, white blood cell count (WBC), platelet count (PLT), serum albumin (ALB), aspartate aminotransferase (AST), alanine aminotransferase (ALT), total bilirubin (TBIL), liver function grade (Child-Pugh), carcinoembryonic antigen (CEA), cancer antigen 19-9 (CA19-9), protein induced by vitamin K absence-II (PIVKA-II), tumor staging (TNM), portal vein cancer thrombus, vascular invasion, distant metastasis, whether percutaneous transhepatic cholangial drainage (PTCD) was placed before treatment.

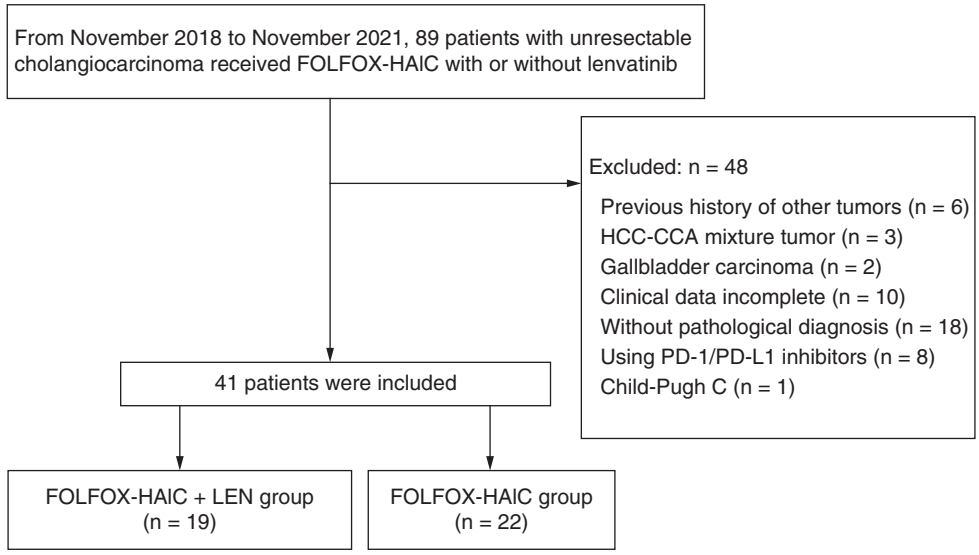


Figure 1. Flow diagram of study design.
CCA: Cholangiocarcinoma; FOLFOX-HAIC: Hepatic arterial infusion chemotherapy of oxaliplatin, fluorouracil, and leucovorin; HCC: Hepatocellular carcinoma; LEN: Lenvatinib.

Treatment procedure

Prior to the initiation of HAIC, patients with CCA received hepatoprotective agents, antiemetics, analgesics, and other symptomatic therapies. In cases where hyperbilirubinemia was present, PTCD drainage was performed as a preparatory measure, with HAIC administered once bilirubin levels dropped below three-times the upper limit of the normal range [10]. One treatment cycle of HAIC is 1 to 3 days. Femoral artery puncture and catheter indwelling were performed in each treatment cycle, and the tumor supplying arteries were accurately super-selected through digital subtraction angiography (DSA). The gastroduodenal artery or the right gastric artery could be embolized with spring coil if necessary. 5-fluorouracil was continuously administered within 12 hours a day with a total dose of 1500 mg. A nightly administration of 50 mg oxaliplatin and 300 mg calcium folinate was conducted over a 2-hour period. The duration between successive HAIC cycles ranged from 2 to 6 weeks, with patients received 1 to 9 cycles of HAIC treatment.

Before or after HAIC treatment, patients orally received lenvatinib (Japan Eisai Co, Ltd) for a duration of 2 weeks, determined by their bilirubin levels. The dosage of lenvatinib ranged from 8 mg (for individuals weighing $\leq$60 kg) to 12 mg (for those weighing >60 kg). In cases where patients experienced intolerance toward HAIC, lenvatinib administration was continued. Alternatively, if lenvatinib was not well-tolerated, patients reduced or halted its intake and proceeded with HAIC treatment. The discontinuation of treatment is contingent upon several factors, including progressive disease (PD), the inability to tolerate toxic side effects, patient refusal of treatment, or a change in the treatment plan. Enhanced computed tomography (CT) or MRI scans were conducted at intervals of 2–3 months, with follow-up assessments scheduled every 3 months.

Outcomes & assessments

The primary end points were OS and PFS. OS referred to the time interval from initial therapy to death from any cause or the last follow-up. PFS denotes the duration starting from the initiation of the initial therapy until disease progression, bridging therapy, transplantation, or the most recent follow-up. Tumor response assessments were conducted by radiologists and hepatobiliary surgeons using the modified Response Evaluation Criteria in Solid Tumors (mRECIST) and RECIST version 1.1. Tumor responses were categorized as complete response (CR), partial response (PR), stable disease (SD), or PD. The objective response rate (ORR) is the combined total of CR and PR, while the disease control rate (DCR) encompasses CR, PR, and SD. The evaluation of treatment-related adverse events (AEs) followed the National Cancer Institute Common Terminology Criteria for Adverse Events (CTCAE) version 5.0.

Statistical analysis

Baseline characteristics of patients with unresectable cholangiocarcinoma treated with FOLFOX-HAIC + LEN and FOLFOX-HAIC were compared using Pearson's chi-square test, Fisher's exact test, or Wilcoxon rank sum test. Mean ± standard error (SE) was used to describe normally distributed variables, while median was used for non-normally distributed variables. Kaplan-Meier method was employed for survival analysis, and log-rank test was used to assess differences in survival curves. Variables exhibiting a univariate p-value of less than 0.05 or deemed to have a potential impact on patient prognosis were incorporated into a multivariate Cox proportional hazards regression analysis. This analysis encompassed various factors, including patients' demographic information, treatment history, tumor characteristics, and additional relevant parameters, to derive hazard ratios (HRs) and corresponding confidence intervals (CIs). All descriptive and multivariate analyses were carried out using R4.2.2 software. A two-tailed p-value of <0.05 was deemed statistically significant.

