Observational Study

Medicine

OPEN

The efficiency and safety of immune checkpoint inhibitors for advanced biliary tract cancers based on gene profiles

A retrospectively controlled study

G.L. Yao, MD[a], Y.G. Fan, MD[a,*]

Abstract

Immune checkpoint inhibitors are potential agents to improve the survival of advanced biliary tract cancers (ABTCs). The current results are controversial because the predictors are imprecise. We present our primary experience with ABTCs based on gene landscape with exciting outcomes. ABTCs who were admitted to The First Affiliated Hospital of Henan University of Science and Technology from October 2019 to March 2021 were enrolled. They were divided into chemotherapy group or immunotherapy group according to the treatment. The primary endpoints were overall survival (OS) and progression-free survival (PFS), and the secondary endpoints were response and toxicities. SSPS 16.0 was used for statistical analysis. A total of 33 patients were enrolled, including 25 in the chemotherapy group and 8 in the immunotherapy group. The median OS and PFS of the chemotherapy group were 2 and 4 months, respectively. The estimated median OS and PFS of immunotherapy were 10 + and 10 + months, respectively. The differences of OS and PFS between the 2 groups were significant ($P = .000$; $P = .003$). Stratified analysis showed that these differences were mainly from those patients with high expression of PD-L1 > 10%. The difference in the overall response was significant between 2 groups ($\chi^2 = 9.275$; $P = .026$). The difference in adverse events between the 2 groups was not significant. Immune checkpoint inhibitors were effective and safe for ABTCs with high expression of PD-L1. The threshold should be precise.

Abbreviation: ABTC = advanced biliary tract cancer, AE = adverse event, BTC = biliary tract cancer, CR = completed response, ECOG = Eastern Cooperation Oncology Group, ICI = immune checkpoint inhibitors, NGS = new generation sequencing, ORR = objective response rate, OS = overall survival, PFS = progression-free survival, PR = partial response, SD = stable disease, TMB = tumor mutational burden.

Keywords: biliary tract cancer, chemotherapy, immune checkpoint inhibitor, overall survival, progression-free survival

1. Introduction

Biliary tract cancers (BTCs) include a series of cancers with poor prognosis. Radical resection is the only procedure for patients striving for long-term survival. Unfortunately, most patients are diagnosed at later stage without the opportunity for surgery.[1,2] Even worth, no chemotherapeutic schedule produced exciting outcomes for advanced biliary tract cancers (ABTCs).[3,4] Target therapy and immunotherapy have high hopes for improving outcomes, but the results of current clinical trials were disappointed.[5,6] Immune checkpoint inhibitors (ICIs) are recommended by the FDA for solid tumors with positive expression of PD-L1. KEYNOTE-028[7] showed a slight improvement in progression-free survival (PFS) and overall survival (OS) in ABTC patients treated with ICIs. Few patients had significant long-term survival, while, as the main predictors, their genetic landscape was not clear. We also observed several patients who significantly benefited from ICIs. This study presents our primary experience using ICIs for ABTC patients with exciting results.

2. Materials and methods

2.1. Inclusion and exclusion criteria

We retrospectively analyzed all patients who were admitted to The First Affiliated Hospital of Henan University of Science and Technology from October 2019 to March 2021. All patients diagnosed of ABTCs were with local advanced disease or distant metastasis. Pathological diagnosis was performed via histological or cytological testing. Enhanced computed tomography (CT) scan and enhanced magnetic resonance imaging were routinely recommended with positron emission tomography-CT, if necessary. A multidisciplinary team ultimately confirmed the later stage. Next-generation sequencing (NGS) was recommended

The authors have no conflicts of interest to disclose.

All data generated or analyzed during this study are included in this published article [and its supplementary information files].

[a] Department of General Surgery, The First Affiliated Hospital of Henan University of Science and Technology, Luoyang, China.

**Correspondence: Y.G. Fan, Department of general surgery, The First Affiliated Hospital of Henan University of Science and Technology, 24 Jinghua Road, Luoyang 471000, China (e-mail:15237917026@163.com).*

How to cite this article: Yao GL, Fan YG. The efficiency and safety of immune checkpoint inhibitors for advanced biliary tract cancers based on gene profiles: A retrospectively controlled study. Medicine 2022;101:37(e30443).

