Efficacy and safety of a triple combination of atezolizumab, bevacizumab plus GEMOX for advanced biliary tract cancer: a multicenter, single-arm, retrospective study

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Abstract

Background: Anti-programmed cell death ligand 1/vascular endothelial growth factor inhibition, coupled with chemotherapy, may potentiate antitumor immunity leading to enhanced clinical benefit, but it has not been investigated in advanced biliary tract cancer (BTC).

Objectives: We investigated the efficacy and safety of atezolizumab, bevacizumab, and gemcitabine plus oxaliplatin (GEMOX) in advanced BTC and explore the potential biomarkers related to the response.

Design: Multicenter, single-arm, retrospective study.

Methods: Advanced BTC patients, who received a triple combination therapy at three medical centers between 18 March 2020 and 1 September 2021, were included. Treatment response was evaluated *via* mRECIST and RECIST v1.1. Endpoints included the overall response rate (ORR), disease control rate (DCR), progression-free survival (PFS), overall survival (OS), and safety. The whole exome sequencing of pathological tissues was conducted for bioinformatic analysis.

Results: In all, 30 patients were enrolled. The best ORR was 76.7% and the DCR was 90.0%. The median PFS was 12.0 months, and the median OS was not reached. During the treatment, 10.0% (3/30) of patients suffered from ⩾grade 3 treatment-related adverse events (TRAEs). Furthermore, fever (73.3%), neutropenia (63.3%), increased aspartate transaminase and alanine aminotransferase levels (50.0% and 43.3%, respectively) are the most common TRAEs. Bioinformatics analysis revealed patients with altered ALS2CL had a higher ORR.

Conclusion: The triple combination of atezolizumab, bevacizumab, and GEMOX may be efficacious and safe for patients with advanced BTC. ALS2CL may be a potential predictive biomarker for the efficacy of triple combination therapy.

Keywords: atezolizumab, bevacizumab, biliary tract cancer, combination therapy gemcitabine and oxaliplatin

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Introduction

Biliary tract cancer (BTC) is an aggressive type of malignant tumor consisting of 3% of all gastrointestinal malignancies in adults.[1] As the symptoms at the early stage are non-specific, most patients are diagnosed at an advanced stage, which induces a poor prognosis with a 5-year survival of around 5–15%.[2] Although gemcitabine plus oxaliplatin (GEMOX) is the first-line regimen for these advanced patients, the progression-free survival

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(PFS) and median overall survival (OS) of only 5.3 months and 8.9 months, respectively, and the objective response rate (ORR) was only 26.1%.[3–5] Thus, it is of great importance to improve the outcomes for patients in an advanced stage.

Immune checkpoint inhibitors (ICIs) have shown clinical activity in a subgroup of patients, but the efficacy of monotherapy is limited due to the highly heterogeneous and immunosuppressed tumor microenvironment (TME).[6] Combining programmed death protein 1 (PD-1)/programmed cell death ligand 1 (PD-L1) with anti-angiogenics or chemotherapy which both possess immunomodulatory capabilities is one way to target TME immunosuppression, and the superiority of the combination therapy to standard treatments has been proved.[7–9] A single-arm phase II clinical trial has demonstrated that lenvatinib plus pembrolizumab promotes the tumor response with an ORR of 25% for advanced BTC, and the median PFS and OS were 4.9 and 11.0 months, respectively.[10] Another single-arm phase II clinical trial of carelizumab plus GEMOX revealed an ORR of 54% and the median PFS and OS were 6.1 and 11.8 months, respectively.[11] The exciting advances in the treatment of BTC have been brought about by the combination of ICIs with either anti-angiogenics or chemotherapy, which intrigues further research on whether the whole combination of those three could increase more potential to reverse the tumor immunosuppressed microenvironment.

A combination of atezolizumab and bevacizumab has shown enormous therapeutic potential for many cancers, and the astounding efficacy even makes it a possibility to be used as a first-line therapy for advanced HCC.[12–16] Considering the critical role of vascular endothelial growth factor (VEGF) in regulating the immunosuppressive mechanism in BTC TME and the enhancement of cytotoxic chemotherapies on antitumor immunity, atezolizumab and bevacizumab may improve the efficacy of the first-line GEMOX chemotherapy in advanced BTC. Furthermore, the triple combination has been under investigation in multiple randomized studies among various cancer types and the efficacy is diverse in different malignancies.[7,17] The triple combination was found to be effective in non-small-cell lung cancer (Impower 150) but not in ovarian cancer (Imagyn050), and the incidence of immune-related adverse events (AEs) of the triple combination was similar and manageable.[14,18] However, whether this triple combination is effective in advanced BTC has not been reported.

Hence, this multicenter, single-arm, and retrospective study enrolled advanced BTC patients who accepted atezolizumab and bevacizumab combined with GEMOX, and investigated the efficacy and safety of the combined therapy. In addition, further exploration was conducted to search novel biomarkers for predicting responses to combined therapy by integrative bioinformatics methods.