Results

Patient & tumor characteristics

As of the end of follow-up, a total of 41 patients with unresectable CCA were included in this study. Table 1 demonstrates the characteristics of the study patients. The study population consisted of 25 male (61.0%) and 16 female (39.0%) patients who had a median age (and range) at the time of the treatment procedure of 61 (26–80) years. 19 patients received FOLFOX-HAIC plus lenvatinib treatment, and 22 patients only received FOLFOX-HAIC treatment. The HAIC + LEN group received 1 to 6 cycles of HAIC with a median of three cycles, while the HAIC group received one to nine cycles of HAIC with a median of three cycles. There were no significant differences in clinical variables between the two groups. The study was followed until 17 October 2022, and 27 patients died and 14 survived by the end of follow-up. In the HAIC + LEN group, one case underwent surgical resection, of which was pathologically confirmed complete necrosis of the tumor, three cases received liver transplantation, of which two cases were pathologically confirmed 70–80% necrosis. In the HAIC group, three cases underwent surgical resection, with one case undergoing palliative surgery (pathological confirmation of only 10–15% necrosis) and two cases undergoing R0 resection (pathological confirmation of 80–90% necrosis). All of them died of disease progression or recurrence.

Treatment efficacy

The median follow-up time was 21.0 (4–39) months. During follow-up, 9 (47.4%) patients in the HAIC + LEN group and 18 (81.8%) patients in the HAIC group died. The median OS of the HAIC + LEN and HAIC groups were 32.0 months and 10.0 months, respectively (p = 0.0094, Figure 2A). The median PFS in the HAIC + LEN and HAIC groups were 20.0 and 6.0 months, respectively (p = 0.041, Figure 2B). The 1-year OS rates in the HAIC + LEN and HAIC groups were 73.7% and 41.0%, respectively (95% CI: 0.53–12.50, p = 0.31).

According to RECIST version 1.1, at the first imaging assessment 3 months after treatment, in the HAIC + LEN group, 3 patients showed PD, 13 patients showed SD, and 3 patients achieved PR, with an ORR of 15.8% and DCR of 84.2%. In the HAIC group, 8 patients showed PD, 11 patients showed SD, and 3 patients achieved PR, with ORR of 13.6% and DCR of 63.6% (Table 2).

Safety & tolerability

According to CTCAE 5.0, the most common grade 1-2 AEs in HAIC + LEN group were transaminase elevation (89.5%), thrombocytopenia (57.9%), and leukocytopenia (52.6%), and the incidence of grade 1-2 AEs was 94.7% (18/19). The most common grade 3-4 AEs was hyhertension (15.8%), and the incidence of grade 3-4 AEs was 36.8% (7/19). In the HAIC group, the most common grade 1-2 AEs were transaminase elevation (61.9%), nausea (57.1%), and thrombocytopenia (38.1%), and the incidence of grade 1-2 AEs and grade 3-4 AEs were 86.4% (19/22) and 13.6% (3/22), respectively. The incidence of hypertension (p = 0.02) and elevated aminotransferase (p = 0.04) was significantly higher in the HAIC + LEN group than in the HAIC group among grade 1-2 AEs, while there was no significant difference in the incidence of grade 3-4 AEs between the two groups. However, most AEs in both groups were improved after symptomatic treatment. No grade 5 AEs or treatment-related deaths occurred in either group (Table 3).

Table 1. Demographics of patients included in the study.

Characteristic	HAIC + LEN, n (%)	HAIC, n (%)	p-value
Age (years)			0.6
<65	12 (63%)	12 (55%)	
≥65	7 (37%)	10 (45%)	
Sex			0.6
Female	3 (16%)	2 (9.1%)	
Male	16 (84%)	20 (91%)	
HBV			0.6
Positive	3 (16%)	2 (9.1%)	
Negative	16 (84%)	20 (91%)	
ECOG			0.7
1	14 (74%)	18 (82%)	
2	5 (26%)	4 (18%)	
WBC (10^9/l), mean (SD)	6.35 (3.13)	6.87 (2.39)	0.4
PLT (10^9/l), mean (SD)	257 (237)	215 (84)	0.5
ALB (10^9/l), mean (SD)	35.7 (5.1)	35.2 (3.1)	>0.9
AST (U/l), mean (SD)	45 (36)	36 (23)	0.6
ALT (U/l), mean (SD)	43 (40)	33 (20)	0.9
TBIL (μmol/l)			>0.9
<26	9 (47%)	10 (45%)	
≥26	10 (53%)	12 (55%)	
Child-Pugh			0.6
A	9 (47%)	12 (55%)	
B	10 (53%)	10 (45%)	
CEA (ng/ml)			0.2
<5	14 (74%)	12 (55%)	
≥5	5 (26%)	10 (45%)	
CA19-9 (U/ml)			0.3
<37	7 (37%)	5 (23%)	
≥37	12 (63%)	17 (77%)	
PVIKA-II (mAU/ml)			>0.9
<40	13 (68%)	15 (68%)	
≥40	6 (32%)	7 (32%)	
Subtype			0.1
iCCA	12 (63%)	8 (36%)	
pCCA	7 (37%)	14 (64%)	
TNM			0.6
I	1 (5.3%)	0 (0%)	
II	3 (16%)	6 (27%)	
III	10 (53%)	12 (55%)	
IV	5 (26%)	4 (18%)	
Tumor thrombus			0.3
Absent	8 (42%)	14 (64%)	
Branch of portal vein	9 (47%)	6 (27%)	
Main portal vein	2 (11%)	2 (9.1%)	
Vascular invasion			0.055
Absent	4 (21%)	11 (50%)	
Present	15 (79%)	11 (50%)	
Metastasis			0.4
Absent	14 (74%)	18 (82%)	
Present	5 (26%)	4 (18%)	

