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Phase Ib study of anlotinib combined with TQB2450 in pretreated advanced biliary tract cancer and biomarker analysis

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Abstract

Background and Aims: We evaluated the efficacy and safety of the antiangiogenic tyrosine kinase inhibitor anlotinib plus TQB2450, a programmed death-ligand 1 inhibitor in pretreated advanced biliary tract cancers (BTCs).

Approach and Results: In this pooled analysis of two single-center, phase Ib clinical trials (TQB2450-Ib-05 and TQB2450-Ib-08 trials), 66 patients with advanced BTCs who had progressed or declined or were ineligible for first-line chemotherapy were included. With the treatment of anlotinib plus TQB2450, two patients achieved complete response, and 12 had a partial response assessed by Response Evaluation Criteria in Solid Tumors 1.1, yielding an objective response rate of 21.21%, a disease control rate (DCR)

Abbreviations: AE, adverse event; BTC, biliary tract cancer; CBR, clinical benefit rate; DCR, disease control rate; DoR, duration of response; EHCC, extrahepatic cholangiocarcinoma; FGF, fibroblast growth factor; GBC, gallbladder cancer; ICI, immune checkpoint inhibitor; IHCC, intrahepatic cholangiocarcinoma; IQR, interquartile range; NGS, next-generation sequencing; NR, not reached; ORR, objective response rate; OS, overall survival; PD-1, programmed cell death protein 1; PD-L1, programmed death-ligand 1; PFS, progression-free survival; RECIST, Response Evaluation Criteria in Solid Tumors; SD, stable disease; TKI, tyrosine kinase inhibitor; TMB, tumor mutational burden; TRAE, treatment-related AE.

Jun Zhou and Yongkun Sun contributed equally to this work.

Trial registration: The TQB2450-Ib-05 and TQB2450-Ib-08 trials were registered on ClinicalTrials.gov (Identifiers: NCT03825705 and NCT03996408, respectively).

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of 72.73%, and a clinical benefit rate (CBR) of 42.42%. With a median follow-up of 19.68 months, median progression-free survival (PFS) and overall survival (OS) were 6.24 (95% confidence interval [CI], 4.11–8.25) and 15.77 (95% CI, 10.74–19.71) months, respectively. Adverse events (AEs) were reported in 64 (96.97%) patients, and the most common grade 3 or worse treatment-related AEs included elevated levels of aspartate aminotransferase (7.58%), alanine aminotransferase (6.06%), and hypertension (6.06%). Patients with high tumor mutational burden (TMB; $\geq$5 mutations/Mbp) had a better CBR (70.8% vs. 22.2%), longer OS (14.32 vs. 9.64 months), and a trend toward longer PFS (7.03 vs. 4.06 months). Patients with *kirsten rat sarcoma viral oncogene homolog* (*KRAS*) mutations showed a lower CBR (12.5% vs. 58.8%) and shorter PFS (2.02 vs. 6.80 months) and OS (10.53 vs. 13.13 months).

Conclusions: Anlotinib combined with TQB2450 showed promising efficacy and was well tolerated in advanced BTCs. *KRAS* mutation and high TMB might serve as predictors of treatment efficacy.

INTRODUCTION

Biliary tract cancers (BTCs), a heterogeneous group of cancers consisting of intrahepatic cholangiocarcinoma (IHCC), extrahepatic cholangiocarcinoma (EHCC), and gallbladder cancer (GBC), are characterized by a relatively low but increasing incidence. Late diagnosis, a high relapse rate after surgery, and refractoriness to treatments contribute to poor prognosis in patients with BTCs, with a 5 year survival rate below 20%.[1] Advanced BTCs are often refractory to chemotherapy, and the response rate for first-line chemotherapy combining gemcitabine and cisplatin is only about 25%–30%, with a median overall survival (OS) of 11–13 months.[2,3] The efficacy of second-line chemotherapy for BTCs remains dismal. The ABC-06 study showed that combination therapy of leucovorin, fluorouracil, and oxaliplatin (FOLFOX) only achieved an objective response rate (ORR) of 5% and an OS of 6.2 months,[4] and the NIFTY study reported that liposomal irinotecan in combination with 5-fluorouracil/leucovorin achieved a median OS of 8.6 months.[5]

Targeted therapies have shown promising therapeutic effects in patients with specific gene alterations, as pemigatinib monotherapy for patients with advanced BTC with *fibroblast growth factor (FGF) receptor 2* fusion/rearrangement achieved an ORR of 37.0% and a median OS of 17.5 months,[6] whereas ivosidenib exhibited a significantly longer OS than the placebo (10.3 vs. 5.1 months, $p < 0.0001$) in patients with refractory BTC with isocitrate dehydrogenase-1 mutation.[7] As for *v-raf murine sarcoma viral oncogene homolog B1* (*BRAF*) *V600E*-mutated BTCs, dabrafenib plus trametinib showed an ORR of 47% in the ROAR study.[8] The results of the MyPathway trial demonstrated that the combination of trastuzumab and pertuzumab reached an ORR of 23% in *human epidermal growth factor receptor 2*-positive BTCs.[9] However, only a small proportion of patients harboring these gene alterations could benefit from the targeted therapy accordingly. Therefore, there is still an urgent need for developing therapeutic strategies for patients with advanced BTCs, especially in second-line treatments and later line treatments.

