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Real-world outcomes of fluorouracil-based second-line therapy in patients with advanced biliary tract cancer refractory to gemcitabine and cisplatin-based treatment

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Abstract

Background: The prognosis for patients with advanced biliary tract cancer (BTC) who have not responded to gemcitabine and cisplatin (GP)-based therapy is dismal. Fluorouracil (5-FU)-based chemotherapy could be considered for those patients who are refractory to GP-based treatments. Our study aimed to evaluate the real-world efficacy and safety of 5-FU-based chemotherapy for BTC patients who had progressed after gemcitabine-based treatment.

Methods: This study analyzed patients from Seoul St. Mary's Hospital and St. Vincent's Hospital with advanced BTC who had previously failed treatment with GP-based chemotherapy. From June 2020 and May 2024, these patients received 5-FU-based chemotherapy as a second-line treatment. The 5-FU-based systemic treatments encompassed 5-FU, leucovorin, and oxaliplatin (FOLFOX); 5-FU, leucovorin, and liposomal irinotecan (Nal-IRI/FL); and 5-FU, leucovorin, irinotecan, and oxaliplatin (FOLFIRINOX). Our investigation focused on evaluating the survival outcomes and safety profiles of each regimen within this cohort.

Results: In our analysis of 147 patients, the primary tumor sites were distributed with 56 (38.1%) having intrahepatic cholangiocarcinoma, 51 (34.7%) with extrahepatic cholangiocarcinoma, and 40 (27.2%) with gallbladder cancer. Regarding the 5-FU-based regimens, 57 patients (38.8%) were treated with FOLFOX, 56 (38.1%) with Nal-IRI/FL, and 34 (23.1%) with FOLFIRINOX. The median progression-free survival (PFS) and overall survival (OS) were 2.3 months (95% confidence interval (CI), 2.0–2.6) and 4.8 months (95% CI, 3.8–5.8), respectively. Poor performance status and higher histologic grade were associated with worse PFS and OS, while female gender and prior surgery were linked to improved OS. FOLFOX and Nal-IRI/FL demonstrated comparable efficacy, with a median OS of 5.4 months (95% CI, 3.5–7.3) for FOLFOX and 4.7 months (95% CI, 2.6–6.9) for Nal-IRI/FL, and no significant differences were observed across subgroups. Grade 3 or higher neutropenia and biliary events were less frequent with FOLFOX, which also showed a lower incidence of adverse events and higher relative dose intensity than Nal-IRI/FL or FOLFIRINOX.

Conclusion: In patients with advanced BTC who failed GP treatment, the FOLFOX regimen demonstrated comparable efficacy, and a more favorable safety profile compared to other 5-FU-based treatments. Given its favorable toxicity profile in a real-world setting, FOLFOX should be considered a standard second-line treatment option.

Keywords: biliary tract cancer, fluorouracil, real-world evidence, second-line chemotherapy

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Introduction

Biliary tract cancers (BTCs) are highly lethal, including a spectrum of invasive carcinomas originating in the bile ducts (intrahepatic and extrahepatic cholangiocarcinoma) and gall bladder.[1] BTC is typically diagnosed at advanced stages, for which curative surgery is not feasible, leading to a poor prognosis.[1] For many years, the combination of gemcitabine and cisplatin (GP) has been the standard of care for first-line chemotherapy for patients with advanced or metastatic BTCs.[2] Recent phase III trials have shown that the addition of durvalumab or pembrolizumab to GP significantly improves overall survival (OS) compared to the regimen with placebo, establishing this combination of chemotherapy and immunotherapy as the new standard first-line treatment.[3,4]

In the context of second-line therapy, fluorouracil-based chemotherapy can be considered if druggable molecular alterations are absent. Findings from the randomized phase III ABC-06 trial revealed that adding folinic acid, fluorouracil, and oxaliplatin (FOLFOX) to active symptom control in patients previously treated with GP moderately improved survival outcomes compared to active symptom control alone.[5]

The folinic acid, fluorouracil, and irinotecan (FOLFIRI) regimen demonstrated some benefits as a second-line treatment.[6] Furthermore, a phase II trial indicated that the combination of liposomal irinotecan with fluorouracil and leucovorin (Nal-IRI/FL) significantly enhanced progression-free survival (PFS) in patients with advanced BTCs who experienced disease progression on GP, compared to treatment with fluorouracil and leucovorin (FL).[7] However, in an updated analysis, while the median PFS for the Nal-IRI/FL group remained significantly superior to that of the FL group, it exhibited a numerical decrease relative to the previous analysis.[8] In addition, another phase II trial assessing the efficacy of Nal-IRI/FL revealed that the addition of liposomal irinotecan to the FL regimen did not improve survival outcomes and was associated with increased toxicity compared to the FL regimen.[9]

A previous phase II trial investigating the use of fluorouracil, leucovorin, irinotecan, and oxaliplatin (FOLFIRINOX) as salvage treatment for advanced BTC patients who had been pretreated with GP reported a median PFS of 6.2 months (95% confidence interval (CI), 3.0–3.1), suggesting a relatively favorable outcome.[10] However, the frequent occurrence of treatment-related adverse events and the small sample size in the study limit the strength of the recommendation for this regimen in BTC patients.

For most BTC patients without targetable molecular alterations, cytotoxic chemotherapy remains the only second-line treatment option. However, the benefits of second-line chemotherapy for advanced BTCs are modest, and there remains no consensus on the optimal regimen. In addition, the gap between clinical trial populations and real-world patients, who are typically older and tend to have poorer performance status, further complicates treatment decisions. Given these challenges, our study aims to investigate the real-world efficacy and safety of fluorouracil-based chemotherapy as a second-line treatment for patients with advanced BTC.

Methods

Patients

Patients with histologically or cytologically verified advanced BTCs were eligible for inclusion in this study if they demonstrated disease progression following previous gemcitabine-based chemotherapy, including neoadjuvant, adjuvant, or palliative treatments. This study retrospectively analyzed medical records of patients from Seoul St. Mary's Hospital and St. Vincent's Hospital with advanced BTCs who had experienced treatment failure with gemcitabine-based chemotherapy. The study complied with Korean regulations and the principles of the Declaration of Helsinki. The reporting of this study conforms to the European Society for Medical Oncology-Grow statement.[11] The Institutional Review Board of The Catholic University of Korea, Seoul St. Mary's Hospital, approved the acquisition of data (approval ID: KC23RASI0602), granting a waiver of informed consent due to the retrospective nature of the analysis.

