Received: 18 July 2024 | Revised: 4 October 2024 | Accepted: 7 October 2024

DOI: 10.1111/cas.16376

ORIGINAL ARTICLE

Cancer Science WILEY

Camrelizumab combined with gemcitabine and apatinib in treating advanced PD-L1-positive biliary tract cancers

Yitong Tian[1,2] | Changxian Li[3] | Ke Jin[4] | Ling Ma[1] | Jiaguang Zhang[1] | Xinyi Zhang[1] | Wei You[3] | Haoyang Shen[1] | Yuting Ding[1] | Hao Qian[1] | Xiangcheng Li[3] | Xiaofeng Chen[1,5,6,7]

[1]Department of Oncology, The First Affiliated Hospital with Nanjing Medical University, Nanjing, China

[2]Department of Oncology, The Fourth Affiliated Hospital of Nanjing Medical University, Nanjing, China

[3]Hepatobiliary Center, The First Affiliated Hospital with Nanjing Medical University, Nanjing, China

[4]Department of Medical Oncology, Liyang People's Hospital, Liyang, China

[5]Gusu School, Nanjing medical University, Suzhou, China

[6]Jiangsu Key Lab of Cancer Biomarkers, Prevention and Treatment, Collaborative Innovation Center for Cancer Personalized Medicine, Nanjing Medical University, Nanjing, China

[7]Department of Oncology, Nanjing Pukou People's Hospital, Nanjing, China

Correspondence
Xiaofeng Chen and Xiangcheng Li, Jiangsu Provincial People's Hospital, 300 Guangzhou Road, Nanjing 210029, Jiangsu, China.
Email: chenxiaofengnjmu@163.com and drxcli@njmu.edu.cn

Funding information
Beijing Xisike Clinical Oncology Research Foundation, Grant/Award Number: Y-HR2019-0367; Science and Technology Development Fund project of Pukou Branch of Jiangsu Provincial People's Hospital in 2021, Grant/Award Number: KJ2021-1

Abstract

The efficacy of combined chemotherapy and immunotherapy has previously been demonstrated in patients with biliary tract cancer. The aim of this study was to assess the efficacy and safety of camrelizumab in combination with gemcitabine and apatinib as a first- or second-line treatment for advanced programmed death-ligand 1 (PD-L1)-positive biliary tract cancer. This prospective, single-arm, and exploratory clinical trial aimed at recruiting 20 PD-L1-positive patients (tumor proportion score ≥1% or combined positive score ≥1) who met the inclusion criteria. Camrelizumab (200 mg) was administered in combination with gemcitabine (800 mg/m^2) and apatinib (250 mg). The primary endpoint was the objective response rate (ORR), and the secondary endpoints were progression-free survival (PFS), overall survival (OS), disease control rate (DCR), and safety. Fourteen patients were enrolled between September 2, 2020, and December 15, 2022. At the data cutoff on August 16, 2023, the median follow-up time was 11.4 months (interquartile range, 4.5–15.4), with one patient still undergoing treatment. Among the enrolled patients, six achieved a partial response, and four had stable disease. The ORR was 42.9% (95% confidence interval [CI], 17.7–71.1), and the DCR was 71.4% (95% CI, 41.9–91.6). The median PFS was 5.4 months (95% CI, 2.8–not reached), and the median OS was 13.5 months (95% CI, 5.7–not reached). The most frequent grade 3 or 4 treatment-related adverse event was neutropenia ($n = 4$, 29%). The combination of camrelizumab, gemcitabine, and apatinib showed promising efficacy and acceptable safety in patients with advanced PD-L1-positive biliary tract cancer.

KEYWORDS
advanced biliary tract cancer, apatinib, camrelizumab, gemcitabine, PD-L1 positive

Yitong Tian, Changxian Li and Ke Jin contributed equally to this work.

1 | INTRODUCTION

Biliary tract cancer (BTC) is an invasive adenocarcinoma that includes cholangiocarcinoma (cancers originating in the intrahepatic, perihilar, or distal biliary tree) and gallbladder carcinoma.[1] Despite varying prognoses and molecular characteristics between tumor sites, previous drug trials have often grouped all BTC subtypes together.

Gemcitabine was initially shown to be effective in treating advanced BTC in 2001.[2] In 2010, the ABC-02 study[3] established the cisplatin + gemcitabine (GC) chemotherapy regimen as the first-line treatment for advanced BTC. This study demonstrated that the GC regimen significantly prolonged the median progression-free survival (PFS) and overall survival (OS) compared with gemcitabine monotherapy. However, during the following decade, therapeutic progress was limited for BTC, and the survival benefit provided by the GC regimen remained relatively modest, with a median OS of less than 1 year.

Recently, immunotherapy using immune checkpoint inhibitors has led to breakthroughs in the treatment of various malignancies, including advanced BTC. The KEYNOTE-028 and KEYNOTE-158[4–7] trials revealed that pembrolizumab monotherapy achieved objective response rates (ORR) of 5.8%–13.0% in patients with advanced BTC. Similarly, nivolumab monotherapy demonstrated comparable outcomes, with an ORR of 22% in a phase II trial.[8] However, immunotherapy alone has limited efficacy in only a small subset of patients with advanced BTC, which is unsatisfactory as a standard of care treatment.

In 2018, our research team conducted a study on the use of chemotherapy combined with immunotherapy as first-line treatment for advanced BTC.[9] The results indicated an ORR of 52.8%, a disease control rate (DCR) of 91.7%, a median PFS of 6.2 months, and a median OS of 12.1 months. This study was incorporated into the Chinese Society of Clinical Oncology Guidelines for BTC Research. However, the incidence of adverse events in grade 3 and above was high during the study period.

Recent phase III studies, such as TOPAZ-1[10] and KEYNOTE-966,[11] support the combination of immunotherapy and the GC regimen as the standard first-line treatment for advanced BTC. Despite these advancements, the overall improvement in efficacy remains moderate, and identifying patients who can derive the greatest benefit from immunotherapy for the treatment of BTC remains a clinical need. Notably, our previous study demonstrated that patients with programmed death-ligand 1 (PD-L1)-positive BTC had a higher ORR (80%) and significant survival benefits.[9] These results are consistent with other studies[8,12,13] that indicate patients with PD-L1-positive BTC may derive greater benefits from immunotherapy.

