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Efficacy analysis of transcatheter arterial chemoembolization combined with anlotinib and PD-1 inhibitors in advanced unresectable intrahepatic cholangiocarcinoma: a multicenter retrospective cohort study based on propensity score matching

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Abstract

Objective To investigate the efficacy and safety of transcatheter arterial chemoembolization (TACE) combined with anlotinib and PD-1 inhibitors in advanced unresectable intrahepatic cholangiocarcinoma (ICC).

Methods In this retrospective cohort study, clinical data were collected from 154 patients with unresectable advanced ICC treated at the First Affiliated Hospital of Zhengzhou University, Henan Cancer Hospital, and Henan Provincial People's Hospital between January 2021 and January 2025. Patients were divided into an observation group and a control group according to

treatment regimen. The observation group received TACE combined with anlotinib and PD-1 inhibitors, whereas the control group received TACE combined with anlotinib. Propensity score matching (PSM) was used to evaluate clinical efficacy, survival prognosis, and the incidence of adverse reactions.

Results Sixty-six patients in the observation group received TACE plus anlotinib and PD-1 inhibitors, and 88 patients in the control group received TACE plus anlotinib. After 1:1 PSM to balance age, sex, tumor size, tumor number, Child-Pugh grade, ECOG score, baseline CA19-9, and TNM stage, 51 matched pairs were created. The objective response rate (ORR) and disease control rate (DCR) in the observation group were 70.6% and 94.1%, respectively, which were significantly higher than those in the control group (41.2% and 72.5%). Median progression-free survival (PFS) and overall survival (OS) were 11.6 months and 19.9 months in the observation group, respectively, compared with 7.9 months and 10.4 months in the control group. Cox proportional hazards analysis showed that tumor size, ECOG score, and treatment modality were independent predictors of PFS and OS. Treatment-related adverse reactions were similar between the two groups, with no statistically significant difference.

Conclusions Compared with TACE plus anlotinib, TACE combined with anlotinib and PD-1 inhibitors significantly prolonged survival and improved tumor response in patients with advanced ICC, with tolerable safety. This triplet regimen may serve as a preferred first-line treatment option for advanced ICC.

Keywords Intrahepatic cholangiocarcinoma, transcatheter arterial chemoembolization, anlotinib, immune checkpoint inhibitors, propensity score matching

Background

Intrahepatic cholangiocarcinoma (ICC) is a primary liver malignancy, with an incidence second only to hepatocellular carcinoma, accounting for 10%–20% of all primary liver cancers. It originates from biliary epithelial cells and is a highly invasive tumor that is often multifocal and poorly responsive to treatment. ICC usually has no typical symptoms in its early stages, and 60%–88% of patients are already at an advanced stage at diagnosis, missing the opportunity for radical resection [1,2].

Comprehensive treatment is considered the key to improving the survival prognosis of patients with advanced ICC. These treatments include chemotherapy, targeted therapy, and immunotherapy [3]. TACE is an effective non-surgical treatment for ICC. Compared with systemic chemotherapy, TACE can kill tumor cells by increasing local drug concentration, reduce systemic adverse reactions, and block the tumor-feeding artery; therefore, it is an important local treatment modality for advanced ICC [4]. TACE alone is insufficient for systemic control of advanced ICC. TACE combined with anlotinib is a commonly used local plus systemic treatment strategy in clinical practice, but its efficacy still needs further improvement [5,6]. In recent years, immune checkpoint inhibitors (ICIs) have been widely used in the clinical treatment of solid tumors. For example, the KEYNOTE-966 and TOPAZ-1 studies demonstrated that adding pembrolizumab or durvalumab to gemcitabine plus cisplatin modestly prolonged median OS in patients with advanced biliary tract cancer [7,8].

Therefore, for advanced ICC, TACE combined with anlotinib and PD-1 inhibitors may be superior to TACE combined with anlotinib alone [9,10]. However, reports on this triplet regimen are scarce worldwide, and head-to-head real-world controlled studies with matched baseline

confounders are lacking. In this study, PSM was used to eliminate baseline bias and to preliminarily investigate the safety and efficacy of the triplet regimen in advanced ICC.

Methods

Case selection

This retrospective cohort study collected clinical data from 154 patients with unresectable ICC admitted to the First Affiliated Hospital of Zhengzhou University, Henan Cancer Hospital, and Henan Provincial People's Hospital between January 2021 and January 2025. Among them, 66 patients received TACE combined with anlotinib and PD-1 inhibitors, and 88 patients received TACE combined with anlotinib alone.

Inclusion criteria: ① age 18–75 years; ② histologically or cytologically confirmed ICC; ③ TNM stage III–IV; ④ ECOG score ≤2; ⑤ Child-Pugh class A or B; ⑥ at least one measurable lesion on baseline CT or MRI; ⑦ adequate bone marrow, hepatic, and renal reserve, with total bilirubin within 2 times the upper limit of normal and normal cardiopulmonary function; ⑧ complete follow-up data.

Exclusion criteria: ① hepatocellular carcinoma, mixed hepatocellular-cholangiocarcinoma, or liver metastases; ② contraindications to TACE or targeted/immunotherapy; ③ uncontrolled extrahepatic metastasis; ④ concurrent other primary malignancies; ⑤ Child-Pugh class C; ⑥ severe cardiopulmonary dysfunction or hepatic/renal insufficiency; ⑦ prior antitumor therapy; ⑧ lack of clinical and follow-up data.

