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RESEARCH

Real-world cohort study of PD-1 blockade plus lenvatinib for advanced intrahepatic cholangiocarcinoma: effectiveness, safety, and biomarker analysis

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Abstract

Background In clinical practice, some patients with advanced intrahepatic cholangiocarcinoma (ICC) cannot tolerate or refuse chemotherapy due to the toxicity, necessitating alternative treatments. PD-1 blockade combined with lenvatinib showed promising results in phase II studies with small sample size, but there is a lack of data on the routine use with this regimen. This study aimed to evaluate the effectiveness and safety of the regimen in patients with advanced ICC, and to identify predictors for treatment response and prognosis.

Methods We conducted a retrospective cohort study of patients treated with PD-1 inhibitors plus lenvatinib for advanced ICC between July 2017 and August 2022. The study endpoints were progression-free survival (PFS), overall survival (OS), objective response rate (ORR), disease control rate (DCR), and safety. Biomarker analysis for CA19-9 and PD-L1 expression was performed. Exploratory analysis for genetic alternation was conducted.

Results The study included 103 patients. It demonstrated a median PFS of 5.9 months and a median OS of 11.4 months. ORR was 18.4% and DCR was 80.6%. The incidence of grade 3 or 4 adverse events was 50.5%. Positive PD-L1 expression (TPS ≥ 1%) was associated with higher ORR ($P=0.013$) and prolonged PFS ($P=0.023$). Elevated CA19-9 (> 37 U/ml) was associated with decreased ORR ($P=0.019$), poorer PFS ($P=0.005$) and OS ($P=0.034$). Patients with *IDH1* mutations exhibited a favorable response to the treatment ($P=0.011$), and patients with *TP53* mutations tended to have worse OS ($P=0.031$).

Conclusions PD-1 blockade plus lenvatinib is effective and safe in routine practice. PD-L1 expression and CA19-9 level appear to predict the treatment efficacy. *IDH1* mutations might indicate a better treatment response.
Clinical trial registration: NCT03892577.

Keywords Liver cancer · Biliary tract cancer · Targeted therapy · Immunotherapy · Programmed death 1

Abbreviations

AE	Adverse event
BTC	Biliary tract cancer
CA19-9	Carbohydrate antigen 19-9
CI	Confidence interval

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CR	Complete response
CSCO	Chinese Society of Clinical Oncology
CTCAE	Common Terminology Criteria for Adverse Events
DCR	Disease control rate
ECOG PS	Eastern Cooperative Oncology Group performance status
ICC	Intrahepatic cholangiocarcinoma
IHC	Immunohistochemistry
MMR	Mismatch repair
MSI-H/L	Microsatellite instability-high/low
NCCN	National Comprehensive Cancer Network
NGS	Next-generation sequencing
ORR	Objective response rate
OS	Overall survival
PD-1	Programmed death 1
PFS	Progression-free survival
PR	Partial response
PUMCH	Peking Union Medical College Hospital
RECIST	Response evaluation criteria in solid tumors
SD	Stable disease
TPS	Tumor proportion score

Introduction

Intrahepatic cholangiocarcinoma (ICC) is a rare malignancy, accounting for 15% of hepatic malignancies and 3% of gastrointestinal malignancies [1]. As a subtype of biliary tract cancer (BTC), ICC is different from others in terms of anatomical location, epidemiology, cellular origin, and molecular landscape [2]. The incidence of ICC has been increasing globally over the decades [3]. Unfortunately, approximately 70% to 80% of ICC patients present with locally advanced or metastatic diseases, which results in a dismal prognosis with a 5-year overall survival (OS) of less than 10% [1]. Systemic chemotherapy represented by GemCis (gemcitabine plus cisplatin) and FOLFOX (folinic acid, fluorouracil and oxaliplatin) is a key component for the treatment of advanced ICC, but it causes significant toxic effects. In well-known phase III trials (ABC-02, ABC-06, TOPAZ-1, and KEYNOTE-966), more than 10% of patients who received chemotherapy experienced treatment discontinuation due to adverse events (AEs) [4–7]. In clinical practice, some patients cannot tolerate chemotherapy or refuse it for the fear of toxicity, highlighting the need for alternative treatments.

The tumor microenvironment in ICC is characterized by immunosuppressive features, and high PD-L1 expression supports the rationale of using PD-1 blockade immunotherapy in ICC [8]. Prior studies of PD-1 blockade monotherapy in refractory BTC demonstrated modest benefits, but did not lead to regulatory approval [9, 10]. Lenvatinib, a tyrosine kinase inhibitor, are known to exert immunomodulatory effects and reshape the immunosuppressive microenvironment [11, 12]. Lenvatinib inhibits the vascular endothelial growth factor (VEGF) pathway by targeting VEGF receptors 1–3, which promotes vascular normalization and enhances the delivery of cytotoxic T cells, resulting in increased accumulation of T cells within the tumor microenvironment [12]. Lenvatinib also induces dendritic cell maturation and reduces Treg infiltration [11]. On the other hand, PD-1 blockade inhibits the PD-1/PD-L1 pathway to boost T cell-mediated antitumor immunity by "releasing the brake". Consequently, a strong rationale supports the combination of lenvatinib and PD-1 blockade [13]. LEAP-005 study evaluated pembrolizumab plus lenvatinib in 31 patients with previously treated BTC, which demonstrated a median progression-free survival (PFS) of 6.1 months, and manageable toxicity with grade 3 or 4 AEs reported in 48% of patients [14]. As a result, the National Comprehensive Cancer Network (NCCN) guidelines (Biliary Tract Cancers, version 1.2023) and the Chinese Society of Clinical Oncology (CSCO) guidelines (Biliary Tract Carcinoma, version 2022) both recommend pembrolizumab plus lenvatinib as a subsequent-line option for patients with unresectable or metastatic BTC. A phase II study assessed toripalimab (PD-1 inhibitor) plus lenvatinib as first-line treatment for 31 patients with advanced ICC, which showed promising antitumor activity with an ORR of 32.3% and a reasonable safety profile [15]. Notably, the above clinical trials adhered to strict inclusion and exclusion criteria (e.g., only included patients with good physical conditions), which may compromise their representativeness due to the small sample size. In contrast, real-world studies employ more flexible inclusion and exclusion criteria, which utilizes real-world data collected during routine practice and permits to evaluate medications across a broader patient population [16]. There is currently no data confirming the effectiveness and safety of PD-1 blockade plus lenvatinib in real-world settings. To address this issue, we conducted a retrospective real-world cohort study of patients with advanced ICC who treated with PD-1 blockade plus lenvatinib, and identified predictive biomarkers for tumor response and prognosis.