Received: 12 May 2022 / Received in final form: 27 July 2022 / Accepted: 29 July 2022

http://dx.doi.org/10.1097/MD.0000000000030443

for each patient with unresectable BTC. Some patients received NGS, while others rejected primarily for economic reasons. Patients with positive PD-L1 were assigned to the immunotherapy group, and other patients were assigned to the chemotherapy group. The ethics committee of The First Affiliated Hospital of Henan University of Science and Technology approved the trial. The inclusion criteria were age >18 years, Eastern Cooperative Oncology Group (ECOG) performance status of 0 to 1, and an expected survival of >3 months. The exclusion criteria were patients without pathological diagnosis or with poor ECOG score of > 1. Patients who were unwilling to be involved in this trial were excluded either. Each included patient provided written informed consent.

2.2. Tumor sample collection for molecular analysis

Formalin-fixed paraffin-embedded tumor tissues were sent to an authoritative third party (Jiangsu Simcere Diagnostics Co., Ltd) for immunohistochemistry and gene sequencing according to the manufacturers' protocol. PD-L1 expression was tested by immunohistochemistry using a monoclonal antibody targeting PD-L1 (SP263), and PD-L1 positivity was defined as a combined positive score ≥ 1. Combined positive score is the number of PD-L1-stained cells (tumor cells, lymphocytes, and macrophages) divided by the total number of tumor cells multiplied by 100.

Tumor mutational burden (TMB) was defined as the number of somatic, coding, base substitution, and indel mutations per megabase of genome examined. The 539 cancer genes targeted NGS panel. TMB was counted by summing all base substitutions and indels in the coding region of targeted genes, excluding synonymous alterations, alterations of allele frequency < 0.02 and alterations listed as known somatic alterations in COSMIC.

To determine microsatellite instability (MSI) status, 334 homopolymer repeat loci with adequate coverage on the panel were selected, and reads that were successfully mapped to each of the 334 loci were extracted from the deduplicated BAM file. Msisensor[8] was used to evaluate the distribution of read counts among various repeat lengths and determine the stability of each locus. An MSI score was defined as the percentage of unstable loci. Any sample with an MSI score ≥ 0.15 was classified MSI-high, and an MSI scores ≥ 0.05 and < 0.15 was classified as MSI-low. Otherwise, it was classified as microsatellite stable.

2.3. Treatment

When comprehensive assessment was completed, chemotherapy was scheduled according to NCCN guidelines. Gemcitabine or fluorouracil alone or in combination was recommended. Irinotecan was recommended for patients with progression. ICI (sintilimab, 200 mg injection, per 3 weeks) was only recommended additionally to patients with PD-L1-positive tumors. Tumor markers and enhanced CT scans and/or magnetic resonance imaging were scheduled every 2 months, with additional examination if necessary. Treatment was terminated upon confirmation of completed response, intolerable toxicity, or the patients or investigators decision to withdraw. Patients with a complete response (CR) were recommended for at least 3 months of additional treatment. Adverse events (AEs) were monitored throughout the treatment and 1 month after the last dose.

2.4. Outcomes

The primary endpoints were OS and PFS. The secondary endpoints were response, including CR, partial response (PR), stable disease (SD), and progressive disease. The response was assessed using mRESIST 1.1.[9] The efficiency was assessed by 2 independent investigators with a discussion when unconformity existed. AEs were graded by the criteria of the National Cancer Institute Common Terminology Criteria for Adverse Events (version 5.0).[10]

2.5. Statistical analyses

SPSS 16.0 was used for statistical analyses. Time-to-event endpoints were estimated using the Kaplan–Meier method, with censoring at the last date of assessment for patients with missing data. Enumeration data, such as gender, ECOG, diagnosis, disease status, response, and toxicities, were statistically analyzed using chi square tests. Measurement data, such as age and cycles of chemotherapy, were statistically analyzed using Student *t* test. $P < .05$ was considered significant. The threshold of PD-L1 was calculated using cluster analysis.