ALT: Alanine aminotransferase; AST: Aspartate aminotransferase; CEA: Carcinoembryonic antigen; CA19-9: Carbohydrate antigen 19-9; HAIC: Hepatic artery infusion chemotherapy; HBV: Hepatitis B virus; iCCA: Intrahepatic cholangiocarcinoma; LEN: Lenvatinib; PLT: Blood platelet; PIVKA-II: Protein induced by vitamin K absence II; pCCA: Perihilar cholangiocarcinoma; TBIL: Total bilirubin; TNM: Tumor node metastasis stage; WBC: White blood cell.

10.2217/hep-2023-0006

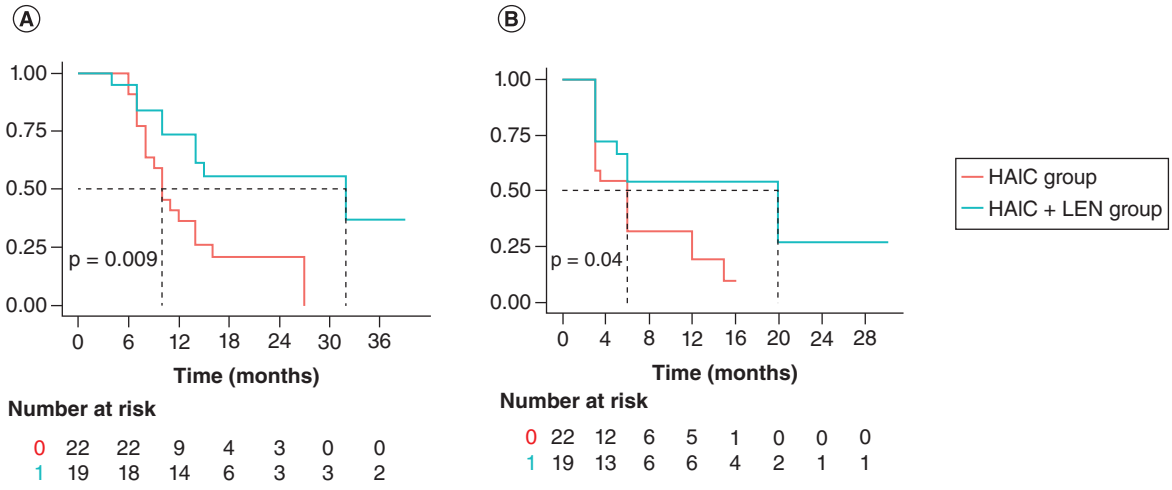


Figure 2. Kaplan-Meier plot for overall survival (A) and progression-free survival (B) based on the hepatic artery infusion chemotherapy + lenvatinib group compared with the hepatic artery infusion chemotherapy alone group.
HAIC: Hepatic artery infusion chemotherapy; LEN: Lenvatinib.

Table 2. Tumor response rate between the two groups.

Tumor response	HAIC + LEN (n = 19)	HAIC (n = 22)	p-value	95% CI
CR	0/0†	0/0	1	–
PR	2/1	2/1	1	0.13–10.11
SD	7/6	3/8	0.34	0.51–9.56
PD	3/0	3/5	0.17	0.048–1.76
ORR	15.68%	13.63%	1	0.12–10.12
DCR	84.21%	63.63%	0.16	0.56–20.78

†Intrahepatic cholangiocarcinoma/perihilar cholangiocarcinoma.
CR: Complete response; DCR: Disease control rate; HAIC: Hepatic artery infusion chemotherapy; LEN: Lenvatinib; ORR: Objective response rate; PR: Partial response; PD: Progressive disease; SD: Stable disease.

Table 3. The adverse events in the two groups according to Common Terminology Criteria for Adverse Events version 5.0.

AEs	Grade 1–2 AEs			Grade 3–4 AEs		
	HAIC + LEN	HAIC	p-value	HAIC + LEN	HAIC	p-value
Nausea	7	12	0.35	0	0	1
Emesis	3	6	0.47	0	0	1
Abdominal pain	1	5	0.19	0	1	1
Abdominal distension	3	1	0.32	0	0	1
Diarrhea	5	2	0.42	1	0	0.46
Fever	4	2	0.38	2	0	0.21
Hyhertension	5	0	0.02†	3	0	0.09
Rash	4	1	0.16	0	0	1
Gastric mucosal bleeding	1	0	0.46	0	0	1
Arthrodynia	1	0	0.46	0	0	1
Thrombcytopenia	11	8	0.22	2	1	0.32
Leukocytopenia	10	6	0.12	2	1	0.32
Transaminase elevation	17	13	0.04†	1	0	0.46
Increasde bilirubin	4	3	0.68	2	0	0.21

†$p < 0.05$.
AE: Adverse event; HAIC: Hepatic artery infusion chemotherapy; LEN: Lenvatinib.

Table 4. COX regression analysis on the relationship between candidate prognostic factors and progression-free survival and overall survival.