The application of immune checkpoint inhibitors (ICIs) is a breakthrough in the treatment of malignancies. Pembrolizumab has been proposed for the treatment of microsatellite instability-high solid tumors including BTCs,[10] whereas most clinical studies of ICIs as monotherapy have shown limited therapeutic efficacy in all-comer patients with advanced BTCs, with an ORR of 3%–13% and an OS of 5.2–7.4 months.[11] To date, a phase II nivolumab trial reported an OS of 14.2 months in patients with advanced BTCs (with 43% of patients having programmed death-ligand 1 [PD-L1]+).[12] Combination therapy, including ICIs, is expected to be an effective strategy for addressing this dilemma, and the TOPAZ-1 trial just demonstrated the advantages of durvalumab combined with chemotherapy in first-line therapy.[13] Furthermore, the combination of ICIs and angiogenesis inhibitors is considered another promising strategy for patients with advanced BTCs based on its synergistic effects and successful application in hepatocellular carcinoma (HCC),[14] renal cell carcinoma,[15] and non–small cell lung cancer (NSCLC).[16] Although several single-arm trials have explored the preliminary efficacy of

such combinations,[17–19] the associated survival benefits require further investigation.

Anlotinib is a multitargeted antiangiogenic tyrosine kinase inhibitor (TKI) approved for advanced lung cancer and soft-tissue sarcoma and has shown a noteworthy efficacy as monotherapy in patients with advanced HCC.[20] Furthermore, in vivo studies[21] and case reports[22,23] have preliminarily demonstrated the application of anlotinib for the treatment of patients with BTCs, providing a rationale for further evaluation of the efficacy of anlotinib in patients with advanced BTCs. TQB2450 is a PD-L1 inhibitor developed by Chia Tai Tianqing Pharmaceutical Group Co. Ltd. (Nanjing, China), which is currently undergoing several clinical studies in China involving BTCs and other solid tumors.

Thus, two phase Ib trials of anlotinib in combination with TQB2450 for pretreated advanced BTCs were conducted at the Cancer Hospital of the Chinese Academy of Medical Sciences and Peking University Cancer Hospital. Given that these two trials were conducted with almost the same regimen during the same time frame, we aimed to conduct a pooled analysis and to preliminarily assess the efficacy and safety of anlotinib plus TQB2450 for pretreated advanced BTCs. In addition, biomarker analysis was performed to identify potential predictors of efficacy for enlightening the precise therapeutic strategies.

PATIENTS AND METHODS

Study design

This study was a pooled analysis of two single-center, phase Ib clinical trials (identifiers: TQB2450-Ib-05 and TQB2450-Ib-08); only patients with advanced BTCs had progressed after first-line chemotherapy or who were ineligible for or declined first-line chemotherapy were enrolled.

The inclusion criteria for the original trials were as follows: (1) age $\geq$ 18 years; (2) Eastern Cooperative Oncology Group performance status score of 0 or 1[24]; (3) life expectancy of at least 3 months; (4) histologically or pathologically confirmed unresectable or metastatic BTCs, including IHCC, EHCC, and GBC, with at least one measurable lesion according to the Response Evaluation Criteria in Solid Tumors (RECIST) version 1.1 criteria[25]; (5) occurrence of disease progression after first-line systemic therapy or intolerance because of adverse events (AEs). The TQB2450-Ib-08 trial additionally included patients with BTCs who were not eligible for or declined the first-line standard treatment. The details of TQB2450-Ib-05 and TQB2450-Ib-08 trials are shown in the supplementary materials.

The TQB2450-Ib-05 and TQB2450-Ib-08 trials were both approved by the institutional review boards (NCC1848: December 25, 2018, and 2019YW41: March 25, 2019), and all patients provided signed informed consent. The two trials were conducted according to the principles of the Declaration of Helsinki and Good Clinical Practice requirements. They were registered on ClinicalTrials.gov (identifiers: NCT03825705 and NCT03996408).

Procedure

Patients in both trials received the same treatment, consisting of an intravenous infusion of TQB2450 (1200 mg, on day 1) and oral administration of anlotinib (once a day, from day 1 to day 14), a regimen that was repeated every 3 weeks until disease progression, occurrence of intolerable toxicities, or the patient withdrew from the study. Briefly, 1200 mg of TQB2450 was dissolved in 250 ml of saline, and the infusion time was around 60 min. The initial dose of anlotinib was 10 mg; if it was well tolerated during the course of the trial, the dosage was increased to 12 mg. The dosage of 12 mg was considered a safe dose if one sixth or fewer of the patients developed dose-limiting toxicity; otherwise, 10 mg was ultimately recommended as a safe dosage. In the case of two sixths or more of patients who were administered a 10-mg dose developed dose-limiting toxicities, the dose was reduced to 8 mg; if the 8-mg dose was still intolerable, the trial would be terminated.

Disease progression (evaluated according to RECIST 1.1) was re-evaluated by investigators according to a modified RECIST 1.1 for immune-based therapeutics. The treatment would continue in case the patients might benefit from it.

Endpoints

The efficacy endpoints included the ORR, disease control rate (DCR), clinical benefit rate (CBR; defined as the proportion of patients with a complete response [CR], a partial response [PR], and stable disease [SD] persisting for $\geq$24 weeks), duration of response (DoR), progression-free survival (PFS), and OS. The ORR was evaluated by RECIST 1.1.[25] Tumor assessments were performed every 6 weeks until week 54 and then every 9 weeks. Safety was assessed using Common Terminology Criteria for AEs (Ver. 5.0).

Detection of PD-L1 expression

PD-L1 expression was determined by immunohistochemistry using VENTANA PD-L1 (SP263) primary antibodies (Roche Diagnostics, Basel, Switzerland) and

stratified based on the combined positive score (CPS) < 5 or CPS ≥ 5. CPS was calculated as the number of PD-L1–positive cells (tumor cells, lymphocytes, and macrophages) divided by the total number of tumor cells and multiplied by 100.

Next-generation sequencing detection

Formalin-fixed, paraffin-embedded tissue specimens were analyzed by next-generation sequencing (NGS; Cancer Sequencing YS panel [CSYS][26]), which targeted all the exons of 450 genes and introns of 39 genes with an average coverage of at least 1000×. The test was carried out by OrigiMed, a College of American Pathologists–accredited and Clinical Laboratory Improvement Amendments–certified laboratory (Shanghai, China).