Ethics approval and consent to participate

This study was conducted in accordance with the Declaration of Helsinki and was approved

by the Clinical Trial Ethics Committee of the First Affiliated Hospital of Zhengzhou University (approval No. ChiECRCT20220109). The requirement for informed consent was waived by the committee because this was a retrospective study.

Baseline information

According to treatment modality, patients were divided into the TACE + anlotinib + PD-1 inhibitor group and the TACE + anlotinib group. Baseline data were collected, including sex, age, ECOG score, Child-Pugh grade,baseline CA19-9tumor size, tumor number, tumor stage, presence or absence of extrahepatic metastasis, survival time, and treatment-related adverse reactions.

Treatment methods

TACE ： The right femoral artery was punctured using the Seldinger technique, and a 5-F sheath was placed. A 5-F hepatic artery catheter and a 0.035-inch hydrophilic guidewire were used for angiography of the celiac artery, superior mesenteric artery, and inferior phrenic artery to confirm tumor blood supply, followed by catheterization into the feeding artery and its branches. After angiographic confirmation, chemotherapeutic agents (routinely gemcitabine and cisplatin) were infused locally through the catheter. Individualized embolization was performed with microspheres of different diameters according to tumor location, size, and number. Angiography was repeated until tumor staining disappeared, at which point embolization was stopped and the procedure was completed. All patients received postoperative supportive treatment, including hepatoprotective, anti-inflammatory, analgesic, and antiemetic agents. Follow-up was performed every 4–6 weeks, and TACE was repeated as needed if active lesions were confirmed by CT or

MRI.

Systemic therapy：Systemic therapy was suspended during TACE and resumed 3–5 days after TACE. Anlotinib was administered orally at 12 mg once daily on a 2-week-on/1-week-off schedule. For grade 1–2 adverse events (AEs), symptomatic treatment was given. For grade 3 or higher AEs, the dose was reduced to 8 mg once daily or the frequency was reduced to once every 2 days until the AE improved or resolved. If AEs persisted, anlotinib was discontinued until the adverse reaction resolved or improved. PD-1 inhibitors included sintilimab (Innovent Biologics, Suzhou, China), tislelizumab (BeiGene, Shanghai, China), and camrelizumab (Hengrui Pharmaceuticals, Lianyungang, China), administered intravenously at 200 mg every 3 weeks.

Postoperative follow-up and efficacy evaluation

The first follow-up evaluation was performed one month after treatment initiation, and subsequent imaging assessments were conducted every 4–6 weeks to evaluate treatment response. Treatment response was assessed according to the modified Response Evaluation Criteria in Solid Tumors (mRECIST) by two radiologists with 10 years of experience, including complete response (CR), partial response (PR), stable disease (SD), and progressive disease (PD). The objective response rate (ORR) was calculated as (CR + PR) / total cases × 100%, and the disease control rate (DCR) was calculated as (CR + PR + SD) / total cases × 100%. PFS was defined as the time from treatment initiation to PD or death, or from treatment initiation to the last follow-up. OS was defined as the time from treatment initiation to death or the last follow-up. Treatment-related AEs were graded according to the National Cancer Institute Common Terminology Criteria for Adverse Events (CTCAE) version 5.0, with grades 0–4.

Statistical analysis

All statistical analyses were performed using R software version 4.2.2. Continuous variables were described as mean ± standard deviation and compared using independent-sample t-tests. Categorical variables were expressed as frequencies (percentages) and compared using Fisher's exact test. To control potential confounders and reduce selection bias,Propensity scores were estimated using logistic regression based on eight baseline covariates(age, sex, tumor size, tumor number, ECOG performance status, CA19-9 level, TNM stage) .Patients were matched 1:1 by nearest-neighbor matching without replacement (caliper: 0.2 standard deviations of the logit of the propensity score), yielding 51 pairs. Balance was assessed using standardized mean differences.Kaplan-Meier analysis was used to estimate median OS and PFS and to plot survival curves, and differences in survival were compared using the log-rank test. Prognostic factors were analyzed using Cox proportional hazards regression models, with univariate screening followed by multivariate analysis of significant variables.

Results

Patient characteristics

From January 2021 to January 2025, a total of 232 patients with advanced ICC were admitted to the participating hospitals. After excluding patients with resectable ICC (n = 23), mixed hepatocellular-cholangiocarcinoma (cHCC-CCA, n = 6), incomplete follow-up data (n = 14), and prior antitumor therapy (n = 35), 154 patients were included in the data analysis (Fig. 1). Among these 154 patients, 66 received TACE combined with anlotinib and PD-1 inhibitors, and

88 received TACE combined with anlotinib. A 1:1 PSM was performed between the two groups. After PSM, 51 patients in each group were included in this study (Table 1).

Tumor response evaluation

Before PSM, among 66 patients in the observation group, the ORR was 71.2% (47/66) and the DCR was 93.9% (62/66). Among 88 patients in the control group, the ORR was 34.1% (30/88) and the DCR was 69.3% (61/88). Both ORR and DCR in the observation group were significantly higher than those in the control group ($P < 0.001$). After PSM, among 51 patients in the observation group, the ORR was 70.6% (36/51) and the DCR was 94.1% (48/51). Among 51 patients in the control group, the ORR was 41.2% (21/51) and the DCR was 72.5% (37/51). Both ORR and DCR in the observation group were significantly higher than those in the control group ($P = 0.003$; $P = 0.007$) (Table 2).