Methods

Patients

Consecutive patients in this multi-center, retrospective, single-arm study came from Eastern Hepatobiliary Hospital, Yueyang Hospital of Integrated Traditional Chinese and Western Medicine, and The First Hospital of Jiaxing Affiliated Hospital of Jiaxing University between 18 March 2020 and 1 September 2021. The diagnosis of advanced BTC was confirmed by imaging data [computerized tomography (CT), magnetic resonance imaging (MRI), or magnetic resonance cholangiopancreatography] and pathological biopsy (the cytological sampling of perihilar cholangiocarcinoma was obtained through brushing or endoscopic retrograde cholangiopancreatography).[19] Patients, who had a Child-Pugh level A or B and an Eastern Cooperative Oncology Group Performance Status Score of 0–1, accepted the combination therapy of atezolizumab and bevacizumab plus GEMOX. And biliary drainage was performed first to ensure the safety of the regimen if jaundice exists.[20,21] Then patients who accepted the combination therapy of atezolizumab and bevacizumab plus GEMOX and had at least one measurable lesion per mRECIST and RECIST v1.1 were enrolled in this study, while those who had received previous therapies or other regimens were excluded.

Treatment

Patients received a combination of atezolizumab, bevacizumab, and GEMOX. Specifically, each treatment cycle lasted for 6 weeks; atezolizumab plus bevacizumab was administered every 3 weeks, and GEMOX was administered every 2 weeks. A fixed dose of 1200 mg of atezolizumab

injection was intravenously administered on Day 1 and Day 22 (60 min of initial IV infusion, followed by 30 min if tolerated), and bevacizumab 15 mg/kg was intravenously administered after an interval of at least 5 min (90 min of initial IV infusion, followed by 60 min and 30 min if tolerated); gemcitabine 1000 mg (100 min of IV infusion) and oxaliplatin 100 mg (120 min of IV infusion) was intravenously administered on Days 2, 16, and 30. The treatment continued until disease progression or unacceptable toxicity occurred or some other conditions were judged by the investigator as inappropriate for continuing the treatment. Once severe toxicity occurred, the administration would be delayed and/or the dose would be reduced according to the drug's instructions.

Assessments

Tumor response was evaluated by three veteran radiologists individually per RECIST v1.1 and mRECIST using imaging evaluations (MRI was preferred and CT was adopted if MRI was not available) which were conducted at baseline and every 4–8 weeks after each therapy session. Complete response (CR) and partial response (PR) had to be confirmed radiologically at least 4 weeks later. AEs were recorded from the first day of treatment until 1 month after the end of treatment and graded according to the National Cancer Institute Common Terminology Criteria for Adverse Events version 4.03.

Endpoints

The primary endpoint was ORR, defined as the proportion of patients with CR or PR. The secondary endpoints included PFS, OS, disease control rate (DCR), and safety. The ORRs and DORs will be calculated under the RECIST 1.1 and modified RECIST (mRECIST), respectively.

Biomarker analysis

Of 30 BTC patients, 9 patients' pathological tissues were available, including 5 PR patients, 3 stable disease (SD) patients, and 1 progressive disease (PD) patient. Optional biopsy specimens from these nine patients were obtained for exploratory biomarker assessment. To be more specific, genomic DNA from tumor tissues was extracted, followed by the whole exome sequencing and analysis of the tumor mutational burden (TMB), and then further bioinformatics analysis was conducted to explore the predictive biomarkers.[22–24] Based on the tumor response, 230 mutations per megabase was adopted as the TMB cutoff value, which was defined by the receiver operating characteristic curve, to differentiate high from low TMB.[25] The difference in gene mutations between the effective group (5 PR patients) and the non-effective group (3 SD and 1 PD patients) was analyzed.

Statistical analyses

Safety and efficacy analyses were performed for all patients who underwent at least one cycle of the combined therapy and clinical evaluation. For baseline characteristics, variables were expressed as frequencies and percentages. SPSS version 23 and R version 4.1.2 were used for data analyses. OS and PFS were depicted by Kaplan–Meier plots. The log-rank test was applied to compare the survival function among different subgroups.

Results

Patients

Between 18 March 2020 and 1 September 2021, a total of 33 patients were screened, of whom three lost follow-up data or refused the combined treatments for personal issues. The follow-up ended on 14 March 2022, with a median follow-up of 10.0 (range, 2.0–24.0) months. Ultimately, 30 patients, pathologically diagnosed as BTC and accepting atezolizumab and bevacizumab combined with GEMOX without other standard chemotherapy first, were included in the analysis (Supplemental Figure S1). The demographic and disease characteristics revealed that all enrolled BTC patients were advanced (Table 1).

