

Gemcitabine-based chemotherapy plus PD-1/PD-L1 inhibitors and tyrosine kinase inhibitors for initially unresectable intrahepatic cholangiocarcinoma: A multicenter, retrospective cohort study

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Abstract

Background

Patients with initially unresectable intrahepatic cholangiocarcinoma (iCCA) have poor prognoses and the current first-line treatments remain unsatisfactory. Our study aimed to evaluate gemcitabine-based chemotherapy plus PD-1/PD-L1 inhibitors and tyrosine kinase inhibitors (TKIs) for iCCA.

Methods

In this multicenter retrospective cohort study, 392 patients were included in the full cohort analysis. For the primary analysis, 88 patients received gemcitabine-based chemotherapy (GEMCIS or GEMOX), and 177 patients received the same chemotherapy backbones combined with PD-1/PD-L1 inhibitors and TKIs. The primary outcome was to evaluate overall survival (OS). The propensity score matching (PSM) method was utilized to reduce potential confounders.

Results

Overall, 392 patients were included from 12 hospitals across China, with data from January 2016 to December 2024. In the full cohort analysis, the combination of GEMCIS/GEMOX with PD-1/PD-L1 inhibitors and TKIs significantly prolonged median OS compared to GEMCIS/GEMOX alone (26.7 vs. 15.3 months; hazard ratio [HR] 0.47, 95% CI: 0.35–0.63, $P < 0.001$). In the primary analysis, the combination strategy was associated with a median OS of 24.8 months and median progression-free survival (PFS) of 11.0 months, with HRs of 0.56 (95% CI: 0.38–0.83, $P = 0.004$) for OS and 0.46 (95% CI: 0.32–0.66, $P < 0.001$) for PFS after PSM. Importantly, our treatment strategy increased the rate of conversion surgery to 42% for locally advanced disease, ultimately resulting in significantly improved overall outcomes (HR for OS: 0.17 [95% CI: 0.05–0.61]).

Conclusion

Combining gemcitabine-based chemotherapy with PD-1/PD-L1 inhibitors and TKIs may provide a promising new first-line treatment option for patients with initially unresectable iCCA.

Introduction

Intrahepatic cholangiocarcinoma (iCCA) is the second most prevalent primary liver cancer after hepatocellular carcinoma [1]. Recognized as an almost universally lethal malignancy, iCCA accounts for approximately 20% of all hepatic cancers and 3% of all gastrointestinal malignancies [2, 3]. Although iCCA is considered a rare cancer, its global incidence is steadily increasing [2–4]. Moreover, over 70% of patients present with locally unresectable or distant metastatic disease, resulting in the loss of the opportunity for curative surgical resection [2, 4]. While systemic therapy may help delay disease progression, the overall survival rate remains limited to one year [5]. Thus, novel biological insights and therapeutic strategies are urgently needed to break through the bottleneck for iCCA.

The combination chemotherapy of gemcitabine and cisplatin (GEMCIS) has been established as the standard first-line therapy for advanced iCCA for over a decade, based on the results of ABC-02 trial. In that phase 3 study, the median overall survival (OS) was 11.7 months (95% confidence interval [CI]: 9.5–14.3) for GEMCIS compared to 8.1 months (95% CI: 7.1–8.7) for gemcitabine alone (hazard ratio [HR]: 0.64, $P < 0.001$) [5]. In Asia, gemcitabine and oxaliplatin (GEMOX) is a common treatment option, offering median OS comparable for patients with locally advanced or metastatic iCCA [6]. Importantly, recent evidences indicate that immunotherapy has become a highly promising treatment option for iCCA. TOPAZ-1 trial demonstrated that the combination of GEMCIS and durvalumab (a programmed death-ligand 1 [PD-L1] inhibitor) provided significant survival benefits for patients with locally advanced or metastatic biliary tract cancer (BTC) when compared to GEMCIS chemotherapy alone, with a median OS of 12.8 months versus 11.5 months (HR: 0.80 [95% CI: 0.66–0.97]; two-sided $P = 0.021$) [7, 8]. Similarly, KEYNOTE-966 study found that the combination of GEMCIS and pembrolizumab (a programmed cell death protein 1 [PD-1] inhibitor) also significantly improved survival outcomes for patients with BTC compared to GEMCIS

alone, showing a median OS of 12.7 months versus 10.9 months (HR: 0.83 [95% CI: 0.72–0.95]; one-sided $P = 0.003$) [9]. Although these regimens have been recommended as preferred first-line therapies for advanced iCCA, the improvement in OS with PD-1/PD-L1 inhibitors combined with chemotherapy as first-line treatment remains modest and unsatisfactory.

With the advent of molecular profiling revealing frequent targetable alterations, molecular targeted therapy for iCCA has become a reality and has shown clinically meaningful activity [10, 11]. Up to 70% of cases may harbor at least one targeted gene mutation [12]. Although the etiology of iCCA is diverse, the abnormal activation of receptor tyrosine kinase signaling pathways is a prevalent mutation observed in this cancer [12]. Thus, tyrosine kinase inhibitors (TKIs), such as pemigatinib [13], infigratinib [14], and futibatinib [15] have gained prominence as the effective targeted therapies in the fight against iCCA. However, TKIs were evaluated in multiple trials in advanced CCA, and without actionable targets (e.g., FGFR2 fusions, BRAF mutations, HER2 amplifications), they are not effective alone or in combination with chemotherapy, it is not effective alone or in combination with chemotherapy [16].

Based on the evidence mentioned above and clinical experience, we firstly reported on the use of GEMCIS in combination with tislelizumab and lenvatinib for the treatment of advanced iCCA. The hepatic lesion was reduced significantly without overlapping adverse effects after four cycles of treatment, and the patient achieved long-term survival after resection of the tumor and lymph nodes [17]. Shortly thereafter, Zhang et al. reported a similar systemic therapeutic regimen consisting of GEMCIS in combination with pembrolizumab and lenvatinib for iCCA, demonstrating high efficacy [18]. Moreover, the clinical benefits of GEMCIS or GEMOX in combination with PD-1/PD-L1 inhibitors and TKIs have been further explored in prospective studies for the first-line treatment of iCCA [19–21]. However, these studies had limited sample sizes or were designed as single-arm trials.

In light of these clinical practices, we conducted a multicenter, large-scale retrospective cohort study to investigate a novel treatment strategy for initially unresectable iCCA, which involved combining GEMCIS or GEMOX with PD-1/PD-L1 inhibitors and TKIs. Furthermore, we examined the benefits of surgery following GEMCIS or GEMOX plus PD-1/PD-L1 inhibitors and TKIs for patients with locally advanced disease. Thereby, this study might provide new clinical evidence to support treatment options for iCCA.

Methods

Study design and patients

This multicenter retrospective cohort study was carried out across twelve hospitals in China, including: The Second Affiliated Hospital Zhejiang University School of Medicine, Zhejiang Cancer Hospital, The First Affiliated Hospital of Zhengzhou University, Cancer Hospital Chinese Academy of Medical Science, Peking Union Medical College Hospital, The First Hospital of Jiaying, The First Affiliated Hospital of Wenzhou Medical University, Huzhou Central Hospital, China-Japan Friendship Hospital, The Fourth Affiliated Hospital of Zhejiang University School of Medicine, Xinjiang Medical University Affiliated Tumor Hospital, and Shangrao People's Hospital. We conducted a retrospective review of data from patients diagnosed with iCCA between January 2016 and December 2024 by the electronic medical record system. The inclusion criteria were as follows: (1) histologically or clinically diagnosed iCCA; (2) previous untreated unresectable disease; (3) received GEMCIS or GEMOX, either alone or in combination with PD-1/PD-L1 inhibitors and TKIs, as first-line treatment. The exclusion criteria were as follows: (1) received only one cycle of treatment; (2) received more than one cycle of treatment but did not have imaging evaluations after treatment, which affected the assessment of response; (3) lacked clinical laboratory data; (4) loss to follow-up.

To protect patient privacy, all personal data were de-identified before analysis. This research report complied with the STROBE guidelines and this study was registered with ClinicalTrials.gov (NCT06718257).