Survival analysis

Follow-up was conducted until January 1, 2026, with a median follow-up time of 18.1 months (95% CI: 17.3–19.2). Before PSM, the median PFS was 10.9 months (95% CI: 10.1–11.9) in the observation group and 7.7 months (95% CI: 7.1–8.3) in the control group; the log-rank test showed $P < 0.001$. The survival curves are shown in Fig. 2a. After PSM, the median PFS was 11.6 months (95% CI: 10.0–12.4) in the observation group and 7.9 months (95% CI: 7.2–9.2) in the control group; the log-rank test showed $P < 0.001$. The survival curves are shown in Fig. 2b. Before PSM, the median OS was 19.9 months (95% CI: 16.8–21.5) in the observation group and 10.0 months (95% CI: 9.3–11.1) in the control group; the log-rank test showed $P < 0.001$. The

177 survival curves are shown in Fig. 2c. After PSM, the median OS was 19.9 months (95% CI: 16.0–
178 NR) in the observation group and 10.4 months (95% CI: 9.1–12.4) in the control group; the
179 log-rank test showed $P < 0.001$. The survival curves are shown in Fig. 2d. Representative cases
180 are shown in Fig. 3.
181
182 **Prognostic factor analysis**
183 Univariate analysis showed that age, ECOG score, Child-Pugh grade, tumor size, tumor
184 number, tumor stage, and treatment modality were factors affecting PFS ($P < 0.01$). In
185 multivariate analysis, variables with $P < 0.1$ from univariate analysis were included, and ECOG
186 score and treatment modality were independent factors affecting PFS ($P < 0.01$). Univariate
187 analysis showed that age, ECOG score, Child-Pugh grade, tumor size, tumor number, tumor stage,
188 and treatment modality were factors affecting OS ($P < 0.01$). In multivariate analysis, variables
189 with $P < 0.1$ from univariate analysis were included, and ECOG score and treatment modality
190 were independent factors affecting OS ($P < 0.01$). The univariate and multivariate analyses after
191 matching are shown in Table 3.
192
193 **Safety**
194 After PSM, TACE-related adverse reactions in the observation group, such as nausea and
195 vomiting and liver function impairment, were all grade ≤2 and were completely relieved after
196 symptomatic treatment. No severe complications such as liver abscess or hepatorenal failure
197 occurred after TACE in the observation group. Systemic therapy-related adverse reactions in both
198 groups were grade ≤2, and there was no statistically significant difference in the incidence of

systemic therapy-related adverse reactions between the two groups (P > 0.05) (Table 4).