Materials and methods

Patients

Patients with advanced ICC who received PD-1 inhibitors and lenvatinib between July 2017 and August 2022 at Peking Union Medical College Hospital (PUMCH) were retrospectively screened. The inclusion criteria were: (i) histologically confirmed unresectable locally advanced or metastatic ICC, (ii) disease measurable per Response Evaluation Criteria in Solid Tumors (RECIST) v1.1, (iii) treated

with PD-1 inhibitors and lenvatinib at least one dose, (iv) Eastern Cooperative Oncology Group performance status (ECOG PS) 0–2, and (v) at least 18 years old. The exclusion criteria were (i) concurrent combination with other systemic drugs, (ii) no concurrent use of lenvatinib and PD-1 inhibitors, and (iii) treatment duration of fewer than two cycles (one course).

This study was performed in accordance with the Declaration of Helsinki and approved by the Ethics Committee of PUMCH (No. JS-1391). The study was registered at ClinicalTrials.gov (NCT03892577).

Treatment, assessment, and endpoints

Patients received a combined regimen of PD-1 blockade and lenvatinib. PD-1 inhibitors encompassed pembrolizumab, nivolumab, camrelizumab, sintilimab, tislelizumab, and toripalimab, all of which are available in mainland China. PD-1 inhibitors were administrated intravenously every 3 weeks with a fixed dose of 200 mg (pembrolizumab, nivolumab, camrelizumab, sintilimab, and tislelizumab) or 240 mg (toripalimab). Lenvatinib was given orally once daily at a dose of 8 mg (for body weight < 60 kg) or 12 mg (for body weight ≥ 60 kg).

Tumor assessments were performed every 6–9 weeks using CT or MRI and evaluated by experienced radiologists per RECIST v1.1. Safety profiles included monitoring of vital signs, physical exams, hematological and biochemical parameters, etc. AEs were assessed at every contact with the patient and were graded by investigators according to National Cancer Institute Common Terminology Criteria for Adverse Events (CTCAE) v5.0.

The study endpoints were PFS, OS, ORR, disease control rate (DCR), and safety. PFS was defined as the time from initiation of the combination regimen to either radiological progression or death. OS was defined as the time from the start of combination therapy to death. ORR was defined as the proportion of patients with complete response (CR) or partial response (PR). DCR was defined as the proportion of patients with disease control (CR, PR, or stable disease [SD]). Subgroup analyses stratified by treatment lines (first-line and subsequent-line) were performed to evaluate the effectiveness in different settings.

Biomarker analysis

Biomarkers analyses were conducted to identify predictors associated with tumor response and prognosis. Carbohydrate antigen 19-9 (CA19-9) is recommended by guidelines as tumor marker for early detection and diagnosis of ICC, and CA19-9 level may predict treatment response and prognosis [17, 18]. CA19-9 levels at baseline were acquired from electronic medical records, and stratified as ≤ 37 U/ml and > 37 U/ml (the normal upper limit of CA19-9 is 37 U/mL). PD-L1 expression was evaluated using immunohistochemistry (IHC) with a PD-L1 IHC 22C3 pharmDx kit (Agilent Technologies, USA) on formalin-fixed, paraffin-embedded (FFPE) tumor specimens. PD-L1 expression was reported as tumor proportion score (TPS), defined as the percentage of tumor cells with membranous PD-L1 staining. TPS were classified as TPS ≥ 1% and < 1%, as suggested by previous reports [19–21]. Microsatellite instability/mismatch repair (MSI/MMR) status was determined from tumor samples by IHC detection of MMR-related proteins (MLH1/MSH2/MSH6/PMS2) or computational analysis of tumor microsatellite loci using next-generation sequencing (NGS) data [22–24]. Tumor genetic alterations were revealed using targeted panel sequencing on NGS platforms provided by OrigiMed (China) [25], Genecast (China) [26], and 3D Medicines (China) [27]. Exploratory biomarker analysis was performed based on mutated genes, and genes with mutation frequencies greater than 15% in our cohort were included.

Statistical analysis

Continuous variables were presented as median and range. Categorical variables were shown as frequency and percentage and were compared by chi-square test or Fisher's exact test as appropriate. Survival analyses were conducted using the Kaplan–Meier method and compared by the log-rank test. Univariate and multivariate Cox regression was applied to identify risk factors for PFS and OS and to estimate hazard ratios (HR) and 95% confidence intervals (95% CI). A two-sided $P < 0.05$ was considered statically significant. All statistical analyses were done with SPSS version 27.