All patients enrolled in the analysis had BTC considered advanced for three reasons: (1) the pathological biopsy of puncture suggested poorly differentiated; (2) the main portal vein or inferior vena cava was invaded; and (3) presented with multiple lymph node metastases or distance metastases.[19] All enrolled patients included 5 (16.7%) gallbladder cancer, 15 (50.0%) intrahepatic cholangiocarcinoma, and 10 (33.3%) perihilar cholangiocarcinoma. CT-guided puncture and drainage therapy were conducted in all perihilar cholangiocarcinoma. Of 30 patients, 3

Table 1. Baseline demographic and clinical characteristics ($n = 30$).

Characteristics	Patients
Age, years	
Median	58.5
Range	37–74
Gender	
Male	21
Female	9
Hepatitis B virus infection	5
Hepatitis C virus infection	0
Child-Pugh classification	
A	26
B	4
Serum AFP level, ng/ml	
<400	29
⩾400	1
Serum CA 19-9 level, u/ml	
<200	12
⩾200	18
Anatomic subtypes	
iCCA	15
pCCA	10
GBC	5
Histology	
Adenocarcinoma	24
Squamous	3
Adenosquamous	3
Grade of differentiation	
Poorly	27
Moderately	1
Not specified	2
Regional lymphatic	
<2	10
⩾2	20
Distant metastasis	7
Target lesion size, cm	
Median	4.5
Range	0.9–16.5
TNM stage	
III A	3
III B	5
IV	22

AFP, alpha-fetoprotein; BCLC, Barcelona Clinic Liver Cancer; CA, carbohydrate antigen; GBC, gallbladder cancer; iCCA, intrahepatic cholangiocarcinoma; pCCA, perihilar cholangiocarcinoma.

(10.0%) had macroscopic vascular invasion, 28 (93.3%) had multiple lymph node metastases, among whom 20 (66.7%) patients had two or more different regional lymphatic metastases. The pathological biopsy results revealed that 27 (90%) were poorly differentiated, and 19 (63.3%) were adenocarcinoma. In addition, 26 (86.7%) had Child-Pugh grade A liver function and 4 (13.3%) had Child-Pugh grade B.

Efficacy

Tumor response was evaluated based on the investigator's assessment *via* RECIST v1.1 and mRECIST. However, for consistency, only assessments using RECIST v1.1 are summarized in the following section. For all patients in the analysis, the median follow-up duration was 10.0 (range, 2.0–24.0) months. During this time, the median treatment duration was 7.2 months (range, 1.8–15.1) and the median regimen session was five times (Figure 1(a)), 24 (80.0%) patients exhibited a reduction from baseline in target lesion size (Figure 1(b)). The median OS was not reached and the median PFS was 12.0 months (95% CI, 6.1–17.9) (Figure 1(c)