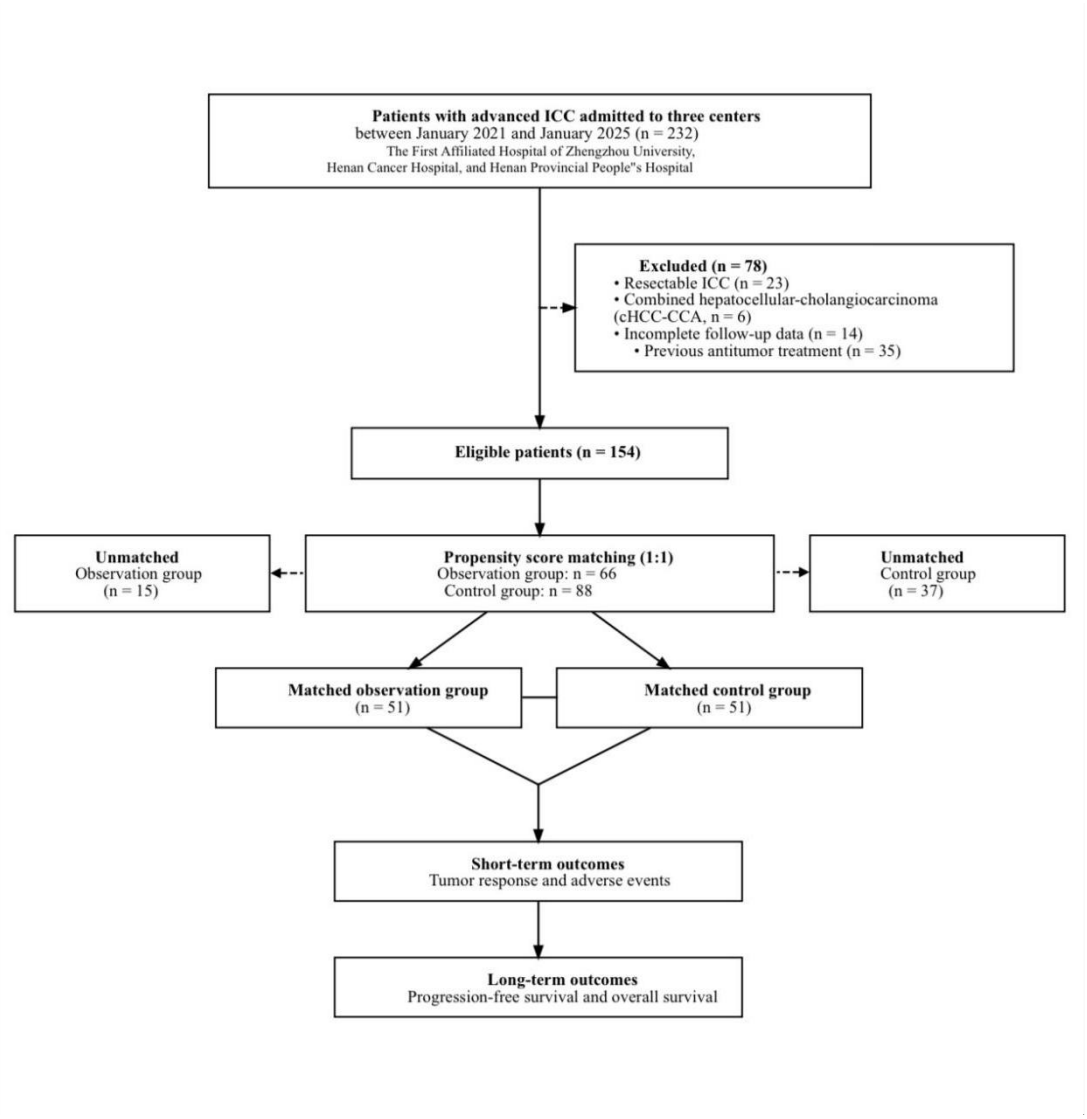


Fig. 1 Flowchart of this study. A total of 232 patients who underwent treatment in three centers from January 2021 to January 2025 were collected and divided into control and observation groups. After propensity-score matching, short- and long-term outcomes were compared.

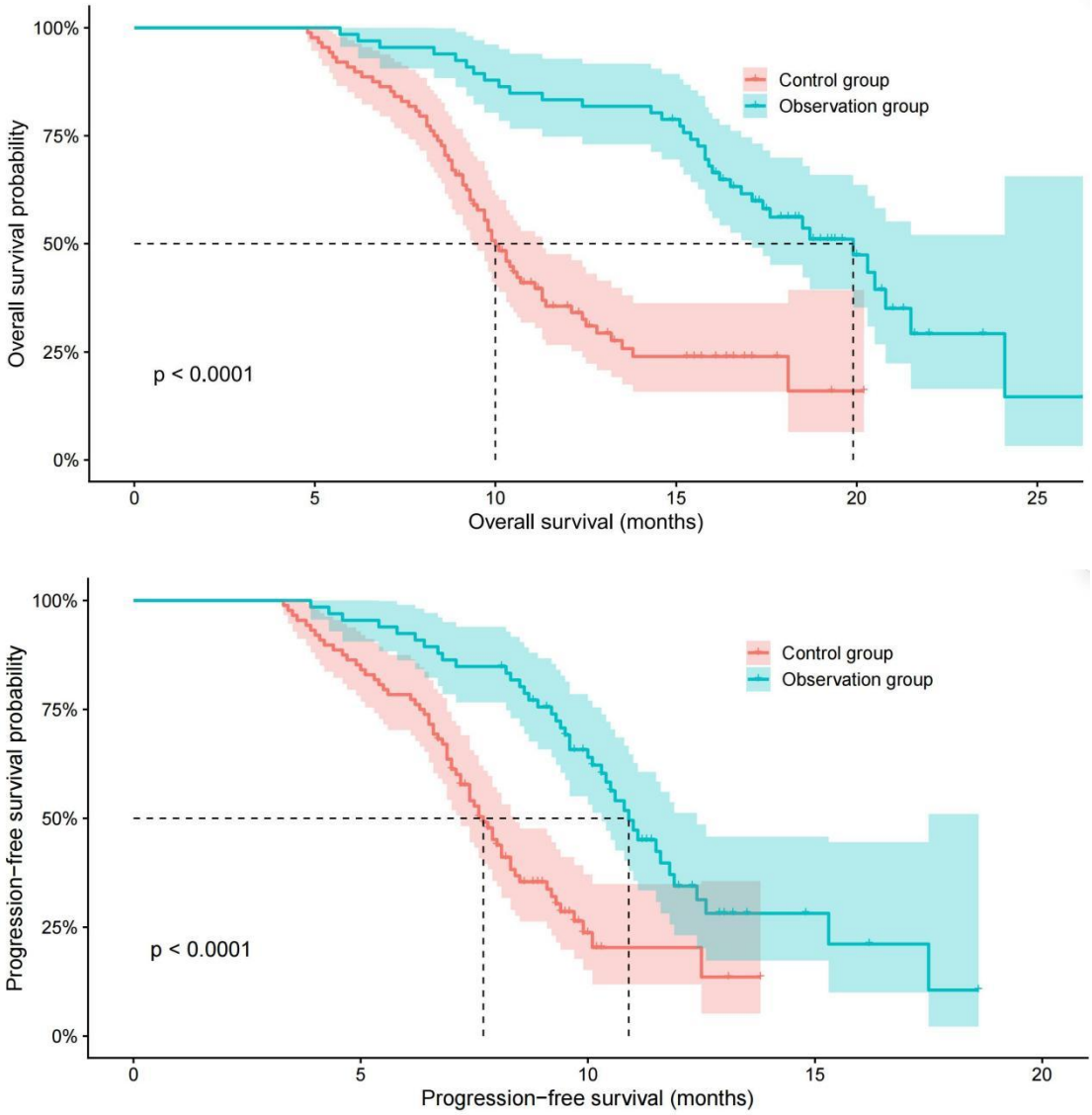
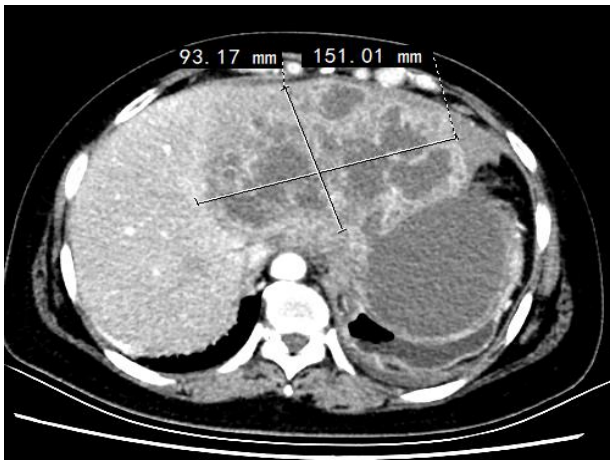
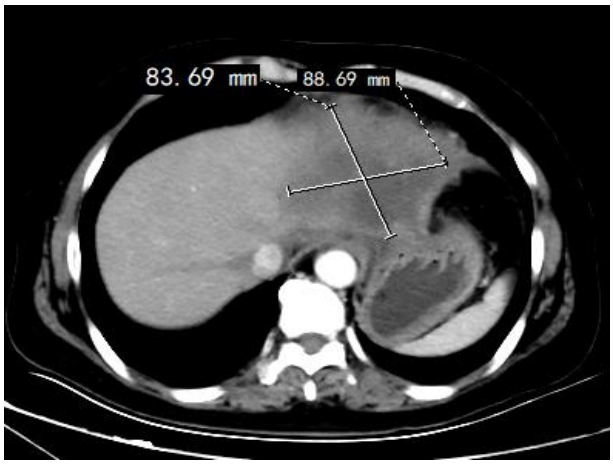