Characteristic	Progression-free survival			Overall survival		
	HR	95% CI	p-value	HR	95% CI	p-value
Treatment: HAIC + LEN vs HAIC	0.12	0.03–0.56	0.007†	0.15	0.04–0.55	0.004†
Age: <65 years vs ≥65 years	0.64	0.25–1.65	0.4	0.62	0.24–1.64	0.3
Sex: Female vs male	0.55	0.06–4.92	0.6	0.83	0.08–8.44	0.9
HBV: Positive vs negative	3.38	0.03–4.82	0.5	1.41	0.09–21.2	0.8
TBIL: ≥17.1 vs <17.1	4.52	0.81–25.3	0.086	2.59	0.55–12.1	0.2
CEA: ≥5 vs <5	1.27	0.50–3.25	0.6	1.76	0.67–4.64	0.3
CA19-9: ≥37 vs <37	13.6	2.68–68.7	0.002†	9.07	1.86–44.1	0.006†
Chlid-Pugh: A vs B	0.39	0.09–1.76	0.2	0.51	0.13–2.03	0.3
Thrombosis: Present vs absent	4.55	0.97–21.3	0.054	2.68	0.62–11.6	0.2
Vasclar invasion: Absent vs present	0.58	0.15–2.19	0.4	1.09	0.30–3.92	0.9
Distant metastasis: Present vs absent	6.25	1.60–24.4	0.009†	5.67	1.42–22.7	0.014†
PTCD: Absent vs present	0.35	0.07–1.83	0.2	1.26	0.28–5.66	0.8

†$p < 0.05$.
HAIC: Hepatic artery infusion chemotherapy; HR: Hazard ratio; HBV: Hepatitis B virus; LEN: Lenvatinib; PTCD: Percutaneous transhepatic cholangial drainage; TBIL: Total bilirubin.

Prognostic factor analysis

COX regression analysis showed that FOLFOX-HAIC plus lenvatinib (versus FOLFOX-HAIC alone, HR: 0.15, 95% CI: 0.04–0.55, p = 0.004) was the independent prognostic factor for longer OS, while high serum CA19-9 level (HR: 9.07, 95% CI: 1.86–44.1, p = 0.006) and distant metastasis (HR: 5.67, 95% CI: 1.42–22.7, p = 0.014) were significantly associated with shorter OS. Meanwhile, FOLFOX-HAIC plus lenvatinib (versus FOLFOX-HAIC alone, HR: 0.12, 95% CI: 0.03–0.56, p = 0.007) was also the independent prognostic factor for longer PFS, while high serum CA19-9 level (HR: 13.6, 95% CI: 2.68–68.7, p = 0.002) and distant metastasis (HR: 6.25, 95% CI: 1.60–24.4, p = 0.009) were also significantly associated with shorter PFS (Table 4).

The forest plot analysis results for factors associated with OS and PFS are presented in the Figures 3 & 4. In CCA patients with portal vein thrombosis or extrahepatic metastasis, the HAIC + LEN group provided more clinical benefits compared with the HAIC group. However, there was a significant interaction between treatment and PIVKA-II levels exceeding 40 mAU/ml for patient prognosis ($p < 0.05$). For OS, the HAIC + LEN group reduced the risk of death by 83% in patients with PIVKA-II <40 mAU/ml compared with those the HAIC group. In contrast, in patients with PIVKA-II ≥40 mAU/ml, the HAIC + LEN group increased the risk of death by 34% compared with the HAIC group, with a significant difference between the two groups (p for interaction = 0.023). For PFS, the HAIC + LEN group reduced the risk of progression by 76% in patients with PIVKA-II <40 compared with the HAIC group. However, in patients with PIVKA-II ≥40 mAU/ml, the HAIC + LEN group increased the risk of progression by 43% compared with the HAIC group, with a significant difference between the two groups (p for interaction = 0.041).

Discussion

In our retrospective study, FOLFOX-HAIC plus lenvatinib was significantly superior to FOLFOX-HAIC alone in terms of OS and PFS in unresectable CCA. The one-year survival rate of FOLFOX-HAIC + LEN group was not statistically significant compared with FOLFOX-HAIC group, but this may be related to the small number of participants. In addition, although the incidence of AEs in the HAIC + LEN group was higher than that in the HAIC group, all AEs were controllable after favorable management, and no treatment-related deaths occurred. Previous studies have reported that CA19-9 and distant metastasis are related factors for poor prognosis, which is consistent with our findings [22]. Interestingly, we found an interaction between PIVKA-II and our treatment regimen, which might be related to the invasion of liver tissue by CCA, resulting in impaired liver function or intrhepatic tissue invasion.

In advanced or unresectable CCA, local treatment options include TACE, radiofrequency ablation, HAIC, radiotherapy, etc. However, compared with other local treatments, HAIC can target diffuse lesions and multiple lesions, and is not limited by tumor size, tumor location or even hyperbilirubinemia [10,15,17,23–25]. FOLFOX