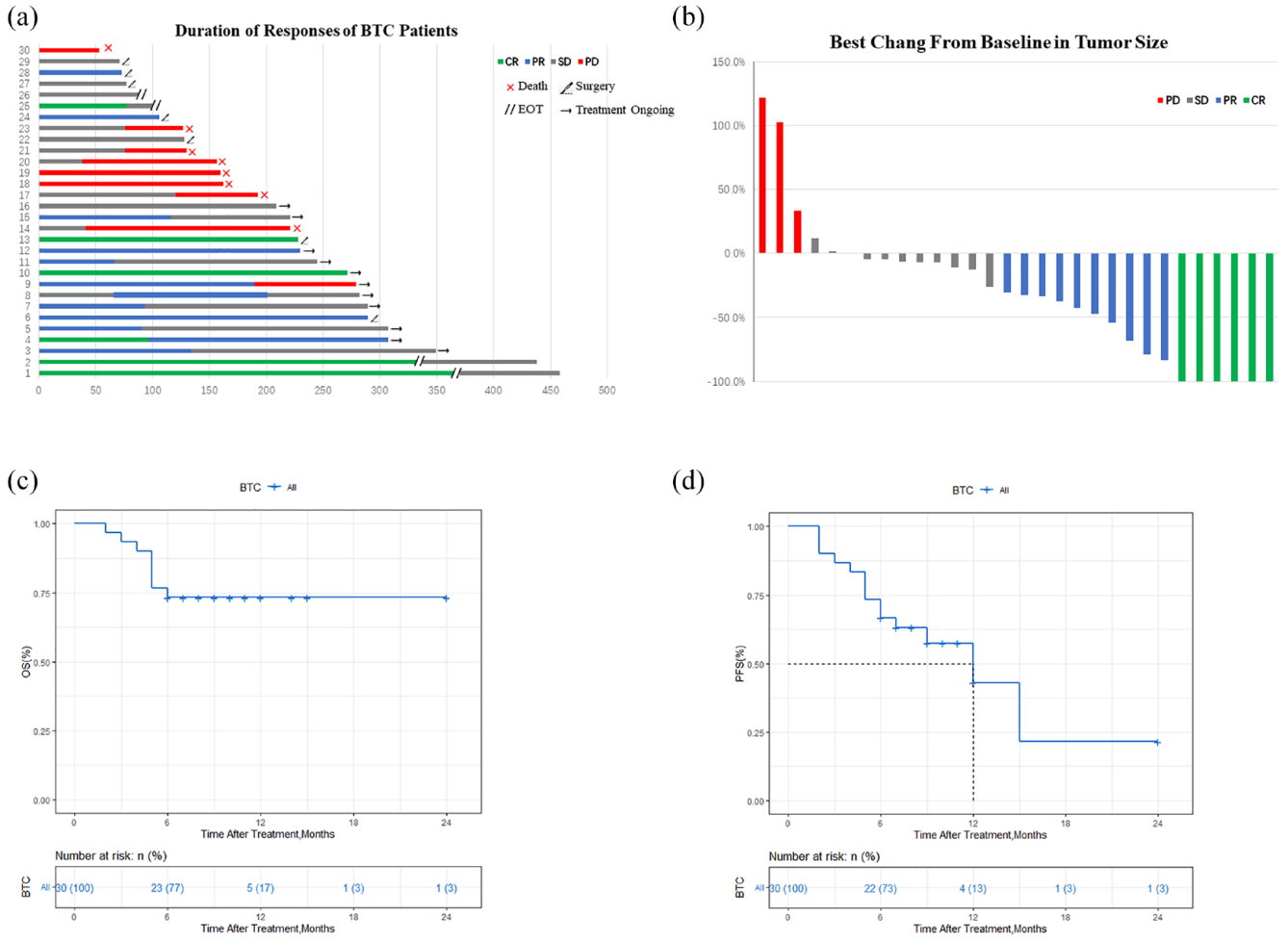

Figure 1. Antitumor activity. (a) Duration of responses of 30 enrolled patients with advanced BTC. The length of each bar represents the follow-up of each patient. (b) Best percentage change from baseline in target lesion size per RECIST v1.1. The Kaplan–Meier curves of (c) OS and (d) PFS in 30 patients.
BTC, biliary tract cancer; EOT, end of treatment; OS, overall survival; PFS, progression-free survival.

and (d)). The best ORR was 76.7%, 6 (20.0%) patients achieved CR and 17 (56.7%) patients achieved PR. An SD was observed in four (13.3%) patients, leading to a DCR of 90.0% (27 patients) (Table 2). At the end of follow-up, 11 (36.7%) patients were still receiving the combined therapy, 7 (23.3%) patients accepted surgical therapy, 12 (40.0%) patients discontinued treatment, including 8 (26.7%) patients because of PD, 2 (6.7%) due to AEs, and 2 (6.7%) for personal reasons. More specifically, examples of patients who could be downstaged to the surgical intervention are shown in Supplemental Figure S2, and the details of all seven patients are presented in Supplemental Table S1.

The ORR was 40.0% (2 of 5) in gallbladder carcinoma, 73.3% (11 of 15) in intrahepatic cholangiocarcinoma, and 100.0% (10 of 10) in perihilar cholangiocarcinoma (Table 2). The median OS and PFS of gallbladder carcinoma were 5.0 months and 4.0 months, respectively, of intrahepatic cholangiocarcinoma not reached and 15.0 months, respectively, of perihilar cholangiocarcinoma not reached neither (Figure 2(a) and (b)).

The carbohydrate antigen (CA) 19-9 level decreased significantly in all 30 patients while the alpha-fetoprotein (AFP) level did not (Figure 2(c) and (d)). The median CA 19-9 level was 348.0 u/ml before treatment and decreased to 59.6 u/ml after

Table 2. Summary of efficacy outcomes.

Variables, *n* (%)	All BTC (*n* = 30)		iCCA (*n* = 15)		pCCA (*n* = 10)		GBC (*n* = 5)	
	Best overall response (mRECIST)	Best overall response (RECIST v1.1)	Best overall response (mRECIST)	Best overall response (RECIST v1.1)	Best overall response (mRECIST)	Best overall response (RECIST v1.1)	Best overall response (mRECIST)	Best overall response (RECIST v1.1)
CR	6 (20.0%)	6 (20.0%)	3 (20.0%)	2 (13.3%)	3 (30.0%)	4 (40.0%)	0	0
PR	17 (56.7%)	10 (33.3%)	8 (53.3%)	5 (33.3%)	7 (70.0%)	4 (40.0%)	2 (40.0%)	1 (20.0%)
SD	4 (13.3%)	11 (36.7%)	3 (20.0%)	7 (46.7%)	0	2 (20.0%)	1 (20.0%)	2 (40.0%)
PD	3 (10.0%)	3 (10.0%)	1 (6.7%)	1 (6.7%)	0	0	2 (40.0%)	2 (40.0%)
ORR	23 (76.7%)	16 (53.3%)	11 (73.3%)	7 (46.7%)	10 (100.0%)	8 (80.0%)	2 (40.0%)	1 (20.0%)
DCR	27 (90.0%)	27 (90.0%)	14 (93.3%)	14 (93.3%)	10 (100.0%)	10 (100.0%)	3 (60.0%)	3 (60.0%)
Surgical resection	7 (23.3%)	7 (23.3%)	3 (20.0%)	3 (20.0%)	3 (30.0%)	3 (30.0%)	1 (20.0%)	1 (20.0%)