Procedures

Patients received fluorouracil-based chemotherapy, FOLFOX, Nal-IRI/FL, or FOLFIRINOX, with each regimen administered as follows: FOLFOX comprised oxaliplatin 85 mg/m^2 as a 2 h intravenous infusion, leucovorin 350 mg/m^2 as a 2-h intravenous infusion concurrently with oxaliplatin, and fluorouracil 400 mg/m^2 (intravenous

bolus) on day 1, followed by fluorouracil 2400 mg/m^2 as a continuous intravenous infusion over 46 h, administered every 2 weeks. The Nal-IRI/FL regimen included 70 mg/m^2 of intravenous liposomal irinotecan over 90 min, followed by leucovorin 400 mg/m^2 intravenously over 30 min, and fluorouracil 2400 mg/m^2 as a continuous intravenous infusion over 46 h, administered every 2 weeks. The FOLFIRINOX regimen consisted of oxaliplatin 85 mg/m^2 as a 2-h intravenous infusion, leucovorin 400 mg/m^2 as a 2-h infusion concurrently with oxaliplatin, irinotecan 150 mg/m^2 over 90 min, and fluorouracil 400 mg/m^2 (intravenous bolus), followed by continuous infusion of fluorouracil 2400 mg/m^2 over 46 h, administered every 2 weeks. Treatment data, including duration and reasons for discontinuation, such as radiological or clinical disease progression, physician's decision, or unacceptable toxicities, were retrospectively analyzed. In addition, data on dosing and treatment schedules were collected, and reasons for treatment discontinuation were documented. Relative dose intensity (RDI) was defined as the ratio of the actual chemotherapy dose delivered to the planned dose over a specified time, expressed as a percentage.

Assessments

Tumor assessments were conducted in accordance with Response Evaluation Criteria in Solid Tumors version 1.1. These assessments utilized imaging data acquired through computed tomography or magnetic resonance imaging of the thoracic, abdominal, and pelvis, performed at 6- to 8-week intervals following the initiation of chemotherapy. Additional imaging was implemented as clinically indicated. Human epidermal growth factor receptor 2 (HER2) status was assessed at each institution's CLIA-certified laboratories through the pathological examination of archival tumor tissue using immunohistochemistry (IHC) and in situ hybridization (ISH). Testing and scoring followed guidelines from the American Society of Clinical Oncology and the College of American Pathologists for gastroesophageal adenocarcinoma, with HER2 positivity defined as IHC 3+ or IHC 2+ with ISH amplification.[12] Adverse events were evaluated at each clinic visit and categorized according to the National Cancer Institute Common Terminology Criteria for Adverse Events, version 5.0. Radiologic response-evaluable subjects are defined as patients who received at least one cycle of study treatment and had at least one post-baseline efficacy disease assessment through an imaging modality. Platinum sensitivity was classified into three categories: sensitive, defined as progression occurring more than 90 days after day 1 of the final cycle of first-line GP; refractory, indicated by progression during first-line treatment with GP; and resistant, characterized by progression within 90 days following day 1 of the final cycle of first-line GP.

Statistical analysis

Descriptive statistics are reported as proportions or medians with interquartile ranges. Categorical variables were compared using the Chi-square or Fisher's exact test, and across multiple groups using a one-way analysis of variance, while continuous variables were compared using the Student's *t*-test. We used Fisher's exact test for pairwise comparisons of objective response and disease control rate across the treatment groups. OS was defined as the time between the initiation of chemotherapy and death due to any cause. PFS was defined as the time from the date of initiation of chemotherapy until the date of disease progression or death. Kaplan–Meier analyses were conducted for each treatment group to obtain non-parametric estimates of median OS and PFS. Pairwise comparisons of OS and PFS between study treatments were performed using the unstratified log-rank test. Unstratified Cox proportional hazards regression was used to estimate hazard ratios (HRs) and their corresponding 95% CIs. A Cox regression model with forward stepwise selection was employed to estimate the effect of treatment and potential baseline prognostic factors for survival outcomes. All tests were two-sided, and p-values <0.05 were considered statistically significant. Statistical analysis was performed using SPSS for Windows version 24.0 (IBM SPSS Inc., Armonk, NY, USA) and GraphPad Prism version 10.3 (GraphPad Software Inc., San Diego, CA, USA).

Results

Patients

Between June 2020 and May 2024, a cohort of 147 patients with advanced BTCs who had received fluorouracil-based chemotherapy as second-line or subsequent treatment were evaluated for eligibility and subsequently enrolled in this study. The cohort was stratified into three treatment groups: FOLFOX ($n=57$), Nal-IRI/FL