Data collection and definitions

All patient data were collected from the electronic medical record system and primarily encompassed the following items: age, gender, history of hypertension and diabetes, smoking habits, alcohol consumption, presence of viral hepatitis, lymphocyte and neutrophil counts, tumor markers such as carbohydrate antigen 19 – 9 (CA 19 – 9) and carcinoembryonic antigen (CEA), number of tumors, and sites of metastatic disease. "Viral hepatitis" encompassed both hepatitis B and hepatitis C. "neutrophil-to-lymphocyte ratio (NLR)" referred to the ratio of lymphocyte counts to neutrophil counts. "Site of metastatic disease" included both lymph node metastases and distant metastases. Regional and distant lymph node involvement were classified under lymph node metastases. In contrast, distant metastases specifically referred to those occurring in other sites, such as the peritoneum, lungs, or bones. The disease was classified as locally advanced when there were no signs of distant metastases in this study.

Treatment regimens

All patients received at least two cycles of gemcitabine-based chemotherapy, either as a standalone treatment or in combination with immuno-targeted therapy. The chemotherapy regimens included GEMCIS (gemcitabine 1000 mg/m² and cisplatin 25 mg/m² on Days 1 and 8) or GEMOX (gemcitabine 1000 mg/m² on Days 1 and 8, and oxaliplatin 85 mg/m² on Day 1). For patients receiving PD-1/PD-L1 inhibitors, the treatment was administered on Day 1 or Day 8. Moreover, TKIs were taken orally every day, with Lenvatinib serving as an example. The dosages of PD-1/PD-L1 inhibitors and TKIs vary depending on the specific medication used.

Follow-up and outcomes

Follow-up was conducted either in the outpatient or inpatient settings, or through telephone consultations. All patients participated in several follow-up sessions, with the last follow-up occurring in July 2025.

The primary outcome was OS, defined as the time from the date of diagnosis to either the date of death from any cause or the most recent follow-up. The secondary outcomes were progression-free survival (PFS), tumor response to the treatment and the occurrence of adverse events (AEs). PFS was defined as the time from the date of diagnosis to disease progression, death from any cause. The tumor response to the treatment was assessed according to the Response Evaluation Criteria in Solid Tumors (RECIST) version 1.1. The objective response rate (ORR) was defined as the percentage of patients who achieved either a complete response (CR) or a partial response (PR). In comparison, the disease control rate (DCR) was defined as the percentage of patients who achieved a CR, PR, or stable disease (SD) as their best overall response. Additionally, AEs in the entire study were recorded in accordance with the Common Terminology Criteria for Adverse Events (CTCAE) version 5.0. All data were evaluated by two or more clinicians and radiologists.

Statistical analysis

To minimize potential confounders, logistic regression model was utilized to adjust for all covariates involved in the PSM procedure. In detail, PSM was conducted in a 1:1 ratio with a caliper width set at 0.2. In this study, we conducted two rounds of PSM. The first round aimed to adjust for potential confounding factors between the GEMCIS/GEMOX cohort and the GEMCIS/GEMOX cohort that included PD-1/PD-L1 inhibitors and TKIs. The second round focused on minimizing confounding factors between the surgical and non-surgical groups.

Continuous variables for all cohorts were reported as median and interquartile range (IQR), and categorical variables were presented as number and frequency. For continuous variables, the independent samples *t* test was used for those that followed a normal distribution, whereas the Mann-Whitney U test was applied for those that did not, in order to compare differences between cohorts. To compare the categorical variables, the chi-square test or Fisher's exact test was employed as appropriate. Survival based on treatment was assessed using the Kaplan-Meier method, and differences were evaluated through the Log-rank test. Multivariate Cox regression analyses were conducted to examine the effects on OS and PFS after adjusting for baseline prognostic parameters. Additionally, subgroup analyses were conducted stratified by age, gender,

history of hypertension and diabetes, smoking habits, alcohol consumption, presence of viral hepatitis, NLR, CA 19 – 9 and CEA levels, number of tumors, lymph node metastasis, and distant metastasis to investigate potential effect modification by these stratification variables using log-likelihood ratio tests to compare models with and without the cross-product term.

All statistical analyses and result generation were performed using SPSS 27.0 (IBM, Armonk, NY, USA) and R 4.2.2 software (R Foundation, Vienna, Austria). Differences were deemed statistically significant at the two-sided P value < 0.05 .

Results

Workflow

The workflow of this retrospective study is illustrated in Fig. 1. From January 2016 to December 2024, a total of 4,062 patients were screened across 12 hospitals in China. Among these, 392 patients met the inclusion criteria and were included in the full cohort analysis. According to the predefined exclusion criteria, 127 patients were excluded for meeting one or more of the exclusion criteria. Ultimately, a total of 265 patients were included in the primary analysis: 88 patients received GEMCIS or GEMOX, 177 patients were treated with GEMCIS or GEMOX in combination with PD-1/PD-L1 inhibitors and TKIs; after the matching process, a total of 164 patients remained in the study, with 82 patients in each cohort. Details of patient enrollment by participating institutions were presented in *Supplementary Table 1*.

Patient characteristics

In the full cohort analysis, the patient demographics and disease characteristics for the full cohort were summarized in *Supplementary Table 2*. Among the 392 patients, 159 received GEMCIS or GEMOX, while 233 were treated with GEMCIS or GEMOX in combination with PD-1/PD-L1 inhibitors and TKIs. Overall, the clinical baseline characteristics of the two cohorts were generally comparable. However, patients in the GEMCIS/GEMOX cohort exhibited a higher proportion of number of tumors ≥ 3 ($P = 0.033$) and a greater incidence of distant metastasis ($P = 0.048$) compared to those in the GEMCIS/GEMOX combined with PD-1/PD-L1 inhibitors and TKIs cohort.

Of the 265 patients in the primary analysis, 113 (43%) were aged over 65 years, 160 (60%) were male, and 105 (40%) were female. Additionally, 57 patients (22%) had viral hepatitis, 219 (83%) had lymph node metastasis, and 192 (72%) presented with metastatic disease (*Supplementary Table 3*). Moreover, as presented in Table 1, Patients in the GEMCIS/GEMOX cohort exhibited a higher proportion of tumors number ≥ 3 ($P = 0.033$) and an increased incidence of distant metastasis ($P = 0.048$), compared with those in the GEMCIS/GEMOX in combination with PD-1/PD-L1 inhibitors and TKIs cohort before propensity score matching (PSM). After PSM, the baseline characteristics of patients were well-balanced between the two cohorts (all $P > 0.05$) and the standardized mean difference showed in *Supplementary Fig. 1A*.

Table 1
Baseline Demographics and Clinical Characteristics of Patients Receiving GEMCIS/GEMOX Alone or with PD-1/PD-L1 Inhibitors and TKIs (Primary Analysis)

Characteristics	Before PSM			After PSM		
	GEMCIS/GEMOX (N = 88)	GEMCIS/GEMOX+ PD-1/PD-L1 inhibitors +TKIs (N = 177)	P	GEMCIS/GEMOX (N = 82)	GEMCIS/GEMOX+ PD-1/PD-L1 inhibitors +TKIs (N = 82)	P
Age, years	62 (55–67)	62 (55–69)	0.900	62 (55–68)	62 (54–69)	0.753
< 65	50 (57)	102 (58)		47 (57)	45 (55)	
≥ 65	38 (43)	75 (42)		35 (43)	37 (45)	
Gender			0.973			0.524
Male	53 (60)	107 (60)		51 (62)	47 (57)	
Female	35 (40)	70 (40)		31 (38)	35 (43)	
Hypertension			0.406			0.609
No	65 (74)	122 (69)		59 (72)	56 (68)	
Yes	23 (26)	55 (31)		23 (28)	26 (32)	
Diabetes			0.216			0.633
No	79 (90)	149 (84)		73 (89)	71 (87)	
Yes	9 (10)	28 (16)		9 (11)	11 (13)	
Smoking			0.084			0.323
No	55 (63)	129 (73)		51 (62)	57 (70)	
Yes	33 (38)	48 (27)		31 (38)	25 (30)	
Alcohol consumption			0.307			0.388
No	62 (70)	135 (76)		56 (68)	61 (74)	
Yes	26 (30)	42 (24)		26 (32)	21 (26)	
Viral hepatitis			0.330			> 0.999
No	66 (75)	142 (80)		62 (76)	62 (76)	
Yes	22 (25)	35 (20)		20 (24)	20 (24)	

Data are median (IQR) or n (%).