BTC, biliary tract cancer; CR, complete response; DCR, disease control rate; GBC, gallbladder cancer; iCCA, intrahepatic cholangiocarcinoma; ORR, objective response rate; pCCA, perihilar cholangiocarcinoma; PD, progressive disease; PR, partial response; SD, stable disease.

the treatment, and in 12 patients CA 19-9 level had returned to the normal range (40 u/ml). The AFP level in all patients was normal except one for multiple intrahepatic metastases, but the AFP level in this patient decreased a lot after the treatment.

Safety

Most patients (80.0%, 24/30) experienced at least one treatment-related AE (TRAE) with varying severity (Table 3), the most common of which were fever (73.3%), neutropenia (63.3%), and increased level of aspartate transaminase (AST) and alanine aminotransferase (ALT) (50.0% and 43.3%, respectively). Two (6.7%) patients experienced grade 3 AEs and one (3.3%) experienced grade 4 AEs, and all were neutropenia. Two (6.7%) patients discontinued the combined therapy because of TRAEs. No treatment-related deaths occurred.

Biomarker analysis

In nine available patients, an exploratory analysis showed that patients with high TMB ($n = 3$) and low TMB ($n = 6$) exhibited no significant difference in ORR (33.3% and 66.7%, $p = 0.343$; Figure 3(a)). Next-generation sequencing revealed that 25 gene mutations were significantly different between the effective group and the non-effective group, and the most common mutation type was missense mutation. Of note, the most significant difference between effective group and non-effective group was the missense mutation of ALS2CL, and the ORR was 100.0% (5 of 5) in patients with altered ALS2CL *versus* 0% (0 of 4) in their wild-type counterparts ($p < 0.01$; Figure 3(b)).

Discussion

To our knowledge, the study is the first retrospective analysis of a cohort of patients with advanced BTC treated with triple combination therapy (atezolizumab, bevacizumab, and GEMOX) and the results suggest this regimen may have good efficacy.

Many studies have reported that the combination therapy prolonged the median OS and the median PFS in advanced BTC,[10,26,27] among which a phase II trial and a phase III study revealed the great potential efficacy of GEMOX plus ICIs.[28,29] However, our findings demonstrated this triple combination could further provide a better prognosis and tumor response compared favorably with these previous trials[11,26,30–33] (Supplemental Table S2). The majority of BTCs harboring either immune-excluded (T-cell effectors confined to the tumor margin unable to penetrate the tumor bed) or immune-desert phenotypes (absence of scarce T-cell infiltration in the stroma and tumor bed) are considered poorly responsive