Vascular normalization is crucial to create an optimal microenvironment for effective immunotherapy. Preclinical studies of lung cancer[14] and breast cancer[15] models have demonstrated that low-dose apatinib can induce tumor vascular normalization, reduce hypoxia levels, and enhance the efficacy of immunotherapy by facilitating $CD8^+$ T lymphocyte infiltration. Importantly, these activated immune cells promote further vascular normalization, establishing a positive feedback loop. These findings provide a solid theoretical foundation for combining anti-angiogenic therapy with immune checkpoint inhibitors for the treatment of malignant tumors. Several studies[16–18] have suggested that immunotherapy combined with angiogenesis inhibitors and first-line chemotherapy may be more effective than immunotherapy combined with chemotherapy alone for the treatment of BTC.

Based on these previous findings, this single-arm, exploratory clinical trial aimed to evaluate camrelizumab in combination with gemcitabine and apatinib mesylate as a first- or second-line treatment for patients with PD-L1-positive advanced BTC.

2 | MATERIALS AND METHODS

2.1 | Study design and participants

This was a prospective, single-arm, exploratory clinical trial that prospectively enrolled eligible patients aged 18–75 years with pathologically confirmed biliary tract malignancies, including intrahepatic cholangiocarcinoma, extrahepatic cholangiocarcinoma, and gallbladder cancer. To be included in the study, patients had to have PD-L1-positive tumors (tumor proportion score [TPS] ≥1% or combined positive score [CPS] ≥1), an Eastern Cooperative Oncology Group (ECOG) performance status of 0 or 1, at least one measurable lesion assessed using the Response Evaluation Criteria in Solid Tumors (RECIST) version 1.1, an estimated life expectancy of at least 12 weeks, and adequate organ function. Patients who previously received immune-targeted therapy or had previous or concurrent malignancies (not including those successfully treated for skin basal cell carcinoma or cervical carcinoma in situ) were excluded. Full inclusion and exclusion criteria can be found in the protocol (Appendix A).

The study protocol, along with its amendments and informed consent form, received approval from the independent ethics committee at the participating research center. All participants provided written informed consent before enrollment. This study was registered in the Chinese Clinical Trial Registry (ChiCTR2000040181).

2.2 | Procedures

Before the study, PD-L1 expression in tumor and immune cells was evaluated using immunohistochemistry, with TPS and CPS as the criteria used for interpretation. TPS represents the percentage of tumor cells showing partial or complete membranous staining; CPS is the total number of cells (lymphocytes, macrophages, and tumor cells) with partial or complete membranous staining divided by the total number of viable tumor cells in the sample, multiplied by 100.

Each enrolled patient received 800 mg/m^2 gemcitabine intravenously on days 1 and 8 of the treatment cycle, 200 mg camrelizumab intravenously on day 1, and 250 mg apatinib orally daily. The

treatment cycle lasted for 21 days. The chemotherapy cycles and dosages were adjusted based on patient tolerance. Patients who were intolerant to chemotherapy or achieved 4–6 cycles with stable disease (SD) or objective response received continued treatment with camrelizumab at a dose of 200 mg every 3 weeks and apatinib at a dose of 250 mg daily until disease progression, intolerable toxicity, or other conditions determined by the investigator, for up to 2 years of treatment.

Tumor assessments via computed tomography (CT) or magnetic resonance imaging (MRI) were conducted at baseline and approximately every 6 weeks throughout the trial, with consistent imaging techniques and retention of all data. Response assessments were performed by the investigators using RECIST version 1.1. Patients who achieved complete response (CR), partial response (PR), or SD were required to undergo re-examination 4 weeks after the initial evaluation to confirm the response. Patients who withdrew from the study owing to intolerable toxicities were evaluated for efficacy at the time of withdrawal. Survival monitoring was performed every 3 months following treatment discontinuation until death, loss to follow-up, consent withdrawal, or study termination.

Safety was continuously monitored; adverse events were documented, graded according to the National Cancer Institute Common Terminology Criteria for Adverse Events (version 5.0), and summarized according to type and severity.

2.3 | Outcomes

The primary endpoint was ORR, as assessed by the investigators according to RECIST version 1.1. The secondary endpoints were PFS, OS, DCR, and safety. ORR was calculated by determining the percentage of participants who achieved a confirmed CR or PR. The DCR represented the proportion of participants who had a confirmed CR, PR, or SD for at least 4 weeks. PFS was defined as the time from treatment initiation to disease progression or death, using the last response evaluation date as the censoring date if neither occurred. OS was defined as the time from treatment initiation to death from any cause, using the participant's last known survival date as the censoring date. Safety endpoints included the incidence and severity of adverse events. The exploratory endpoints assessed included PD-L1 status, CA199, tumor mutation burden (TMB), and other prognostic biomarkers.

2.4 | Sample size estimation

In 2001, a phase II trial[2] demonstrated an ORR of 22% for gemcitabine monotherapy as a first-line treatment for advanced BTC. Furthermore, a previous study[9] combining camrelizumab and gemcitabine + oxaliplatin (GEMOX) showed an ORR of approximately 50% for the treatment of advanced BTC. Based on this information, we hypothesized that combining gemcitabine with camrelizumab and apatinib as first-line or second-line treatment for PD-L1-positive advanced BTC could potentially achieve an ORR of 50%. To test this hypothesis, a one-sided test with an alpha level of 0.05 and a power (1-beta) of 0.8 would require a minimum sample size of 18 cases. Considering a potential dropout rate of 10%, we aimed to enroll approximately 20 patients.