Tumor mutational burden (TMB) scores were calculated from CSYS data for each sample by counting somatic mutations, including coding single nucleotide variants and indels, per megabase of the sequence examined. Known somatic mutations in the Catalog of Somatic Mutations in Cancer and known germline polymorphisms in the US National Center for Biotechnology Information's Single Nucleotide Polymorphism Database were not counted.[27] The best cutoff value of TMB in this study was obtained from the optimal critical value calculated by the receiver-operating characteristic (ROC) curve analysis of CBR.

Statistical analysis

In phase Ib trials, the sample size of both trials was not based on efficacy benefit and type I error considerations. The sample size of 20–30 patients in the dose-expansion phase was designed to evaluate preliminary efficacy and safety.

Statistical analysis was performed with the SAS 9.4 software (SAS Institute, Cary, North Carolina), and NGS data were analyzed with the R 3.5.7 software (R Foundation for Statistical Computing, Vienna, Austria). Differences in continuous variables were assessed by two-tailed unpaired t-test, and differences in categorical variables were examined by the chi-square test or Fisher's exact test. Median PFS, OS, and DoR were calculated by the Kaplan-Meier method and displayed as median (95% CI). ORR and DCR were evaluated with point estimates and 95% CIs. Duration of follow-up was calculated by the reverse Kaplan-Meier estimate of OS. A value of $p < 0.05$ was considered statistically significant.

RESULTS

Baseline characteristics and treatment

A total of 66 patients with BTC were included, including 34 from the TQB2450-Ib-05 trial and 32 from the TQB2450-Ib-08 trial (Figure 1), with a median age of 58 (range, 35–75) years. The primary tumor locations in most cases were the intrahepatic bile duct (46.97%),

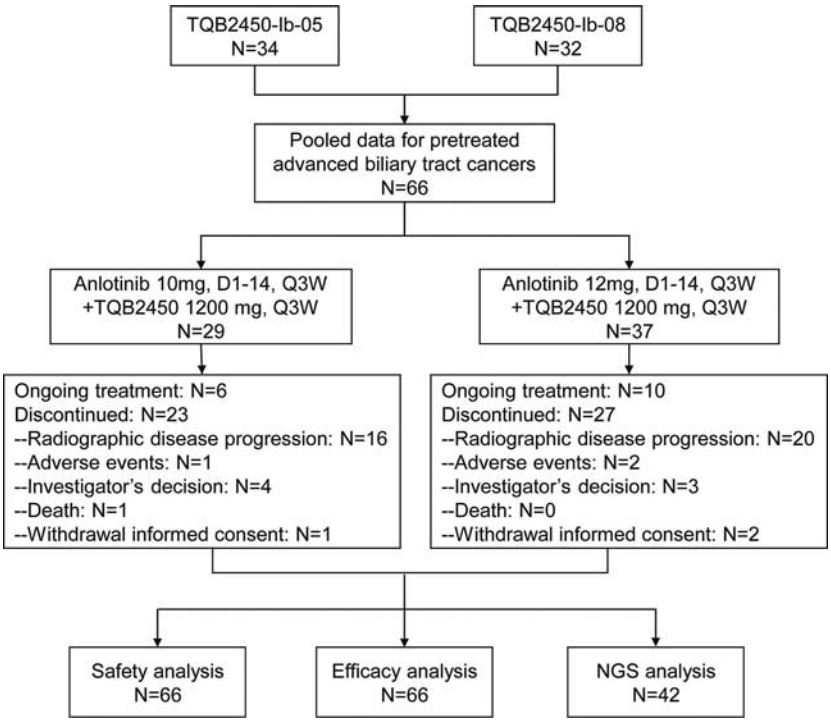

FIGURE 1 Study flowchart. NGS, next-generation sequencing. D1–14, from day 1 to day 14; Q3W, every 3 weeks.

TABLE 1 Baseline characteristics

Characteristics	Total (n = 66)	Anlotinib (10 mg) (n = 29)	Anlotinib (12 mg) (n = 37)
Age, years old, median (range)	58 (35–75)	57 (37–75)	58 (35–74)
Age group, n (%)			
<65	52 (78.79)	22 (75.86)	30 (81.08)
≥65 years old	14 (21.21)	7 (24.14)	7 (18.92)
Male, n (%)	31 (46.97)	15 (51.72)	16 (43.24)
ECOG PS, n (%)			
0	43 (65.15)	19 (65.52)	24 (64.86)
1	23 (34.85)	10 (34.48)	13 (35.14)
HBV infection[b], n (%)	10 (15.15)	4 (13.79)	6 (16.22)
Tumor location, n (%)			
Gallbladder	20 (30.30)	6 (20.69)	14 (37.84)
Intrahepatic bile duct	31 (46.97)	19 (65.52)	12 (32.43)
Extrahepatic bile duct	15 (22.73)	4 (13.79)	11 (29.73)
Sum of the diameter of target lesions at baseline, mm, median (range)	54 (10–201)	51 (10–201)	57 (11–186)
CA199 level at baseline, U/ml, median (range)	81.5 (1.1–11,647)	61.2 (1.1–11,647)	148.3 (2–3093)
PD-L1 expression[a], n (%)			
CPS < 5	23 (58.97)	8 (66.67)	15 (55.56)
CPS ≥ 5	16 (41.03)	4 (33.33)	12 (44.44)
Number of metastatic sites, n (%)			
1	26 (39.39)	9 (31.03)	17 (45.95)
2	19 (28.79)	12 (41.38)	7 (18.92)
3	14 (21.21)	6 (20.69)	8 (21.62)
4	4 (6.06)	1 (3.45)	3 (8.11)
Missed data	3 (4.55)	1 (3.45)	2 (5.41)
Metastatic site, n (%)			
Liver	30 (45.45)	16 (55.17)	14 (37.84)
Lung	23 (34.85)	14 (48.28)	9 (24.32)
Bone	5 (7.58)	2 (6.9)	3 (8.11)
Number of previous treatments, n (%)			
0	2 (3.03)	0	2 (5.41)
1	53 (80.3)	24 (82.76)	29 (78.38)
2	9 (13.64)	4 (13.79)	5 (13.51)
≥3	2 (3.03)	1 (3.45)	1 (2.7)
Prior surgery, n (%)	51 (77.27)	20 (68.97)	31 (83.78)
Prior radiotherapy, n (%)	13 (19.70)	8 (27.59)	5 (13.51)