3. Results

A total of 33 patients were included in this trial. Twenty-five patients were divided into the chemotherapy group as no NGS or negative expression of PD-L1 and the other 8 patients with PD-L1-positive tumors were divided into the immunotherapy group. Differences in the baseline characteristics, including age, gender, ECOG, diagnosis, and disease status, did not reach significance. These results are shown in Table 1. Eight patients had positive PD-L1 tumors as measured by 539-panel NGS. The PD-L1, TMB, and MSI statuses are listed in Table 2. Four patients had high PD-L1 expression (>10%). Two of these patients were diagnosed with multiple metastases in the liver and lung, and both were recommended to receive sintilimab 200 mg injections as a second-line schedule every 3 weeks and additional capecitabine orally twice daily for 14 days continuously with a 7-day break. Another 2 patients with high expression of PD-L1 were recommended to receive sintilimab 200 mg injection as a first-line schedule every 3 weeks with oral capecitabine. All 4 patients had PR based on the criteria of mRECIST 1.1, including 1 CR at the 6-month follow-up, which was diagnosed with mucinous adenocarcinoma of the bile duct. All 4 patients were followed up without progression. One patient who reached CR discontinued treatment 3 months after CR, and another patient discontinued because the tumor did not continue to shrink after 10 cycles of therapy. The other 2 patients continued as planned. Four patients with 1% or 5% expression of PD-L1 reached SD. Three of them experienced endpoint events at 6 to 10 months. Twenty-five patients in the chemotherapy group

Table 1

Baseline characteristics of included patients.

	Chemotherapy (25)	Immunotherapy (8)	*P*
Age	60.0 ± 15.0	53.8 ± 16.5	.301
Gender			.678
Male	10	2	
Female	15	6	
ECOG			.616
0	4	2	
1	21	6	
Diagnosis			.833
GBC	10	4	
ECC	6	2	
ICC	9	2	
Disease status			.687
Local advanced	9	2	
Metastatic	19	6	

ECC = extrahepatic cholangiocarcinoma, ECOG = Eastern Cooperative Oncology Group, GBC = gallbladder cancer, ICC = intrahepatic cholangiocarcinoma.

Table 2

Results of next-generation sequencing.

Patient's number	PD-L1 (%)	TMB (Muts/Mb)	MSI
1	>1	9.3	MSS
2	>1	7.1	MSS
3	>1	5.3	MSS
4	5	13.8	MSS
5	10	22.5	MSI-H
6	10	9.7	MSS
7	25	14.9	MSI-H
8	30	7.6	MSS

MSI = microsatellite instability, MSI-H = microsatellite instability-high, MSI-L = microsatellite instability-low, MSS = microsatellite stable, TMB = tumor mutational burden.

Table 3

The response to therapy.

	Chemotherapy (N = 25)	Immunotherapy (N = 8)	*P*
Cycles of therapy	4.6 ± 2.5	9.5 ± 3.8	.018
Response (%)			.026
CR	0 (0.0)	1 (12.5)	
PR	4 (16.0)	3 (50.0)	
SD	11 (44.0)	4 (37.5)	
PD	10 (40.0)	0 (0.0)	
mPFS (m)	2	10+	.003
mOS (m)	4	10+	.000
6-mo PFS (%)	3/25 (12.0)	4/8 (50.0)	.042
6-mo OS (%)	7/25 (28.0)	6/8 (75.0)	.035

CR = completed response, mPFS = median progression-free survival, mOS = median overall survival, PD = progressive disease, PR = partial response, SD = stable disease.

without PD-L1 expression or rejection of NGS testing were recommended to receive chemotherapy based on gemcitabine or fluorouracil alone or in combination. Four patients reached PR, and 11 patients reached SD without CR.