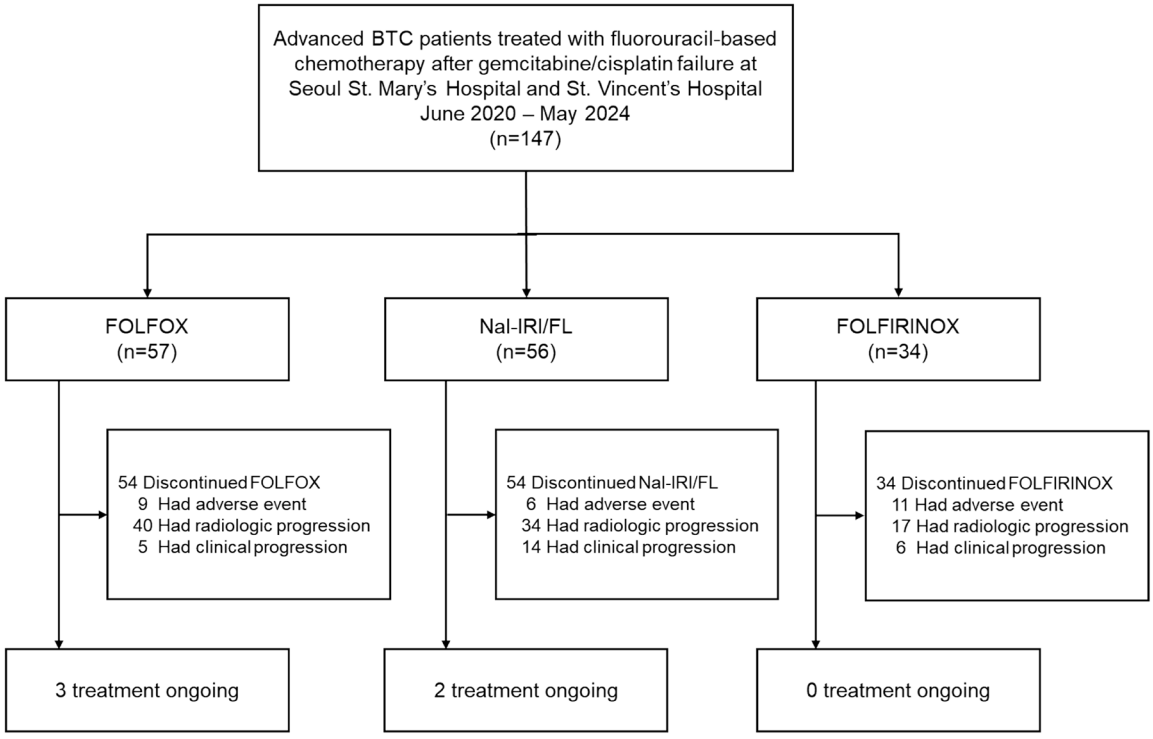

Figure 1. Visual outline of this study.
BTC, biliary tract cancer; FOLFIRINOX, 5-FU, leucovorin, irinotecan, and oxaliplatin; FOLFOX, 5-FU, leucovorin, and oxaliplatin; Nal-IRI/FL, 5-FU, leucovorin, and liposomal irinotecan.

($n=56$), and FOLFIRINOX ($n=34$; Figure 1). The clinical characteristics of the patients are summarized in Table 1. The primary tumor locations encompassed intrahepatic cholangiocarcinoma in 56 patients (38.1%), extrahepatic cholangiocarcinoma in 51 patients (34.7%), and gallbladder cancer in 40 patients (27.2%). The majority of patients presented with metastatic disease ($n=142$, 96.2%), and a significant proportion ($n=121$, 82.3%) were classified as platinum-resistant or refractory. Baseline characteristics were generally well-balanced across the treatment groups, except for the FOLFIRINOX group, which included patients with comparatively superior performance status. At the time of analysis, 5.3% of patients in the FOLFOX group and 3.6% in the Nal-IRI/FL group were still undergoing treatment. In each treatment group, the most common reason for treatment discontinuation was disease progression, either radiological or clinical (Figure 1).

Efficacy

The median follow-up duration for the entire cohort was 4.3 months (95% CI, 3.9–5.2). Among the 147 patients, disease progression was observed in 134 (91.2%), and 127 (86.4%) had died at the time of analysis. The median PFS for the entire cohort was 2.3 months (95% CI, 2.0–2.6), and the median OS was 4.8 months (95% CI, 3.8–5.8; Figure S1). In the subset of 123 patients (83.7%) evaluable for radiologic response, the median PFS was 2.6 months (95% CI, 2.2–2.9), and the median OS was 6.2 months (95% CI, 4.5–7.8). For survival outcomes based on tumor location, the median PFS was 2.3 months (95% CI, 1.9–2.7) for intrahepatic, 2.5 months (95% CI, 2.1–3.0) for extrahepatic cholangiocarcinoma, and 1.6 months (95% CI, 1.1–2.1) for gallbladder cancer (Figure 2(a)). The median OS was 4.9 months (95% CI, 3.8–6.1) for intrahepatic, 5.5 months (95% CI, 3.7–7.3) for extrahepatic cholangiocarcinoma, and 4.6 months (95% CI, 2.7–6.4) for gallbladder cancer (Figure 2(b)). The HER2-negative group had a significantly longer median PFS of 2.1 months (95% CI, 1.8–2.5) compared to 1.4 months (95% CI, 0.9–1.9) in the HER2-positive group (HR = 0.53; 95% CI, 0.23–1.25; $p=0.048$, Figure S2). However, there was no significant difference in median OS between the two groups.

Table 1. Baseline characteristics.

Variable	FOLFOX (*n* = 57)	Nal-IRI/FL (*n* = 56)	FOLFIRINOX (*n* = 34)	*p* Value
Age, years	67 (61–72)	65 (59–74)	63 (58–71)	0.402
Gender				
Male	36 (63.1)	35 (62.5)	20 (58.8)	0.912
Female	21 (36.9)	21 (37.5)	14 (41.2)	
ECOG performance status				
0–1	43 (75.4)	39 (69.6)	31 (91.2)	0.060
2	14 (24.6)	17 (30.4)	3 (8.8)	
Disease stage				
Locally advanced	4 (7.0)	1 (1.7)	2 (5.9)	
Metastatic	53 (93.0)	55 (98.3)	32 (94.1)	
Primary tumor location				
Intrahepatic	17 (29.8)	21 (37.5)	18 (52.9)	0.246
Extrahepatic	24 (42.1)	18 (32.1)	9 (26.5)	
Gallbladder	16 (28.1)	17 (30.4)	7 (20.6)	
Histology				
Adenocarcinoma	56 (98.2)	53 (94.6)	30 (88.2)	
Other[a]	1 (1.8)	3 (5.4)	4 (11.8)	
Grade of differentiation				
Well to moderately	41 (71.9)	37 (66.1)	23 (67.6)	0.666
Poorly	15 (26.3)	15 (26.8)	10 (29.4)	
Not specified	1 (1.8)	4 (7.1)	1 (3.0)	
HER2 status				
Positive	9 (15.8)	0	3 (8.8)	
Negative	34 (59.6)	45 (80.3)	29 (85.3)	
Not available	14 (24.6)	11 (19.7)	2 (5.9)	
Had previous surgery	30 (52.6)	25 (44.6)	22 (64.7)	0.181
Previous GP				
Duration, months	4.9 (2.8–6.8)	4.5 (2.3–6.5)	4.8 (3.8–7.9)	0.753
⩾6 months	17 (29.8)	17 (30.4)	11 (32.4)	0.967