CA 19 – 9, carbohydrate antigen 19 – 9; CEA, carcinoembryonic antigen; GEMCIS, gemcitabine and cisplatin; GEMOX, gemcitabine and oxaliplatin; NLR, neutrophil-to-lymphocyte ratio; PD-1/PD-L1, programmed cell death protein 1/programmed death-ligand 1; PSM, propensity score matching; TKIs, tyrosine kinase inhibitors.

^a Regional or distant lymph node metastasis.

^β Including metastases to other sites such as the peritoneum, lungs, and bones, while excluding lymph nodes and intrahepatic metastases.

*P < 0.05.

Characteristics	Before PSM			After PSM		
	GEMCIS/GEMOX (N = 88)	GEMCIS/GEMOX+ PD-1/PD-L1 inhibitors +TKIs (N = 177)	P	GEMCIS/GEMOX (N = 82)	GEMCIS/GEMOX+ PD-1/PD-L1 inhibitors +TKIs (N = 82)	P
NLR			0.189			0.740
< 3	28 (32)	71 (40)		26 (32)	28 (34)	
≥ 3	60 (68)	106 (60)		56 (68)	54 (66)	
CA 19 – 9, U/mL			0.901			> 0.999
< 500	52 (59)	106 (60)		47 (57)	47 (57)	
≥ 500	36 (41)	71 (40)		35 (43)	35 (43)	
CEA, ng/mL			0.313			0.638
< 5	48 (55)	108 (61)		43 (52)	46 (56)	
≥ 5	40 (45)	69 (39)		39 (48)	36 (44)	
Number of tumors			0.033*			0.875
< 3	38 (43)	101 (57)		37 (45)	36 (44)	
≥ 3	50 (57)	76 (43)		45 (55)	46 (56)	
Lymph node metastasis^a			0.259			0.828
No	12 (14)	34 (19)		12 (15)	13 (16)	
Yes	76 (86)	143 (81)		70 (85)	69 (84)	
Distant metastasis^β			0.048*			0.492
No	57 (65)	135 (76)		56 (68)	60 (73)	
Yes	31 (35)	42 (24)		26 (32)	22 (27)	
Data are median (IQR) or n (%).						
CA 19 – 9, carbohydrate antigen 19 – 9; CEA, carcinoembryonic antigen; GEMCIS, gemcitabine and cisplatin; GEMOX, gemcitabine and oxaliplatin; NLR, neutrophil-to-lymphocyte ratio; PD-1/PD-L1, programmed cell death protein 1/programmed death-ligand 1; PSM, propensity score matching; TKIs, tyrosine kinase inhibitors.						
^a Regional or distant lymph node metastasis.						
^β Including metastases to other sites such as the peritoneum, lungs, and bones, while excluding lymph nodes and intrahepatic metastases.						
*P < 0.05.						

Efficacy of GEMCIS or GEMOX in combination with PD-1/PD-L1 inhibitors and TKIs

The follow-up for this study was completed in July 2025. In the full cohort analysis, the median number of treatment cycles was 3 (IQR 2–6) in the GEMCIS/GEMOX cohort, which was lower than that in the GEMCIS/GEMOX plus PD-1/PD-L1 inhibitors and TKIs cohort (median 4 cycles, IQR 3–6; $P < 0.001$; *Supplementary Fig. 2* and *Supplementary Table 4*). This suggests that patients receiving combination therapy might be able to tolerate treatment for a longer duration. According to *Supplementary Table 4*, a total of 87 patients were not evaluable for tumor response, and no CR was achieved. The GEMCIS/GEMOX plus PD-1/PD-L1 inhibitors and TKIs cohort demonstrated higher ORR (43% [85/198] vs. 16% [17/107]; $P < 0.001$) and DCR (95% [188/198] vs. 72% [77/107]; $P < 0.001$) compared to the GEMCIS/GEMOX cohort. Furthermore, 87 patients were lost to follow-up and were censored at the date of their last confirmed follow-up (Fig. 1). The median OS was longer in the GEMCIS/GEMOX plus PD-1/PD-L1 inhibitors and TKIs cohort at 26.7 months (95% CI: 21.3–32.2) compared with 15.3 months (95% CI: 12.6–17.9) in the GEMCIS/GEMOX cohort, corresponding to a HR of 0.47 (95% CI: 0.35–0.63; $P < 0.001$; Fig. 2A). The estimated OS rates for the GEMCIS/GEMOX plus PD-1/PD-L1 inhibitors and TKIs cohort and the GEMCIS/GEMOX cohort were 80% and 68% at 12 months, 57% and 33% at 24 months, and 41% and 14% at 36 months, respectively (*Supplementary Table 4*). In addition, the median PFS was 12.2 months (95% CI: 10.7–13.7) in the GEMCIS/GEMOX plus PD-1/PD-L1 inhibitors and TKIs cohort versus 7.0 months (95% CI: 5.4–8.6) in the GEMCIS/GEMOX cohort (HR 0.46 [95% CI: 0.35–0.59]; $P < 0.001$; Fig. 2B). The combination therapy cohort showed higher PFS rates at all time points: 54% vs. 35% at 12 months, 28% vs. 17% at 24 months, and 15% vs. 8% at 36 months (*Supplementary Table 4*). Furthermore, we performed subgroup analyses based on the chemotherapy regimen (*Supplementary Fig. 3*). The results demonstrated that GEMCIS combined with PD-1/PD-L1 inhibitors and TKIs significantly improved OS (HR: 0.49 [95% CI: 0.32–0.75]; $P < 0.001$) and PFS (HR: 0.49 [95% CI: 0.33–0.71]; $P < 0.001$) compared to GEMCIS alone. Similarly, GEMOX combined with PD-1/PD-L1 inhibitors and TKIs also showed superior OS (HR: 0.49 [95% CI: 0.32–0.75]; $P < 0.001$) and PFS (HR: 0.43 [95% CI: 0.30–0.63]; $P < 0.001$) compared to GEMOX alone.

In the primary analysis, after PSM, the median number of treatment cycles were 5 (IQR 3–6) for both the GEMCIS/GEMOX cohort and the GEMCIS/GEMOX plus PD-1/PD-L1 inhibitors and TKIs cohort (Table 2). No statistically differences were observed between the two cohorts across the treatment cycles ($P = 0.600$). The ORR in the GEMCIS/GEMOX plus PD-1/PD-L1 inhibitors and TKIs cohort was 40%, with a DCR of 95%. These rates were higher than those observed in the GEMCIS/GEMOX cohort, which had an ORR of 18% ($P = 0.003$) and a DCR of 77% ($P = 0.001$).

Table 2

Treatment Cycles, Best Objective Response, and Survival Outcomes in Patients Receiving GEMCIS/GEMOX Alone or with PD-1/PD-L1 Inhibitors and TKIs after PSM (Primary Analysis).

Treatment cycle	GEMCIS/GEMOX (N = 82)	GEMCIS/GEMOX+ PD-1/PD-L1 inhibitors+TKIs (N = 82)
	5 (3–6)	5 (3–6)
Response		
CR	0	0
PR	15 (18)	33 (40)
SD	48 (59)	45 (55)
PD	19 (23)	4 (5)
ORR	15 (18)	33 (40)
DCR	63 (77)	78 (95)
Overall survival		
Median OS	14.8 (11.7–17.9)	24.8 (20.8–28.8)
1-year OS	63	72
2-year OS	27	54
3-year OS	12	33
Progression-free survival		
Median PFS	7.0 (5.0–9.0)	11.0 (8.5–13.5)
1-year PFS	22	46
2-year PFS	9	26
3-year PFS	3	22
Data are median (IQR) or n (%).		
CR, complete response; DCR, disease control rate; GEMCIS, gemcitabine and cisplatin; GEMOX, gemcitabine and oxaliplatin; ORR, objective response rate; OS, overall survival; PD, progression disease; PD-1/PD-L1, programmed cell death protein 1/programmed death-ligand 1; PFS, progression-free survival; PR, partial response; PSM, propensity score matching; SD, stable disease; TKIs, tyrosine kinase inhibitors.		