Abbreviations: CA-199: carbohydrate antigen 199; CPS: combined positive score; ECOG: Eastern Cooperative Oncology Group Performance Score; HBV: hepatitis B virus; PD-L1: programmed death-ligand 1.

[a]The proportions of patients with different PD-L1 expression levels were calculated based on the number of evaluable patients.

[b]All the enrolled patients were without hepatitis C virus infection.

gallbladder (30.30%), and extrahepatic bile duct (22.73%). A total of 39 patients had available PD-L1 data, with 23 (58.97%) and 16 (41.03%) patients showing CPS < 5 and CPS ≥ 5, respectively (Table 1). Among 66 patients, 29 and 37 received 10 and 12 mg anlotinib, respectively. Patients' demographic and clinical characteristics are presented in Table 1. The data cutoff date was June 15, 2021, with a median follow-up of 19.68 (95% CI, 16.33–20.44) months. The median duration of treatment was 4.98 (interquartile range [IQR]: 2.53–9.86) months (including nine patients who continued treatment one or more cycles after progression, according to the investigator's decision), which was 4.60 (IQR: 2.53–9.86) and 5.85 (IQR: 2.53–9.43) months in the 10-mg and 12-mg anlotinib subgroups, respectively.

TABLE 2 Efficacy

	Total (n = 66)	Anlotinib (10 mg, n = 29)	Anlotinib (12 mg, n = 37)
ORR	14 (21.21)	3 (10.34)	11 (29.73)
DCR	48 (72.73)	21 (72.41)	27 (72.97)
CBR	28 (42.42)	9 (31.03)	19 (51.35)
CR	2 (3.03)	0	2 (5.41)
PR	12 (18.18)	3 (10.34)	9 (24.32)
SD	34 (51.52)	18 (62.07)	16 (43.24)
PD	13 (19.70)	6 (20.69)	7 (18.92)
NA	5 (7.58)	2 (6.90)	3 (8.11)

Note: All data were presented as n (%).
Abbreviations: CR, complete response; DCR, disease control rate; NA, not available for efficacy assessment; ORR: objective response rate; PD, progressive disease; PR: partial response; SD: stable disease.

Efficacy

As shown in Table 2, two patients achieved a CR, and 12 achieved a PR; the ORR for the total population was 21.21% (95% CI, 12.11%–33.02%). Figure 2 shows the best diameter changes from baseline of target lesions, with the target lesions decreasing in 67.21% (41/61) of the evaluated patients. Besides, 34 patients achieved SD, including 28 cases, with SD lasting for ≥24 weeks, indicating a DCR of 72.73% (95% CI, 60.36%–82.97%) and a CBR of 42.42% (95% CI, 30.34%–55.21%). Median PFS was 6.24 (95% CI, 4.11–8.25) months (Figure 3A). The DoR was not reached (NR). Accordingly, the median OS was 15.77 (95% CI, 10.74–19.71) months (Figure 3B).

Efficacy was further evaluated according to anlotinib dosage (Table 2). It was found that the ORR of patients administered 10 mg anlotinib was 10.34% (95% CI, 2.19%–27.35%), and median PFS and OS were 4.86 (95% CI, 3.15 to NR) and 13.54 (95% CI, 8.94–19.71) months, respectively. In patients who received 12 mg anlotinib, the ORR was 29.73% (95% CI, 15.87%–46.98%), and median PFS and OS were 6.87 (95% CI, 3.45–14.32) and 18.92 (95% CI, 10.61 to NR) months, respectively.

According to PD-L1 expression, median PFS was 6.80 (95% CI, 4.11 to NR) months in patients with CPS ≥ 5 and CPS ≥ 6.24 (95% CI, 2.76 to NR) months in those with CPS < 5. The longest OS was noted in patients with GBC, with a median OS of 19.71 (95% CI, 6.08–21.16) months; in patients with IHCC, the median OS was 15.51 (95% CI, 10.32 to NR) months versus 14.32 (95% CI, 4.63–22.54) months in patients with EHCC.

Safety

AEs were reported in 96.97% (64/66) of patients, including 89.39% (59/66) with treatment-related AEs (TRAEs). The incidence rates of TRAEs were 96.55% and 83.78% in patients administered 10 and 12 mg of anlotinib, respectively. The most common TRAEs were hypothyroidism (54.55%), leukopenia (42.42%), elevated aspartate aminotransferase (40.91%), diarrhea (40.91%), and elevated bilirubin (39.39 %). The incidence of TRAEs with grade 3 or higher was 25.76% (17/66). The most common TRAEs with grade 3 or higher were elevated aspartate aminotransferase (7.58%, n = 5), alanine aminotransferase (6.06%, n = 4), and hypertension (6.06%, n = 4). The incidence of immune-related AEs was 46.97% (n = 31), whereas 16.67% of patients experienced immune-related AEs with grade 3 or higher.