Results

Patient characteristics

A total of 103 patients were included in this study (Fig. 1). The median age of patients was 57 years old (range, 30–82), 38.8% of patients (40/103) were female, and 77.7% of patients (80/103) had an Eastern Cooperative Oncology Group performance status (ECOG PS) of 1 or 2. Additionally, 64.1% of patients (66/103) presented with distant metastasis. Forty-four patients (42.7%) were in the first-line setting. Fifty-nine patients (57.3%) experienced prior systemic treatments, among whom 41 (69.5%) received chemotherapy. Baseline characteristics were summarized in Table 1.

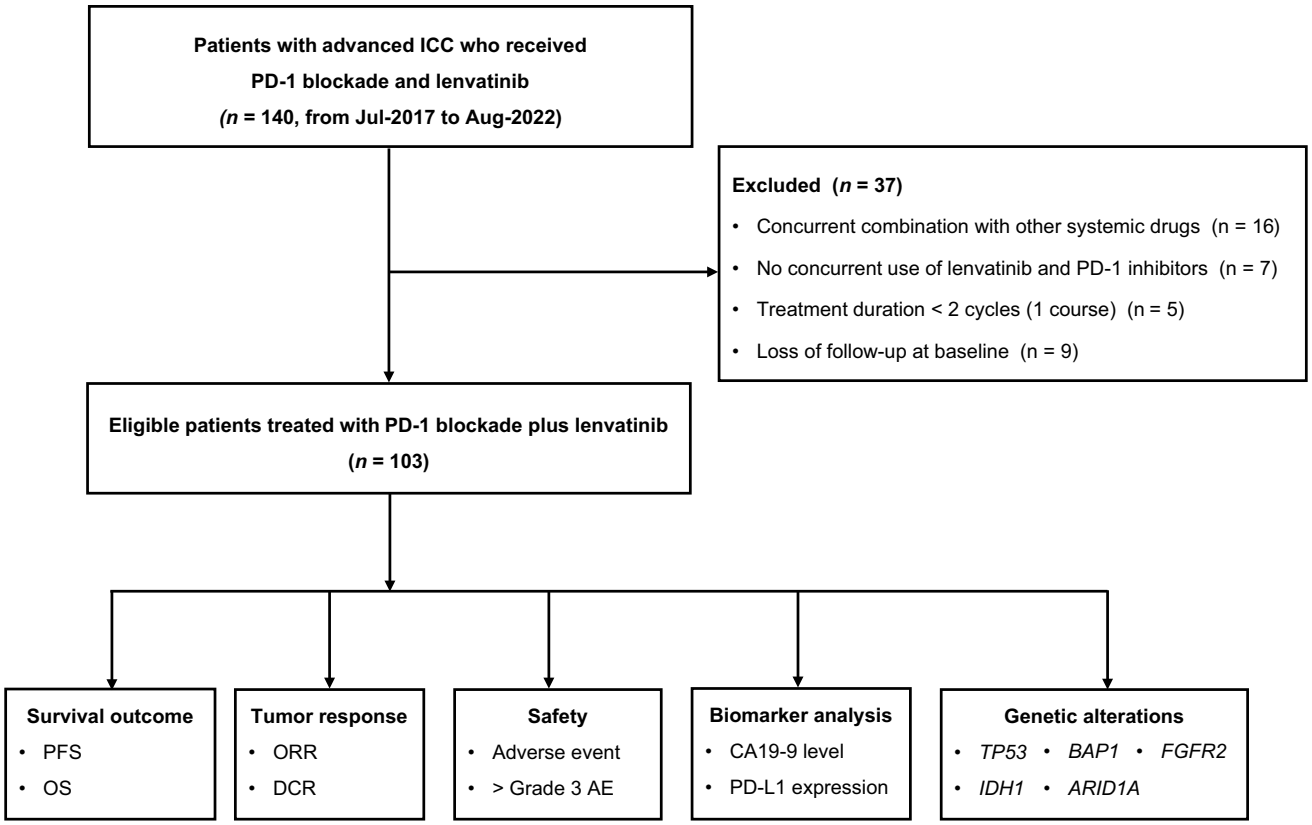

Fig. 1 Flowchart of the study

Table 1 Baseline characteristics

	Total (n = 103)	1st line (n = 44)	≥ 2nd line (n = 59)
Median age (range), yr	**57** (30–82)	**59** (33–77)	**57** (30–73)
Female, n (%)	**40** (38.8%)	**14** (31.8%)	**26** (44.1%)
ECOG PS, n (%)			
0	**23** (22.3%)	**10** (22.7%)	**13** (22.0%)
1	**61** (59.2%)	**26** (59.1%)	**35** (59.3%)
2	**19** (18.4%)	**8** (18.2%)	**11** (18.6%)
Metastatic site, n (%)			
Liver	**87** (84.5%)	**38** (86.4%)	**49** (83.1%)
Lymph nodes	**83** (80.6%)	**35** (79.5%)	**48** (81.4%)
Lung	**25** (24.3%)	**6** (13.6%)	**19** (32.2%)
Bone	**24** (23.3%)	**10** (22.7%)	**14** (23.7%)
Extent of disease, n (%)			
Locally advanced	**37** (35.9%)	**16** (36.4%)	**21** (35.6%)
Distant metastasis	**66** (64.1%)	**28** (63.6%)	**38** (64.4%)
Treatment line, n (%)			
1	**44** (42.7%)	**44** (100%)	–
2	**52** (50.5%)	–	**52** (88.1%)
≥ 3	**7** (6.8%)	–	**7** (11.9%)
Prior treatment, n (%)			
Surgery	**37** (35.9%)	**12** (27.3%)	**25** (42.4%)
Locoregional therapy	**48** (46.6%)	**12** (27.3%)	**36** (61.0%)
Systemic therapy	**59** (57.3%)	**–**	**59** (100%)

