Ther Adv Gastroenterol

2022, Vol. 15: 1–10

DOI: 10.1177/17562848221117638

Expression of PD-L1 as a predictive marker of sensitivity to immune checkpoint inhibitors in patients with advanced biliary tract cancer

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Abstract

Background: Expression of programmed death-ligand 1 (PD-L1) has been reported to correlate with response to immune checkpoint inhibitors (ICIs) in various tumor types. However, there are few data on the role of PD-L1 expression as a predictive and prognostic biomarker of sensitivity to ICIs in patients with advanced biliary tract cancer (BTC).

Objectives: We evaluated the role of PD-L1 expression as a predictive and prognostic biomarker of response to ICIs in patients with advanced BTC.

Design: We retrospectively analyzed data from 83 advanced BTC patients who received ICIs as second- or third-line treatment between February 2018 and April 2021.

Methods: All patient data analysis included evaluation of PD-L1 expression by the combined positive score (CPS).

Results: Among 83 patients, 56 (67.5%) had PD-L1 positivity (CPS $\geqslant$ 1). The objective response rate (ORR) to ICIs was significantly higher in advanced BTC patients with PD-L1 expression compared to those without PD-L1 expression (17.8% *versus* 0%, $p = 0.026$). However, there were no significant differences in median progression-free survival (PFS; 2.9 *versus* 2.6 months, $p = 0.330$) and median overall survival (OS; 8.1 *versus* 6.3 months, $p = 0.289$) as a response to ICIs between patients with and without PD-L1 expression. Also, there were no significant differences in ORR, PFS, and OS as a response to ICIs in conjunction with a response to a prior gemcitabine plus cisplatin regimen ($p = 0.654$, $p = 0.278$, and $p = 0.302$, respectively).

Conclusions: The present study suggests that the expression of PD-L1 alone was not sufficient as a novel marker to select advanced BTC patients who might benefit from ICIs. Additional comprehensive studies of biomarkers that can assist in predicting BTC patient responses to pembrolizumab and/or nivolumab therapy are required.

Keywords: biliary tract neoplasms, biomarker, chemotherapy, immunotherapy, programmed death-ligand 1 expression

Received: 15 December 2021; revised manuscript accepted: 18 July 2022.

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Introduction

Biliary tract cancers (BTCs) represent rare, aggressive, and heterogeneous malignancies arising from the bile duct system, including cholangiocarcinoma (CCA), gallbladder cancer, and ampulla of Vater cancer.[1–3] Surgical resection is the only curative treatment for patients with BTC, but metastasis usually prevents surgical intervention. In patients with advanced BTCs, systemic treatment is the only therapeutic option; and the

combination of gemcitabine plus cisplatin (GP) has been the standard first-line therapy. However, the prognosis for these patients is poor, and the median overall survival (OS) is less than 1 year.[4–6]

The expression of programmed death-ligand 1 (PD-L1) assessed by immunohistochemistry (IHC) has been reported to correlate with response to immune checkpoint inhibitors (ICIs) as a potential predictive biomarker in several tumor types.[7–9] In patients with advanced BTC, ICIs have shown modest efficacy in several trials. The KEYNOTE-028 (phase Ib) and KEYNOTE-158 (phase II) studies showed that the clinical efficacy of pembrolizumab, a human IgG4 monoclonal antibody inhibitor of PD-1, was more favorable in advanced BTC patients with expression of PD-L1 compared to those without expression of PD-L1.[10,11] In a phase II trial, nivolumab, another PD-1 inhibitor, showed similar clinical efficacy in patients with advanced BTC regardless of the expression of PD-L1.[12] Previous data from testing PD-L1 as a biomarker of sensitivity to ICI in BTCs were discordant.

In the era of immunotherapy, several biomarkers have been associated with response to ICIs in many solid tumors, including tumor mutational burden (TMB) and high microsatellite instability (MSI-H).[13,14] However, there has been little study on the role of biomarkers in advanced BTCs.

In the present analysis, we retrospectively evaluated the role of the expression of PD-L1 as a predictive and prognostic biomarker of response to ICIs in patients with advanced BTC.

Patients and methods

Patients

From February 2018 to April 2021, 83 advanced BTC patients received ICIs as second- or third-line treatment at Samsung Medical Center, Korea. All 83 patients were histologically or cytologically confirmed to have advanced BTC and were evaluated for PD-L1 expression with IHC staining. The pathologic specimens were obtained in primary tumor sites (90.4%) and metastatic sites (9.6%) and were examined by two dedicated pathologists (SYH and KTJ).

All clinical, laboratory, and radiological data were collected for all patients from electronic medical records. Patients in the database were identified by patient number only and the patient information kept as confidential information according to IRB protocol. The reporting of this study conforms to the STARD statement.[15]

PD-L1 immunohistochemistry

Formalin-fixed, paraffin-embedded tumor samples were obtained at the time of diagnosis of advanced or metastatic BTC. IHC staining was performed with a Dako PD-L1 IHC 22C3 pharmDx kit (Agilent Technologies, Santa Clara, CA, USA) using the Dako Autostainer Link 48 system (Agilent Technologies) and an EnVision FLEX visualization system according to the manufacturer's instructions. PD-L1 expression was calculated as the combined positive score (CPS) and number of PD-L1-stained cells (tumor cells, lymphocytes, and macrophages) divided by the total number of viable tumor cells, multiplied by 100.[16] The specimen was considered to have PD-L1 expression at CPS $\geqslant 1$.