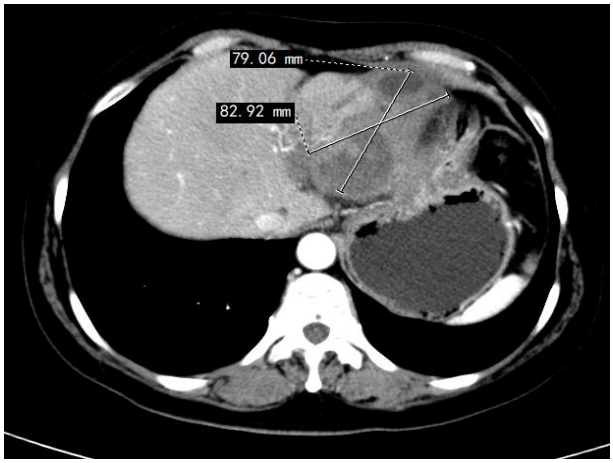
Fig. 2 Kaplan-Meier analyses of progression-free survival and overall survival in the two groups before PSM and after PSM



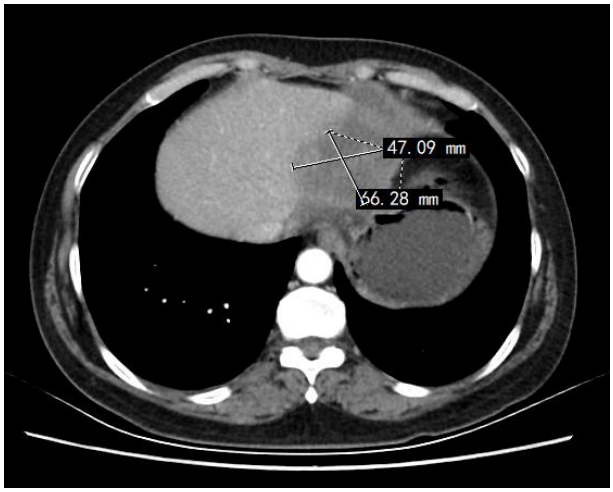
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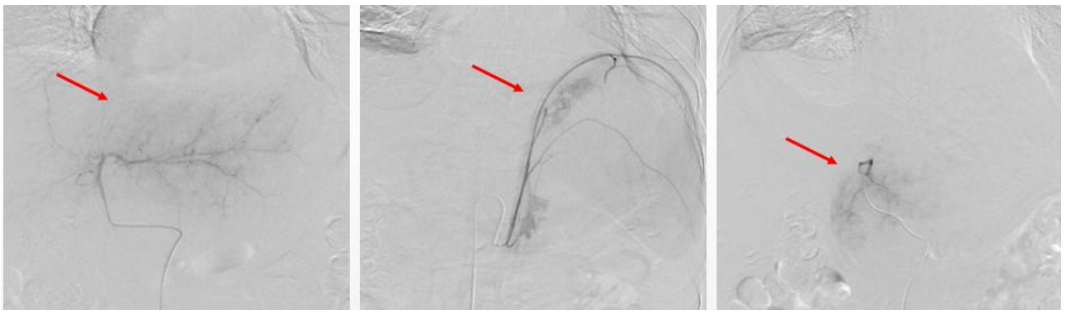
B