At the cutoff date, 4.6 ± 2.5 cycles of chemotherapy were completed for the chemotherapy group, and 9.5 ± 3.8 cycles of immunotherapy were completed for the immunotherapy group (P = .018). A total of 4 of 25 (16.0%) patients in the chemotherapy group achieved PR, and 4 of 8 (50.0%) patients in the immunotherapy group achieved PR, including 1 CR. A total of 11 of 25 (44%) patients in the chemotherapy group reached SD, and 4 of 8 (50%) patients in the immunotherapy group reached SD. Progressive disease was observed in 10 of 25 (40%) patients in the chemotherapy group. The difference in response between the 2 groups was significant (χ^2 = 9.275; P = .026), as listed in Table 3. The median OS (mOS) and median PFS (mPFS) were 4 and 2 months, respectively, for the chemotherapy group. The mOS and mPFS were not reached for the immunotherapy group because 4 of the 8 patients had continuous PR or CR at the date cutoff. The estimated mOS and mPFS were 10+ months. The difference between the 2 groups was significantly related to mOS and mPFS, with P values of .000 and .003, respectively, as shown in Figures 1 and 2. The 6-month OS rates for the chemotherapy group

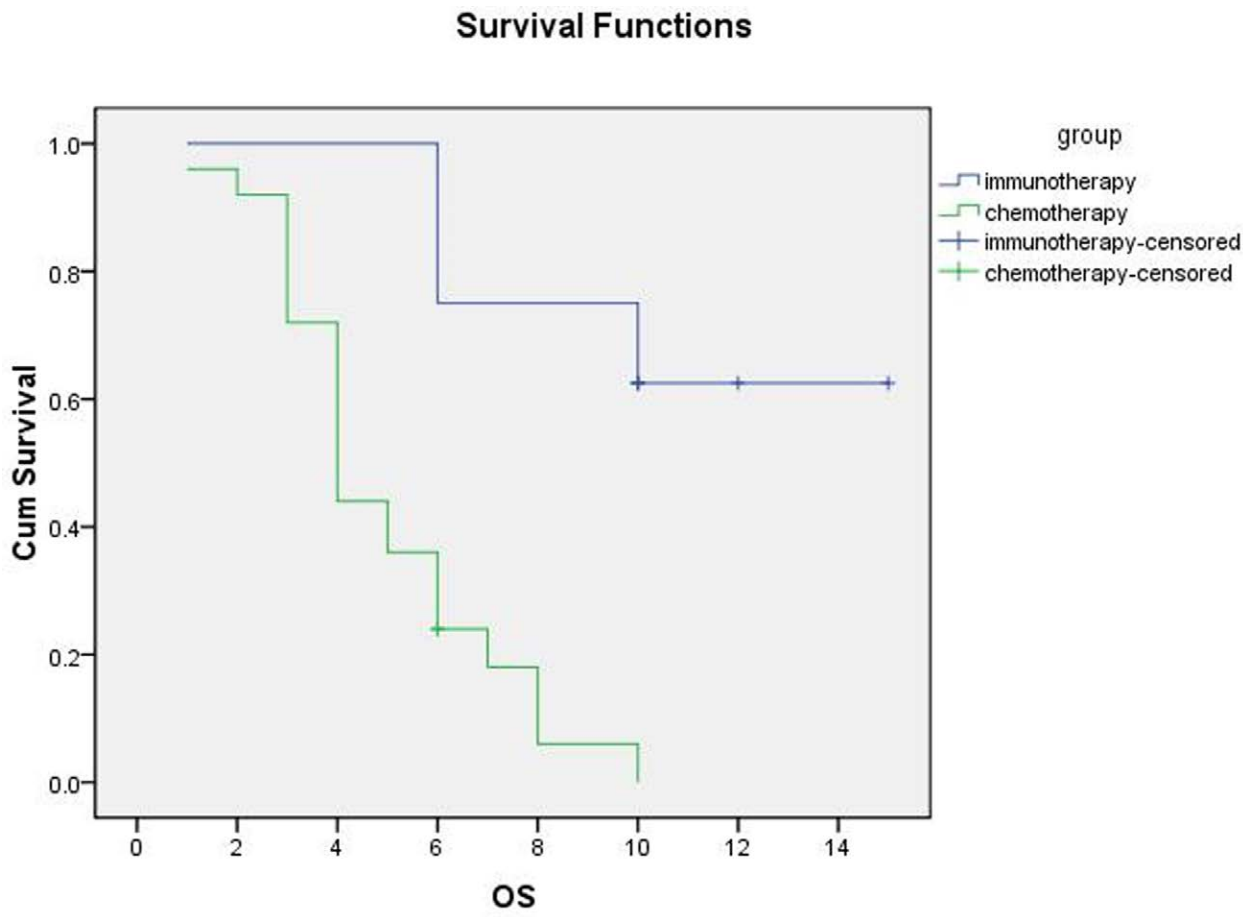

Figure 1. Overall survival. Immunotherapy group had longer overall survival (χ^2 = 13.266; P = .000).

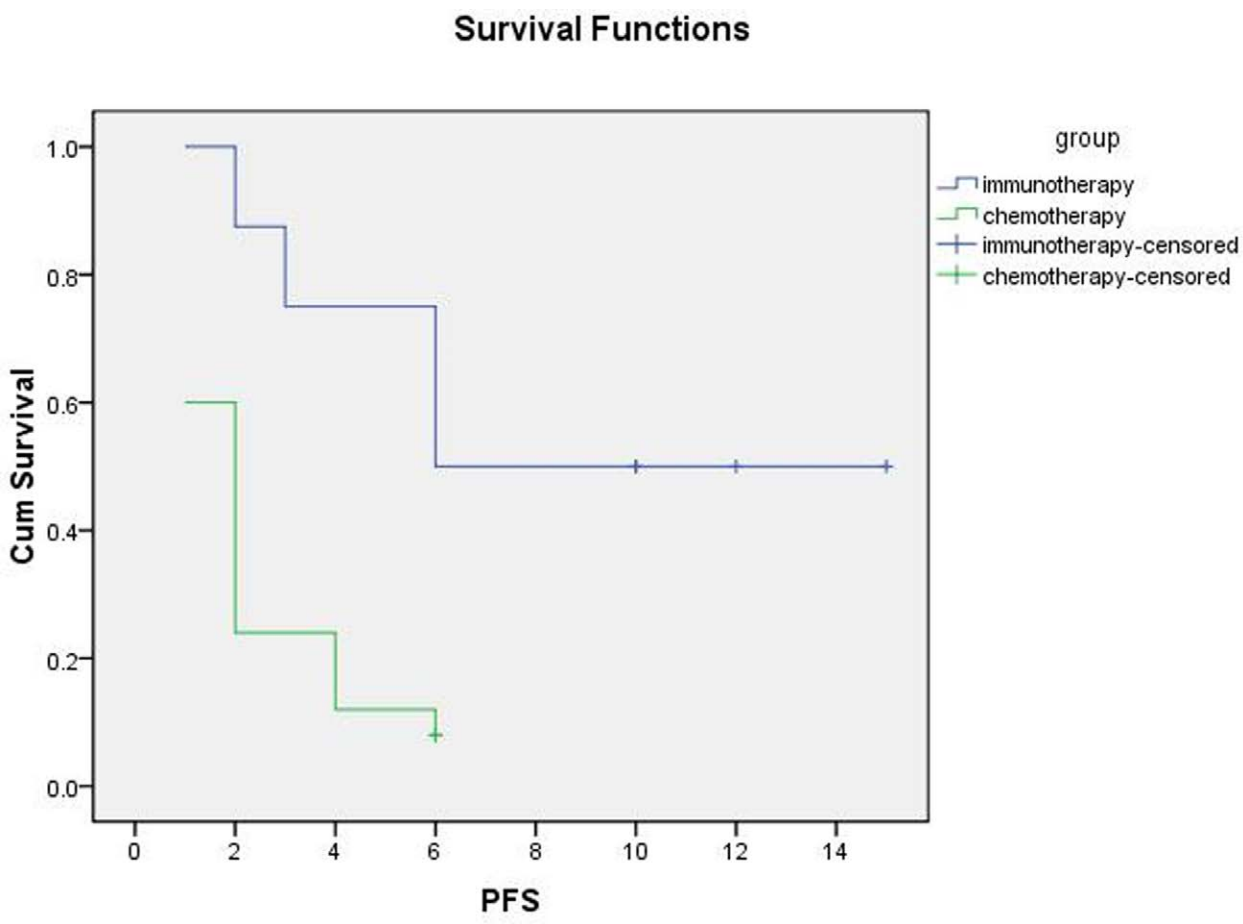

Figure 2. Progression-free survival. Immunotherapy group had longer progression-free survival (χ^2 = 8.774; *P* = .003).