Moreover, survival outcomes were evaluated both before and after PSM. Before PSM, OS and PFS was significantly longer in the GEMCIS/GEMOX plus PD-1/PD-L1 inhibitors and TKIs cohort compared to the GEMCIS/GEMOX cohort, with a HR for OS of 0.47 (95% CI: 0.34–0.64; $P < 0.001$; Fig. 3A) and a HR for PFS of 0.42 (95% CI: 0.31–0.56; $P < 0.001$; Fig. 3B). After PSM, a total of 39 patients (48%) in the GEMCIS/GEMOX plus PD-1/PD-L1 inhibitors and TKIs cohort had died, compared to 73 patients (89%) in the GEMCIS/GEMOX cohort. As shown in Fig. 3C and Table 2, the median OS in the GEMCIS/GEMOX plus PD-1/PD-L1 inhibitors and TKIs cohort was 24.8 months (95% CI: 20.8–28.8), while the GEMCIS/GEMOX cohort had a median OS of 14.8 months (95% CI: 11.7–17.9); the HR was 0.56 (95% CI: 0.38–0.83) with a statistically significant difference ($P = 0.004$). The estimated OS rates at 12, 24, and 36 months were 72%, 54%, and 33% in the GEMCIS/GEMOX plus PD-1/PD-L1 inhibitors and TKIs cohort, compared with 63%, 27%, and 12% in the GEMCIS/GEMOX cohort. Additionally, as presented in Fig. 3D and Table 2, the median PFS was 11.0 months (95% CI: 8.5–13.5) in the GEMCIS/GEMOX plus PD-1/PD-L1 inhibitors and TKIs cohort, whereas it was only 7.0 months (95% CI: 5.0–9.0) in the GEMCIS/GEMOX cohort; the HR for PFS was 0.46 (95% CI:

0.32–0.66; $P < 0.001$). The estimated PFS rates for the GEMCIS/GEMOX plus PD-1/PD-L1 inhibitors and TKIs cohort were 46% at 12 months, 26% at 24 months, and 22% at 36 months. In comparison, the PFS rates for the GEMCIS/GEMOX cohort were 22% at 12 months, 9% at 24 months, and 3% at 36 months, respectively.

Univariable and multivariable analyses were conducted to evaluate the association between individual factors and OS as well as PFS, as detailed in *Supplementary Table 5*. The results indicated that smoking habits (HR: 1.58 [95% CI: 1.08–2.32]; $P = 0.017$) and serum CA 19 – 9 ≥ 500 U/mL (HR: 2.28 [95% CI: 1.56–3.34]; $P < 0.001$) were associated with worse OS. Additionally, smoking habits (HR: 1.48 [95% CI: 1.03–2.14]; $P = 0.034$) and serum CA 19 – 9 ≥ 500 U/mL (HR: 1.61 [95% CI: 1.10–2.37]; $P = 0.014$) were linked to worse PFS. Notably, the treatment strategy of GEMCIS/GEMOX plus PD-1/PD-L1 inhibitors and TKIs emerged as an independent prognostic protective factor for both OS (HR: 0.54 [95% CI: 0.36–0.80]; $P = 0.002$) and PFS (HR: 0.49 [95% CI: 0.34–0.71]; $P < 0.001$).

The analyses of clinically relevant subgroups demonstrated generally consistent OS and PFS benefits for the GEMCIS/GEMOX combined with PD-1/PD-L1 inhibitors and TKIs cohort across most subgroups (Fig. 4A-B). In patients with serum CEA ≥ 5 U/mL and CEA < 5 ng/mL, the HR for OS in the combination cohort, compared to the GEMCIS/GEMOX alone cohort, was 1.14 (95% CI: 0.64–2.05) and 0.33 (95% CI: 0.16–0.67). A significant interaction was observed in the CEA subgroup, with a P-value of 0.014.

Safety of GEMCIS or GEMOX in combination with PD-1/PD-L1 inhibitors and TKIs

Given the retrospective nature of the study, the analysis of treatment-related AEs was restricted to specific hematological indicators, which could be clearly obtained from the electronic medical record system.

In the full cohort analysis, 54 patients were excluded due to unavailable treatment-related AEs data, leaving 338 patients included in the final analysis. Any grade AEs occurred in 85% (180/212) of the GEMCIS/GEMOX plus PD-1/PD-L1 inhibitors and TKIs cohort versus 83% (104/126) of the GEMCIS/GEMOX cohort; Grade 3–4 AEs were reported in 29% (62/212) and 28% (35/126) of patients, respectively (*Supplementary Table 6*). No statistically differences were observed between the cohorts for either any grade AEs ($P = 0.645$) or grade 3–4 AEs ($P = 0.805$).

For the primary safety analysis, 164 patients were included after PSM, with 82 receiving GEMCIS/GEMOX plus PD-1/PD-L1 inhibitors and TKIs, and 82 receiving GEMCIS/GEMOX alone. Any grade of AEs was observed in 66 patients (80%) in the GEMCIS/GEMOX plus PD-1/PD-L1 inhibitors and TKIs cohort, compared to 65 patients (79%) in the GEMCIS/GEMOX cohort, with grade 3 or 4 AEs reported in 25 patients (30%) in the combination cohort and 22 patients (27%) in the GEMCIS/GEMOX cohort (Table 3). No significant differences were observed between the two cohorts regarding either any grade AEs ($P = 0.846$) or grade 3/4 AEs ($P = 0.604$). In the GEMCIS/GEMOX plus PD-1/PD-L1 inhibitors and TKIs cohort, the most frequently reported grade 3 or 4 AEs included thrombocytopenia (15%), neutropenia (12%), and anemia (9%), while the GEMCIS/GEMOX cohort alone reported as thrombocytopenia (16%), neutropenia (11%), and leukopenia (6%).

Table 3

Summary of Adverse Events in Patients Treated with GEMCIS/GEMOX plus PD-1/PD-L1 Inhibitors and TKIs Compared to Those Treated with GEMCIS/GEMOX Alone after PSM (Primary Analysis).

	GEMCIS/GEMOX (N = 82)			GEMCIS/GEMOX plus PD-1/PD-L1 inhibitors and TKIs (N = 82)		
	Grade 1–2	Grade 3–4	Any grade	Grade 1–2	Grade 3–4	Any grade
Leukopenia	24 (29)	5 (6)	29 (35)	30 (37)	4 (5)	34 (41)
Neutropenia	21 (26)	9 (11)	30 (37)	24 (29)	10 (12)	34 (41)
Thrombocytopenia	20 (24)	13 (16)	33 (40)	21 (26)	12 (15)	33 (40)
Anemia	50 (61)	4 (5)	54 (66)	52 (63)	7 (9)	59 (72)
ALT/AST increased	17 (21)	1 (1)	18 (22)	21 (26)	3 (4)	24 (29)
Blood bilirubin increased	6 (7)	3 (4)	9 (11)	10 (12)	3 (4)	13 (16)
Blood creatinine increased	4 (5)	0	4 (5)	8 (10)	1 (1)	9 (11)
Hypoalbuminaemia	22 (27)	0	22 (27)	19 (23)	0	19 (23)
Total	65 (79)	22 (27)	65 (79)	66 (80)	25 (30)	66 (80)
Data are n (%).						
ALT, alanine aminotransferase; AST, aspartate aminotransferase; GEMCIS, gemcitabine and cisplatin; GEMOX, gemcitabine and oxaliplatin; PD-1/PD-L1, programmed cell death protein 1/programmed death-ligand 1; PSM, propensity score matching; TKIs, tyrosine kinase inhibitors.						

Treatment details

The detailed treatment regimens were provided in *Supplementary Table 7*. In the chemotherapy cohort, 54 and 34 patients received GEMCIS and GEMOX, respectively. In contrast, the chemotherapy combined with PD-1/PD-L1 inhibitors and TKIs cohort received various regimens, with the most common being GEMCIS plus tislelizumab and lenvatinib (18%), GEMOX plus tislelizumab and lenvatinib (16%), and GEMOX plus toripalimab and lenvatinib (12%). Additionally, 47 (27%) patients were treated with GEMCIS plus PD-1 inhibitors and lenvatinib, while 74 (42%) patients received GEMOX plus PD-1 inhibitors and lenvatinib.