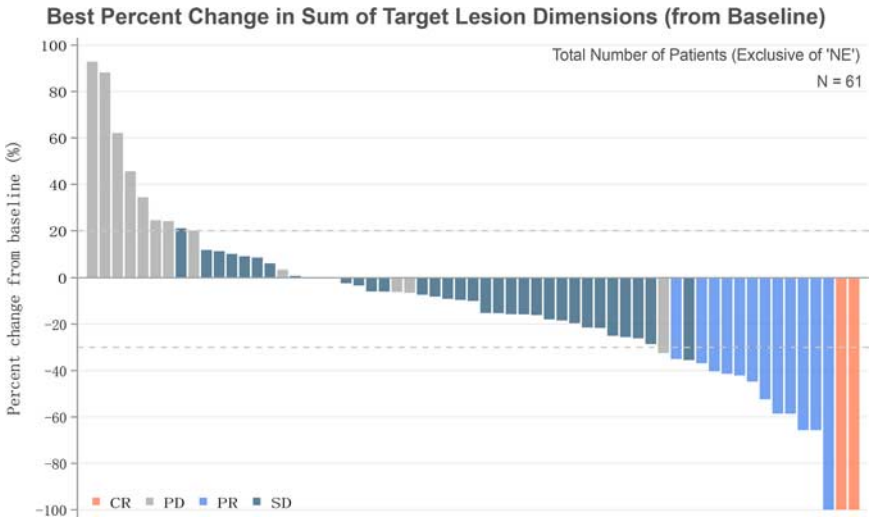

FIGURE 2 Diameter changes of target lesions from baseline. CR, complete response; NE, not evaluable; PD, progressive disease; PR, partial response; SD, stable disease.

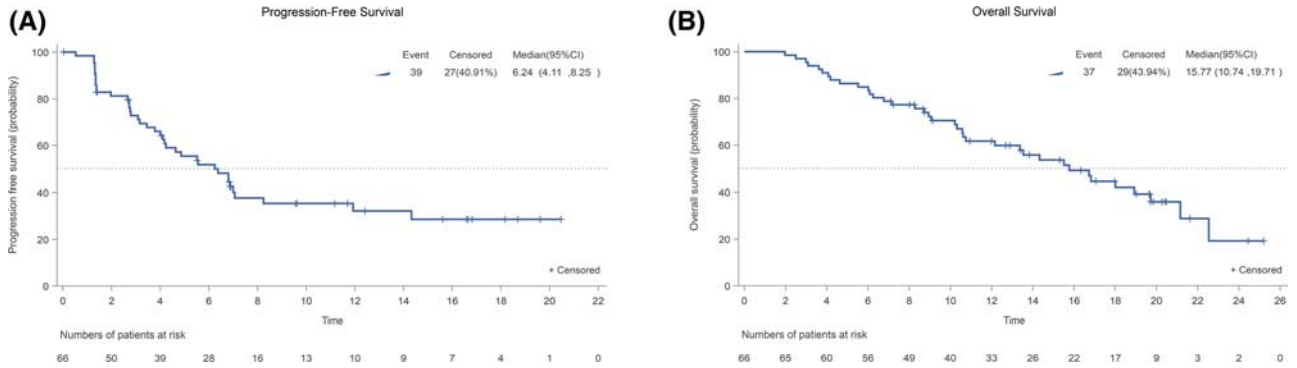

FIGURE 3 Progression-free survival (A) and overall survival (B) in the total population. CI, confidence interval.

TABLE 3 Treatment-related adverse events

Treatment-related adverse events	Total (n = 66) Any grade	Grades 3–5	Anlotinib (10 mg, n = 29) Any grade	Grades 3–5	Anlotinib (12 mg, n = 37) Any grade	Grades 3–5
Hypothyroidism	36 (54.55)	0	21 (72.41)	0	15 (40.54)	0
Leukopenia	28 (42.42)	0	12 (41.38)	0	16 (43.24)	0
Elevated aspartate aminotransferase	27 (40.91)	5 (7.58)	13 (44.38)	2 (6.90)	14 (37.84)	3 (8.11)
Diarrhea	27 (40.91)	1 (1.52)	19 (65.52)	0	8 (21.62)	1 (2.70)
Elevated bilirubin	26 (39.39)	1 (1.52)	14 (48.28)	0	12 (32.43)	1 (2.70)
Hand-foot syndrome	26 (39.39)	0	17 (58.62)	0	9 (24.32)	0
Asthenia	26 (39.39)	2 (3.03)	17 (58.62)	0	9 (24.32)	2 (5.41)
Elevated alanine aminotransferase	25 (37.88)	4 (6.06)	12 (41.38)	1 (3.45)	13 (35.14)	3 (8.11)
Elevated low-density lipoprotein	25 (37.88)	0	15 (51.72)	0	10 (27.03)	0
Hypertension	23 (34.85)	4 (6.06)	15 (51.72)	2 (6.90)	8 (21.62)	2 (5.41)
Proteinuria	21 (31.82)	0	12 (41.38)	0	9 (24.32)	0
Neutrophil count decreased	20 (30.30)	0	8 (27.59)	0	12 (32.43)	0
Prolonged QT interval	19 (28.79)	1 (1.52)	16 (55.17)	1 (3.45)	3 (8.11)	0
Gingival bleeding	19 (28.79)	0	12 (41.38)	0	7 (18.92)	0
Hyperthyroidism	18 (27.27)	0	6 (20.69)	0	12 (32.43)	0
Hypertriglyceridemia	18 (27.27)	0	11 (37.93)	0	7 (18.92)	0
Platelet count decreased	16 (24.24)	1 (1.52)	7 (24.14)	0	9 (24.32)	1 (2.70)
Rash	15 (22.73)	1 (1.52)	6 (20.69)	1 (3.45)	9 (24.32)	0
Hematuria	15 (22.73)	0	10 (34.48)	0	5 (13.51)	0
Hypercholesterolemia	12 (18.18)	0	8 (27.59)	0	4 (10.81)	0
Hyperglycemia	9 (13.64)	0	6 (20.69)	0	3 (8.11)	0
Abdominal pain	9 (13.64)	0	8 (27.59)	0	1 (2.70)	0
Dysphonia	8 (12.12)	0	4 (13.79)	0	4 (10.81)	0
Decreased appetite	5 (7.58)	0	4 (13.79)	0	1 (2.70)	0
Vomiting	5 (7.58)	0	5 (17.24)	0	0	0
Oropharyngeal pain	5 (7.58)	0	4 (13.79)	0	1 (2.70)	0
Back pain	5 (7.58)	0	5 (17.24)	0	0	0
Creatinine increased	4 (6.06)	0	1 (3.45)	0	3 (8.11)	0
Subungual bleeding	4 (6.06)	0	2 (6.90)	0	2 (5.41)	0
Myalgia	4 (6.06)	1 (1.52)	2 (6.90)	1 (3.45)	2 (5.41)	0