(Continued)

Table 1. (Continued)

Variable	FOLFOX (*n* = 57)	Nal-IRI/FL (*n* = 56)	FOLFIRINOX (*n* = 34)	*p* Value
Prior gemcitabine-based therapy				
GP	47 (82.5)	48 (85.8)	29 (85.3)	
GP/nab-paclitaxel	7 (12.3)	4 (7.1)	5 (14.7)	
GP/durvalumab	3 (5.2)	4 (7.1)	0	
Platinum sensitivity				
Resistant or refractory	49 (86.0)	43 (76.8)	29 (85.3)	0.386
Sensitive	8 (14.0)	13 (23.2)	5 (14.7)	
Prior 5-FU exposure				
No	49 (86.0)	49 (87.5)	27 (79.4)	0.562
Yes	8 (14.0)	7 (12.5)	7 (20.6)	
Baseline CA 19-9				
<172 U/mL	18 (31.6)	24 (42.9)	18 (52.9)	0.124
⩾172 U/mL	39 (68.4)	32 (57.1)	16 (47.1)	

Data are *n* (%) or median (IQR).
[a]Other included squamous, adenosquamous, and not specified.
5-FU, fluorouracil; CA 19-9, carbohydrate antigen 19-9; ECOG, Eastern Cooperative Oncology Group; FOLFIRINOX, 5-FU, leucovorin, irinotecan, and oxaliplatin; FOLFOX, 5-FU, leucovorin, and oxaliplatin; GP, gemcitabine and cisplatin; HER2, human epidermal growth factor receptor 2; IQR, interquartile range; Nal-IRI/FL, 5-FU, leucovorin and liposomal irinotecan.

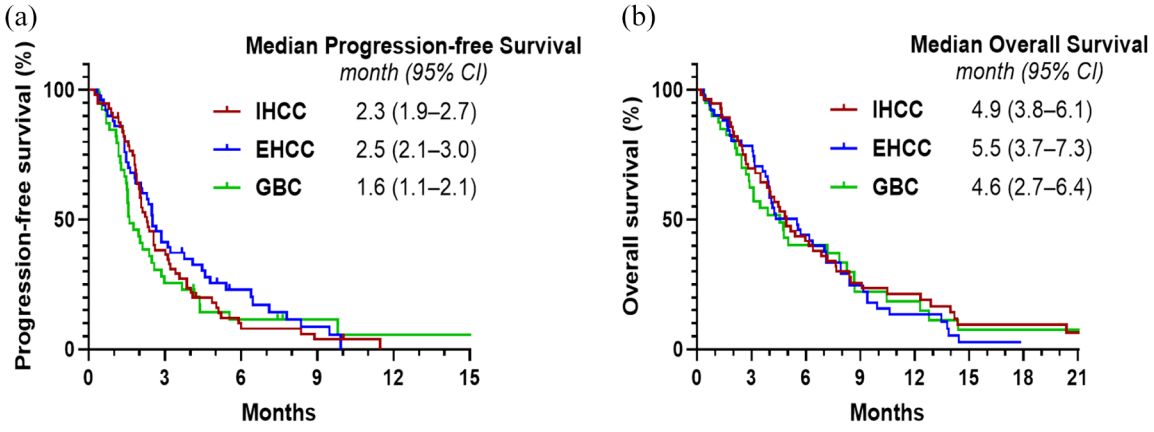

Figure 2. Kaplan–Meier estimates of (a) progression-free survival and (b) overall survival, according to primary tumor location.

Table 2 presents the findings from the univariable, and multivariable analyses aimed at identifying predictors of survival outcomes. In the multivariable analysis, poor performance status (HR = 1.77; 95% CI, 1.17–2.67; $p = 0.007$), higher histologic grade (HR = 1.58; 95% CI, 1.04–2.39; $p = 0.032$), and platinum resistance (HR = 1.70; 95% CI, 1.05–2.75; $p = 0.032$) were significantly associated with worse PFS. In addition, poor performance status (HR = 2.31; 95% CI, 1.51–3.54; $p < 0.001$)

Table 2. Univariate and multivariate assessments of clinicopathologic features in relation to PFS and OS in patients with advanced biliary tract cancer.

Variables	PFS				OS			
	Univariate analysis		Multivariate analysis		Univariate analysis		Multivariate analysis	
	HR (95% CI)	*p* Value	HR (95% CI)	*p* Value	HR (95% CI)	*p* Value	HR (95% CI)	*p* Value
Age ⩾70 (vs <70 years)	1.04 (0.72–1.50)	0.825			1.36 (0.94–1.97)	0.106		
Female (vs male)	0.84 (0.59–1.19)	0.330			0.66 (0.47–0.95)	0.027	0.49 (0.32–0.73)	0.001
ECOG PS 2 (vs PS 0–1)	1.87 (1.25–2.79)	0.002	1.77 (1.17–2.67)	0.007	2.56 (1.69–3.88)	<0.001	2.31 (1.51–3.54)	<0.001
Primary tumor location EHCC (vs IHCC) GBC (vs IHCC)	0.86 (0.58–1.28) 1.12 (0.73–1.72)	0.458 0.609			1.13 (0.75–1.70) 1.12 (0.72–1.74)	0.545 0.619		
Grade 3 histology (vs grade 1–2)	1.70 (1.14–2.51)	0.008	1.58 (1.04–2.39)	0.032	1.85 (1.25–2.74)	0.002	2.02 (1.33–3.09)	0.001
Previous surgery (vs none)	0.73 (0.51–1.03)	0.076	0.97 (0.66–1.44)	0.889	0.63 (0.44–0.90)	0.011	0.69 (0.47–1.02)	0.064
Previous GP ⩾6 months (vs <6 months)	0.90 (0.62–1.30)	0.563			0.73 (0.50–1.08)	0.115		
Platinum resistance (vs none)	1.78 (1.11–2.86)	0.016	1.70 (1.05–2.75)	0.032	1.89 (1.15–3.08)	0.012	1.55 (0.93–2.59)	0.090
Prior 5-FU exposure (vs none)	0.80 (0.49–1.29)	0.359			0.63 (0.38–1.04)	0.069		
CA 19-9 ⩾172 U/mL (vs <172 U/mL)	1.07 (0.76–1.52)	0.695			1.21 (0.85–1.73)	0.294		