2.5 | Statistical analysis

SPSS (version 25; IMB, Armonk, NY, USA) and R software (version 4.3.1; The R Foundation, Vienna, Austria) were used for statistical analyses and data visualization. Descriptive statistics, including mean, standard deviation, median, and maximum and minimum values, were used to summarize continuous variables. Frequency and percentages were used to summarize categorical variables. The Kaplan–Meier method was used to estimate the survival distribution of time-event variables, and the median survival time and two-sided 95% confidence intervals (CIs) were calculated. The log-rank test was used to evaluate the differences in survival between the two groups. The ORR and DCR were calculated as percentages with 95% CIs using the Clopper–Pearson method. Fisher's exact test was used to assess the differences in response among the clinical subgroups. Significant prognostic factors associated with survival were evaluated using the Cox proportional hazards regression model, reporting hazard ratios (HR) and 95% CIs in univariate analyses. A two-sided p-value of <0.05 was considered statistically significant.

3 | RESULTS

3.1 | Patient population

Fourteen patients were enrolled in the study between September 2, 2020, and December 15, 2022. Owing to the limited number of eligible participants and slow enrollment progress, enrollment was terminated ahead of schedule. The baseline characteristics of the study participants are presented in Table 1. The median age of the participants was 53 years (interquartile range [IQR], 45–60 years), with the majority having an ECOG performance status of 1 ($N = 12$, 86%). All patients underwent PD-L1 testing according to standardized criteria, with CPS values ≥1. Additionally, eight (57%) patients had a TPS <1%. Of the 14 patients, six (43%) had primary tumors located in the intrahepatic bile ducts, six (43%) had gallbladder involvement, and two (14%) had a primary tumor in the extrahepatic bile ducts. Seven (50%) patients underwent surgery prior to treatment. Most patients ($n = 11$, 79%) received first-line treatment after enrollment in the study.

As of the data cutoff on August 16, 2023, the median follow-up time was 11.4 months (IQR, 4.5–15.4). At the data cutoff, one (7%) patient continued to receive the study regimen; of the remaining participants, 12 (92%) had discontinued treatment due to radiological disease progression and one (8%) due to non-study-related death. All 14 patients were included in the treatment response evaluation.

TABLE 1 Baseline participants' characteristics.

	Participants ($N = 14$)
Age, year	53 (45–60)
Sex	
Male	7 (50%)
Female	7 (50%)
Eastern Cooperative Oncology Group performance status	
0	2 (14%)
1	12 (86%)
Primary tumor type	
Intrahepatic cholangiocarcinoma	6 (43%)
Extrahepatic cholangiocarcinoma	2 (14%)
Gallbladder cancer	6 (43%)
History of surgery	
Yes	7 (50%)
No	7 (50%)
Therapeutic regimen	
First-line treatment	11 (79%)
Second-line treatment	3 (21%)
PD-L1 status	
TPS <1%	8 (57%)
TPS ≥1%	6 (43%)
CPS <5	5 (36%)
CPS ≥5	9 (64%)
CPS <10	10 (71%)
CPS ≥10	4 (29%)
TMB status	
TMB-L	11 (79%)
TMB-H	2 (14%)
Not available	1 (7%)
MSS status	
MSI	0
MSS	13 (93%)
Not available	1 (7%)
Baseline carbohydrate antigen 19-9[a]	
< 30 units per mL	5 (36%)
≥ 30 units per mL	9 (64%)

Note: Data shown as *n* (%) or median (IQR).
[a]Carbohydrate antigen 19-9 units are grouped by the upper limit of the normal range.

TABLE 2 Clinical antitumor activity.

	Overall ($N = 14$)
Confirmed objective response rate, *n* (%; 95% CI)	6 (42.9%; 17.7–71.1)
Disease control rate, *n* (%; 95% CI)	10 (71.4%; 41.9–91.6)
Best overall response	
Complete response	0
Partial response	6 (42.9%)
Stable disease	4 (28.6%)
Progressive disease	4 (28.6%)

Note: Data are *n* (%), unless otherwise specified. Responses were assessed in accordance with the Response Evaluation Criteria in Solid Tumors version 1.1.

3.2 | Efficacy

The confirmed ORR was 42.9% (95% CI, 17.7–71.1), with six participants having a PR. Four (28.6%) participants had SD, with a DCR of 71.4% (95% CI, 41.9–91.6; Table 2). Changes in the diameter of the target lesion and the duration of treatment are depicted in Figure 1. The ORR was 45.5% among the 11 patients receiving first-line treatment and 33.3% among those receiving second-line treatment. The ORR was 50.0% (95% CI, 11.8–88.1) for both patients with gallbladder cancer and those with intrahepatic cholangiocarcinoma, whereas no response was observed in patients with extrahepatic cholangiocarcinoma. Figure S1 displays a forest plot illustrating the ORR for each subgroup.

Median PFS was 5.39 months (95% CI, 2.83–not reached [NR]), with a 6-month PFS rate of 42.9% (95% CI, 23.4–78.5) and 1-year PFS rate of 19.0% (95% CI, 6.0–60.2; Figure 2A). Median OS was 13.5 months (95% CI, 5.65–NR), with a 6-month OS rate of 64.3% (95% CI, 43.5–95.0) and a 1-year OS rate of 57.1% (95% CI, 36.3–89.9; Figure 2B). The median OS for patients receiving first-line treatment was 13.5 months (95% CI, 5.65–NR), with a median PFS of 5.13 months (95% CI, 2.53–NR). Of all the participants, those with intrahepatic cholangiocarcinoma had the longest survival, with a median PFS of 5.1 months (95% CI, 0.9–9.4) and a median OS of 13.6 months (95% CI, 2.8–24.4).

Figure 2C illustrates the outcomes for all 14 patients. No significant differences were observed in PFS or OS when stratifying according to baseline clinical characteristics (Tables S1 and S2).