Note: All data were presented as n (%).

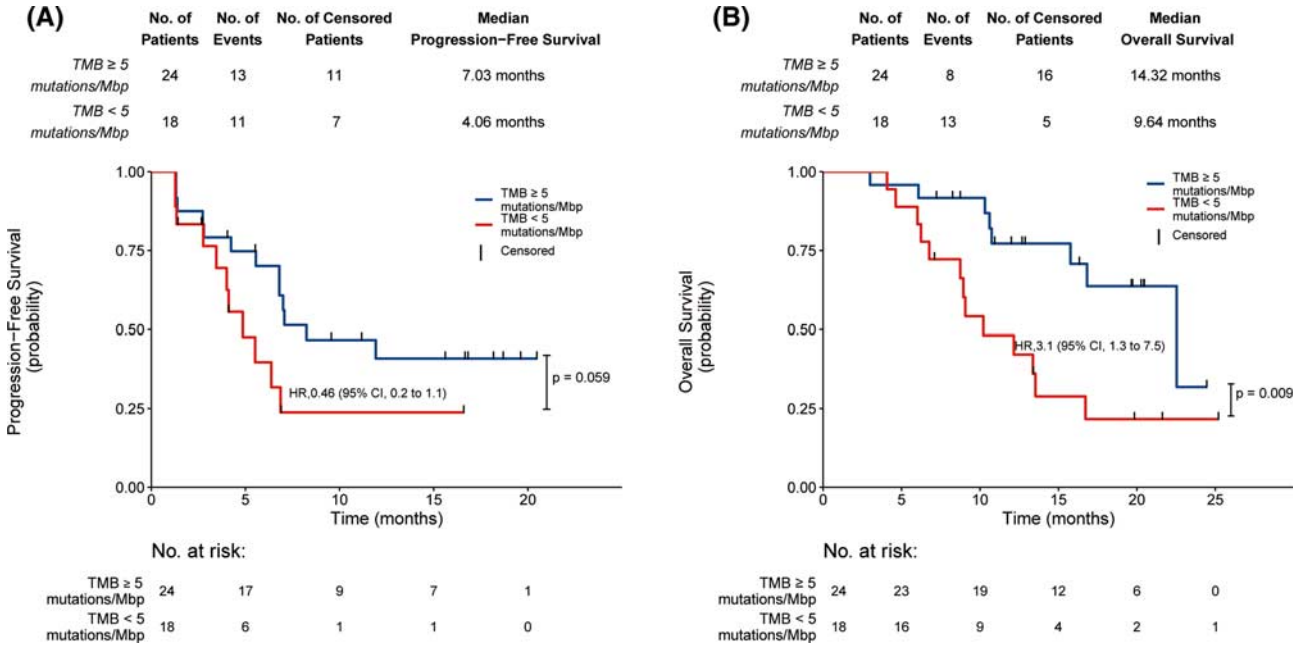

FIGURE 4 Progression-free survival (A) and overall survival (B) in patients with TMB ≥ 5 and TMB < 5 mutations/Mbp. CI, confidence interval; HR, hazard ratio; TMB, tumor mutational burden.

The detailed characteristics of TRAEs, including anlotinib dosage subgroup analysis, are shown in Table 3.

Severe AEs were reported in seven (10.61%) patients. Dosage modification or interruption of any treatment because of AEs occurred in 22 (33.33%) patients. In addition, seven (10.61%) patients discontinued any treatment with anlotinib or TQB2450 because of AEs.

The landscape of genomic alternations and potential predictive factors of treatment response

According to NGS, *tumor protein 53* (*TP53*; 45%), *epidermal growth factor receptor* (36%), *mucins 16* (35%), *kirsten rat sarcoma viral oncogene homolog* (*KRAS*; 19%), and *obscurin* (19%) were the most frequently mutated genes (Figure S1).

The median TMB was 6.45 mutations/Mbp. ROC curve analysis of CBR indicated that the best cutoff value for TMB was 5.05 (Figure S2). For the convenience of clinical application, the value of 5 was chosen as the best prediction cutoff. Patients with high TMB (≥5 mutation/Mb, $n = 24$) had a better CBR than those with low TMB ($n = 18$; CBR, 70.8% vs. 22.2%; $p = 0.004$), as well as a longer median OS (14.32 vs. 9.64 months, $p = 0.009$) and a trend of longer median PFS (7.03 vs. 4.06 months, $p = 0.059$) (Figure 4). Based on the significant correlation between TMB and treatment efficacy, gene and pathway mutation profiles were analyzed between patients with CBR and patients without CBR. Significant differences were found in *AT-rich interaction domain 1B* (*ARID1B*) and *KRAS* mutations ($p = 0.0207$ and 0.0448, respectively). It is noteworthy that the Switch/Sucrose Nonfermentable (SWI/SNF) pathway was found to be the most affected, and its mutation rate was higher in the CBR group (Figure S3).