Role of surgery in patients with locally advanced disease

In this study, a total of 192 patients with locally advanced disease were included. Among these, 57 patients received GEMCIS/GEMOX, while 135 patients received GEMCIS/GEMOX plus PD-1/PD-L1 inhibitors and TKIs (*Supplementary Fig. 3*). As shown in *Supplementary Table 8*, the surgical rate in the GEMCIS/GEMOX plus PD-1/PD-L1 inhibitors and TKIs cohort was significantly higher than that in the GEMCIS/GEMOX cohort (42% vs 5%; $P < 0.001$). Among the patients who underwent surgery following treatment with GEMCIS/GEMOX plus PD-1/PD-L1 inhibitors and TKIs, 36 patients (63%) achieved PR, while 21 patients (37%) had SD. Clinically, no patients experienced PD before undergoing surgery following treatment with GEMCIS/GEMOX, either alone or in combination with PD-1/PD-L1 inhibitors and TKIs. Moreover, all 3 patients in the GEMCIS/GEMOX cohort received adjuvant therapy, whereas 37 out of 57 patients in the GEMCIS/GEMOX plus PD-1/PD-L1 inhibitors and TKIs cohort received adjuvant therapy. The details of the adjuvant therapy strategy were also presented in *Supplementary Table 8*.

To further explore the role of surgery, we selected patients treated with GEMCIS/GEMOX plus PD-1/PD-L1 Inhibitors and TKIs and conducted PSM as well, and the baseline characteristics were presented in *Supplementary Table 9*. Before PSM, compared to the surgery group, patients in the non-surgery group had significantly higher proportions of the following: NLR ≥ 3 ($P = 0.002$), CA 19 – 9 ≥ 500 U/mL ($P = 0.003$), CEA ≥ 5 ng/mL ($P = 0.001$), and number of tumors ≥ 3 ($P < 0.001$). After PSM,

both the surgery group and the non-surgery group included 33 patients, and their baseline clinical features were consistent and the standardized mean difference showed in *Supplementary Fig. 1B*.

Patients in the surgery group had significantly better outcomes than those in the non-surgery group, demonstrating longer OS and PFS (Fig. 5A-B). Specifically, the HR for OS was 0.17 (95% CI: 0.05–0.61; $P = 0.002$) and for PFS was 0.53 (95% CI: 0.28–1.00; $P = 0.045$). For instance, a previously reported patient who underwent surgery after receiving GEMCIS combined with Tislelizumab and Lenvatinib conversion therapy[17], has survived for more than five years.

Discussion

This study was the first multicenter, large-scale retrospective cohort study to demonstrate that the combination of GEMCIS or GEMOX with PD-1/PD-L1 inhibitors and TKIs resulted in satisfactory OS, PFS, ORR, and DCR in patients with previous untreated unresectable iCCA. A trend indicating improved OS and PFS benefits was observed across prespecified subgroups with the combination of PD-1/PD-L1 inhibitors and TKIs. The addition of PD-1/PD-L1 inhibitors and TKIs to gemcitabine-based chemotherapy did not result in an increase in hematological-related AEs. Importantly, we found that the combination of GEMCIS or GEMOX with PD-1/PD-L1 inhibitors and TKIs could enhance the surgical rate for patients with locally advanced iCCA, ultimately leading to improved overall outcomes significantly. These findings may provide valuable insights into the new combination strategy for iCCA.

In advanced iCCA cases, GEMCIS chemotherapy remains the cornerstone of systemic therapy and has been the first-line treatment recommended worldwide since 2010 based on the sensational findings from the ABC-02 clinical trial [5]. In this study, the median PFS was 7.0 months (95%CI: 5.0–9.0) and the ORR was 18% in the GEMCIS/GEMOX cohorts, both of which were comparable to historical studies of GEMCIS [5, 7, 9]; however, the median OS was 14.8 months (95%CI: 11.7–17.9), which exceeded the studies reported in those historical findings [5, 7, 9]. Several potential factors might contribute to these results. Clinically, we observed that once patients in the GEMCIS or GEMOX cohorts experienced tumor progression, they would receive subsequent antitumor treatments. Indeed, these patients had numerous options for second-line and later-line treatment, which might help to extend their long-term survival [22, 23]. For example, a recent Phase 3 randomized trial found that the OS in the GEMCIS group was 13.6 months (95% CI: 9.7–16.6), with iCCA accounting for 67% of the cases [24]. Additionally, another randomized Phase 2 trial reported an OS of 13.7 months (95% CI: 10.2–15.3) in the GEMCIS group, where iCCA comprised 68% of the participants [20]. The OS results from these two trials are slightly lower than those found in our study. This may be attributed to the fact that patients with iCCA tend to have better OS compared to those with BTC originating from other primary sites [25–27].

Recent studies, TOPAZ-1 and KEYNOTE-966, have confirmed the efficacy of PD-1 or PD-L1 inhibitors in combination with GEMCIS as a first-line treatment for advanced iCCA. However, the findings showed a median OS of less than 13 months and an ORR of below 30% [7–9]. These results suggest that the benefits of these treatments may be limited. Thus, there is a growing trend in clinical trials favoring multi-drug combination therapies. These approaches include the combination of chemotherapy with immunotherapy and targeted therapy [19–21, 28, 29], as well as chemotherapy paired with dual immunotherapy [30, 31]. Our study revealed an improvement in OS and PFS for the patients receiving GEMCIS/GEMOX in combination with PD-1/PD-L1 inhibitors and TKIs, when compared to the cohort treated with GEMCIS/GEMOX alone. The median OS for the cohort receiving GEMCIS/GEMOX plus PD-1/PD-L1 inhibitors and TKIs was 24.8 months (95% CI: 20.8–28.8), while the median PFS for the same cohort was 11.0 months (95% CI: 8.5–13.5). These outcomes were higher than currently reported OS and PFS. For instance, in the phase 2 IMbrave-151 study, patients with advanced BTC receiving gemcitabine and cisplatin combined with atezolizumab and bevacizumab exhibited a median OS of 14.9 months (95% CI: 11.6–18.0) and a median PFS of 8.3 months (95% CI: 6.8–10.6) [28]. This result may also be related to the study population, as our research specifically targeted iCCA, whereas most other studies encompassed BTC as a whole. As evidenced by a single-center, single-arm, phase 2 study reported, patients with advanced iCCA attained a median OS of 22.5 months (95% CI: 15.6–29.3) and a median PFS of 10.2 months (95% CI: 9.3–16.8) when treated with a combination of toripalimab, lenvatinib, and GEMOX [19]. In addition, this study noted higher ORR and DCR. The ORR for the GEMCIS/GEMOX plus PD-1/PD-L1

inhibitors and TKIs cohort was 40%, significantly higher than the ORR observed in the GEMCIS/GEMOX cohort alone (18%). Previous studies showed that the ORR of chemotherapy with PD-1/PD-L1 inhibitors and targeted therapy is 27–80% [19–21, 28]. In summary, the current results provide additional evidence supporting the efficacy and feasibility of using gemcitabine-based chemotherapy in combination with PD-1/PD-L1 inhibitors and TKIs as a first-line treatment for patients with initially unresectable iCCA.

Several potential reasons for the enhanced anti-tumor efficacy and improved long-term prognosis associated with this combination strategy include the following: Firstly, endogenous antitumor responses, indicated by the presence of tumor-infiltrating lymphocytes, were observed in all iCCA [32, 33]. Secondly, chemotherapy may help mitigate the immunosuppressive effects of the tumor microenvironment, promote antigen cross-presentation, and enhance the infiltration of immune cells [34, 35]. TKIs have gained prominence as the most effective pathway-directed targeted therapies in the fight against cancer since the early 2000s [36]. By targeting pathways, TKIs can induce rapid tumor cell death and facilitate the release of antigens, thereby enhancing the immune response and improving the efficacy of immunotherapy [37, 38]. Therefore, the combination of chemotherapy with PD-1/PD-L1 inhibitors and TKIs may offer synergistic benefits, potentially enhancing the overall response to anti-tumor therapy.