5-FU, fluorouracil; CA 19-9, carbohydrate antigen 19-9; ECOG, PS Eastern Cooperative Oncology Group Performance Status; EHCC, extrahepatic cholangiocarcinoma; GBC, gallbladder cancer; GP, gemcitabine with cisplatin; HR, hazard ratio; IHCC, intrahepatic cholangiocarcinoma; OS, overall survival; PFS, progression-free survival.

Table 3. Efficacy outcomes of second-line systemic chemotherapy in patients with advanced biliary tract cancer.

Variable	FOLFOX (*n* = 57)	Nal-IRI/FL (*n* = 56)	FOLFIRINOX (*n* = 34)
Best overall response, *n* (%)			
Partial response	5 (8.8)	5 (8.9)	5 (14.7)
Stable disease	23 (40.4)	20 (35.7)	10 (29.4)
Progressive disease	29 (50.8)	31 (55.4)	19 (55.9)
Objective response rate, *n* (%)	5 (8.8)	5 (8.9)	5 (14.7)
p Value vs FOLFOX		1.000	0.492
Disease control rate, *n* (%)	28 (49.1)	25 (44.6)	15 (44.1)
p Value vs FOLFOX		0.707	0.670
Median PFS, months (95% CI)	2.4 (1.9–2.8)	2.2 (1.7–2.6)	2.0 (1.5–2.6)
p Value vs FOLFOX		0.817	0.563
16-Weeks PFS, % (95% CI)	24.0 (13.7–35.9)	26.5 (15.7–38.5)	34.7 (19.0–50.9)
Median OS, months (95% CI)	5.4 (3.5–7.3)	4.7 (2.6–6.9)	4.3 (3.5–5.1)
p Value vs FOLFOX		0.901	0.397
6-months OS, % (95% CI)	43.6 (30.2–56.3)	45.1 (31.5–57.7)	35.3 (20.0–51.0)

CI, confidence interval; FOLFIRINOX, 5-FU, leucovorin, irinotecan, and oxaliplatin; FOLFOX, 5-FU, leucovorin, and oxaliplatin; Nal-IRI/FL, 5-FU, leucovorin and liposomal irinotecan; OS, overall survival; PFS, progression-free survival.

and higher histologic grade (HR = 2.02; 95% CI, 1.33–3.09; $p = 0.001$) were significantly correlated with worse OS. By contrast, female gender (HR = 0.49; 95% CI, 0.32–0.73; $p = 0.001$) and a history of prior surgery (HR = 0.69; 95% CI, 0.47–1.02; $p = 0.064$) were associated with more favorable OS outcomes.

Effectiveness of treatment regimens

Table 3 presents the efficacy outcomes according to the treatment regimen. The objective response rates were 8.8% for FOLFOX, 8.9% for Nal-IRI/FL ($p = 1.000$ vs FOLFOX), and 14.7% for FOLFIRINOX ($p = 0.492$ vs FOLFOX). Although the FOLFIRINOX group exhibited a numerically higher response rate, the objective response rates were comparable across the three treatment groups, with no statistically significant differences. Similarly, the disease control rates showed no significant variation between the groups. Median PFS was also comparable, with 2.4 months (95% CI, 1.9–2.8) for FOLFOX, 2.2 months (95% CI, 1.7–2.6) for Nal-IRI/FL ($p = 0.817$ vs FOLFOX, Figure 3(a)), and 2.0 months (95% CI, 1.5–2.6) for FOLFIRINOX ($p = 0.563$ vs FOLFOX, Figure 3(b)). The median OS was 5.4 months (95% CI, 3.5–7.3) for FOLFOX, 4.7 months (95% CI, 2.6–6.9) for Nal-IRI/FL ($p = 0.901$ vs FOLFOX, Figure 3(c)), and 4.3 months (95% CI, 3.5–5.1) for FOLFIRINOX ($p = 0.397$ vs FOLFOX, Figure 3(d)), with no statistically significant differences observed between the treatment groups.

Subgroup analyses of PFS and OS, comparing the efficacy of FOLFOX and Nal-IRI/FL, are presented in Figures S2 and S3. Across all subgroups, no statistically significant differences in survival outcomes between the two treatment regimens were observed. In patients with intrahepatic cholangiocarcinoma, Nal-IRI/FL was associated with numerically longer survival, whereas FOLFOX showed a better survival outcome compared to Nal-IRI/FL in patients with gallbladder cancer. However, neither difference reached statistical significance (Figure 4).