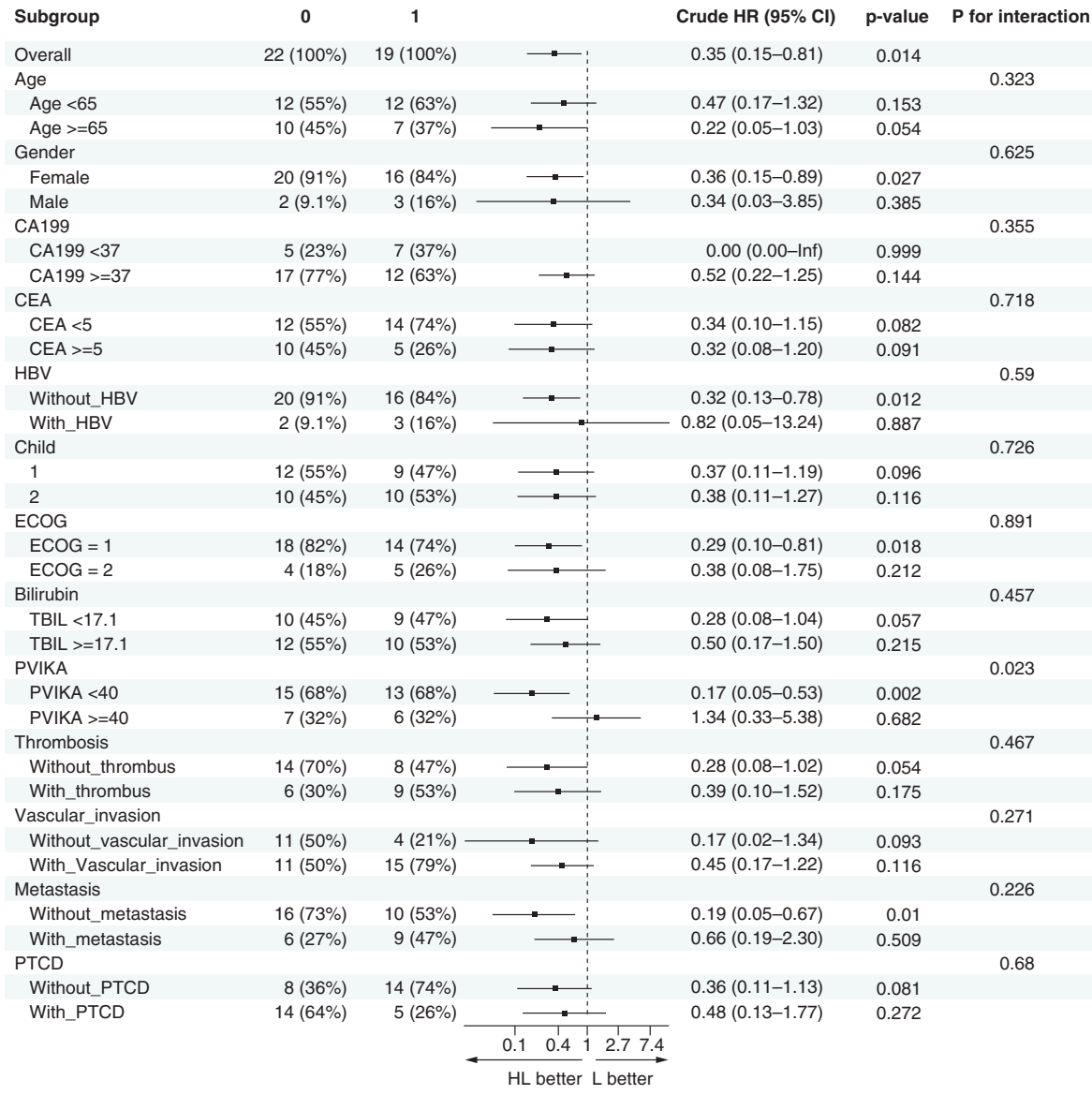


Figure 3. Subgroup analyses of overall survival in the entire cohort.
0: The HAIC group; 1: The HAIC + LEN group; ECOG: Eastern cooperative oncology group; HR: Hazard ratio; HBV: Hepatitis B virus; PIVKA: Protein induced by vitamin K absence; PTCD: Percutaneous transhepatic cholangial drainage; TBIL: Total bilirubin.

(oxaliplatin, fluorouracil, and leucovorin) was proposed as the standard second-line treatment for advanced CCA in the ABC-06 clinical study [16]. The arterial administration of FOLFOX not only mitigated the toxic and side effects, but also achieved a higher concentration of the drug and a stronger antitumor effect than systemic administration [15,17]. We have also achieved good clinical outcomes in the treatment of unresectable hepatocellular carcinoma using FOLFOX-HAIC combined with targeted therapy or immunotherapy in previous study [10]. Massani *et al.* [26] used fluorouracil, oxaliplatin-HAIC to treat unresectable intrahepatic CCA and reported a median OS of 17.6 months. Cercek *et al.* [4] reported that local combined systemic therapy in unresectable CCA showed a significant survival benefit compared with systemic chemotherapy or local therapy alone. The latest National Comprehensive Cancer Network (NCCN) Guidelines in Hepatobiliary Oncology also recommends HAIC as a treatment option for patients with unresectable CCA. Multiple studies have proposed that HAIC can not only control the progression of local bile duct diseases, but also is associated with longer survival of patients [4,10,14–17]. This study also confirmed the effectiveness of FOLFOX-HAIC in CCA.