C



D



E



F

Fig. 3 A: Enhanced CT image before interventional treatment. **B:** Enhanced CT at 1.5 months after treatment showed significant reduction of the lesion, without obvious enhancement. **C:** Enhanced CT at 3 months after treatment showed further reduction of the lesion, but without significant changes. **D:** Enhanced CT at 6 months after treatment showed significant reduction of the lesion, without obvious enhancement. **E:** Digital subtraction angiography during the

interventional procedure showed that the hepatic lesion was stained and supplied by the left hepatic artery branch and the left phrenic artery. **F:** Image after interventional embolization, showing complete disappearance of tumor staining.

Table 1 Baseline characteristics of patients in the two groups before and after PSM

Characteristics	Before PSM				After PSM			
	Observation group (n=66)	Control group (n=88)	SMD	*P*	Observation group (n=51)	Control group (n=51)	SMD	*P*
Age,(x±s)	59.2±7.3	60.1±8.0	0.112	0.489	58.6±7.8	59.1±7.7	0.071	0.693
Gender,n(%)								
Male	38 (57.6%)	47 (53.4%)	0.083	0.607	26 (51.0%)	28 (54.9%)	0.079	0.832
Female	28 (42.4%)	41 (46.6%)			25 (49.0%)	23 (45.1%)		
Tumor size, (x±s)	4.8±1.4	4.8±1.6	0.008	0.959	4.7±1.5	4.7±1.5	0.001	0.994
Tumor number, (x±s)	1.7±0.9	1.9±1.0	0.159	0.333	1.7±0.9	1.7±0.9	0.066	0.684
Child,n(%)								
A	44 (66.7%)	54 (61.4%)	0.110	0.498	35 (68.6%)	35 (68.6%)	0.000	1.000
B	22 (33.3%)	34 (38.6%)			16 (31.4%)	16 (31.4%)		

Characteristics	Before PSM				After PSM			
	Observation group (n=66)	Control group (n=88)	SMD	*P*	Observation group (n=51)	Control group (n=51)	SMD	*P*
ECOG,n(%)								
0	41 (62.1%)	50 (56.8%)	0.107	0.508	32 (62.7%)	34 (66.7%)	0.082	0.824
1	25 (37.9%)	38 (43.2%)			19 (37.3%)	17 (33.3%)		
2	0 (0.0%)	0 (0.0%)			0 (0.0%)	0 (0.0%)		
CA199,(x±s)	47.0±23.9	49.1±26.8	0.084	0.609	46.1±25.3	45.5±24.8	0.022	0.900
TNM,n(%)								
III	42 (63.6%)	51 (58.0%)	0.116	0.476	34 (66.7%)	34 (66.7%)	0.000	1.000
IV	24 (36.4%)	37 (42.0%)			17 (33.3%)	17 (33.3%)		

PSM: Propensity Score Matching; ECOG: Eastern Cooperative Oncology Group; SMD: Standardized Mean Difference. SMD<0.1 considered as well balanced among baseline variables

244

245 **Table 2** Summary of response rates before and after PSM

All response, n(%)	Before PSM			After PSM		
	Observation group (n=66)	Control group (n=88)	*P*	Observation group (n=51)	Control group (n=51)	*P*
CR	5 (7.6%)	3 (3.4%)	0.289	3 (5.9%)	3 (5.9%)	1.000
PR	42 (63.6%)	27 (30.7%)	<0.001	33 (64.7%)	18 (35.3%)	0.004
SD	15 (22.7%)	31 (35.2%)	0.093	12 (23.5%)	16 (31.4%)	0.541
PD	4 (6.1%)	27 (30.7%)	<0.001	3 (5.9%)	14 (27.5%)	0.007
ORR	47 (71.2%)	30 (34.1%)	<0.001	36 (70.6%)	21 (41.2%)	0.003
DCR	62 (93.9%)	61 (69.3%)	<0.001	48 (94.1%)	37 (72.5%)	0.007

246 CR: Complete Response; PR: Partial Response; SD: Stable Disease; PD: Progression Disease; ORR: Objective

247 Response Rate; DCR: Disease Control Rate; PSM: Propensity Score Matching

248

249 **Table 3** Prognostic factors associated with PFS and OS after PSM

Variables	Progression-free survival				Overall survival			
	Univariate analysis		Multivariate analysis		Univariate analysis		Multivariate analysis	
	HR	95%CI *P*	HR	95%CI *P*	HR	95%CI *P*	HR	95%CI *P*