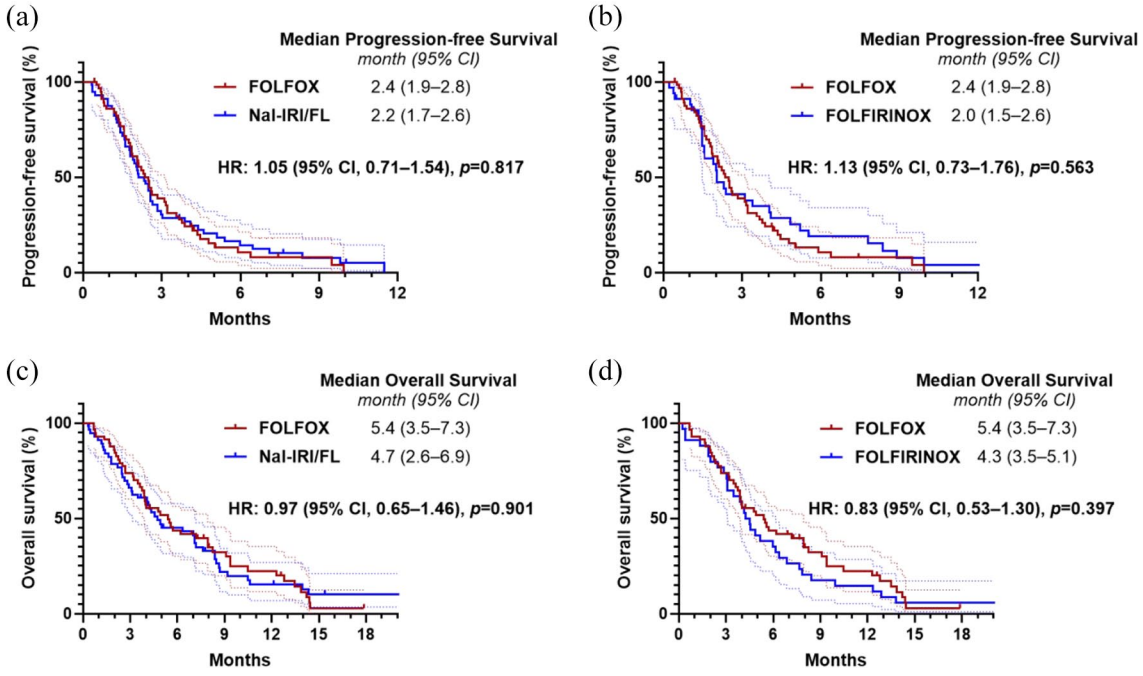

Figure 3. Kaplan–Meier analysis of survival outcomes. (a) PFS comparing Nal-IRI/FL to FOLFOX. (b) PFS comparing FOLFIRINOX to FOLFOX. (c) OS comparing Nal-IRI/FL to FOLFOX. (d) OS comparing FOLFIRINOX to FOLFOX.
FOLFIRINOX, fluorouracil, leucovorin, irinotecan, and oxaliplatin; FOLFOX, fluorouracil, leucovorin, and oxaliplatin; Nal-IRI/FL, fluorouracil, leucovorin, and liposomal irinotecan; OS, overall survival; PFS, progression-free survival.

Safety

Grade 3–5 adverse events occurred in 35 patients (61.4%) in the FOLFOX group, 43 patients (76.8%) in the Nal-IRI/FL group, and 29 patients (85.3%) in the FOLFIRINOX group (Table 4). Five chemotherapy-related deaths were reported, three due to sepsis and two due to febrile neutropenia, with one occurring in the FOLFOX group and two each in the Nal-IRI/FL and FOLFIRINOX groups. Neutropenia was the most frequently reported Grade 3–5 toxicity, affecting 38.6% of patients in the FOLFOX group, 39.3% in the Nal-IRI/FL group, and 64.7% in the FOLFIRINOX group. Febrile neutropenia was observed in 3.6%, 8.9%, and 17.6% of patients in the FOLFOX, Nal-IRI/FL, and FOLFIRINOX groups, respectively. Among non-hematologic toxicities, Grade 3 or higher biliary events were most frequently reported, occurring in 15.8% of FOLFOX, 25.0% of Nal-IRI/FL, and 26.5% of FOLFIRINOX patients.

Treatment dose intensity

The median treatment duration was 1.8 months (IQR, 1.3–3.5) in the FOLFOX group, 1.7 months (IQR, 0.9–3.4) in the Nal-IRI/FL group, and 1.6 months (IQR, 1.0–3.5) in the FOLFIRINOX group (Table S1). The median RDI for FOLFOX, Nal-IRI/FL, and FOLFIRINOX were 0.78, 0.69, and 0.66, respectively, with 54.4%, 35.7%, and 41.2% of patients achieving an RDI of ⩾80%. Permanent treatment discontinuation after one cycle occurred in 30 of 147 patients (20.4%), including 10 in the FOLFOX group, 13 in the Nal-IRI/FL group, and 7 in the FOLFIRINOX group. The main reasons for early discontinuation were disease progression (radiological in five patients, clinical in eight patients), intolerable toxicity (10 patients), intercurrent illness (three patients), and patient decision (four patients).

Subsequent treatment

Of the 147 patients, 55 (37.4%) received subsequent systemic anticancer treatment following fluorouracil-based therapy (Table S2), with the highest proportion observed in the FOLFOX group (45.6%). Among patients receiving further fluorouracil-based regimens, Nal-IRI/FL was the most frequently administered in the FOLFOX group (31.6%), whereas 26.8% of patients in the Nal-IRI/FL group were subsequently treated with FOLFOX. A smaller proportion of patients