In this study, the overall rate of any grade AEs was 80% in the GEMCIS/GEMOX plus PD-1/PD-L1 inhibitors and TKIs cohort, and 79% in the GEMCIS/GEMOX cohort. Additionally, the incidence of grade 3 or 4 AEs was 30% and 27% respectively in the aforementioned cohorts. The treatment-related AEs reported in this study were lower than those observed in previous studies, where grade 1–2 adverse effects approached nearly 100% and grade 3–4 adverse effects were exceeding 60% [7–9, 20, 28]. This discrepancy can primarily be attributed to the limited range of adverse event metrics assessed, which was constrained by the retrospective nature of the available data. However, as previously reported [20, 30], the most common AEs were hematologic toxicities, which were included in our analysis. Thus, the AEs reported in our study may offer valuable reference significance. Notably, the addition of PD-1/PD-L1 inhibitors and TKIs did not increase both any grade and grade 3–4 treatment-related AEs among patients. These results align with findings from a previous study [20]. Overall, the safety profile of gemcitabine-based chemotherapy plus PD-1/PD-L1 inhibitors and TKIs seems to be acceptable.

Importantly, radical tumor resection remains crucial for achieving long-term survival. For patients with locally advanced iCCA, conversion therapy involving tumor downsizing and subsequent resection, has shown promising results in several case reports [17, 18, 39]. This study further found that the novel treatment strategy combining GEMCIS or GEMOX with PD-1/PD-L1 inhibitors and TKIs could increase the rate of conversion surgery to 42%. Previous retrospective study has indicated that as the efficacy of systemic therapy improves, patients with initially unresectable BTC may derive greater benefits from conversion surgery rather than solely from systemic treatment [40]. A phase 2 study reported that resection following treatment with GEMOX plus Tislelizumab and Lenvatinib resulted in significantly longer survival, with a median OS of 30.8 months, compared to just 13.4 months for locally advanced BTC patients who were unable to undergo surgery [29]. And in our study, along with significant benefits (HR for OS: 0.17 [95% CI: 0.05–0.61]; HR for PFS: 0.53 [95% CI: 0.28–1.00]) in the surgery subgroup, these results may guide future clinical management of patients undergoing conversion surgery with this treatment strategy.

To the best of our knowledge, this study represents the largest cohort study to date exploring the combination of chemotherapy with immunotherapy and targeted therapy for iCCA. The IMbrave151 trial reported on 46 iCCA patients who were treated with GEMCIS in combination with atezolizumab and bevacizumab [28]. Another phase 2 trial involved 23 iCCA patients who received GEMCIS along with sintilimab and anlotinib [20]. Furthermore, three additional studies explored the combination of GEMCIS or GEMOX with PD-1/PD-L1 inhibitors and TKIs, all of which employed single-arm designs, with each study involving no more than 40 iCCA patients [19, 21, 29]. Notably, our study specifically targets iCCA, while many current studies treat biliary tract cancer BTC as a single entity [5, 7–9, 20, 21, 24]. Given the heterogeneity among cholangiocarcinoma subtypes from various anatomical origins [4, 41], we argue that iCCA, perihilar cholangiocarcinoma, and distal cholangiocarcinoma should be explored separately to yield more representative and clinically relevant insights [19].

Our study had several limitations that warrant consideration. Firstly, the retrospective nature of this study carries an inherent risk of selection bias and may result in incomplete data collection. Secondly, this design restricts our ability to establish causal relationships, as it prevents the comprehensive control for all potential confounding variables. To enhance the reliability, we collected data from 12 authoritative medical centers across China, aiming to maximize the sample size of all cohorts and reduce the bias. Next, we conducted PSM to adjust for potential confounding factors to the greatest extent possible. Thirdly, the absence of this critical information may diminish the reliability of our conclusions. We only recorded hematological-related AEs because many adverse events, including immune-related adverse events, may not have been systematically captured in the electronic medical record system, resulting in an incomplete assessment of the safety profile and overall tolerability of the treatment. To gain more comprehensive insights into the therapeutic implications for this patient population, future clinical trials should consider prospective study designs with rigorous data collection protocols.

In conclusion, gemcitabine-based chemotherapy in combination with PD-1/PD-L1 inhibitors and TKIs showed a clinically meaningful improvement when compared to gemcitabine-based chemotherapy alone. This combination therapy did not present any new safety risks for patients with previously untreated unresectable iCCA. In addition, our strategy could improve the surgical rates and overall outcomes for patients locally advanced disease. All the evidence suggested that this new strategy may have the potential to be a viable first-line treatment option for iCCA.

Abbreviations

Adverse events

AEs

alanine aminotransferase

ALT

aspartate aminotransferase

AST

biliary tract cancer

BTC

carbohydrate antigen 19 – 9

CA 19 – 9

carcinoembryonic antigen

CEA

Common Terminology Criteria for Adverse Events

CTCAE

complete response

CR

confidence interval

CI

disease control rate

DCR

gemcitabine and cisplatin

GEMCIS

gemcitabine and oxaliplatin

GEMOX

hazard ratio

HR

interquartile range

IQR

intrahepatic cholangiocarcinoma

iCCA

not available
NA
neutrophil-to-lymphocyte ratio
NLR
objective response rate
ORR
overall survival
OS
partial response
PR
programmed cell death protein 1/programmed death-ligand 1
PD-1/PD-L1
progression disease
PD
progression-free survival
PFS
propensity score matching
PSM
Response Evaluation Criteria in Solid Tumors
RECIST
stable disease
SD
tyrosine kinase inhibitors
TKIs.

Declarations

Data Availability

All data generated during this study are included in this article and its Supplementary Information files.

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Conflicts of interests

All authors declare that they have no competing interests.

Ethics committee approval and patient consent

The study was conducted in accordance with the Declaration of Helsinki and its subsequent amendments. Ethical approval was obtained from the Ethics Committee of the Second Affiliated Hospital Zhejiang University School of Medicine (Approval No. 2024-1395), Zhejiang Cancer Hospital (Approval No. IRB-2025-91), The First Affiliated Hospital of Zhengzhou University (Approval No. 2025-KY-0773-001), Cancer Hospital Chinese Academy of Medical Science (Approval No. 25/438-5384), Peking Union Medical College Hospital (Approval No. I-25PJ0654), The First Hospital of Jiaxing (Approval No. 2025-LP-449), The First Affiliated Hospital of Wenzhou Medical University (Approval No. 2025-R097), Huzhou Central Hospital (Approval No. 202503016-01), China-Japan Friendship Hospital (Approval No. 2025-KY-138), The Fourth Affiliated Hospital of Zhejiang University School of Medicine (Approval No. 20250324-3), Xinjiang Medical University Affiliated Tumor Hospital (Approval No. K-2024221), and Shangrao People's Hospital (Approval No. 2025-609). Before treatment, informed consent was obtained from all participants, and their data were anonymized for clinical research purposes. This report adhered to the Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) statement.