Treatment and assessment

All patients were treated with pembrolizumab or nivolumab as ICI according to physician preference. Pembrolizumab was administered 200 mg intravenously every 3 weeks, and nivolumab was administered 3 mg/kg intravenously every 2 weeks. All patients were evaluated radiologically through computed tomography or magnetic resonance imaging for clinical outcomes of objective response rate (ORR), disease control rate (DCR), progression-free survival (PFS), and OS according to Response Evaluation Criteria in Solid Tumors version 1.1.

Statistics

The cutoff date for data collection was 30 April 2021. Descriptive statistics were used to summarize patient and tumor characteristics and treatment history and were reported as proportion and median. Data are presented as numbers (%) for categorical variables. Correlations between PD-L1 expression and clinicopathologic features were analyzed using the Fisher exact test. PFS was defined as the time from the start of ICI until the date of disease progression or death from any cause. OS was defined as the time from the start of ICI until death from any cause. Survival analyses were performed using the Kaplan–Meier method, and

differences were analyzed by log-rank test. Hazard ratios and corresponding 95% confidence intervals (CIs) were calculated using the Cox proportional hazards model. Univariate analysis of predictive and prognostic factors was performed using Cox proportional hazards models for PFS and OS. Significant prognostic variables in univariate analysis for survival were included in the multivariate analysis. Statistical analysis was performed using IBM SPSS Statistics 25 (Armonk, NY, USA).

Results

Patient characteristics

The baseline characteristics of patients at treatment initiation are presented in Table 1. The median age was 64 (range, 35–84 years). Intrahepatic CCA was the most common type (57.8%), and most patients (91.6%) had metastatic lesions. Pembrolizumab (90.4%) was most frequently used as ICI.

Among the 83 patients, 56 (67.5%) had PD-L1 CPS positivity. PD-L1 positivity was not significantly associated with specific clinical characteristics except the kind of ICI ($p = 0.021$). All patients received GP as first-line therapy; and ICI was used as second-line (73.5%), third-line (25.3%), or fourth-line therapy (1.2%).

Treatment outcomes with ICIs according to status of PD-L1

We evaluated the relationship between the status of PD-L1 expression and the efficacy of ICIs. The ORR and DCR were 17.8% (10/56) and 42.9% (24/56), respectively, in patients with PD-L1 expression and 0% (0/0) and 44.4% (12/27) in patients without PD-L1 expression (Figure 1). There was a significant difference in the ORR to ICIs according to the status of PD-L1 expression ($p = 0.026$) (Table 2).

The median duration of follow-up was 4.8 months (range, 0.1–36.7 months). The median PFS to ICIs was 2.9 months (95% CI, 2.4–3.4 months) in patients with PD-L1 expression and 2.6 months (95% CI, 2.3–2.9 months) in those without PD-L1 expression ($p = 0.330$) (Figure 2(a)). The median OS was 8.1 months (95% CI, 6.0–10.3 months) in patients with PD-L1 expression and 6.3 months (95% CI, 5.4–7.2 months) in those without PD-L1 expression ($p = 0.289$)

Table 1. Patient characteristics.

Patient characteristics	PD-L1 (+) ($n = 56$)	PD-L1 (–) ($n = 27$)	*p* value
Median age (IQR), years	64 (42–84)	64 (35–77)	0.700
Age ⩾65 years, *n* (%)	26 (46.4%)	12 (44.4%)	0.865
Sex, *n* (%)			0.974
Male	37 (66.1%)	17 (63.0%)	
Female	19 (33.9%)	10 (37.0%)	
Tumor site, *n* (%)			0.098
Intrahepatic	28 (50.0%)	20 (74.1%)	
Extrahepatic	18 (32.1%)	6 (22.2%)	
Gallbladder	8 (14.3%)	0 (0.0%)	
Ampulla of Vater	2 (3.6%)	1 (3.7%)	
Pathological type, *n* (%)			0.574
Poorly	18 (32.1%)	11 (40.7%)	
Well/moderate	33 (58.9%)	15 (55.6%)	
Unknown	5 (8.9%)	1 (3.7%)	
Disease status			0.512
Metastasis	50 (89.3%)	26 (96.3%)	
Locally advanced	6 (10.7%)	1 (3.7%)	
No. of metastatic sites			1.000
⩽2	44 (78.6%)	21 (77.8%)	
2<	12 (21.4%)	6 (22.2%)	
Prior curative surgery	28 (50.0%)	7 (25.9%)	0.065
Prior systemic therapies			0.344
1	42 (75.0%)	19 (70.4%)	
2	14 (25.0%)	7 (25.9%)	
⩾3	0 (0.0%)	1 (3.7%)	
ICI			0.021
Pembrolizumab	54 (96.4%)	21 (77.8%)	
Nivolumab	2 (3.6%)	6 (22.2%)	
First-line GP chemotherapy best response			0.509
Complete response	1 (1.8%)	0 (0.0%)	
Partial response	13 (23.2%)	8 (29.6%)	
Stable disease	24 (42.9%)	14 (51.9%)	
Progressive disease	18 (32.1%)	5 (18.5%)	

GP, gemcitabine plus cisplatin; ICI, immune checkpoint inhibitor; IQR, interquartile range; PD-L1, programmed death-ligand 1.