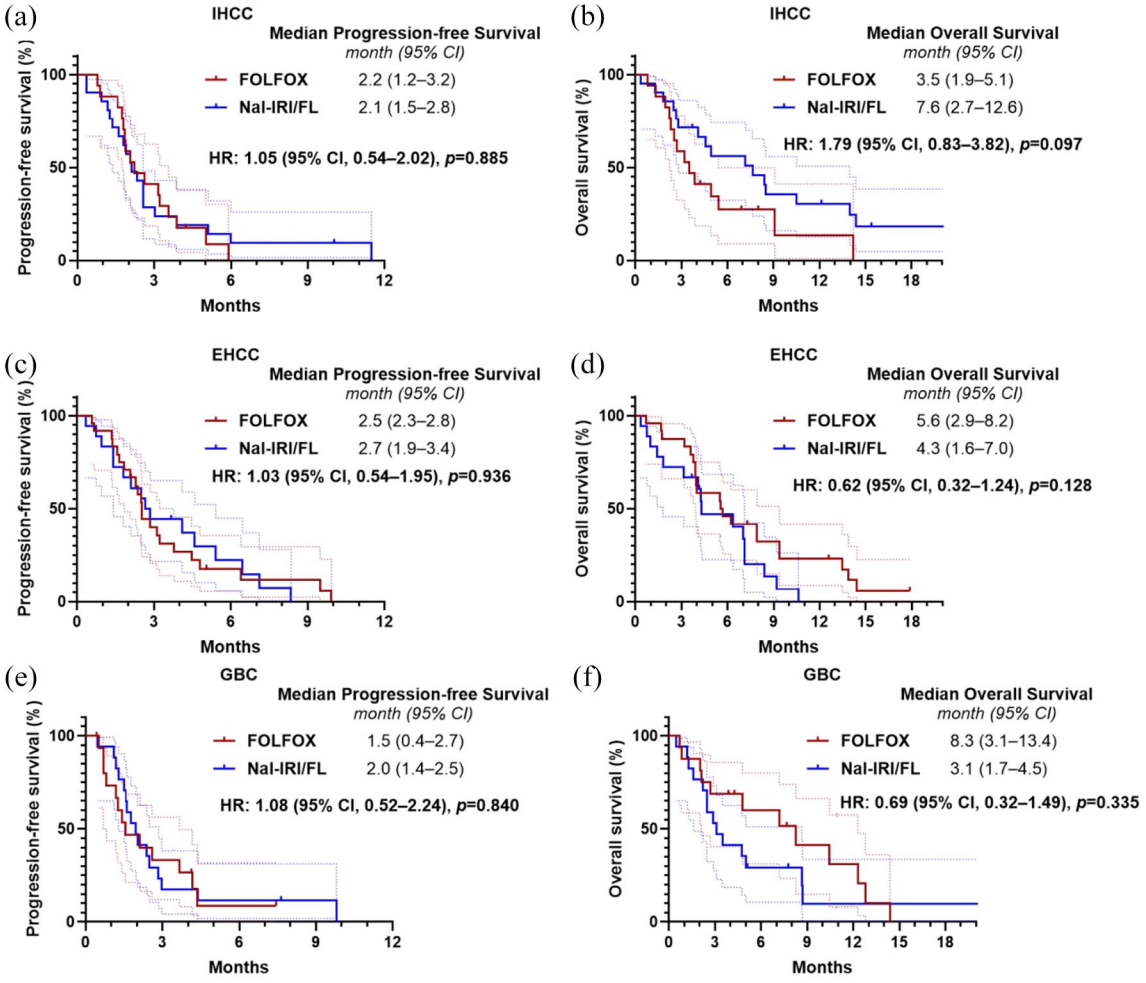

Figure 4. Survival analyses comparing PFS and OS by primary tumor location across different treatment regimens. (a) PFS and (b) OS in patients with intrahepatic cholangiocarcinoma, (c) and (d) in patients with extrahepatic cholangiocarcinoma, and (e) and (f) in patients with gallbladder cancer, comparing FOLFOX with Nal-IRI/FL.
FOLFOX, fluorouracil, leucovorin, and oxaliplatin; Nal-IRI/FL, fluorouracil, leucovorin, and liposomal irinotecan; OS, overall survival; PFS, progression-free survival.

(6.1%) received immunotherapy, with similar rates across all treatment groups. Anti-HER2-targeted therapy was administered to five patients (3.4%), all from the FOLFOX group.

Discussion

This retrospective study evaluated the overall efficacy and safety of fluorouracil-based chemotherapy in a real-world setting for advanced BTC patients refractory to gemcitabine-based treatment. In the overall population, no significant survival differences were observed based on primary tumor location. Poor performance status and higher histologic grade were associated with worse PFS and OS, while female gender and a history of prior surgery were linked to improved OS outcomes. Although FOLFOX and Nal-IRI/FL showed no significant efficacy differences across all subgroups, FOLFOX demonstrated a lower rate of adverse events and maintained a relatively higher dose intensity compared to Nal-IRI/FL and FOLFIRINOX.

The survival outcomes in this study were inferior to those of previous prospective clinical trials that assessed the efficacy of FOLFOX and Nal-IRI/FL.[5,7] This may be attributed to the real-world nature of the study, which included 34 patients (23.1%) with Eastern Cooperative Oncology Group performance status 2. Indeed, among patients with evaluable radiologic response, the

Table 4. Adverse events.

Adverse events	FOLFOX (*n* = 57)		Nal-IRI/FL (*n* = 56)		FOLFIRINOX (*n* = 34)	
	Any grade	Grade ⩾3	Any grade	Grade ⩾3	Any grade	Grade ⩾3
Any	53 (93.0)	35 (61.4)	54 (96.4)	43 (76.8)	34 (100)	29 (85.3)
Diarrhea	2 (3.6)	1 (1.8)	3 (5.3)	0	2 (5.9)	0
Vomiting	3 (5.4)	1 (1.8)	2 (3.6)	0	8 (23.5)	1 (2.9)
Fatigue	25 (43.9)	3 (5.4)	24 (42.9)	8 (14.3)	19 (55.9)	7 (20.6)
Anorexia	12 (21.0)	1 (1.8)	14 (25.0)	1 (1.8)	8 (23.5)	3 (8.8)
Biliary event[a]	11 (19.3)	9 (15.8)	15 (26.8)	14 (25.0)	11 (32.3)	9 (26.5)
Sepsis	5 (8.8)	5 (8.8)	9 (16.1)	9 (16.1)	8 (23.5)	8 (23.5)
Neutropenia[b]	29 (50.9)	22 (38.6)	28 (46.4)	22 (39.3)	23 (67.6)	22 (64.7)
Febrile neutropenia	2 (3.6)	2 (3.6)	5 (8.9)	5 (8.9)	6 (17.6)	6 (17.6)
Anemia	36 (63.2)	7 (12.3)	40 (71.4)	11 (19.6)	26 (76.5)	10 (29.4)
Thrombocytopenia	19 (33.3)	6 (10.5)	13 (23.2)	5 (8.9)	18 (52.9)	8 (23.5)

Data are number of patients (%).
[a]Includes liver infection, increased bilirubin, and hepatitis.
[b]Includes agranulocytosis, febrile neutropenia, granulocytopenia, neutropenia, neutropenic sepsis, decreased neutrophil count, and pancytopenia.
FOLFIRINOX, 5-FU, leucovorin, irinotecan, and oxaliplatin; FOLFOX, 5-FU, leucovorin, and oxaliplatin; Nal-IRI/FL, 5-FU, leucovorin and liposomal irinotecan.

median OS was identical to the 6.2 months reported in the ABC-06 trial. Regarding tumor location, while not statistically significant, patients with gallbladder cancer exhibited a trend toward shorter PFS compared to those with intrahepatic or extrahepatic cholangiocarcinoma. Similar outcomes were observed in previous clinical trials assessing GPs with immunotherapy (TOPAZ-1, KEYNOTE-966), where patients with gallbladder cancer had shorter PFS compared to those with other tumors.[3,4] These results suggest that gallbladder cancer may have more aggressive biological behavior and is associated with a dismal prognosis due to its refractory nature to chemotherapy.