and immunotherapy group were 28% and 75%, respectively (*P* = .035). The 6-month PFS rates for the chemotherapy group and immunotherapy group were 12% and 50%, respectively (*P* = .042).

Cluster analysis showed that the calculated threshold should be set at 10% of PD-L1. Stratified statistical analysis showed that patients with high expression of PD-L1 > 10% had significantly longer OS than the other patients (*P* = .001), as shown in Figure 3.

The AEs for both groups were myelosuppression, gastrointestinal reaction, bleeding, fatigue, and capillary proliferation. No significant difference was observed between the 2 groups. Four grade 3 myelosuppression in the chemotherapy group were observed after 3 to 8 cycles of gemcitabine-based chemotherapy. All of the patients recovered with 1 transfer to capecitabine monotherapy. One grade 3 capillary proliferation in the immunotherapy group was observed and recovered after 2 months of withdrawal from ICIs. The difference did not reach significance, as shown in Table 4.

4. Discussion

BTC is one of the most troublesome diseases with poor prognosis. Most patients were diagnosed at advanced stage on admission and lost the chance of surgery, which is the only opportunity for clinical curation. Even worth, no effective chemotherapy schedule is available for ABTCs. Both contribute to the poor prognosis of ABTCs. Although the NCCN guidelines recommend gemcitabine or fluorouracil alone or in combination as first-line chemotherapy for ABTCs, with irinotecan as second-line treatment, the objective response rate (ORR) is much lower than that of other digestive cancers, especially when first-line therapy failed. Ying and Chen[11] performed a comprehensive meta-analysis of salvage treatment of ABTCs. They found that the response rate as second-line therapy for ABTCs was only 7.7%, with mPFS of 2.6 months and mOS of 6.5 months. S1 used as second-line monotherapy had an ORR of 7.5%, with mPFS of 2.5 months and mOS of 6.8 months.[12] Combined treatment did not reach superiority as second-line therapy.[12] The current outcomes from chemotherapy are disappointed.

ICIs have been recommended for PD-L1-positive tumors with exciting results in certain cancers.[13] ICIs are also recommended to some ABTCs with gentle superiorities,[14–16] but the superiority was not significant. One fact contributing to this result was that the threshold was not clear until now. If the threshold was set to 1% of PD-L1, most patients benefitted little from ICIs, in relation to ORR, OS, and PFS.[14] Our primary experience also showed superiority of ICIs for ABTCs. Eight PD-L1-positive ABTCs were included in our trial. Longer OS and PFS were observed for the immunotherapy group than for the chemotherapy group. The calculated threshold of PD-L1 by cluster analysis was 10%. Four patients were observed with high PD-L1 expression of >10%, and stratified analysis showed that patients with PD-L1 expression >10% had longer OS. This superiority was significant compared to other patients. According to our limited experience, we suggest that ICIs should be recommended to certain patients with high expression of PD-L1>10%. Four patients in our cohort who had high expression of PD-L1 (>10%) were administered ICIs as first-line or second-line therapy. One patient who was diagnosed of mucinous adenocarcinoma of the bile duct had