Effectiveness

At the data cutoff of analysis (February 1, 2023), median follow-up time was 17.3 months (range, 2.3 to 37.3). In the overall population ($n=103$), median PFS (mPFS) was 5.9 months (95% CI 5.1–6.7), and the 6-, and 12-month PFS rates were 46.2% and 14.7%, respectively (Fig. 2a). Median OS (mOS) was 11.4 months (95% CI 10.1–12.7), and the 6-, 12-, and 24-month OS rates were 85.8%, 44.1%, and 15.7%, respectively (Fig. 2b). Among 104 patients, 19 (18.4%) achieved an objective response, all of which were PRs, resulting in an ORR of 18.4%. Disease control was achieved in 83 patients (80.6%), with 47 patients (45.6%) experiencing continued disease control for 6 months or longer (Table 2).

In first-line ($n=44$), mPFS was 6.5 months (95% CI 4.8–8.2), and mOS was 12.0 months (95% CI 10.7–13.3) (Fig. 2c). The 6-, and 12-month PFS rates were 54.5% and 20.1%, respectively. The 6-, 12-, and 24-month OS rates were 88.5%, 47.3%, and 22.1%, respectively. ORR and DCR were 22.7% (10/44) and 81.8% (36/44), respectively (Table 2).

In subsequent-line ($n=59$), mPFS was 5.0 months (95% CI 4.3–5.7), and mOS was 11.0 months (95%CI, 8.3 to 13.7) (Fig. 2d). The 6-, and 12-month PFS rates were 39.3% and 10.1%, respectively. The 6-, 12-, and 24-month OS rates

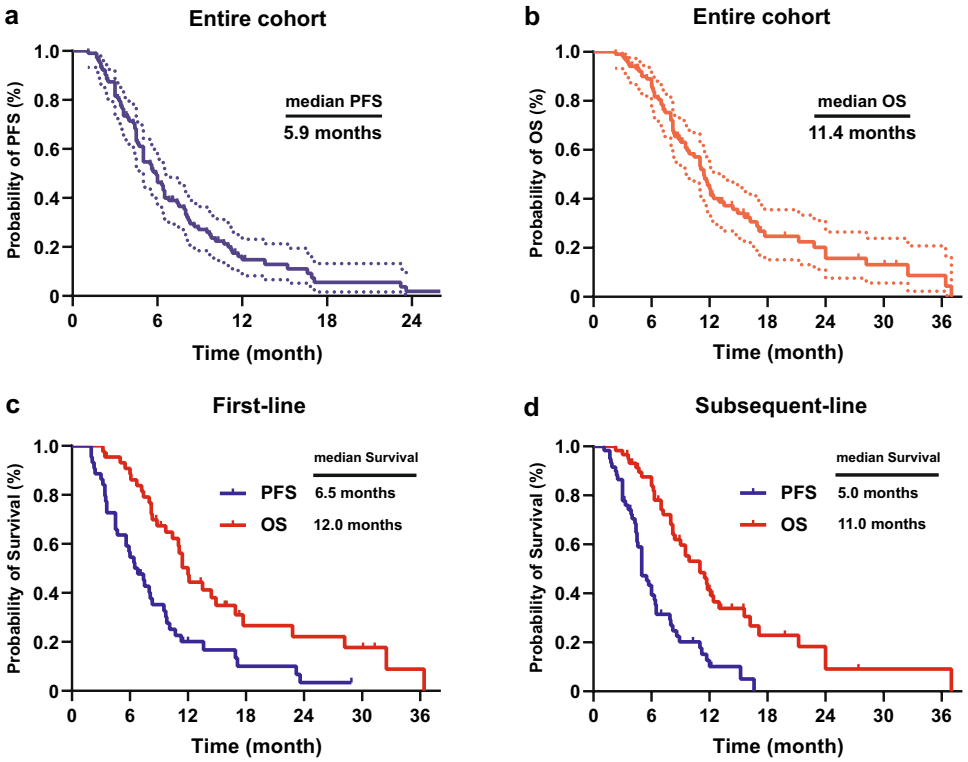

Fig. 2 Survival curves for overall population and subgroups **a** Progression free-survival of the entire cohort. **b** Overall survival of the entire cohort. Survival curves of **c** patients in first-line, and **d** patients in subsequent-line

Table 2 Tumor response

	Total (n = 103)	1st line (n = 44)	≥ 2nd line (n = 59)
Objective response, n (%)	**19** (18.4%)	**10** (22.7%)	**9** (15.3%)
Complete response, n (%)	**0** (0)	**0** (0)	**0** (0)
Partial response, n (%)	**19** (18.4%)	**10** (22.7%)	**9** (15.3%)
Stable disease, n (%)	**64** (62.1%)	**26** (59.1%)	**38** (64.4%)
Disease control rate, n (%)	**83** (80.6%)	**36** (81.8%)	**47** (79.7%)
Continued disease control, n (%)			
≥ 3 months	**89** (86.4%)	**39** (88.6%)	**50** (84.7%)
≥ 6 months	**47** (45.6%)	**25** (56.8%)	**22** (37.3%)
≥ 9 months	**23** (22.3%)	**14** (31.8%)	**9** (15.3%)
Progressive disease, n (%)	**20** (19.4%)	**8** (18.2%)	**12** (20.3%)

were 83.8%, 41.5%, and 9.1%, respectively. ORR and DCR were 15.3% (9/59) and 79.7% (47/59), respectively (Table 2).