3.3 | Safety

Table 3 outlines the treatment-related adverse events recorded during this study. The most frequently reported adverse events were hematologic events, including thrombocytopenia ($n = 10$, 71%), leukopenia ($n = 8$, 57%), and neutropenia ($n = 7$, 50%). The most common treatment-related adverse events of grade 3 or higher were neutropenia ($n = 4$, 29%), leukopenia ($n = 3$, 21%), and elevated aspartate aminotransferase/alanine aminotransferase levels ($n = 3$, 21%). Hypothyroidism ($n = 4$, 29%) and immune rash ($n = 4$, 29%) were the most commonly observed immune-related adverse events. Among the immune-related adverse events of grade 3 or higher, immune rash was the most frequent ($n = 3$, 21%). Two patients (14%) experienced serious adverse events, of which one (7%) was considered a treatment-related serious adverse event (acute kidney injury). Another patient (7%) died of

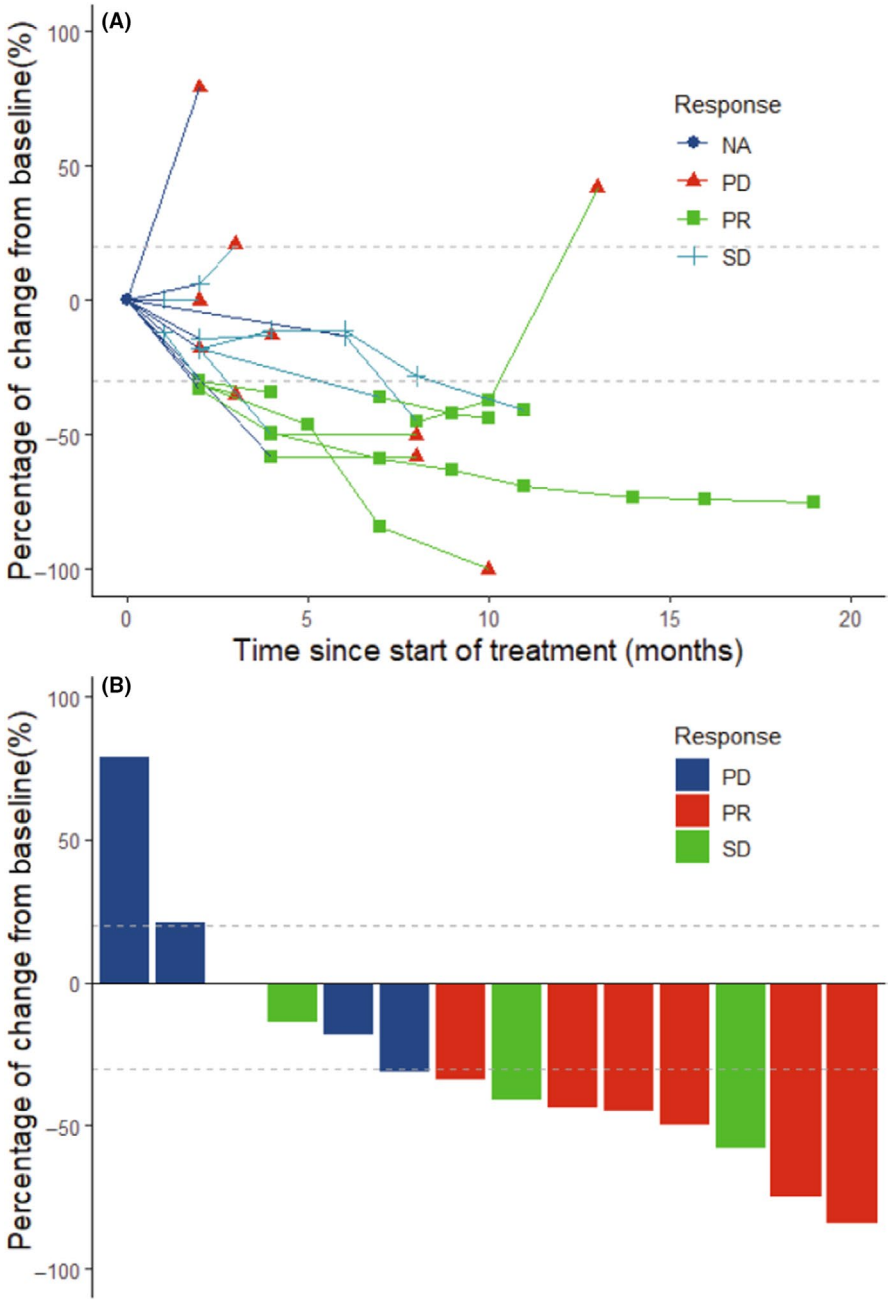

FIGURE 1 Changes in tumour burden. (A) Maximum percentage change from baseline in the size of target lesions with corresponding confirmed best responses based on RECIST version 1.1. The lower dotted line represents the cutoff value for a partial response, and the upper dotted line represents the cutoff value for progression. (B) Percentage change from baseline in the total number of target lesions over time. The dotted lines represent 20% tumour increase and 30% tumour reduction from baseline.

gastrointestinal bleeding, which was considered to be related to disease progression.

4 | DISCUSSION

In this single-arm, exploratory study, the combination of camrelizumab with gemcitabine and apatinib demonstrated promising activity in patients with advanced PD-L1-positive biliary malignancies and maintained a manageable safety profile. To the best of our knowledge, this is the first prospective investigation of chemotherapy combined with immunological and targeted therapy in PD-L1-positive BTC.

This study included patients with PD-L1-positive tumors as previous research[9] suggested that these patients may have benefitted the most from immunotherapy. Kim et al.,[8] evaluating nivolumab for advanced refractory BTC, and Li et al.,[12] evaluating the combination of toripalimab and gemcitabine + S-1 chemotherapy, both found a significant association between PD-L1 expression and prolonged PFS. Similarly, a study[13] on toripalimab in combination with lenvatinib and GEMOX as a first-line treatment for intrahepatic cholangiocarcinoma showed a positive correlation between PD-L1 expression and ORR (PD-L1 positive vs. PD-L1 negative: 100% vs. 68.8%; $p = 0.048$). These studies provide compelling evidence that PD-L1 is a valuable biomarker for predicting the efficacy of immunotherapy in BTC.