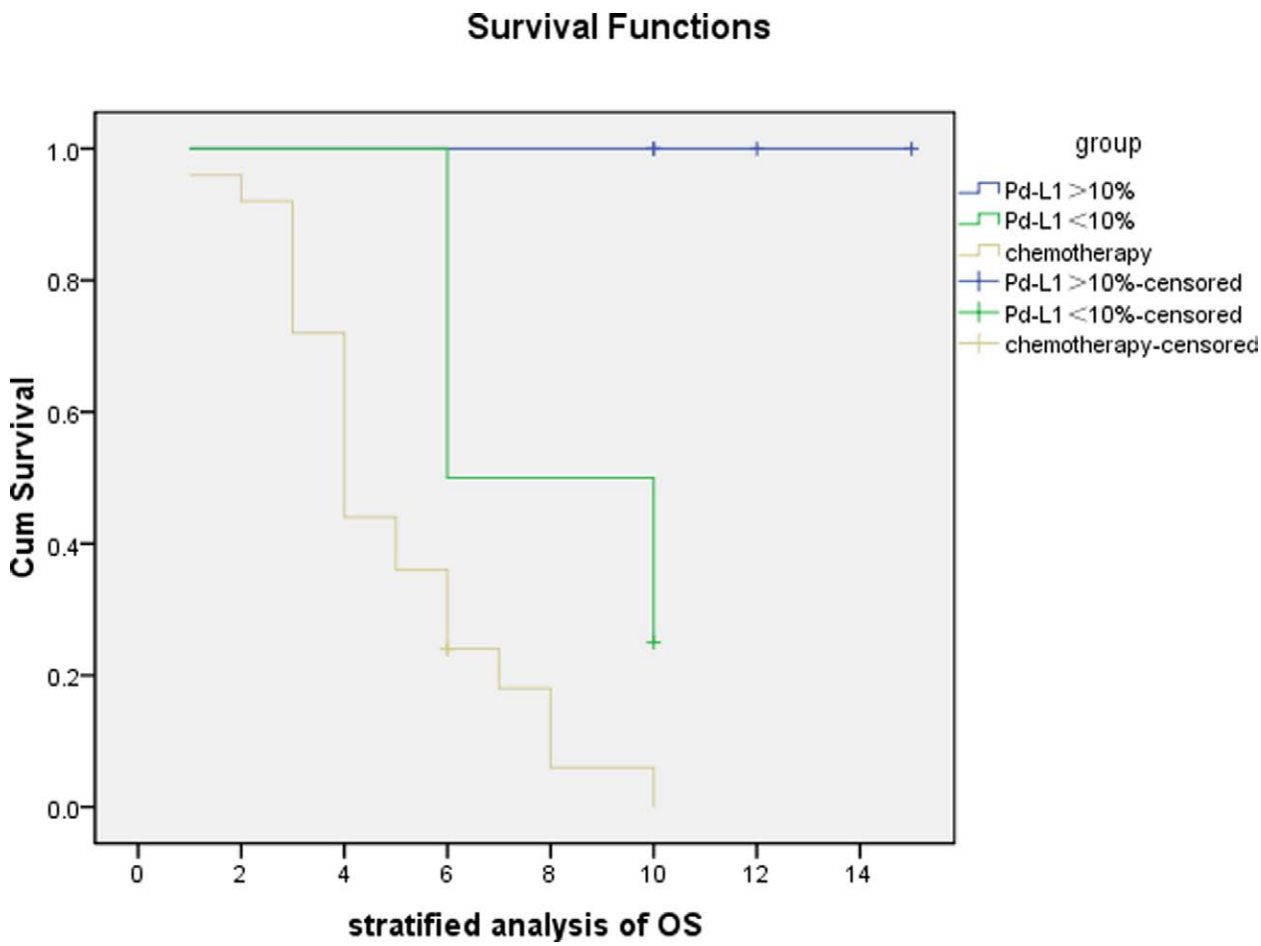

Figure 3. Stratified analysis of overall survival. Stratified analysis showed patients with high PD-L1 of > 10% had significantly longer overall survival (χ^2 = 14.872; *P* = .001).

Table 4

Adverse events.

	Chemotherapy (25)	Immunotherapy (8)	*P*
Myelosuppression	9	1	.382
Gastrointestinal reaction	13	3	.688
Bleeding	0	0	–
Fatigue	8	1	.394
Transaminase elevation	2	1	1.000
Capillary proliferation	0	2	–

CR after 6 months of treatment with ICIs. No progression was observed during the entire follow-up period. The other patients reached PR.