Subgroup	0	1	Crude HR (95% CI)	p-value	P for interaction
Overall	22 (100%)	19 (100%)	0.44 (0.19–1.02)	0.055	
Age					0.43
Age <65	12 (55%)	12 (63%)	0.59 (0.22–1.63)	0.312	
Age >=65	10 (45%)	7 (37%)	0.28 (0.06–1.35)	0.114	
Gender					0.411
Female	20 (91%)	16 (84%)	0.50 (0.21–1.24)	0.134	
Male	2 (9.1%)	3 (16%)	0.17 (0.01–1.99)	0.158	
CA199					0.998
CA199 <37	5 (23%)	7 (37%)	0.00 (0.00–Inf)	0.999	
CA199 >=37	17 (77%)	12 (63%)	0.61 (0.26–1.46)	0.267	
CEA					0.393
CEA <5	12 (55%)	14 (74%)	0.38 (0.11–1.26)	0.113	
CEA >=5	10 (45%)	5 (26%)	0.83 (0.26–2.67)	0.753	
HBV					0.885
Without_HBV	20 (91%)	16 (84%)	0.44 (0.18–1.09)	0.075	
With_HBV	2 (9.1%)	3 (16%)	0.47 (0.03–7.86)	0.6	
Child					0.973
1	12 (55%)	9 (47%)	0.47 (0.15–1.52)	0.209	
2	10 (45%)	10 (53%)	0.46 (0.14–1.52)	0.203	
ECOG					0.897
ECOG = 1	18 (82%)	14 (74%)	0.39 (0.14–1.10)	0.074	
ECOG = 2	4 (18%)	5 (26%)	0.46 (0.10–2.08)	0.312	
Bilirubin					0.196
TBIL <17.1	10 (45%)	9 (47%)	0.26 (0.07–0.96)	0.043	
TBIL >=17.1	12 (55%)	10 (53%)	0.76 (0.25–2.28)	0.626	
PVIKA					0.041
PVIKA <40	15 (68%)	13 (68%)	0.22 (0.07–0.69)	0.009	
PVIKA >=40	7 (32%)	6 (32%)	1.43 (0.36–5.72)	0.616	
Thrombosis					0.77
Without_thrombus	14 (70%)	8 (47%)	0.36 (0.10–1.30)	0.12	
With_thrombus	6 (30%)	9 (53%)	0.50 (0.13–1.87)	0.304	
Vascular_invasion					0.479
Without_vascular_invasion	11 (50%)	4 (21%)	0.23 (0.03–1.83)	0.164	
With_vascular_invasion	11 (50%)	15 (79%)	0.49 (0.18–1.33)	0.161	
Metastasis					0.463
Without_metastasis	16 (73%)	10 (53%)	0.29 (0.08–1.04)	0.057	
With_metastasis	6 (27%)	9 (47%)	0.61 (0.18–2.13)	0.442	
PTCD					0.394
Without_PTCD	8 (36%)	14 (74%)	0.37 (0.12–1.17)	0.091	
With_PTCD	14 (64%)	5 (26%)	0.59 (0.16–2.15)	0.428	

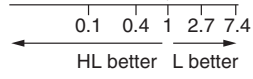


Figure 4. Subgroup analyses of progression-free survival in the entire cohort.
0: the HAIC group; 1: the HAIC + LEN group; ECOG: Eastern cooperative oncology group; HR: Hazard ratio; HBV: Hepatitis B virus; PIVKA: Protein induced by vitamin K absence; PTCD: Percutaneous transhepatic cholangial drainage; TBIL: Total bilirubin.

Due to the popularity of whole genome sequencing or NGS in cancer, a variety of gene mutations, including *FGFR2*, *IDH1/2*, *HER2*, *WNT* and *EGFR* were found in CCA, and the mutation frequency could reach more than 50%. Therefore, drugs targeting these targets may be an effective treatment for CCA [27,28], and targeted therapy combined with systemic or local chemotherapy, or even combined immunotherapy, is considered to be a better treatment pattern for CCA [4,5]. Lenvatinib is a potent anti-angiogenic medication that targets multiple molecular pathways, exhibiting promising anti-tumor effects on angiogenesis across diverse solid malignancies. Furthermore, it has demonstrated the ability to enhance T-cell infiltration within the tumor microenvironment, thereby exerting a positive influence [12,13,19,29]. Numerous investigations focusing on hepatobiliary malignancies have substantiated the synergistic potential of combining lenvatinib with HAIC, showing a good antitumor therapeutic effect [10,20,21,29]. Lenvatinib was used instead of single-target inhibitors in this study, considering that CCA is caused by multi-target gene mutations [2,30,31]. Previous studies have also shown that lenvatinib administered

alone exhibits good antitumor activity and safety in unresectable CCA, but its ORR is only 11.5% [21]. IDH1/2 inhibitors, FGFR2 inhibitors, and immunosuppressants have also been used in CCA and have achieved relatively ideal efficacy. However, currently, the treatment options for unresectable CCA are limited and their therapeutic effects are not satisfactory. A retrospective report has reported that the ORR of lenvatinib combined with PD-1 in advanced CCA with first-line treatment failure was 20.27%, the DCR was 71.62%, and the median OS was 9.5 months [32]. Zhu *et al.* [33] reported that the ORR of lenvatinib + PD-1/PD-L1 inhibitor + GEMOX in advanced CCA was 43.9%, the DCR was 91.2%, the median OS was 13.4 months, and the median PFS was 9.27 months. The outcome of the combined regimen is significantly better than lenvatinib monotherapy or HAIC alone, thus the safety and efficacy of FOLFOX-HAIC combined lenvatinib in unresectable CCA deserve to be investigated.

Certain limitations exist within this study. Firstly, it entails a retrospective analysis conducted at a solitary center, encompassing a restricted sample size. Therefore, the potential for selection bias cannot be disregarded. This study needs to be extended to larger populations in other regions, and the results need to be validated in future prospective multicenter randomized clinical trials. Second, Due to the COVID-19 pandemic, it was not possible to strictly adhere to the treatment frequency of once every 3 weeks. Moreover, considering the actual condition of the patients and clinical laboratory indicators, we made adjustments to the treatment cycles to avoid the cumulative effects of side effects and the occurrence of severe complications. However, this also implies that there are variations in the treatment regimens among each patient, potentially compromising the reliability of the findings in this study. In conclusion, given the limited available data, we believe it is important to present our findings as hypothesis-generating and exploratory in nature. While all statistical analyses conducted should be interpreted as exploratory, the results of this retrospective analysis can provide valuable insights and generate hypotheses for future research in this field.

Conclusion

This study demonstrated that FOLFOX-HAIC plus lenvatinib had a significant therapeutic effect and manageable AEs in patients with unresectable CCA, and can significantly prolong the OS and PFS of patients compared with FOLFOX-HAIC alone. Thus, FOLFOX-HAIC combined with lenvatinib have the potential to be a treatment option for unresectable CCA.