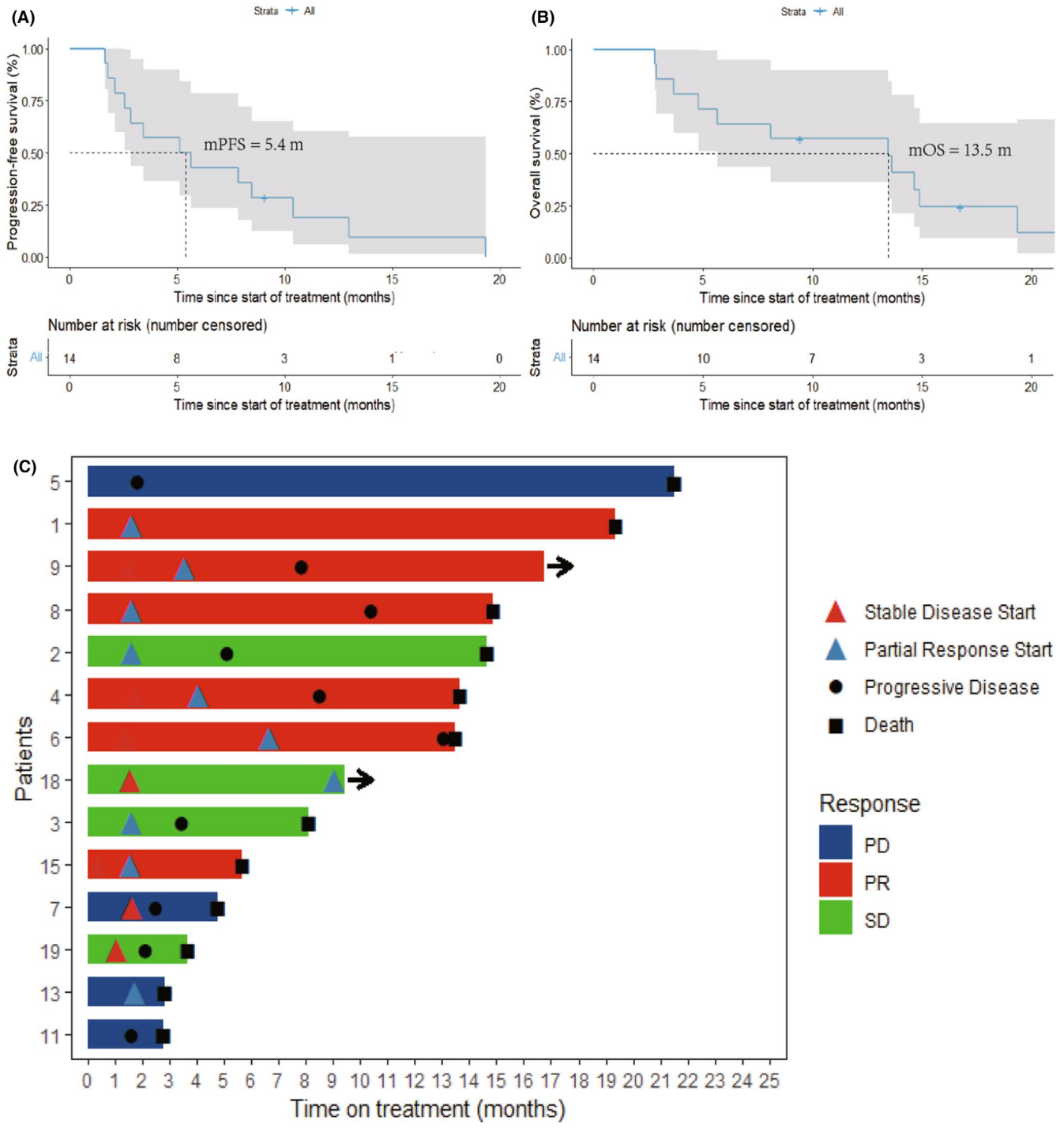

FIGURE 2 Progression-free survival and overall survival. (A) Progression-free survival in all patients over time. (B) overall survival in all patients overtime. (C) A swimmer plot showing outcomes in all patients from the start of treatment to either disease progression or the date of last follow-up.

Interestingly, Kang et al.[19] found that altering the positivity threshold for PD-L1 expression resulted in different outcomes. Specifically, patients with TPS ≥50% achieved an ORR of 37.5%, whereas those with TPS <50% had an ORR of 6.5%. This finding suggests that higher levels of PD-L1 expression within the tumor are associated with a greater likelihood of response to immunotherapy. However, no standardized guidelines exist for assessing PD-L1 expression, leading to confounding and inconsistent research outcomes. In our study, we used CPS ≥1 as the cutoff value and stratified PD-L1 expression levels based on CPS thresholds of 5 and 10. However, due to the limited sample size, we did not observe a clear trend between the efficacy and survival data in these subgroups (Figure S2). Further investigations are necessary to optimize treatment strategies and improve outcomes in patients with PD-L1-positive BTC.

TABLE 3 Treatment-related adverse events (TRAEs).

	Grades 1 and 2	Grades 3 and 4	All grades
Any TRAE	13 (93%)	8 (57%)	13 (93%)
Hematologic			
White blood cell count decreased	5 (36%)	3 (21%)	8 (57%)
Neutrophil count decreased	3 (21%)	4 (29%)	7 (50%)
Anemia	2 (14%)	1 (7%)	3 (21%)
Platelet count decreased	8 (57%)	2 (14%)	10 (71%)
Increased alanine aminotransferase (ALT) or aspartate aminotransferase (AST)	4 (29%)	3 (21%)	7 (50%)
Non-hematologic			
Nausea	4 (29%)	0	4 (29%)
Anorexia or dysgeusia	4 (29%)	0	4 (29%)
Abdominal pain	1 (7%)	0	1 (7%)
Reactive cutaneous capillary endothelial proliferation (RCCEP)	2 (14%)	0	2 (14%)
Fever	5 (36%)	0	5 (36%)
Proteinuria	3 (21%)	1 (7%)	4 (29%)
Hypertension	0	2 (14%)	2 (14%)
Acute kidney injury	0	1 (7%)	1 (7%)
Diarrhea	1 (7%)	0	1 (7%)
Venous thrombosis	1 (7%)	0	1 (7%)
Immune-related toxicities			
Hypothyroidism	4 (29%)	0	4 (29%)
Hyperthyroidism	1 (7%)	0	1 (7%)
Immune rash	1 (7%)	3 (21%)	4 (29%)
Immune myocarditis	1 (7%)	0	1 (7%)
Immune pneumonia	1 (7%)	0	1 (7%)
Hypocorticosis	0	1 (7%)	1 (7%)

Note: Data shown as *n* (%) of all participants ($N = 14$).