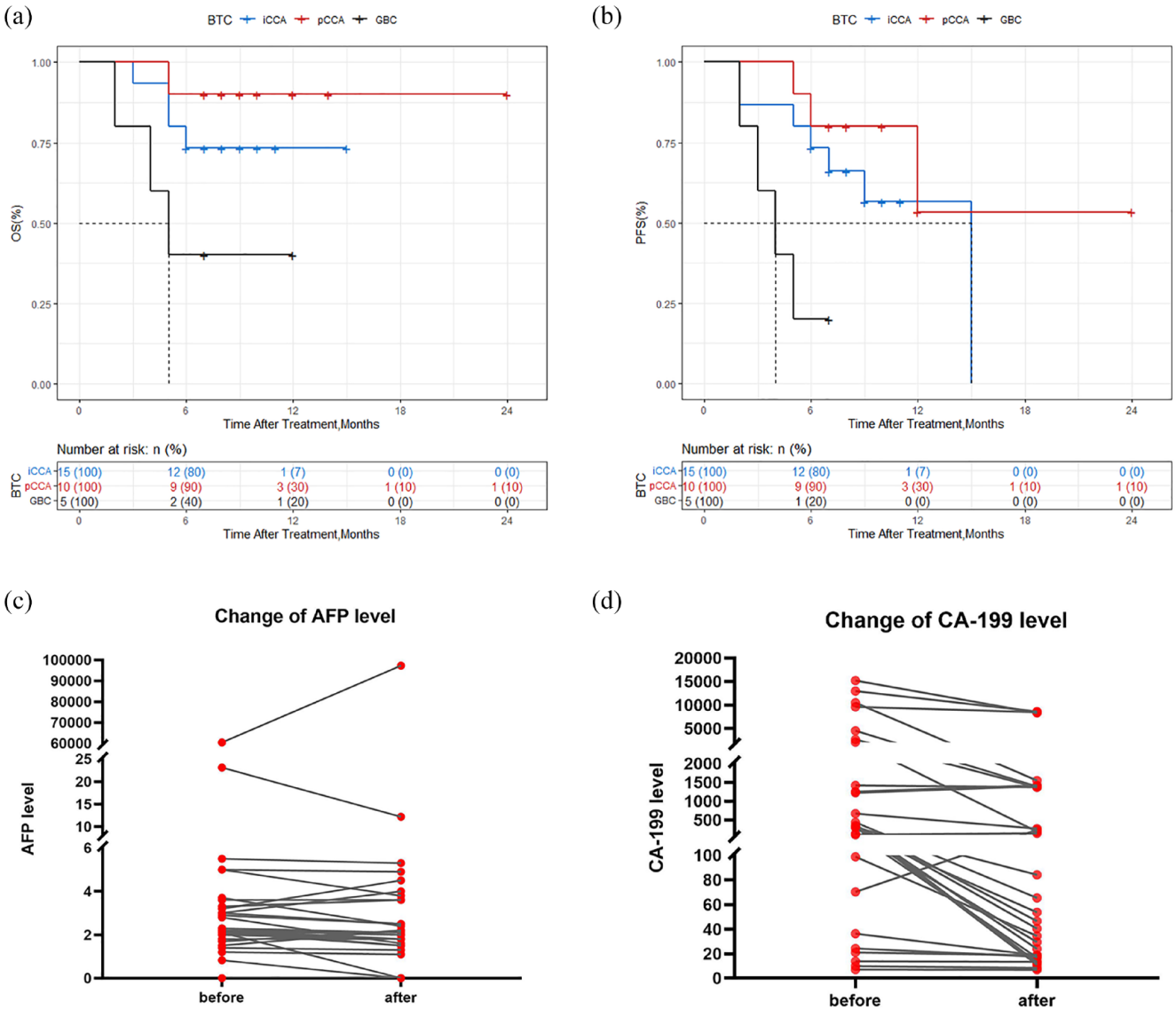

Figure 2. Subgroup analysis and variables change after the regimen. The Kaplan–Meier curves of (a) OS and (b) PFS in patients with iCCA, pCCA, and GBC, respectively. The level change of (c) AFP and (d) CA-19-9.
OS, overall survival; PFS, progression-free survival; iCCA, intrahepatic cholangiocarcinoma; pCCA, perihilar cholangiocarcinoma; GBC, gallbladder carcinoma; AFP, alpha-fetoprotein; CA 19-9, carbohydrate antigen 19-9.

to PD-1/PD-L1 inhibitors.[34,35] Notably, bevacizumab could block or reverse the immunosuppressive effects of VEGF which include upregulation of inhibitory immune checkpoints, damaged $CD8^+$ T-cell infiltration and function, inhibition of dendritic cell function and maturation, and the increase in immunosuppressive cell types, such as regulatory T cells (Treg), myeloid-derived suppressor cells, tumor-associated macrophages, and so on.[7,8,36,37] Similarly, chemotherapy could weaken the immunosuppressive effects of the TME, promote antigen cross-presentation, improve the permeability of immune cells, and increase the proportion of $CD8^+$ T-cell or Treg to enhance the immune system.[38] Based on these potential mechanisms, patients treated with triple combination therapy exhibited better results.

The safety profile of our cohort is also investigated and consistent with that reported in previous trials.[39,40] Of note, only 10.0% of patients suffered from ⩾grade 3 TRAEs, which is

Table 3. Summary of the TRAEs in patients ($n = 30$).

AE term, *n* (%)	Any grade	Grade 1	Grade 2	Grade 3	Grade 4
Fever	22 (73.3)	16 (53.3)	6 (20.0)	0	0
Neutropenia	19 (63.3)	12 (40.0)	4 (13.3)	2 (6.7)	1 (3.3)
AST level increased	15 (50.0)	14 (46.7)	1 (3.3)	0	0
ALT level increased	13 (43.3)	7 (23.3)	6 (20.0)	0	0
Rash	6 (20.0)	3 (10.0)	3 (10.0)	0	0
Anemia	3 (10.0)	3 (10.0)	0	0	0
Serum bilirubin increase	4 (13.3)	4 (13.3)	0	0	0
Hypertension	5 (16.7)	3 (10.0)	2 (6.7)	0	0
Fatigue	5 (16.7)	5 (16.7)	0	0	0
Proteinuria	2 (6.7)	2 (6.7)	0	0	0
Diarrhea	3 (10.0)	3 (10.0)	0	0	0
Pruritus	2 (6.7)	2 (6.7)	0	0	0
Oral ulcer	2 (6.7)	2 (6.7)	0	0	0
lymphadenitis	1 (3.3)	1 (3.3)	0	0	0
Decreased appetite	4 (13.3)	4 (13.3)	0	0	0
Hypothyroidism	1 (3.3)	1 (3.3)	0	0	0
Weight decreased	1 (3.3)	1 (3.3)	0	0	0
Abdominal distention	1 (3.3)	1 (3.3)	0	0	0
Arthralgia	1 (3.3)	1 (3.3)	0	0	0
Gastrohelcoma	1 (3.3)	1 (3.3)	0	0	0
Oulorrhagia	1 (3.3)	1 (3.3)	0	0	0