Safety

All patients experienced AEs of various grade (Table 3). The most common AEs were fatigue (60.2%), hypertension (51.4%), elevated alanine aminotransferase (ALT) or aspartate alanine aminotransferase (AST) (45.6%), decreased appetite (43.7%), and hypothyroidism (34.0%). The incidence of grade 3 or 4 AEs was 50.5%. The most common grade 3 or 4 AEs were hypertension (12.6%), fatigue (6.8%), increased blood bilirubin (6.8%), diarrhea (5.8%), and elevated ALT or AST (4.9%). Grade 5 AEs occurred in 2 patients (1.9%), including pneumonia and arrhythmia (one each). Ten patients (9.6%) discontinued the treatment due to AEs.

Biomarker analysis

Seventy-six patients had records of baseline CA19-9 levels, among whom 50 patients (65.8%) had CA19-9 higher than 37 U/ml. Patients with CA19-9 ≤ 37 U/ml achieved a higher ORR than those with CA19-9 > 37 U/ml (34.6% vs. 12.0%, $P = 0.019$; Fig. 3a). Moreover, patients with CA19-9 ≤ 37 U/ml showed longer PFS and OS than those with CA19-9 > 37 U/ml (PFS, $P = 0.005$; OS, $P = 0.034$; Fig. 3b, c). Evaluation of PD-L1 expression was performed in 64 patients, and 25 patients (39.1%) were classified as TPS ≥ 1%. Patients with TPS ≥ 1% experienced a higher ORR than those with TPS < 1% (36.0% vs. 10.3%, $P = 0.013$; Fig. 3d). Patients with TPS ≥ 1% displayed a longer PFS than those with TPS < 1% ($P = 0.023$; Fig. 3e). Although the difference in OS between the two subgroups was not significant ($P = 0.423$; Fig. 3f), the TPS ≥ 1% subgroup showed an improvement in the tail of the OS curve. Detection of MSI/MMR status was performed in 42 patients. Most of the patients (92.9%) were presented with MSS/MSI-L or pMMR, and only 3 patients (7.1%) were MSI-H/dMMR. Given the small number of patients with MSI-H/dMMR, it would be improper and inaccuracy to evaluate the predictive power of MSI/MMR status, and a further attempt at biomarker analysis for MSI/MMR status was abolished.

Cox regression analyses were done to identify prognostic factors (Supplementary material Table 4). Univariate analyses indicated that CA19-9 level (> 37 vs. ≤ 37 U/ml) and PD-L1 expression (TPS ≥ 1% vs. < 1%) were significantly associated with PFS ($P < 0.05$). Multivariate analyses demonstrated that positive PD-L1 expression (TPS ≥ 1%) was a favorable prognostic factor for PFS (HR = 0.46, $P = 0.047$). Moreover, CA19-9 > 37 U/ml and distant metastasis were independent predictors for worse OS (CA19-9 level: HR = 2.42, $P = 0.008$; Extent of disease: HR = 2.46, $P = 0.039$).

Table 3 Most common adverse events

	Any grade	Grade 3 or 4
Total	**103** (100%)	**52** (50.5%)
Fatigue	**62** (60.2%)	**7** (6.8%)
Hypertension	**53** (51.4%)	**13** (12.6%)
Elevated ALT or AST	**47** (45.6%)	**5** (4.9%)
Decreased appetite	**45** (43.7%)	**4** (3.9%)
Hypothyroidism	**35** (34.0%)	**1** (1.0%)
Proteinuria	**32** (31.1%)	**3** (2.9%)
Increased blood bilirubin	**31** (30.1%)	**7** (6.8%)
Rash	**30** (29.1%)	**4** (3.9%)
Diarrhea	**29** (28.2%)	**6** (5.8%)
Abdominal pain	**28** (27.2%)	**3** (2.9%)
Thrombocytopenia	**25** (24.3%)	**4** (3.9%)
Vomiting	**23** (22.3%)	**2** (1.9%)
Decreased weight	**22** (21.4%)	**0**
Nausea	**19** (18.4%)	**0**
Palmar-plantar erythrodysesthesia	**18** (17.5%)	**3** (2.9%)
Hypoalbuminemia	**18** (17.5%)	**2** (1.9%)
Anemia	**16** (15.5%)	**2** (1.9%)
Dysphonia	**13** (12.6%)	**0**
Upper gastrointestinal hemorrhage	**10** (9.7%)	**3** (2.9%)