In the multivariate analysis, poorly differentiated histology was significantly associated with worse PFS and OS, consistent with previous studies that show a correlation between higher histologic grade and poor prognosis in gallbladder cancer.[13] Therefore, further molecular profiling analysis is urgently needed in patients with poorly differentiated histology to guide the development of appropriate treatment strategies. Patients with prior curative-intent surgery for their primary tumor had better OS outcomes, possibly explained by the lower incidence of poorly differentiated histology compared to those without surgery (19.5% vs 39.7%). Moreover, they experienced fewer biliary events (22.1% vs 28.6%) and a lower frequency of treatment discontinuation due to adverse events (12.9% vs 22.9%), which may have contributed to their improved survival outcomes. Female patients showed significantly better OS than male patients, though the reason for this observed advantage remains unclear.

Patients treated with FOLFOX in this study had a response rate of 8.8% and a median OS of 5.4 months, outcomes that were relatively comparable to those from the ABC-06 trial (5.0%, 6.2 months) and a prior meta-analysis (10.4%, 6.4 months).[5,14] Those treated with Nal-IRI/FL had a response rate of 8.9% and a median OS of 4.7 months, showing worse survival outcomes compared to the NIFTY study (12.5%, 8.6 months) and even the NALIRICC study (14%, 6.9 months).[8,9] Patients receiving FOLFIRINOX demonstrated a response rate of 14.7% and a median OS of 4.3 months, which was consistent with the response rate observed in

previous phase II trials, but with a significant divergence in survival outcomes.[10] In a real-world setting, FOLFOX and Nal-IRI/FL did not show significant differences in efficacy outcomes across all subgroups. However, FOLFOX demonstrated a weaker OS benefit in the gallbladder cancer subgroup, while Nal-IRI/FL showed a weaker OS benefit in the intrahepatic cholangiocarcinoma subgroup. These findings should be interpreted with caution due to insufficient statistical power. The extended OS with FOLFOX in gallbladder cancer, despite no PFS advantage, may be explained by the fact that all five patients who received subsequent anti-HER2 therapy were in the FOLFOX group. Similarly, the longer OS trend with Nal-IRI/FL in intrahepatic cholangiocarcinoma, despite no PFS superiority, could be attributed to molecular biomarker analysis conducted in 37 of 56 intrahepatic cholangiocarcinoma patients, which identified isocitrate dehydrogenase-1 (IDH1) mutations in three patients, all of whom were in the Nal-IRI/FL group. As *IDH1*-mutant cholangiocarcinoma typically has a more favorable prognosis, this may have influenced survival outcomes.[15,16] To date, the role of tumor location in differential responses to FOLFOX and Nal-IRI/FL remains unclear.

Grade 3 or higher neutropenia occurred less frequently with FOLFOX and Nal-IRI/FL compared to FOLFIRINOX, while grade 3 or higher biliary events were less frequent with FOLFOX than with Nal-IRI/FL and FOLFIRINOX. These differences in safety profiles likely resulted in FOLFOX maintaining a higher RDI than the other regimens. A notable proportion of patients (20.4%) experienced early discontinuation of treatment for various reasons, potentially indicating rapid deterioration in some patients before the treatment could exert a biological effect. Therefore, for patients predicted to have poor outcomes, active symptom control should be prioritized over cytotoxic chemotherapy. This is especially crucial in geriatric or vulnerable populations, where overtreatment must be avoided, considering the safety profiles. To date, based on the ABC-06 study, FOLFOX is recommended as the primary second-line option for advanced BTC patients with Nal-IRI/FL, as demonstrated in the NIFTY study, serving as an alternative option. However, given that clinical trial populations do not fully represent real-world patients, applying trial results directly to clinical practice poses certain challenges.

This study has several limitations. A considerable number of patients received subsequent treatments, which limits the assessment of treatment efficacy based on OS outcomes. In addition, the relatively small sample size in this heterogeneous disease resulted in insufficient power for subgroup analyses, complicating certain interpretations. Given the retrospective nature of this study, non-hematological toxicity may have been underreported, especially for low-grade adverse events. Furthermore, this study was conducted exclusively in a single country with an entirely East Asian population, which may limit the generalizability of the findings to more diverse populations. Lastly, due to the lack of molecular biomarker analysis, we were unable to explore the correlation between genetic alterations and efficacy outcomes.

Conclusion

Our study demonstrated the efficacy and safety of three different fluorouracil-based chemotherapy regimens in a real-world setting. For advanced BTC patients without targetable genetic alterations who have failed gemcitabine plus cisplatin, second-line chemotherapy should be considered for highly selected patients with good performance status, who are medically fit and have normal organ function. Further prospective studies, including molecular biomarker analysis and the exploration of targeted therapies, are necessary to determine the optimal treatment sequence to improve clinical outcomes.

Declarations

Ethics approval and consent to participate

The study conformed to the Korean regulations and the Declaration of Helsinki. Ethical approval for the acquisition of data was obtained by the Institutional Review Board (IRB) of The Catholic University of Korea, Seoul St. Mary's Hospital (approval ID: KC23RASI0602) with a waiver of informed consent due to the retrospective nature of the analysis.

Consent for publication

Not applicable.

Author contributions

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Kabsoo Shin: Writing – original draft.

Hyunho Kim: Conceptualization; Data curation; Methodology; Writing – original draft.

Hyung Soon Park: Conceptualization; Data curation; Methodology; Writing – original draft.

Tae Ho Hong: Writing – original draft.

In-Ho Kim: Writing – original draft.

MyungAh Lee: Conceptualization; Data curation; Formal analysis; Methodology; Writing – original draft; Writing – review & editing.

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