Group												
Control/ Observation	0.40	0.25–0.64	<0.001	0.29	0.17–0.49	<0.001	0.31	0.19–0.51	<0.001	0.22	0.13–0.38	<0.001
Age (Years) ≤60/>60	3.60	2.14–6.08	<0.001	2.81	0.50–15.83	0.241	3.39	2.08–5.54	<0.001	0.95	0.28–3.18	0.929
Gender Male/Female	0.88	0.53–1.46	0.618	—	—	—	0.57	0.35–0.92	0.022	0.61	0.37–1.01	0.053
Tumor size (cm) ≤5/>5	2.69	1.55–4.66	<0.001	0.15	0.04–0.49	0.002	2.85	1.67–4.88	<0.001	0.30	0.11–0.85	0.023
Tumor number 1/≥2	2.99	1.72–5.22	<0.001	3.20	0.36–28.71	0.298	3.13	1.82–5.38	<0.001	7.62	1.74–33.38	0.007
Child-Pugh Grade A/B	3.50	2.05–5.97	<0.001	1.12	0.50–2.51	0.788	3.47	2.12–5.71	<0.001	0.94	0.50–1.78	0.852
ECOG Score 0/1–2	3.86	2.14–6.98	<0.001	4.45	2.48–8.00	<0.001	2.67	1.51–4.73	<0.001	2.82	1.60–4.99	<0.001
CA199 ≤37 U/mL/	2.42	1.35–4.34	0.003	1.15	0.41–3.20	0.795	2.52	1.40–4.52	0.002	0.64	0.20–2.00	0.443

>37 U/mL												
TNM III/IV	3.72	2.21–6.27	<0.001	1.83	0.73–4.63	0.200	3.65	5.93	2.25–	<0.001	2.77	0.89–8.61	0.079

250 PFS: Progression-Free Survival; OS: Overall Survival; HR: Hazard Ratio; CI: Confidence Interva; PSM:

251 Propensity Score Matching

252

253 **Table 4** Incidence of adverse reactions of two groups during the treatment with

254 systemic therapy

Adverse Reaction	**Observation Group (*N*=51)**		**Control Group (N=51)**			
	Grades I~II (case)	Incidence (%)	Grades I~II (case)	Incidence (%)	χ^2	***P***
Fatigue	12	23.5	14	27.5	0.06	0.81
Anorexia	21	41.2	11	21.6	3.12	0.08
Vomiting	21	41.2	3	5.9	12.04	<0.01
Diarrhea	5	9.8	5	9.8	0.00	1.00
Hoarseness	12	23.5	13	25.5	0.00	1.00
Hypertension	8	15.7	7	13.7	0.00	1.00
Hand foot syndrome	8	15.7	7	13.7	0.00	1.00
Mucositis	13	25.5	9	17.6	0.41	0.52
Proteinuria	9	17.6	6	11.8	0.31	0.58

Hypoproteinemia	23	45.1	22	43.1	0.00	1.00
Hyperbilirubinemia	20	39.2	17	33.3	0.14	0.71
ALT increased	18	35.3	13	25.5	0.76	0.38
AST increased	20	39.2	13	25.5	2.12	0.15
Anemia	22	43.1	16	31.4	1.14	0.29
Neutropenia	21	41.2	12	23.5	2.78	0.10
Thrombocytopenia	19	37.3	14	27.5	0.76	0.38
Hypothyroidism	14	27.5	1	2.0	9.60	<0.01

Discussion

ICC originates from the epithelial cells of the intrahepatic bile ducts and is highly invasive, with a tendency toward multifocality, lymph node metastasis, and vascular invasion. As a result, most patients are already at an advanced stage at diagnosis, and the resection rate is low. Gemcitabine plus cisplatin is the standard first-line chemotherapy regimen for advanced ICC, with an overall tumor response rate of 10%–30% and a median OS of only 11.7 months. The limited efficacy and potential toxicity of this regimen have prompted the exploration of better treatment approaches.

ICIs can reverse the immunosuppressive state of the tumor microenvironment and have recently shown therapeutic potential in biliary tract malignancies. The emergence of immunotherapy has changed the treatment landscape of various solid tumors, including ICC, and PD-1 inhibitors have shown favorable efficacy in multiple solid tumors [11]. However, PD-1

inhibitor monotherapy has limited efficacy in advanced ICC. In recent years, combination treatment strategies have been extensively explored, including targeted therapy plus immunotherapy, targeted therapy plus immunotherapy plus chemotherapy, and targeted therapy plus TACE, all of which have shown advantages of combination therapy.Guo-Ming Shi et al. conducted a single-center, single-arm, phase II clinical study of toripalimab combined with lenvatinib and GEMOX as first-line treatment for advanced ICC [12]. With a median follow-up of 23.5 months, the ORR was 80%, the DCR was 93.3%, median OS was 22.5 months, and median PFS was 10.2 months. Grade ≥3 AEs occurred in 56.7% of patients but were controllable, including leukopenia in 23.3%. This prospective study suggested that toripalimab combined with lenvatinib and GEMOX is a promising first-line treatment for advanced ICC.Chengpei Zhu et al. conducted a multicenter real-world study of PD-1 inhibitors combined with lenvatinib and GEMOX as first-line treatment for advanced ICC [13]. The study included 53 patients with advanced ICC, with a median follow-up of 13.7 months (95% CI: 12.9–17.2). The ORR and DCR were 52.8% and 94.3%, respectively. Median PFS was 8.63 months (95% CI: 7.17–11.6), and median OS was 14.3 months (95% CI: 11.3–NR). These studies suggest that multimodal treatment combining immunotherapy with targeted therapy and chemotherapy can clearly improve outcomes in patients with ICC. Grade ≥3 AEs occurred in 41.5% of patients but were controllable. However, the incidence of adverse reactions was relatively high, and the regimen still needs optimization.