AE, adverse event; ALT, alanine aminotransferase; AST, aspartate transaminase; TRAE, treatment-related AE.

significantly better than that of the trial (19.2%) combing ramucirumab and pembrolizumab and another trial (70%) combing camrelizumab and GEMOX in advanced BTC,[33,39] demonstrating the triple combination might have a safety advantage over other treatment strategies with similar mechanisms of actions. Furthermore, this study suggested the most common AEs were fever, neutropenia, increased AST level, and increased ALT level, of which the overall incidences were 73.3%, 63.3%, 50.0%, and 43.3%, respectively, for any grade. The use of triple combination therapy might increase the rate of certain toxicities inevitably, but the potential immune-related AEs such as fever, increased AST and ALT, and rash were generally manageable and reversible. Hypertension was the most common AE associated with bevacizumab, but all the cases were grade 1–2 and without significant effects on quality of life and medication. Notably, neutropenia was the most frequent grade 3–4 toxicity associated with chemotherapy occurring in three patients (10.0%) including one case of grade 4, and the severe action induced the discontinuation of the triple combination therapy in two patients. Our finding suggested that advanced BTC

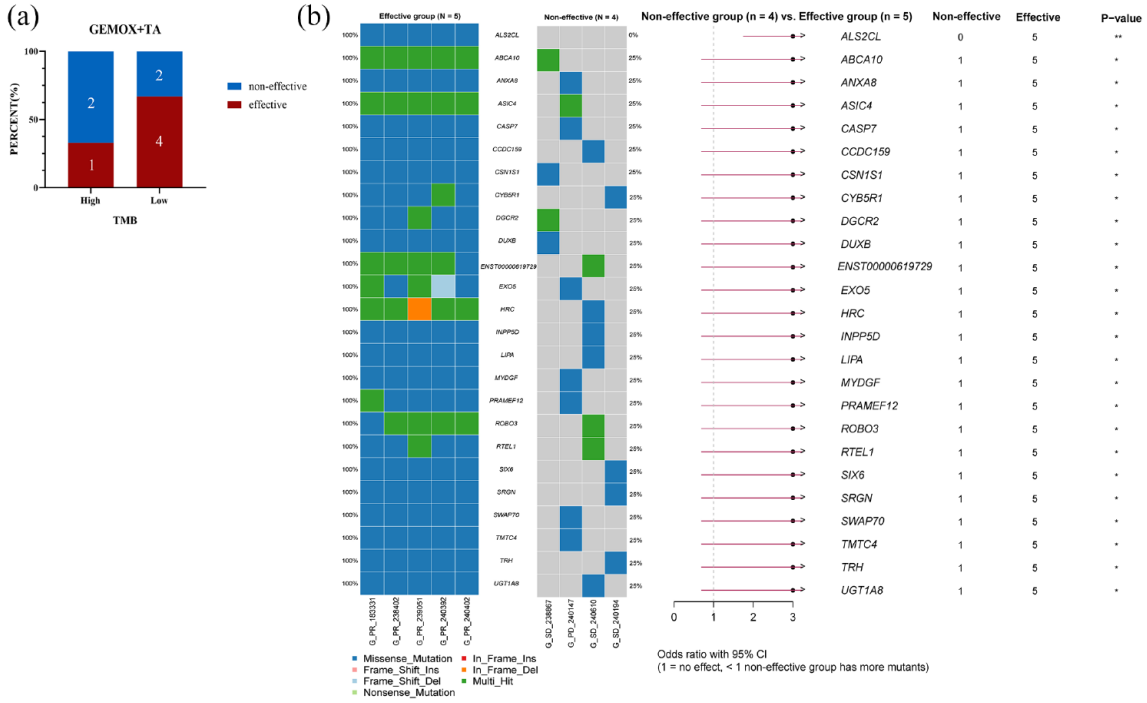

Figure 3. Bioinformatic analysis for the whole-exome sequencing. (a) Treatment response of efficacy-evaluable patients stratified by TMB (high *versus* low). (b) Heatmap of gene mutations in nine patients with advanced BTC.

Rows represent genes, and columns represent samples. Glyphs and color coding are used to summarize distinct genomic alterations. The forest map indicated the univariate linear regression results of 25 genes.

BTC, biliary tract cancer; TMB, tumor mutational burden.

patients should be carefully evaluated for treatment benefits and risk of chemotherapy toxicity after triple combination therapy.