Patients with *KRAS* mutations ($n = 8$) had a lower CBR (12.5 vs. 58.8%, $p = 0.045$) and shorter median PFS (2.02 vs. 6.80 months, $p < 0.001$) and OS (10.53 vs. 13.13 months, $p = 0.038$) (Figure 5). The median PFS and OS of patients with mutated *ARID1B* ($n = 6$) and wild-type gene were 13.08 versus 4.55 months ($p = 0.082$) and 17.81 versus 10.84 ($p = 0.074$) (Figure S4A,B). Patients with mutated *AT-rich interaction domain 1A* (*ARID1A*) ($n = 4$) and wild-type gene showed median PFS of 1.36 and 6.59 months, respectively ($p < 0.001$), and OS of 11.38 and 12.78 months, respectively ($p = 0.023$) (Figure S4C, D). Two patients harboring co-occurrence of *KRAS* and *ARID1A* mutations. Furthermore, the median PFS and OS of patients with abnormal FGF pathway ($n = 10$, 23.8%) were numerically longer than those of cases with normal FGF, but no significant difference was found (median PFS, 6.85 vs. 5.52 months; $p = 0.518$; and median OS, 16.59 vs. 10.84 months; $p = 0.268$).

DISCUSSION

In this pooled analysis of two phase Ib trials, anlotinib plus TQB2450 demonstrated promising clinical activity in pretreated advanced BTCs, with an ORR of 21.21% (14/66), median PFS of 6.24 months, and median OS of 15.77 months. These results seemed to be superior to those of chemotherapy or TKIs or ICIs as monotherapy

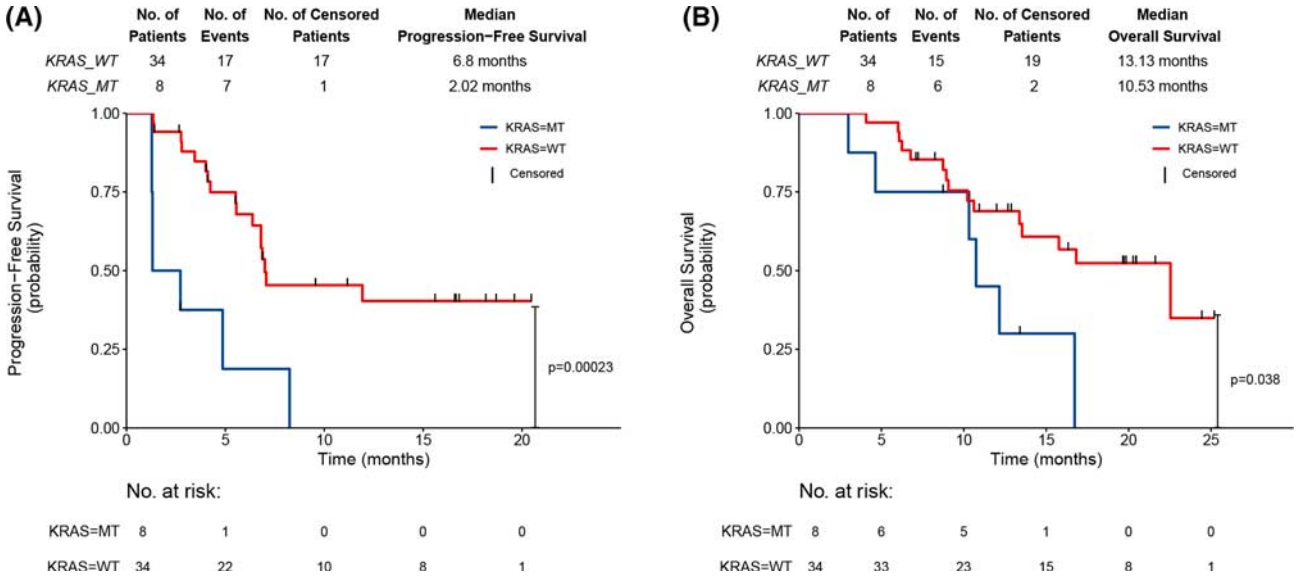

FIGURE 5 Progression-free survival (A) and overall survival (B) of patients with KRAS mutations and patients without KRAS mutations. *KRAS*, *kirsten rat sarcoma viral oncogene homolog*; MT, mutant type; WT, wild type.

in the second-line treatment of advanced BTCs. Although limited, the ABC-06 study proved the benefits of FOLFOX in the second-line treatment of BTCs.[4] There is no consensus regarding the optimal chemotherapy regimen worldwide.[11] In a systematic review of second-line chemotherapies, which included 761 patients and 25 trials, mean PFS, OS, and ORR were merely 3.2 months, 7.2 months, and 7.7%, respectively.[28] Monotherapy with antiangiogenic TKIs, including regorafenib and apatinib, also yielded minimal efficacy in pretreated advanced BTCs, with median OS of 5.3 and 4.81 months,[29,30] respectively. As for ICI monotherapy, existing findings on second-line use of programmed cell death protein 1 (PD-1)/PD-L1 inhibitors in BTCs are controversial, with a discrepancy in median OS ranging from 5.2 to 14.24 months.[12,31]

The combination of angiogenic inhibitors and ICIs has demonstrated certain antitumor effects in patients with advanced BTCs in several single-arm clinical studies. With the results of this study, anlotinib combined with TQB2450 seemed to be a promising regimen for patients with pretreated BTCs. Lenvatinib plus pembrolizumab (a PD-1 inhibitor) achieved an ORR of 10% and a median PFS of 6.1 months with a median OS of only 8.6 months in the second-line treatment of advanced BTCs.[18] Meanwhile, the combination of regorafenib and avelumab, a PD-L1 inhibitor, yielded a median PFS of only 2.5 months, with an ORR of 13.8% and a median OS of 11.9 months, whereas a median PFS of only 2.5 months was documented.[19] Our results provided further insights into the ICI-combination strategy in second-line treatment of advanced BTCs. So far, emerging evidence supports the potential of ICI-combined therapy as initial treatments in several solid tumors, as well as in advanced BTCs. In addition to the combination of durvalumab and chemotherapy proved in the TOPAZ-1 study,[13] chemotherapy plus durvalumab with or without tremelimumab exhibited encouraging efficacy in a phase 2 trial.[32]