Exploratory analysis on genetic alterations

Profiles of genetic alterations were available in 40 patients (Fig. 4a). *TP53* mutations were the most frequently observed alterations (11/40, 27.5%), followed by *BAP1* mutations (9/40, 22.5%), *FGFR2* fusions/rearrangement (8/40, 20%), *IDH1* mutations (8/40, 20%), *ARID1A* mutations (8/40, 20%), *KRAS* mutations (5/40, 12.5%), and *CDKN2A* mutations (5/40, 12.5%). More than half of the patients with PR harbored *IDH1* mutations (5/9, 55.6%). Patients with SD frequently exhibited alterations in *BAP1* (8/26, 30.8%), *TP53* (7/26, 26.9%), *FGFR2* (7/26, 26.9%), and *ARID1A* (7/26, 26.9%). *TP53* mutations were identified in 2 out of 5 (40%) patients with progressive disease. We conducted biomarker analysis for the top 5 altered genes. Patients with mutant *TP53* had a similar ORR and PFS compared to those with wildtype *TP53*, but showed a worse OS ($P = 0.031$) (Fig. 4b). Patients harboring genetic alterations of *BAP1*, *FGFR2,* or *ARID1A* tended to obtain a higher ORR, but with no statistical difference. There was no difference in survival between the mutated and the wildtype of the three genes. Intriguing, none of the patients with alterations in *BAP1* or *FGFR2* responded to the regimen (Fig. 4c, d). Moreover, patients with *IDH1* mutations exhibited a higher ORR when compared to those with wildtype *IDH1* (62.5% vs. 12.5%, $P = 0.011$), and appeared to have a prolonged PFS

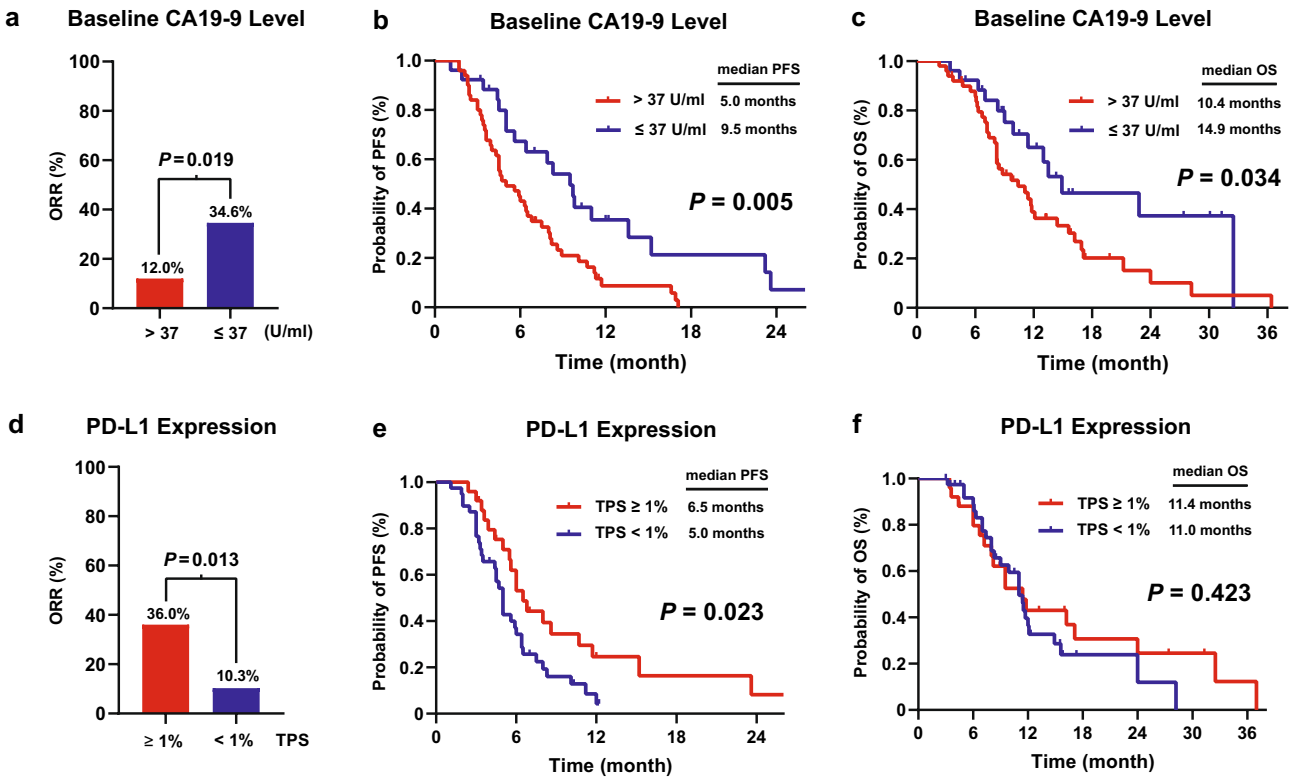

Fig. 3 Treatment-related biomarkers **a**–**c** ORR, PFS, and OS for patients with CA19-9 > 37 U/ml and ≤ 37 U/ml. **d**–**f** ORR, PFS, and OS for patients with TPS ≥ 1% and < 1%

and OS, though the difference was not statistically significant (Fig. 4e).

Discussion

The application of PD-1 blockade combined with lenvatinib for ICC has not been comprehensively assessed in real-world settings. In this study, we evaluated the experience in the use of this therapeutic option in a retrospective real-world cohort, focusing on effectiveness and safety, as well as treatment-related biomarkers. We were able to confirm that the combination treatment is an effective and safe option in patients with advanced ICC. Our study has three strengths: (i) the study population is the largest sample size cohort to date (a total of 103 patients); (ii) a long observation period (from 2017 to 2022); (iii) a comprehensive biomarker analysis.