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Figures

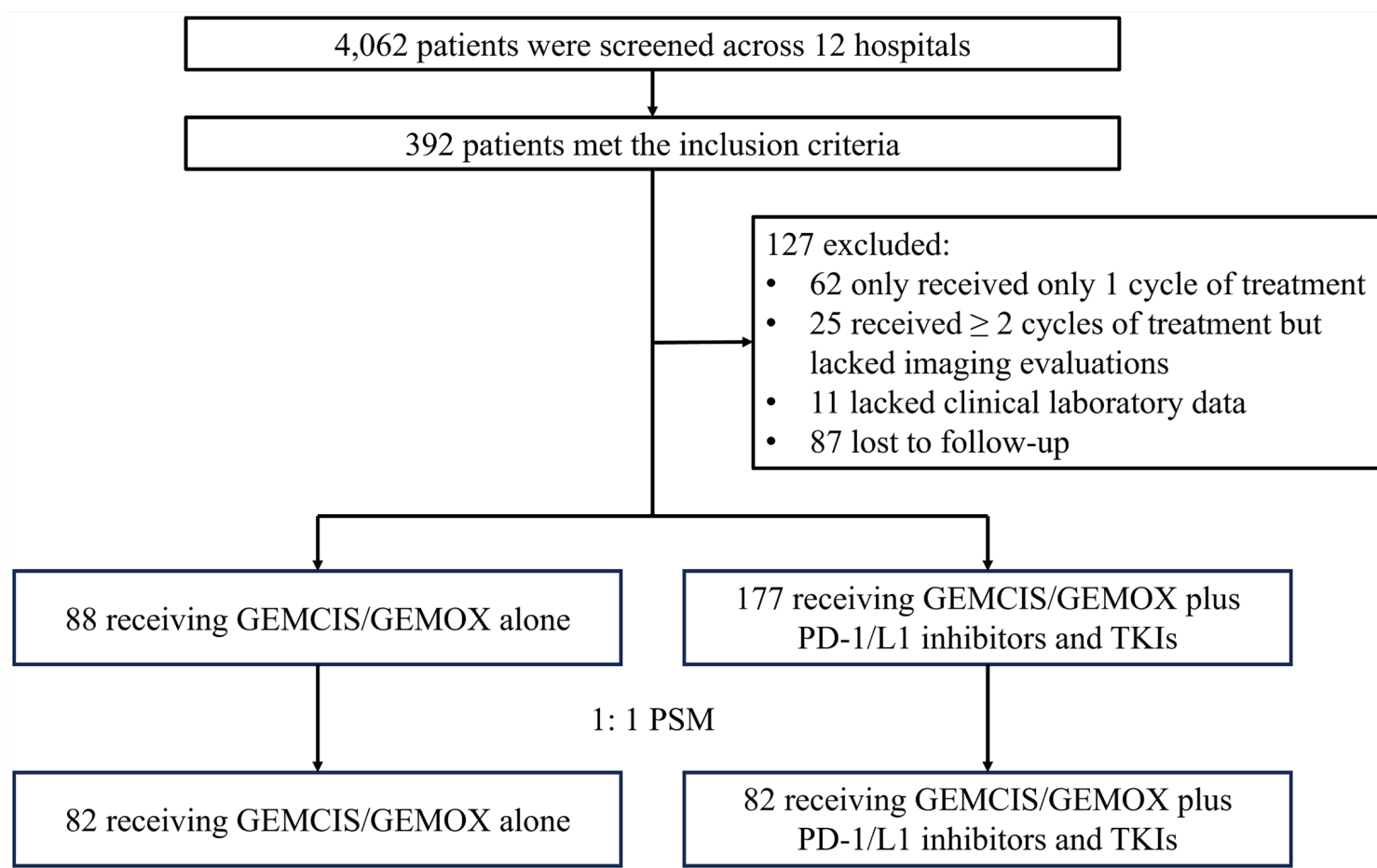


Figure 1

Flowchart of the Study. GEMCIS, gemcitabine and cisplatin; GEMOX, gemcitabine and oxaliplatin; iCCA, intrahepatic cholangiocarcinoma; PD-1/PD-L1, programmed cell death protein 1/programmed death-ligand 1; TKIs, tyrosine kinase inhibitors.

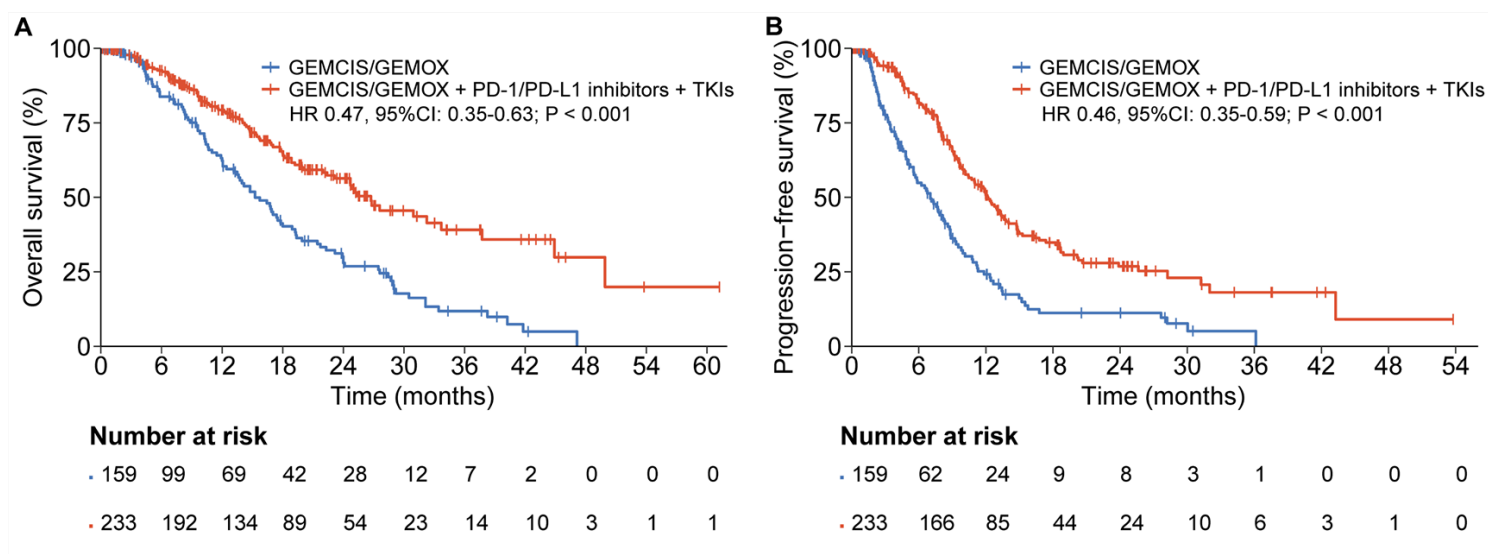


Figure 2

Outcomes of GEMCIS/GEMOX plus PD-1/PD-L1 Inhibitors and TKIs Versus GEMCIS/GEMOX Alone: Full Cohort Analysis. A: Kaplan-Meier curve estimates of overall survival; B: Kaplan-Meier curve estimates of progression-free survival. GEMCIS,

gemcitabine and cisplatin; GEMOX, gemcitabine and oxaliplatin; PD-1/PD-L1, programmed cell death protein 1/programmed death-ligand 1; TKIs, tyrosine kinase inhibitors.