This study demonstrated an ORR of 42.9% and a DCR of 71.4%. In comparison, the ORRs reported in two significant phase III clinical trials, TOPAZ-1[10] and KEYNOTE-966,[11] were 26.7% and 29%, respectively. Previous phase I and II clinical trials[9,12,20,21] investigating first-line immunotherapy combined with chemotherapy for advanced BTC have reported ORRs between 30.6%–73.4%. Second-line immunotherapy with chemotherapy has achieved an ORR of approximately 10%.[22] Another study, SAGC,[16] reported an ORR of 37.5% using sintilimab and anlotinib combined with GC as the first-line treatment for advanced BTC. Although the results of our study are comparable with these findings, we included both first-line and second-line treatments, which may have impacted our results.

The efficacy of immunotherapy varies according to the site of cholangiocarcinoma. TOPAZ-1[10] and KEYNOTE-966[11] subgroup analyses showed survival benefits for intrahepatic cholangiocarcinoma (HR, 0.79 [95% CI 0.58–0.98] and 0.75 [95% CI 0.64–0.91], respectively). A study using toripalimab combined with lenvatinib and GEMOX as first-line treatment for advanced intrahepatic cholangiocarcinoma reported an ORR of up to 80%.[13] This higher ORR may be attributed to the inherent efficacy of intrahepatic cholangiocarcinoma treatment. Consequently, this may have influenced the overall ORR found in the current study as we included patients with intrahepatic cholangiocarcinoma, extrahepatic cholangiocarcinoma, and gallbladder cancer.

Chemotherapy drugs and their intensity can also have an effect on the ORR. Three-drug regimens demonstrate an ORR of 25%–31%,[23,24] two-drug combinations reach 18.7%–29.8%,[10,11,16] and single-agent gemcitabine achieves 11.9%–21.9%.[2,25] However, increasing the number of chemotherapeutic agents often coincides with a reciprocal increase in toxicity. Our previous study examining the GEMOX regimen[9] caused high fever (73%) and grade 3 or higher adverse events (70%). Therefore, instead of the standard dose of 1000 mg/m^2, we opted to use a reduced dose of gemcitabine (800 mg/m^2) in the current study. Apatinib was introduced to mitigate the toxicity of chemotherapy and immunotherapy. Consequently, the ORR achieved herein with a single chemotherapeutic agent plus immune- and anti-angiogenic therapy was comparable with that with a two-drug chemotherapy combination plus immune and anti-angiogenic therapy.[16] These findings suggested that the combination of camrelizumab, gemcitabine, and apatinib had potential antitumor activity against advanced BTC and warrants further study.

In terms of safety, hematologic toxicities were the most common adverse events, primarily due to chemotherapy; however, these were effectively managed with dose adjustments and symptomatic treatments. Grade 3 or higher adverse reactions were less frequent in this study than in our previous studies (70% vs. 56%). The incidence of fever (73% vs. 36%) and reactive cutaneous capillary endothelial proliferation (62% vs. 14%) also decreased, whereas the addition of apatinib led to proteinuria (0 vs. 29%) and hypertension (0 vs. 14%).[9] Immune-related adverse reactions were relatively infrequent and manageable, without a significant influence on quality of life or treatment. Overall, based on the current data, the combination of camrelizumab, gemcitabine, and apatinib showed clinically acceptable safety in patients with advanced BTC.

This study has several limitations that should be acknowledged. First, this was a single-center study with a small sample size, which introduced the potential for bias in multivariate and subgroup analyses. Second, the inclusion of different tumor types increased the heterogeneity and complexity when interpreting treatment responses, particularly considering the small sample size. Last, we initially intended to enroll 20 patients, but ultimately only included 14 owing to several factors. The low incidence of BTC[26] and an overall PD-L1 positivity rate of approximately 8–12%[27–30] in BTC led to a prolonged recruitment period and slow study progress. Additionally, the publications of TOPAZ-1[10] and KEYNOTE-966,[11] which established immunotherapy plus chemotherapy as the standard treatment for BTC, made the enrollment process more challenging. Considering these circumstances, the investigators opted to terminate the study prior to completion.

AUTHOR CONTRIBUTIONS

Yitong Tian: Data curation; resources; writing – original draft. **Changxian Li:** Supervision; writing – review and editing. **Ke Jin:** Supervision; writing – review and editing. **Ling Ma:** Conceptualization; methodology. **Jiaguang Zhang:** Data curation. **Xinyi Zhang:** Data curation. **Wei You:** Supervision; validation. **Haoyang Shen:** Formal analysis; software. **Yuting Ding:** Data curation. **Hao Qian:** Data curation. **Xiangcheng Li:** Project administration; writing – review and editing. **Xiaofeng Chen:** Conceptualization; funding acquisition; resources; supervision; writing – review and editing.

ACKNOWLEDGEMENTS

We thank the enrolled patients and their families for participating in the study.

FUNDING INFORMATION

This study was funded by the Beijing Xisike Clinical Oncology Research Foundation (No. Y-HR2019-0367) and the Science and Technology Development Fund project of Pukou Branch of Jiangsu Provincial People's Hospital in 2021 (No. KJ2021-1).

CONFLICT OF INTEREST STATEMENT

The authors have no relevant financial or non-financial interests to disclose.

ETHICS STATEMENT

Approval of the research protocol by an Institutional Review Board: This study was performed in line with the principles of the Declaration of Helsinki. Approval was granted by the Ethics Committee of the First Affiliated Hospital with Nanjing Medical University (No. 2020-SR-301, No. 2020-SR-301.A1).

Informed Consent: N/A.

Registry and the Registration No. of the study/trial: ChiCTR.gov.cn: ChiCTR2000040181.

Animal Studies: N/A.

CONSENT

Informed consent was obtained from all individual participants included in the study.