The main AEs from our cohort were capillary proliferation and gastrointestinal reactions in immunotherapy group. One patient in immunotherapy group had serious capillary proliferation. He recovered after 2 months of withdrawal and transferred to capecitabine monotherapy due to the patient's rejection to ICIs. This patient progressed at the 6th month follow-up and died at the 10th month. Myelosuppression, gastrointestinal reaction and fatigue were the main AEs for chemotherapy. All of the patients recovered without mortality.

BTC is a relatively rare malignant tumor. Positive expression of PD-L1 was not common in those cancers. Both factors contributed to the small studies that were published and ours. As a small-volume study, bias may exist. The calculated threshold may be imprecise. The relatively short period of follow-up of 10 months may also induce bias, especially for survival. A retrospectively designed trial may induce bias either. Randomized controlled trials with large volumes should be planned to ensure the value of ICIs for ABTCs and the threshold of PD-L1.

5. Conclusions

ICIs are efficient for certain ABTCs with high expression of PD-L1 with acceptable AEs. The threshold of PD-L1 should be more precise.

References

[1] Jung KW, Won YJ, Hong S, et al. Prediction of cancer incidence and mortality in Korea. Cancer Res Treat. 2020;52:351–8.
[2] Siegel RL, Miller KD, Jemal A. Cancer statistics. CA Cancer J Clin. 2020;70:7–30.
[3] Kim BJ, Yoo C, Kim KP, et al. Efficacy of fluoropyrimidine-based chemotherapy in patients with advanced biliary tract cancer after failure of gemcitabine plus cisplatin: retrospective analysis of 321 patients. Br J Cancer. 2017;116:561–7.
[4] Valle JW, Wasan H, Lopes A, et al. Cediranib or placebo in combination with cisplatin and gemcitabine chemotherapy for patients with advanced biliary tract cancer (ABC-03): a randomised phase 2 trial. Lancet Oncol. 2015;16:967–78.
[5] Valle JW, Bai LY, Orlova R, et al. Ramucirumab (RAM) or merestinib (MER) or placebo (PL) plus gemcitabine (GEM) and cisplatin (CIS)

as first-line treatment for advanced or metastatic biliary tract cancer (BTC): a randomized, double-blind, phase II study. J Clin Oncol. 2020;38(4_suppl):477.
[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;147:2190–8.
[7] 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;37:318–27.
[8] Niu B, Ye K, Zhang Q, et al. MSI sensor: microsatellite instability detection using paired tumor-normal sequence data. Bioinformatics. 2014;30:1015–6.
[9] Schwartz LH, Litière S, de Vries E, et al. RECIST 1.1-Update and clarification: from the RECIST committee. Eur J Cancer. 2016;62:132–7.
[10] Freites-Martinez A, Santana N, Arias-Santiago S, et al. Using the Common Terminology Criteria for Adverse Events (CTCAE - Version 5.0) to evaluate the severity of adverse events of anticancer therapies. Actas Dermosifiliogr (Engl Ed). 2021;112:90–2.
[11] Ying J, Chen J. Combination versus mono-therapy as salvage treatment for advanced biliary tract cancer: a comprehensive meta-analysis of published data. Crit Rev Oncol Hematol. 2019;139:134–42.
[12] Suzuki E, Ikeda M, Okusaka T, et al. A multicenter phase II study of S-1 for gemcitabine-refractory biliary tract cancer. Cancer Chemother Pharm. 2013;71:1141–6.
[13] Vaddepally RK, Kharel P, Pandey R, et al. Review of Indications of FDA-approved immune checkpoint inhibitors per NCCN guidelines with the level of evidence. Cancers (Basel). 2020;12:738.
[14] Bang YJ, Ueno M, Malka D, et al. Pembrolizumab (pembro) for advanced biliary adenocarcinoma: results from the KEYNOTE-028 (KN028) and KEYNOTE-158 (KN158) basket studies. J Clin Oncol. 2019;37:4079.
[15] 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;4:611–21.
[16] 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;6:888–94.