A systematic review[33] revealed that combining TKIs and ICIs could result in the increase of treatment-related toxicity, with an overall incidence of around 60% for severe TRAEs. However, the combination of anlotinib and TQB2450 in this study exhibited a favorable safety profile, which was consistent with findings in previous studies of anlotinib plus other ICIs.[34,35] In the present study, the incidence of TRAEs with grade 3 or higher was 25.76% with no unexpected safety concerns. For lenvatinib plus pembrolizumab, the incidence of grade 3 or higher TRAEs was 48%.[18] Additionally, dosage modification or interruption of any treatment because of AEs occurred in 22 (33.33%) patients in this study, whereas it was 85.3% of regorafenib plus avelumab.[19] Taking into consideration the favorable safety profile and the numerically higher ORR and better survival, TQB2450 combined with anlotinib at 12 mg was recommended as the dosage for further assessment in a phase 3 clinical study.

The correlation between PD-L1 expression and efficacy was analyzed based on archived samples, and a similar median PFS was observed in patients with CPS $\geq$ 5 and CPS $<$ 5 (6.80 vs. 6.24 months), indicating that the efficacy of anlotinib and TQB2450 might be independent of PD-L1 expression. However, these findings need to be validated, preferably with new biopsy specimens, at the time of enrollment in larger-scale studies.

The present study assessed the value of genomic mutation signature and TMB in predicting the response to anlotinib plus TQB2450. In this study, patients with

higher TMB (≥5 mutations/Mbp) achieved a better CBR and longer PFS and OS. Mutations of specific tumor genes may impact the sensitivity to ICI therapy. In this study, eight patients bearing *KRAS* mutations exhibited significantly lower CBR (12.5% vs. 58.8%, $p = 0.045$) and shorter median PFS (2.02 vs. 6.80 months, $p < 0.001$) and OS (10.53 vs. 13.13 months, $p = 0.038$) compared with wild-type counterparts. This finding indicated that *KRAS* mutation might be a negative predictor of response to ICI treatment response in BTCs. Yoon et al. also reported that *KRAS* mutation was associated with resistance to immunotherapy in BTCs.[36] However, the role of *KRAS* mutation in ICI therapy remains elusive. Treatment with PD-1 inhibitors revealed significantly better clinical benefits in patients with NSCLC harboring both *KRAS* and *TP53* mutations versus wild-type cases.[37,38] Further investigation would be warranted to confirm the impact of *KRAS* mutation on ICI treatment.

In addition, this study provided interesting information on the correlation of several other gene mutations and ICI treatment. *ARID1B* and *ARID1A*, the subunits of the SWI/SNF chromatin remodeling complex that is pivotal for maintaining genomic stability,[39] were found to have a high mutation rate in this population. Patients with *ARID1B* mutations had longer PFS and OS, which was consistent with findings by Zhu et al.[40] In contrast, patients harboring *ARID1A* mutations seemed to have poorer survival, which is inconclusive because of the small sample size ($n = 4$). In addition, available data revealed that the role of *ARID1A* in predicting the efficacy of ICI therapy remains controversial.[41,42] In this study, patients with mutated FGF pathway had numerically longer survival than those with unaltered pathway. Aberrant FGF signaling pathway was reported to be associated with indolent behavior and favorable prognosis in patients with BTCs.[43,44] Meanwhile, better survival in patients with altered FGF pathway was found but without a significant correlation, which might be attributed to the small sample size. Considering the small number of patients, the exact roles of *ARID1A*, *ARID1B*, and FGF pathway in the prediction of ICI therapy in BTCs deserve further investigation.

The limitations of the present study should be pointed out. Firstly, this was a pooled analysis of two single-arm trials with relatively small sample sizes, and all the results were preliminary, which deserves further investigation. Secondly, tumor specimens were only available in a proportion of patients leading to insufficient detection of PD-L1 expression and NGS analysis. Finally, the archival samples for biomarker analysis were all collected at the initial diagnosis or surgery, which may impact the accurate interpretation of these results.

In conclusion, the pooled results of two single-center, phase Ib clinical trials showed that anlotinib combined with a PD-L1 monoclonal antibody (TQB2450) demonstrated promising antitumor activity and survival benefit, with favorable tolerability in patients with pretreated advanced BTCs. *KRAS* mutations and TMB ≥ 5 mutations/Mbp could serve as potential predictive factors of treatment efficacy. A randomized, parallel-controlled, multicenter phase III study of TQB2450 combined with anlotinib versus chemotherapy as second-line treatment in patients with advanced BTCs is ongoing (identifier: NCT04809142).

AUTHOR CONTRIBUTION

Aiping Zhou and Lin Shen were responsible for the experimental design. Jun Zhou, Yongkun Sun, Wen Zhang, Jiajia Yuan, Zhi Peng, Jifang Gong, Lin Yang, Yanshuo Cao, and Hong Zhao were members of the trial management group and were responsible for clinical trial execution. Jun Zhou, Yongkun Sun, and Wei Wang were responsible for writing the original draft. Chao Chen and Weifeng Wang contributed to data analysis. Aiping Zhou, Lin Shen, Jun Zhou, and Yongkun Sun critically revised the manuscript for important intellectual content. All the authors have read and agreed to the published version of the manuscript.

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CONFLICT OF INTEREST

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