As a local treatment for advanced unresectable ICC, TACE can effectively induce tumor response. By increasing the local concentration of chemotherapeutic drugs and reducing the arterial blood supply to the tumor, TACE achieves local tumor control and has the advantage of

reducing systemic adverse reactions compared with systemic chemotherapy. However, TACE alone has limited efficacy in advanced ICC.

Anlotinib, as a multi-target antiangiogenic drug, can inhibit tumor angiogenesis and improve the hypoxic tumor microenvironment. Combined with TACE, it can achieve a synergistic effect of local tumor debulking and systemic antiangiogenic inhibition [14–17], and it is currently a commonly used comprehensive treatment strategy for advanced cholangiocarcinoma. However, real-world studies have shown that TACE combined with anlotinib still has shortcomings, such as poor control of distant metastasis and limited depth of tumor response, making it difficult to meet the need for long-term survival benefit.

TACE combined with anlotinib and PD-1 inhibitors has multiple synergistic advantages in terms of mechanism. TACE can cause ischemic necrosis of tumor tissue, release a large amount of tumor-specific antigen, and transform “cold tumors” into more immunogenic “hot tumors,” thereby amplifying the immune killing effect of PD-1 inhibitors. Anlotinib, as a multi-target antiangiogenic drug, can inhibit tumor angiogenesis and improve the hypoxic tumor microenvironment. At present, most related studies worldwide are single-arm exploratory studies of TACE + anlotinib + PD-1 inhibitors, and controlled studies with strictly matched baseline characteristics are lacking. Therefore, the real-world survival benefit of adding PD-1 inhibitors to the doublet regimen cannot be objectively evaluated. This study used 1:1 PSM to eliminate baseline confounding bias in a retrospective study, balance key confounders such as age, performance status, liver function, tumor burden, metastatic status, and treatment line, and conduct a head-to-head comparison of the efficacy, safety, and prognostic characteristics of the triplet versus doublet regimens, providing real-world evidence for individualized comprehensive

313 treatment of advanced cholangiocarcinoma.

314 The survival analysis in this study showed that after baseline balancing by PSM, median OS
315 and median PFS in the triplet group were significantly better than those in the TACE plus
316 anlotinib group, with simultaneous improvements in ORR and DCR. These results confirm that
317 adding PD-1 inhibitors to TACE plus anlotinib can further enhance systemic antitumor effects,
318 improving short-term tumor regression and significantly delaying disease progression and
319 prolonging OS. Possible synergistic mechanisms include the following: first, TACE causes local
320 tumor tissue necrosis and releases a large amount of tumor antigen, activating dendritic cells and
321 initiating antitumor immune responses; second, anlotinib remodels tumor blood vessels and
322 promotes the infiltration of CD8+ T cells and NK cells into the tumor; third, PD-1 inhibitors
323 relieve T-cell exhaustion and clear residual intrahepatic micrometastases and circulating
324 micrometastases, forming a complete therapeutic loop of “local tumor debulking, vascular
325 remodeling, and systemic immune clearance,” thereby compensating for the limited efficacy of
326 monotherapy.

327 Safety is an important consideration for the clinical promotion of comprehensive treatment
328 regimens. In this study, the incidence of adverse reactions in the two groups was evaluated
329 according to CTCAE 5.0. The results showed that there was almost no statistically significant
330 difference in the incidence of adverse reactions of any grade between the two groups, with
331 statistical differences only in vomiting and hypothyroidism. The triplet group did not experience
332 immune-related adverse reactions other than hypothyroidism, and no treatment-related deaths
333 occurred. There were also no statistically significant differences between the two groups in the
334 incidence of anlotinib-related hypertension, hand-foot skin reactions, or liver function impairment

after TACE. These findings suggest that the addition of PD-1 inhibitors does not significantly increase severe adverse reactions, and the overall tolerability is good. Patients with Child-Pugh A/B advanced cholangiocarcinoma could complete full-course treatment regularly, indicating strong clinical feasibility.

Multivariate Cox regression analysis in this study showed that the triplet treatment regimen was an independent factor affecting OS in patients with advanced cholangiocarcinoma, further confirming the survival benefit of combined immunotherapy. The direction of the effect of tumor size on OS and PFS reversed between univariate analysis and multivariate adjustment. This phenomenon may be explained by sample confounding, suppression effects, and insufficient model stability; therefore, this factor was not included in the final analysis and discussion.

This study has several limitations. It was a retrospective study with inevitable selection bias, and future multicenter cohort studies are needed for further validation. The types of PD-1 inhibitors included were not completely uniform, and differences in efficacy among different immune drugs were not analyzed by stratification. The follow-up time was limited, and 5-year long-term survival data were lacking, so the impact of the triplet regimen on long-term survival could not be evaluated. Molecular indicators such as genetic testing and immune markers were not included, so the population that would benefit most from the triplet regimen could not be identified.

In conclusion, this study used PSM to eliminate baseline confounders and confirmed that, compared with TACE plus anlotinib, TACE combined with anlotinib and PD-1 inhibitors significantly prolonged PFS and OS, improved ORR, and had controllable and tolerable adverse reactions in patients with advanced cholangiocarcinoma. For unresectable advanced

cholangiocarcinoma, the triplet regimen may be a preferred comprehensive treatment strategy and provides real-world evidence for combined interventional, targeted, and immunotherapy.

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