ORCID

Yitong Tian https://orcid.org/0009-0005-1650-8065

Xiaofeng Chen https://orcid.org/0000-0002-0754-951X

REFERENCES

1. Valle JW, Kelley RK, Nervi B, Oh DY, Zhu AX. Biliary tract cancer. *Lancet*. 2021;397:428-444.
2. Penz M, Kornek GV, Raderer M, et al. Phase II trial of two-weekly gemcitabine in patients with advanced biliary tract cancer. *Ann Oncol*. 2001;12:183-186.
3. Valle J, Wasan H, Palmer DH, et al. Cisplatin plus gemcitabine versus gemcitabine for biliary tract cancer. *N Engl J Med*. 2010;362:1273-1281.
4. Chung HC, Ros W, Delord JP, et al. Efficacy and safety of pembrolizumab in previously treated advanced cervical cancer: results from the phase II KEYNOTE-158 study. *J Clin Oncol*. 2019;37:1470-1478.
5. Ott PA, Bang YJ, Piha-Paul SA, et al. T-cell-inflamed gene-expression profile, programmed death ligand 1 expression, and tumor mutational burden predict efficacy in patients treated with pembrolizumab across 20 cancers: KEYNOTE-028. *J Clin Oncol*. 2019;37:318-327.
6. Piha-Paul SA, Oh DY, Ueno M, et al. Efficacy and safety of pembrolizumab for the treatment of advanced biliary cancer: results from the KEYNOTE-158 and KEYNOTE-028 studies. *Int J Cancer*. 2020;147:2190-2198.
7. Ueno M, Chung HC, Nagrial A, et al. Pembrolizumab for advanced biliary adenocarcinoma: results from the multicohort, phase II KEYNOTE-158 study. *Ann Oncol*. 2018;29:viii210.
8. Kim RD, Chung V, Alese OB, et al. A phase 2 multi-institutional study of nivolumab for patients with advanced refractory biliary tract cancer. *JAMA Oncol*. 2020;6:888-894.
9. Chen X, Wu X, Wu H, et al. Camrelizumab plus gemcitabine and oxaliplatin (GEMOX) in patients with advanced biliary tract cancer: a single-arm, open-label, phase II trial. *J Immunother Cancer*. 2020;8:8.
10. Rimini M, Fornaro L, Lonardi S, et al. Durvalumab plus gemcitabine and cisplatin in advanced biliary tract cancer: an early exploratory analysis of real-world data. *Liver Int*. 2023;43:1803-1812.
11. Kelley RK, Ueno M, Yoo C, et al. Pembrolizumab in combination with gemcitabine and cisplatin compared with gemcitabine and cisplatin alone for patients with advanced biliary tract cancer (KEYNOTE-966): a randomised, double-blind, placebo-controlled, phase 3 trial. *Lancet*. 2023;401:1853-1865.
12. Li W, Wang Y, Yu Y, et al. Toripalimab in advanced biliary tract cancer. *Innovations*. 2022;3:3.
13. Shi GM, Huang XY, Wu D, et al. Toripalimab combined with lenvatinib and GEMOX is a promising regimen as first-line treatment

for advanced intrahepatic cholangiocarcinoma: a single-center, single-arm, phase 2 study. *Signal Transduct Target Ther*. 2023;8:106.
14. Zhao S, Ren S, Jiang T, et al. Low-dose apatinib optimizes tumor microenvironment and potentiates antitumor effect of PD-1/PD-L1 blockade in lung cancer. *Cancer Immunol Res*. 2019;7:630-643.
15. Wang Q, Gao J, Di W, et al. Anti-angiogenesis therapy overcomes the innate resistance to PD-1/PD-L1 blockade in VEGFA-overexpressed mouse tumor models. *Cancer Immunol Immunother*. 2020;69:1781-1799.
16. Jingjing L, Qi X, Wei Q, et al. A phase 2, randomized, open-label, multicenter study of sintilimab and anlotinib in combination with gemcitabine plus cisplatin (GemCis) as first-line therapy in patients (pts) with advanced biliary tract cancer (BTC): SAGC. *J Clin Oncol*. 2022;40:4100.
17. Shi C, Li Y, Yang C, et al. Lenvatinib plus programmed cell death Protein-1 inhibitor beyond first-line systemic therapy in refractory advanced biliary tract cancer: a real-world retrospective study in China. *Front Immunol*. 2022;13:946861.
18. Zhou J, Sun Y, Zhang W, et al. Phase Ib study of anlotinib combined with TQB2450 in pretreated advanced biliary tract cancer and biomarker analysis. *Hepatology*. 2023;77:65-76.
19. Kang J, Jeong JH, Hwang HS, et al. Efficacy and safety of pembrolizumab in patients with refractory advanced biliary tract cancer: tumor proportion score as a potential biomarker for response. *Cancer Res Treat*. 2020;52:594-603.
20. Oh D-Y, Lee K-H, Lee D-W, et al. Phase II study assessing tolerability, efficacy, and biomarkers for durvalumab (D) ± tremelimumab (T) and gemcitabine/cisplatin (GemCis) in chemo-naïve advanced biliary tract cancer (aBTC). *J Clin Oncol*. 2020;38:4520.
21. Ueno M, Ikeda M, Morizane C, et al. Nivolumab alone or in combination with cisplatin plus gemcitabine in Japanese patients with unresectable or recurrent biliary tract cancer: a non-randomised, multicentre, open-label, phase 1 study. *Lancet Gastroenterol Hepatol*. 2019;4:611-621.
22. Sahai V, Griffith KA, Lin BS-L, et al. A multicenter phase Ib/II study of liposomal-irinotecan, 5-fluorouracil (5-FU), and leucovorin (LV) with nivolumab as second-line therapy for patients with advanced biliary tract cancer (BilT-03). *J Clin Oncol*. 2022;40:438.
23. Phelip JM, Desrame J, Edeline J, et al. Modified FOLFIRINOX versus CISGEM chemotherapy for patients with advanced biliary tract cancer (PRODIGE 38 AMEBICA): a randomized phase II study. *J Clin Oncol*. 2022;40:262-271.
24. Shroff RT, Guthrie KA, Scott AJ, et al. SWOG 1815: a phase III randomized trial of gemcitabine, cisplatin, and nab-paclitaxel versus gemcitabine and cisplatin in newly diagnosed, advanced biliary tract cancers. *J Clin Oncol*. 2023;41:LBA490.
25. Furuse J, Okusaka T, Miyazaki M, Taniai H, Nimura Y, BT22 Study Group. A randomized study of gemcitabine/cisplatin versus single-agent gemcitabine in patients with biliary tract cancer. *J Clin Oncol*. 2009;27:4579.
26. Vogel A, Bridgewater J, Edeline J, et al. Biliary tract cancer: ESMO clinical practice guideline for diagnosis, treatment and follow-up. *Ann Oncol*. 2023;34:127-140.
27. Fontugne J, Augustin J, Pujals A, et al. PD-L1 expression in perihilar and intrahepatic cholangiocarcinoma. *Oncotarget*. 2017;8:24644-24651.
28. Mody K, Starr J, Saul M, et al. Patterns and genomic correlates of PD-L1 expression in patients with biliary tract cancers. *J Gastrointest Oncol*. 2019;10:1099-1109.
29. Walter D, Herrmann E, Schnitzbauer AA, et al. PD-L1 expression in extrahepatic cholangiocarcinoma. *Histopathology*. 2017;71:383-392.
30. Weinberg BA, Xiu J, Lindberg MR, et al. Molecular profiling of biliary cancers reveals distinct molecular alterations and potential therapeutic targets. *J Gastrointest Oncol*. 2019;10:652-662.

SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

How to cite this article: Tian Y, Li C, Jin K, et al. Camrelizumab combined with gemcitabine and apatinib in treating advanced PD-L1-positive biliary tract cancers. *Cancer Sci*. 2025;116:204-213. doi:10.1111/cas.16376

APPENDIX A

Supporting information Inclusion criteria and exclusion criteria.

Inclusion criteria.

A. Pathologically confirmed biliary malignancies, including intrahepatic cholangiocarcinoma, extrahepatic cholangiocarcinoma, gallbladder carcinoma, and immunohistochemical evidence of PD-L1 positive (TPS ≥1% or IPS ≥1%).

B. Age 18–75 years old, gender unlimited.

C. Expected survival ≥3 months.

D. ECOG score 0–1.

E. Have at least one measurable lesion according to RECIST 1.1 criteria.

F. No previous treatment with apatinib mesylate, gemcitabine, or PD-1/PD-L1 monoclonal antibody. Patients who had previously received gemcitabine and relapsed more than 6 months after the end of adjuvant chemotherapy, or who had failed to receive platinum/Tiggio/capecitabine and other drugs as first-line therapy could be enrolled, and the number of previous late treatment lines did not exceed one line.

G. The major organs function well, that is, the relevant examination indicators within 14 days before enrollment meet the following requirements:

Hemoglobin ≥90 g/L (no transfusion within 14 days);

Neutrophil count > 1.5 × 109/L;

Platelet count ≥100 × 109/L;

Total bilirubin ≤1.5 × ULN (upper limit of normal);

Serum glutamic pyruvic aminotransferase (ALT) or serum glutamic oxalacetic aminotransferase (AST) ≤ 2.5 × ULN; If there is liver metastasis, ALT or AST ≤5 × ULN;

Endogenous creatinine clearance ≥60 mL/min (Cockcroft–Gault formula);

Cardiac Doppler ultrasound assessment: left ventricular ejection fraction (LVEF) ≥ 50%.

H. Signed informed consent and compliance is good, and family members agree to cooperate with survival follow-up.

Exclusion criteria.

A. Previous or co-existing malignancies other than basal cell carcinoma of the skin and carcinoma in situ of the cervix, which have been cured; Patients with small gastric stromal tumors, as well as other early tumors after radical treatment, other tumors that

investigators judge will not affect the patient's life in the short term can be excluded.

B. Participated in other drug clinical trials within 4 weeks.

C. Patients with known or history of central nervous system metastasis prior to screening. For patients with clinically suspected central nervous system (CNS) metastasis, CT or MRI examination must be performed within 28 days before enrollment to rule out CNS metastasis.

D. Patients with a history of unstable angina pectoris; Patients with newly diagnosed angina pectoris or myocardial infarction within 3 months prior to screening; Arrhythmias (including QTcF: ≥450 ms in men and ≥470 ms in women) requiring long-term use of antiarrhythmic drugs and New York Heart Association grade ≥ II cardiac insufficiency.

E. Urine routine indicated urinary protein ≥++ and confirmed 24-h urinary protein quantification >1.0 g.

F. For female subjects: should be surgically sterilized, postmenopausal, or consent to use a medically approved contraceptive during the study treatment and for 6 months after the end of the study treatment period; Serum or urine pregnancy tests must be negative within 7 days prior to study enrollment and must be non-lactating. Male subjects: patients who should be surgically sterilized or who have consented to use a medically approved contraceptive method during the study treatment period and for 6 months after the end of the study treatment period;

G. Used immunotargeted therapy drugs.

H. A history of immunodeficiency, or other acquired or congenital immunodeficiency diseases, or a history of organ transplantation.

I. Patients with infectious pneumonia, non-infectious pneumonia, interstitial pneumonia and others requiring corticosteroid use.

J. History of chronic autoimmune diseases, such as systemic lupus erythematosus; history of ulcerative enteritis, Crohn's disease and other inflammatory bowel diseases, irritable bowel syndrome and other chronic diarrheal diseases; a history of sarcoidosis or tuberculosis; history of active hepatitis B, hepatitis C and HIV infection.

K. Patients with hypersensitivity to human or murine monoclonal antibodies.

L. Have a history of psychotropic drug abuse and can not quit or have mental disorders.

M. Pleural effusion or abdominal effusion with clinical symptoms requiring clinical intervention.

N. A concomitant disease that, in the judgment of the investigator, seriously endangers the patient's safety or interferes with the patient's completion of the study.