In the entire cohort, PD-1 inhibitors plus lenvatinib demonstrated a mPFS of 5.9 months, a mOS of 11.4 months, an ORR of 18.4%, and a DCR of 80.6%. To better interpret the efficacy data and compare with previous reports, the cohort was divided based on treatment lines. In the first-line setting, this regimen demonstrated a mPFS of 6.5 months, a mOS of 12.0 months, an ORR of 22.7%, and a DCR of 81.8%. Only one phase II study (preliminary results reported as an abstract) evaluated toripalimab plus lenvatinib as a first-line treatment for 31 patients with advanced ICC [15]. The ORR and DCR was 32.3% and 74.2%, and 6-months OS rate was 87.1%. Besides, our efficacy data was comparable to GemCis in the TOPAZ-1 study (mPFS 5.7 months, mOS 11.5 months, ORR 18.7%, and DCR 82.6%), though showed a slight numerical differences compared to GemCis plus durvalumab (mPFS 7.2 months, mOS 12.8 months, ORR 26.7%, and DCR 85.3%) [6]. As a subsequent-line treatment, PD-1 inhibitors plus lenvatinib achieved a mPFS of 5.0 months, a mOS of 11.0 months, an ORR of 15.3%, and a DCR of 79.7%. Lin and his colleagues reported pembrolizumab plus lenvatinib as a subsequent-line therapy in 32 patients with BTC, which demonstrated a mPFS of 4.9 months, a mOS of 11.0 months, an ORR of 25%, and a DCR of 78.1% [28]. The phase II LEAP-005 study assessed lenvatinib plus pembrolizumab in a second-line setting for 31 patients with advanced BTC, demonstrating a mPFS of 6.1 months, a mOS of 8.6 months, an ORR of 10%, and a DCR of 68% [14]. Ding et al. conducted a retrospective study investigating sintilimab plus lenvatinib as second-line therapy for 41 patients with advanced ICC [21]. The median time to progression was 6.6 months, and mOS was 16.6 months. Strikingly, 46.3% of patients achieved an objective response,

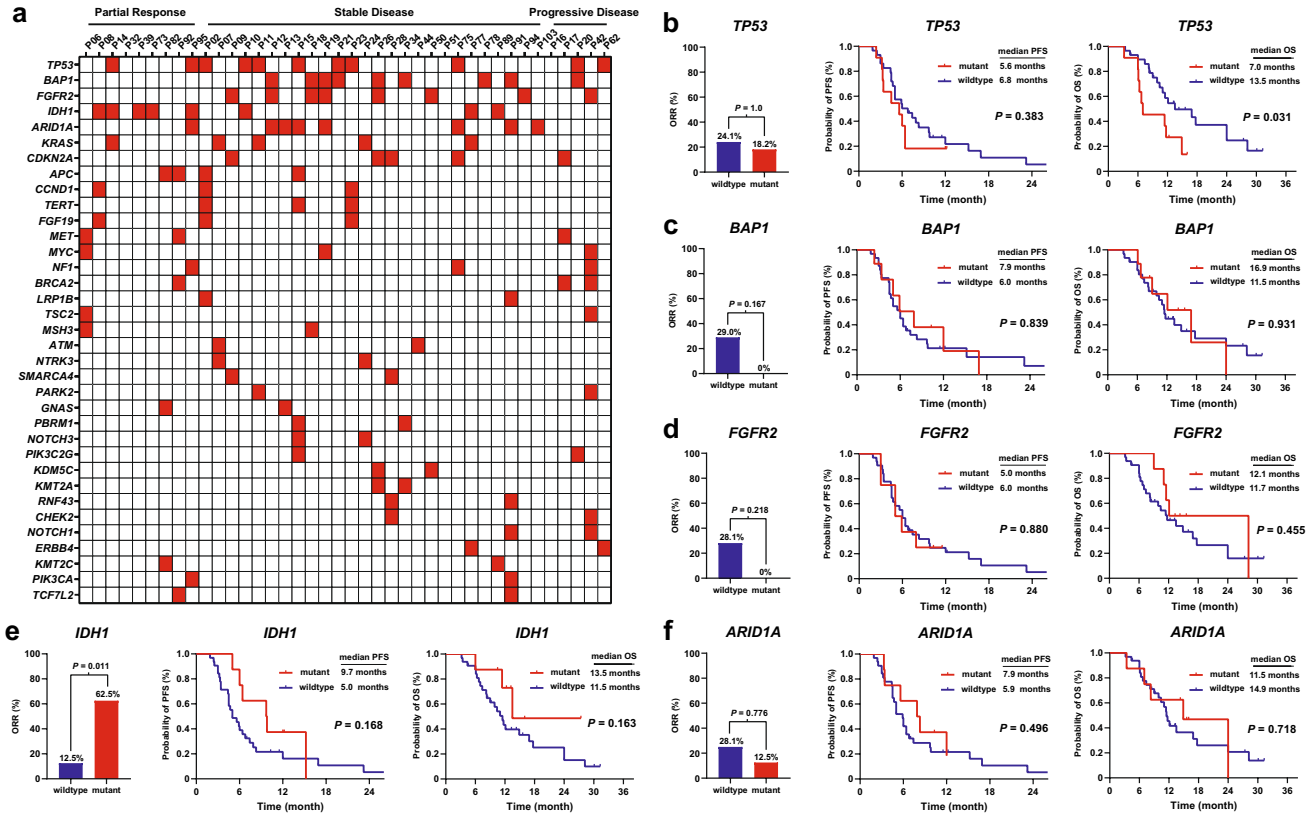

Fig. 4 Exploratory analysis for genetic alternations **a** Profiles of genetic alternations. ORR, PFS, and OS stratified by mutation status for **b** *TP53*, **c** *BAP1*, **d** *FGFR2*, **e** *IDH1*, and **f** *ARID1A*