Summary points

- This study compared the effectiveness and safety of two treatments for unresectable cholangiocarcinoma (CCA): FOLFOX-HAIC plus lenvatinib versus FOLFOX-HAIC alone.
- The combination of FOLFOX-HAIC and lenvatinib significantly prolonged overall survival (OS) and progression-free survival (PFS) compared with FOLFOX-HAIC alone.
- Patients receiving FOLFOX-HAIC plus lenvatinib had a median OS of 32.0 months, while the median OS for the FOLFOX-HAIC group was 10.0 months.
- The median PFS for the FOLFOX-HAIC plus lenvatinib group was 20.0 months, whereas it was 6.0 months for the FOLFOX-HAIC group.
- Grade 1–2 adverse events (AEs), such as hypertension and elevated aminotransferase, were more frequent in the FOLFOX-HAIC plus lenvatinib group, but there was no significant difference in the incidence of grade 3–4 AEs between the two groups.
- Most adverse events in both groups improved with symptomatic treatment, and no treatment-related deaths were reported.
- High serum CA19-9 level and distant metastasis were identified as independent prognostic factors for shorter OS and PFS.
- PIVKA-II levels exceeding 40 mAU/ml had a significant interaction with treatment for patient prognosis.
- The combination of FOLFOX-HAIC plus lenvatinib shows promise as a treatment option for unresectable CCA, with improved clinical outcomes and manageable side effects.

Author contributions

This work was designed by Y Wang, Z Wei and Y Zhang. The data were analyzed by Y Wang, Z Wei, Z Zhang, J Xu, Y Wang. This manuscript was written by Y Wang and Z Wei. All authors approved the final version.

Financial disclosure

This study was funded by Capital's Funds for Health Improvement and Research (2020-2-2242), CAMS Innovation Fund for Medical Sciences (2019-I2M-5-056) and Tsinghua Precision Medicine Foundation (12020B7028). The authors have no other relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript apart from those disclosed.

Competing interests disclosure

The authors have no competing interests or relevant affiliations with any organization or entity with the subject matter or materials discussed in the manuscript. This includes employment, consultancies, honoraria, stock ownership or options, expert testimony, grants or patents received or pending, or royalties.

Writing disclosure

No writing assistance was utilized in the production of this manuscript.

Ethical conduct of research

This study was reviewed and approved by Beijing Tsinghua Changgung Hospital, Tsinghua University, Changping District, Beijing 102218, China. The patients provided their written informed consent to participate in this study. Written informed consent was obtained from the individuals for the publication of any potentially identifiable images or data included in this article.

References

Papers of special note have been highlighted as: • of interest

1. Vithayathil M, Khan SA. Current epidemiology of cholangiocarcinoma in Western countries. *J. Hepatol.* 77(6), 1690–1698 (2022).
2. Banales JM, Marin JJG, Lamarca A *et al.* Cholangiocarcinoma 2020: the next horizon in mechanisms and management. *Nat. Revi. Gastroenterol. Hepatol.* 17(9), 557–588 (2020).
3. Moris D, Palta M, Kim C, Allen PJ, Morse MA, Lidsky ME. Advances in the treatment of intrahepatic cholangiocarcinoma: an overview of the current and future therapeutic landscape for clinicians. *CA Cancer J. Clin.* 73(2), 198–222 (2022).
4. Cercek A, Boerner T, Tan BR *et al.* Assessment of hepatic arterial infusion of floxuridine in combination with systemic gemcitabine and oxaliplatin in patients with unresectable intrahepatic cholangiocarcinoma a phase 2 clinical trial. *JAMA Oncol.* 6(1), 60–67 (2020).
5. Konstantinidis IT, Koerkamp BG, Do RKG *et al.* Unresectable intrahepatic cholangiocarcinoma: systemic plus hepatic arterial infusion chemotherapy is associated with longer survival in comparison with systemic chemotherapy alone. *Cancer* 122(5), 758–765 (2016).
6. Valle J, Wasan H, Palmer DH *et al.* Cisplatin plus gemcitabine versus gemcitabine for biliary tract cancer. *N. Engl. J. Med.* 362(14), 1273–1281 (2010).

• **The standard treatment regimen for advanced cholangiocarcinoma, widely adopted internationally, is based on the findings of this study.**

7. Oh D-Y, He AR, Qin S *et al.* A phase 3 randomized, double-blind, placebo-controlled study of durvalumab in combination with gemcitabine plus cisplatin (GemCis) in patients (pts) with advanced biliary tract cancer (BTC): TOPAZ-1. *J. Clin. Oncol.* 40(4), 378 (2022).

• **The conclusions of this study have provided updated recommendations for the standard treatment regimen of advanced cholangiocarcinoma.**

8. Oh D-Y, Lee K-H, Lee D-W *et al.* Gemcitabine and cisplatin plus durvalumab with or without tremelimumab in chemotherapy-naive patients with advanced biliary tract cancer: an open-label, single-centre, phase 2 study. *Lancet Gastroenterol. Hepatol.* 7(6), 522–532 (2022).
9. Franssen S, Soares KC, Jolissaint JS *et al.* Comparison of hepatic arterial infusion pump chemotherapy vs resection for patients with multifocal intrahepatic cholangiocarcinoma. *JAMA Surg.* 157(7), 590–596 (2022).
10. Wang T, Dong J, Zhang Y *et al.* Efficacy and safety of hepatic artery infusion chemotherapy with mFOLFOX in primary liver cancer patients with hyperbilirubinemia and ineffective drainage: a retrospective cohort study. *Ann. Translat. Med.* 10(7), 411 (2022).
11. Lyu N, Lin Y, Kong Y *et al.* FOXAI: a phase II trial evaluating the efficacy and safety of hepatic arterial infusion of oxaliplatin plus fluorouracil/leucovorin for advanced hepatocellular carcinoma. *Gut* 67(2), 395–396 (2018).
12. Lyu N, Wang X, Li J-B *et al.* Arterial chemotherapy of oxaliplatin plus fluorouracil versus sorafenib in advanced hepatocellular carcinoma: a biomolecular exploratory, randomized, phase III Trial (FOHAIC-1). *J. Clin. Oncol.* 40(5), 468 (2022).