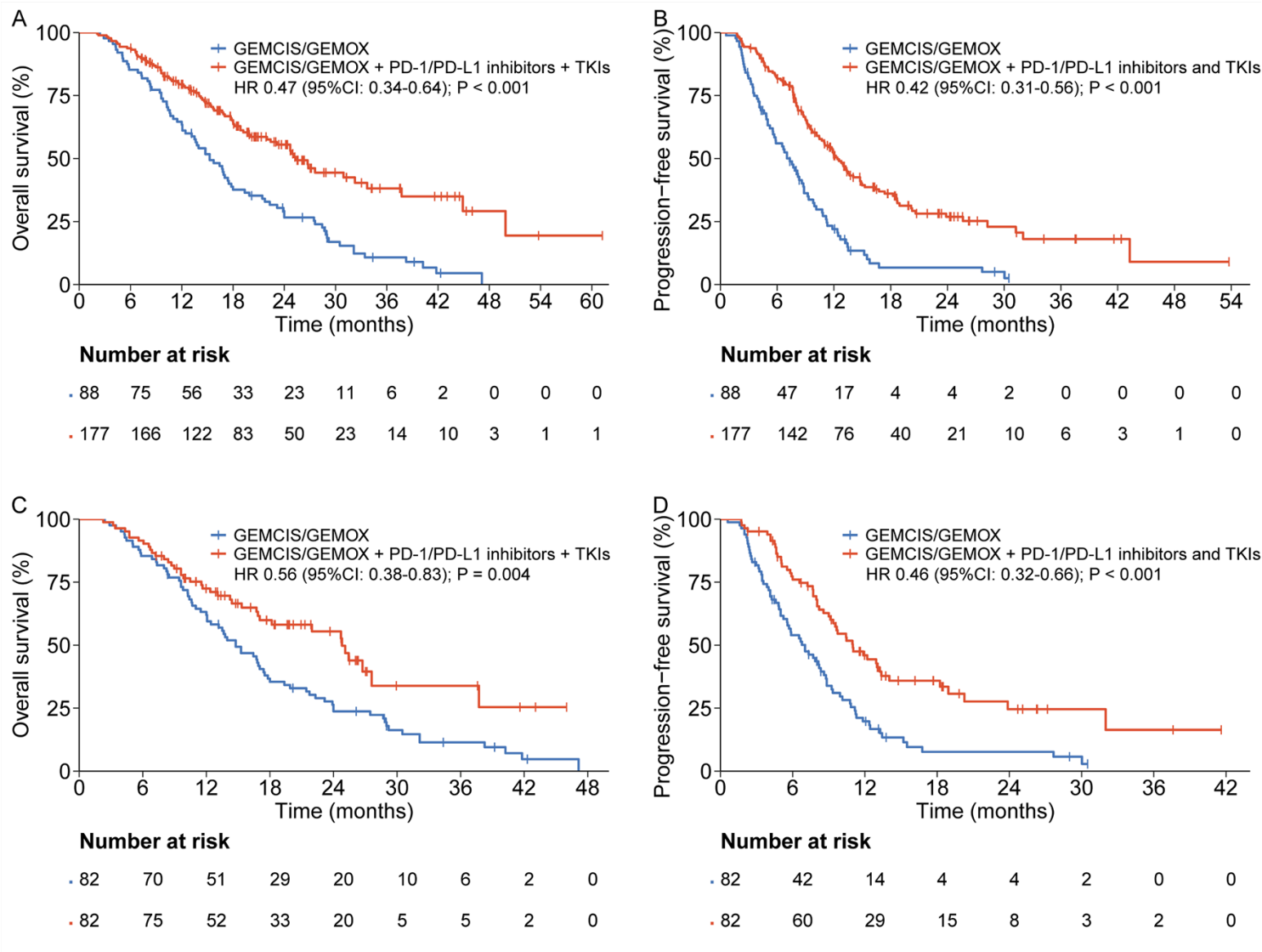


Figure 3

Outcomes of GEMCIS/GEMOX plus PD-1/PD-L1 Inhibitors and TKIs Versus GEMCIS/GEMOX Alone: Before and After PSM. A, B: Kaplan-Meier curve estimates of overall survival and progression-free survival before PSM; C, D: Kaplan-Meier curve estimates of overall survival and progression-free survival after PSM. GEMCIS, gemcitabine and cisplatin; GEMOX, gemcitabine and oxaliplatin; PD-1/PD-L1, programmed cell death protein 1/programmed death-ligand 1; PSM, propensity score matching; TKIs, tyrosine kinase inhibitors.

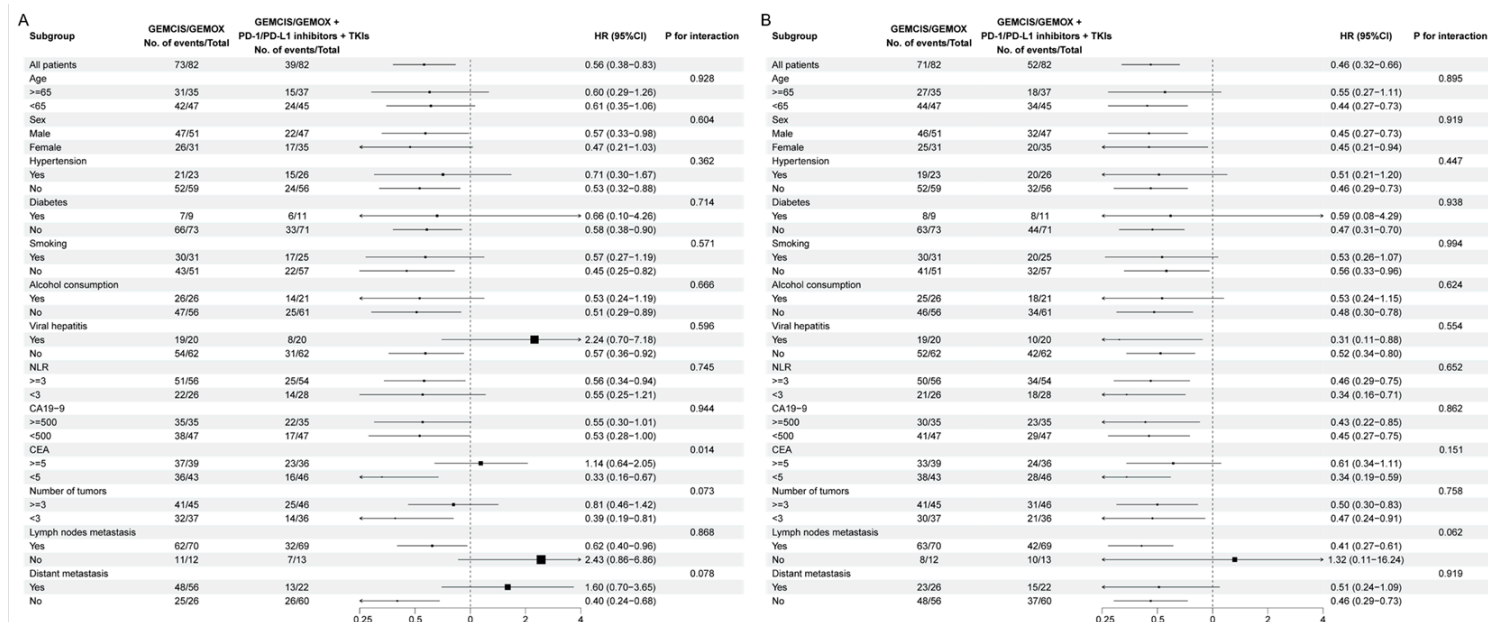


Figure 4

Subgroup Analysis of Survival Outcomes after PSM: GEMCIS/GEMOX plus PD-1/PD-L1 Inhibitors and TKIs versus GEMCIS/GEMOX Alone. A: Forest plot depicted overall survival; B: Forest plot depicted progression-free survival. CA 19-9, carbohydrate antigen 19-9; CEA, carcinoembryonic antigen; GEMCIS, gemcitabine and cisplatin; GEMOX, gemcitabine and oxaliplatin; NLR, neutrophil-to-lymphocyte ratio; programmed cell death protein 1/programmed death-ligand 1; TKIs, tyrosine kinase inhibitors.

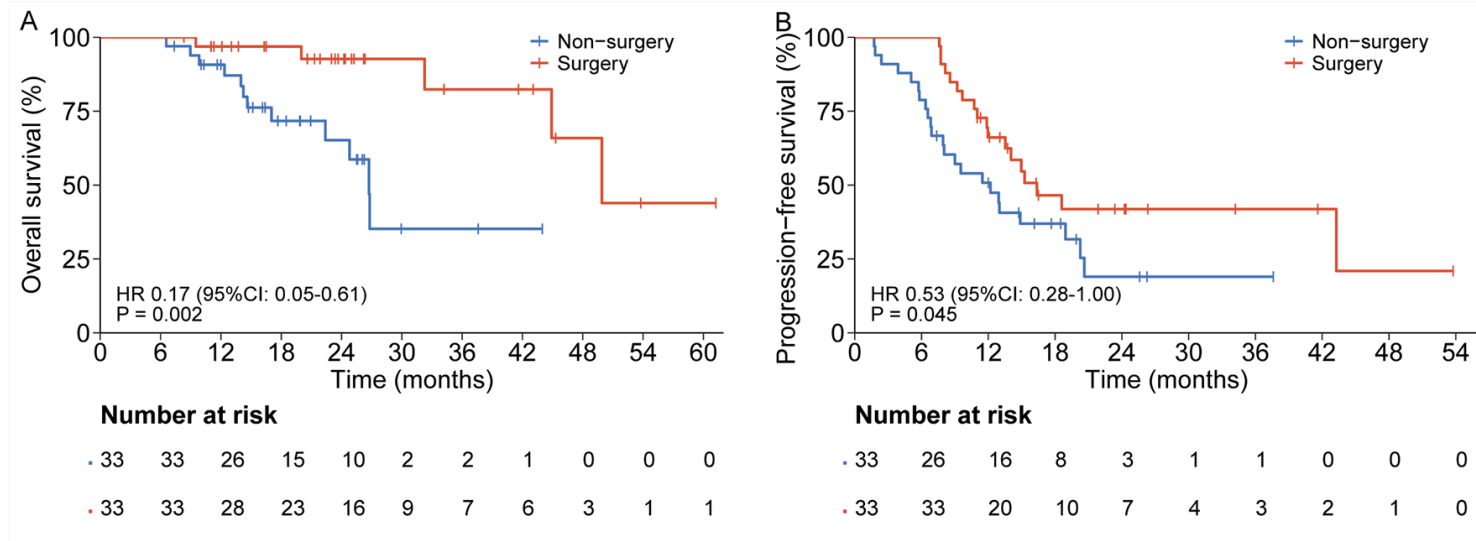


Figure 5

Outcomes in Locally Advanced Patients after PSM: Surgery versus No Surgery. A: Kaplan-Meier curve estimates of overall survival; B: Kaplan-Meier curve estimates of progression-free survival. PSM, propensity score matching.

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