Several studies have reported that TMB is associated with response to ICIs monotherapy across multiple cancers,[41] To further validate the potential association between the TMB and immunotherapy response, next-generation sequencing was conducted. Interestingly, our study found the higher TMB group had a higher ORR but failed to establish a significant association between TMB and ORR. Although the result was consistent with the study of camrelizumab plus GEMOX for advanced BTC and another study of anti-PD-1/cytotoxic T-cell lymphocyte-4 combination immunotherapy for solid tumors,[11,42] a large sample study was desired for further confirmation. Furthermore, the investigation of gene mutation difference between the effective group and the non-effective group was conducted to explore the potential predictive biomarkers. Compared with previous studies, some novel genes were discovered regarding the high heterogeneity of BTC and the particularity of the triple combination regimen, which warranted further investigations. Of concern is the mutation of ALS2CL occurred in all effective patients, while none in non-effective patients. ALS2CL is a protein associated with ALS2 which encodes the RAB5 guanine exchange factor ALSIN 60.[43] Several studies demonstrated that ALS2CL colocalized with Rab5 strongly and regulated the dynamics of Rab5 *in vivo*, while Rab5 could suppress autophagy to promote drug resistance in cancer cells.[44,45] Thus, patients with altered ALS2CL may respond significantly more to the triple combination regimen than their wild-type counterparts.

Overall, this single-arm study is mainly designed to provide a foundation and new insight for future research, which mainly aims to improve the long-term prognosis for advanced BTC patients. GEMOX chemotherapy has been recommended as the first-line treatment, but the efficacy is

limited owing to the high heterogeneity in BTC patients. According to the density of immune-cell infiltrate, the tumor can be characterized as 'hot tumors' (immune inflamed) and 'cold tumors' (immune excluded or immune-desert).[46] These patterns of tumor immune phenotype may induce different response to traditional therapy. Therefore, a higher response to chemotherapy may be observed after converting the immunosuppressive tumor environment. The results of this study revealed that atezolizumab and bevacizumab in combination with GEMOX may be a potential option for advanced BTC patients, but the underlying mechanism needs further exploration.

The interpretation of the findings in this study may be limited by the following fields. First, the interpretation of PFS and ORR may be limited owing to their retrospective nature. But 25 patients (25/30, 83.3%) enrolled were almost followed up every 4–5 weeks, which may avoid the impact of bias caused by an unfixed follow-up period. Second, this study has no control arm to provide a comparator to the triple combination therapy. Finally, the baseline variables of patients were different for the subgroups with regard to anatomic types and histology which may affect the clinical outcome and side effects of the regiment. Furthermore, we have been conducting a prospective study (Chinses Clinical Trial Register, registration number: ChiCTR2100049830) with a larger population to verify the results.

Conclusion

The triple combination regimen of atezolizumab, bevacizumab plus GEMOX has shown inspiring efficacy and acceptable safety in this study, which suggest this regimen may be a promising option for patients with advanced BTC, and ALS2CL may be a potential predictive biomarker for the efficacy. Moreover, the comprehensive genomic profiling of the BTC gene mutational landscape will provide novel insight into future studies involving multiple biomarkers for molecularly stratified therapy of advanced BTC.

Declarations

Ethics approval and consent to participate

This study was conducted in accordance with the Declaration of Helsinki and the International Conference on Harmonisation guidelines for Good Clinical Practice and approved by the Institutional Ethics Committees of the Eastern Hepatobiliary Hospital, Yueyang Hospital of Integrated Traditional Chinese and Western Medicine, and the First Hospital of Jiaxing Affiliated Hospital of Jiaxing University (Ethics code: EHBHKY2022-K-027.1/3/2020). We obtained informed consents from all patients involved, before receiving treatment and for their data to be used in clinical research, and de-identified all patient details such that the identity of the patients may not be ascertained in any way. The reporting of this study conforms to the STROBE statement.

Consent for publication

Not applicable.

Author contribution(s)

Kang Wang: Conceptualization; Data curation; Formal analysis; Methodology; Resources; Writing – review & editing.

Zong-Han Liu: Conceptualization; Data curation; Formal analysis; Software; Writing – original draft.

Hong-Ming Yu: Conceptualization; Data curation; Investigation.

Yu-Qiang Cheng: Conceptualization; Data curation; Investigation; Supervision.

Yan-Jun Xiang: Data curation; Methodology; Software.

Jing-Ya Zhong: Formal analysis; Software.

Qian-Zhi Ni: Data curation; Resources.

Li-Ping Zhou: Data curation.

Chao Liang: Data curation.

Hong-Kun Zhou: Data curation.

Wei-Wei Pan: Data curation.

Wei-Xing Guo: Data curation; Resources.

Jie Shi: Data curation; Resources.

Shu-Qun Cheng: Conceptualization; Data curation; Funding acquisition; Investigation; Visualization; Writing – original draft; Writing – review & editing.

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Competing interests

The authors declare that there is no conflict of interest.

Availability of data and materials

The data that support the findings of this study were available from the corresponding author upon reasonable request.

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Supplemental material

Supplemental material for this article is available online.

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