which might owe to that 80.5% of the patients were positive PD-L1 expression. Our results were similar to these prior studies, especially in terms of survival. In addition, when compared to the FOLFOX regimen in the ABC-06 study (the only phase III trial in second-line) [5], which only achieved a mPFS of 4.0 months, a mOS of 6.2 months, an ORR of 4.9%, and a DCR of 33%, PD-1 blockade combined with lenvatinib exhibited conspicuous advantages. Although direct comparisons between studies may be inappropriate because patient characteristics are different in each study, but helpful in interpreting results. Collectively, these results strongly support the efficacy and reliability of PD-1 blockade plus lenvatinib for advanced ICC. It holds promise as a viable first-line treatment, especially for patients who cannot tolerate or refuse chemotherapy. Moreover, it represents a potential subsequent-line option preferred for patients who experience progression after chemotherapy. To validate our findings, high-level evidence from well-designed trials is still needed. Several ongoing prospective studies (NCT04361331, NCT03797326, NCT04211168) are expected to provide more robust evidence.

Safety profiles are another emphasis. In our cohort, 50.5% of the patients experienced grade 3 or 4 AEs. The vast majority of AEs could be well managed. Fatigue and decreased appetite could cause low quality of life. Some AEs such as gastrointestinal bleeding, pneumonia, hepatitis, myocarditis, and enteritis could be life-threatening and require periodic monitoring and timely management. With the accumulation of experience, general and severe AEs management will gradually improve. PD-1 blockade plus lenvatinib have been widely used in HCC [29]. Oncologists can leverage their experience from treating HCC to promptly detect and manage AEs during the administration of this regimen in ICC.

To aid clinical decision-making, we identified several treatment-related biomarkers. PD-L1 expression is the most widely used biomarker for immunotherapy. High PD-L1 expression is generally considered to associate with better response to immunotherapy and prolonged survival [30]. In our study, positive PD-L1 expression was associated with higher ORR and prolonged PFS, consistent with previous studies [21, 28]. There are few reports exploring the influence of genetic alteration on immunotherapy for ICC. *IDH1* mutations are common molecular alterations of ICC, detected in approximately 20% of the patients [31, 32]. Mutation of *IDH1* results in the accumulation of the oncometabolite 2-hydroxyglutarate, and the 2-hydroxyglutarate is involved in carcinogenesis and immunoevasion [33]. In

our cohort, patients with *IDH1* mutations were found to have higher ORR and a tendency towards longer survival. It seems that *IDH1* mutations might predict a better response to immunotherapy. Genetic alterations of *BAP1* and *FGFR2* occur in 15–20% of patients with ICC [31, 32]. Interestingly, in our cohort, none of the patients with alterations in *BAP1* or *FGFR2* responded to the regimen. *BAP1* is a tumor suppressor gene, and its mutations have been identified in various tumors, including ICC [34]. Downregulation or inactivation of BAP1 can accelerate tumor onset, invasion, recurrence, and progression. *FGFR2* alterations, such as gain-of-function mutations, amplifications, and chromosomal translocations, can activate FGFR2 signaling, leading to tumorigenesis and progression by promoting proliferation and survival of tumor cells [35]. The presence of genetic alterations in *BAP1* and *FGFR2* may contribute to an aggressive biological behavior of tumors, which could partly explain why the regimen was less effective in patients with these altered genes. The underlying molecular mechanisms are fascinating and require further investigation before drawing generalizable conclusions [36]. Besides, considering that lenvatinib inhibits the VEGF/VEGFR pathway by targeting VEGFR1/2/3, future investigation will be conducted to determine whether VEGFR expression could serve as a potential biomarker.

The study has some limitations. Firstly, its retrospective nature may introduce potential bias. Multicenter prospective studies are needed to confirm our results. Secondly, multiple kinds of PD-1 inhibitors may introduce heterogeneity. It is unavoidable in complex real-world situations because many kinds of PD-1 inhibitors are currently available, and various factors may influence patient choices such as economic considerations, insurance coverage, charitable donation and preferences. Thirdly, a proportion of patients received the regimen as first-line treatment instead of standard chemotherapy (e.g., GemCis), which requires explanation. This is because these patients explicitly refused chemotherapy during the visit. The treatment decision was made with patient consent and regulatory approval. It was compliant with ethical guidelines and the principle of compassionate use.

In conclusion, PD-1 blockade plus lenvatinib is effective and safe in routine practice. It presents as a viable treatment option for advanced ICC. PD-L1 expression and baseline CA19-9 level appear to predict the treatment efficacy. *IDH1* mutations might predict a better response to the regimen.

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Author contributions HZ and LL contributed to the conception and design of the study. All authors participated in patient follow-up and contributed to data collection. JC, SW, and HW collated the data and performed analysis. JC, SW, and HW drafted the manuscript. All authors contributed to reviewing or revising the manuscript and approved the final version.

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Data availability The data that support the findings of our study are available from the corresponding author upon request.

Declarations

Conflict of interest All authors declare that they have no conflict of interest.

Ethical approval The study was approved by the Ethics Committee of Peking Union Medical College Hospital (No. JS-1391), and was conducted in accordance with the Declaration of Helsinki. Informed consent was obtained from all patients.

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