13. He M, Li Q, Zou R *et al.* Sorafenib plus hepatic arterial infusion of oxaliplatin, fluorouracil, and leucovorin vs sorafenib alone for hepatocellular carcinoma with portal vein invasion a randomized clinical trial. *JAMA Oncol.* 5(7), 953–960 (2019).
14. Nehls O, Klump B, Arkenau HT *et al.* Oxaliplatin, fluorouracil and leucovorin for advanced biliary system adenocarcinomas: a prospective phase II trial. *Br. J. Cancer* 87(7), 702–704 (2002).
15. Wang X, Hu J, Cao G *et al.* Phase II Study of Hepatic Arterial Infusion Chemotherapy with Oxaliplatin and 5-Fluorouracil for Advanced Perihilar Cholangiocarcinoma. *Radiology* 283(2), 580–589 (2017).
16. Lamarca A, Palmer DH, Wasan HS *et al.* Second-line FOLFOX chemotherapy versus active symptom control for advanced biliary tract cancer (ABC-06): a phase 3, open-label, randomised, controlled trial. *Lancet Oncol.* 22(5), 690–701 (2021).
17. Cai Z, He C, Zhao C, Lin X. Survival comparisons of hepatic arterial infusion chemotherapy with mFOLFOX and transarterial chemoembolization in patients with unresectable intrahepatic cholangiocarcinoma. *Front. Oncol.* 11, 611118 (2021).
18. Sia D, Hoshida Y, Villanueva A *et al.* Integrative molecular analysis of intrahepatic cholangiocarcinoma reveals 2 classes that have different outcomes. *Gastroenterology* 144(4), 829–840 (2013).
19. Al-Salama ZT, Syed YY, Scott LJ. Lenvatinib: a review in hepatocellular carcinoma. *Drugs* 79(6), 665–674 (2019).
20. Zhao Y, Zhang Y-N, Wang K-T, Chen L. Lenvatinib for hepatocellular carcinoma: from preclinical mechanisms to anti-cancer therapy. *Biochim. Biophys. Acta Rev. Cancer* 1874(1), 188391 (2020).
21. Ueno M, Ikeda M, Sasaki T *et al.* Phase 2 study of lenvatinib monotherapy as second-line treatment in unresectable biliary tract cancer: primary analysis results. *BMC Cancer* 20(1), 1105 (2020).
22. Izquierdo-Sanchez L, Lamarca A, La Casta A *et al.* Cholangiocarcinoma landscape in Europe: diagnostic, prognostic and therapeutic insights from the ENSCCA Registry. *J. Hepatol.* 76(5), 1109–1121 (2022).
23. Akinwande O, Dendy M, Ludwig JM, Kim HS. Hepatic intra-arterial injection of irinotecan drug eluting beads (DEBIRI) for patients with unresectable colorectal liver metastases: a systematic review. *Surg. Oncol. Oxford* 26(3), 268–275 (2017).
24. Elganainy D, Holliday EB, Taniguchi CM *et al.* Dose escalation of radiotherapy in unresectable extrahepatic cholangiocarcinoma. *Cancer Med.* 7(10), 4880–4892 (2018).
25. Hong TS, Wo JY, Yeap BY *et al.* Multi-institutional phase II study of high-dose hypofractionated proton beam therapy in patients with localized, unresectable hepatocellular carcinoma and intrahepatic cholangiocarcinoma. *J. Clin. Oncol.* 34(5), 460 (2016).
26. Massani M, Nistri C, Ruffolo C *et al.* Intrahepatic chemotherapy for unresectable cholangiocarcinoma: review of literature and personal experience. *Updates Surg.* 67(4), 389–400 (2015).
27. Park JJH, Hsu G, Siden EG, Thorlund K, Mills EJ. An overview of precision oncology basket and umbrella trials for clinicians. *CA Cancer J. Clin.* 70(2), 125–137 (2020).
28. Lowery MA, Ptashkin R, Jordan E *et al.* Comprehensive molecular profiling of intrahepatic and extrahepatic cholangiocarcinomas: potential targets for intervention. *Clin. Cancer Res.* 24(17), 4154–4161 (2018).
29. Goyal L, Kongpetch S, Crolley VE, Bridgewater J. Targeting FGFR inhibition in cholangiocarcinoma. *Cancer Treat. Rev.* 95, 102170 (2021).
30. Abou-Alfa GK, Macarulla T, Javle MM *et al.* Ivosidenib in *IDH1*-mutant, chemotherapy-refractory Croatia & cholangiocarcinoma (ClarlDHy): a multicentre, randomised, double-blind, placebo-controlled, phase 3 study. *Lancet Oncol.* 21(6), 796–807 (2020).
31. Montal R, Sia D, Montironi C *et al.* Molecular classification and therapeutic targets in extrahepatic cholangiocarcinoma. *J. Hepatol.* 73(2), 315–327 (2020).
32. Shi C, Li Y, Yang C *et al.* Lenvatinib plus programmed cell death protein-1 inhibitor beyond first-line systemic therapy in refractory advanced biliary tract cancer: a real-world retrospective study in China. *Front. Immunol.* 13, 946861 (2022).
33. Zhu C, Xue J, Wang Y *et al.* Efficacy and safety of lenvatinib combined with PD-1/PD-L1 inhibitors plus Gemox chemotherapy in advanced biliary tract cancer. *Front. Immunol.* 14, 1109292 (2023).