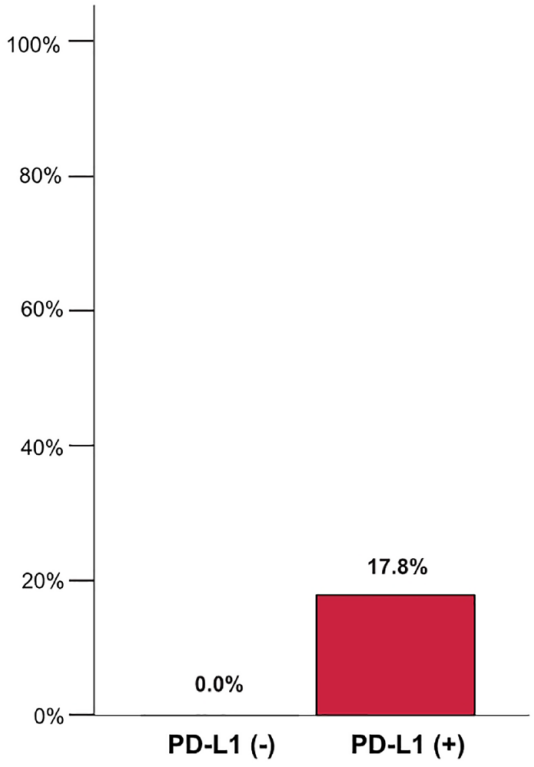

Figure 1. ORR to ICIs by PD-L1 expression.

Table 2. Efficacy outcomes of ICI.

	PD-L1 (+) (*n* = 56)	PD-L1 (–) (*n* = 27)	*p* value
Median cycles (range)	3.0 (1–18)	4.0 (1–17)	0.618
Best response			
Complete response	1 (1.8%)	0 (0.0%)	1.000
Partial response	9 (16.1%)	0 (0.0%)	0.155
Stable disease	14 (25.0%)	12 (44.4%)	0.083
Progressive disease	21 (37.5%)	13 (48.2%)	0.475
Not evaluable	11 (19.6%)	2 (7.4%)	0.205
ORR	10 (17.8%)	0 (0.0%)	0.026
DCR	24 (42.9%)	12 (44.4%)	1.000

DCR, disease control rate; ICI, immune checkpoint inhibitor; ORR, objective response rate; PD-L1, programmed death-ligand 1.

(Figure 2(b)). We additionally analyzed the treatment outcomes with ICIs according to the CPS cutoff at 5% and 10%. The median PFS and OS did not differ according to the cutoff values (5 and 10) of CPS ($p > 0.05$ for all) (Supplemental Figures S1 and S2). There were no significant differences in PFS and OS to ICIs according to the status of PD-L1 expression. However, there were significant differences in PFS and OS between responders and non-responders to ICIs (OS: 23.7 *versus* 6.3 months, $p < 0.001$; PFS: not reached *versus* 2.6 months, $p < 0.001$) (Figure 3(a) and 3(b).

We also conducted univariate analyses for PFS and OS to evaluate the role of PD-L1 expression as an independent biomarker (Table 3). There were no clear associations between survival and variables, except the number of metastatic sites, in univariate analyses. PD-L1 expression was not an independent factor in univariate analyses for PFS ($p = 0.334$) and OS ($p = 0.292$).

Association between efficacy of ICIs and response to GP

We additionally analyzed the efficacy and survival related to ICI administration based on response to GP as first-line therapy. The ORR to GP was 25.0% (14/56) in patients with PD-L1 expression and 29.6% (8/27) in patients without PD-L1 expression ($p = 0.654$). Also, there were no significant differences in PFS and OS as the result of ICI administration between responders and non-responders to GP (OS: 9.0 *versus* 6.5 months, $p = 0.302$; PFS: 2.6 *versus* 2.8 months, $p = 0.278$) (Figure 4(a), (b)).

Discussion

In this study, we analyzed the role of PD-L1 expression as a predictive and prognostic biomarker of response to ICI therapy in patients with advanced BTC. We found that the incidence of expression of PD-L1 in advanced BTC was 67.5% (56/83). In this analysis, the ORR to ICIs was significantly higher in advanced BTC patients with PD-L1 expression compared to those without PD-L1 expression (17.8% *versus* 0%, $p = 0.026$). However, there were no significant differences in median PFS (2.9 *versus* 2.6 months, $p = 0.330$) and median OS (8.1 *versus* 6.3 months, $p = 0.289$) in response to ICI therapy between patients with and without PD-L1 expression. These findings suggest that PD-L1 expression might be a useful biomarker to predict sensitivity to ICIs but not for predicting survival with ICI treatment in advanced BTC patients.

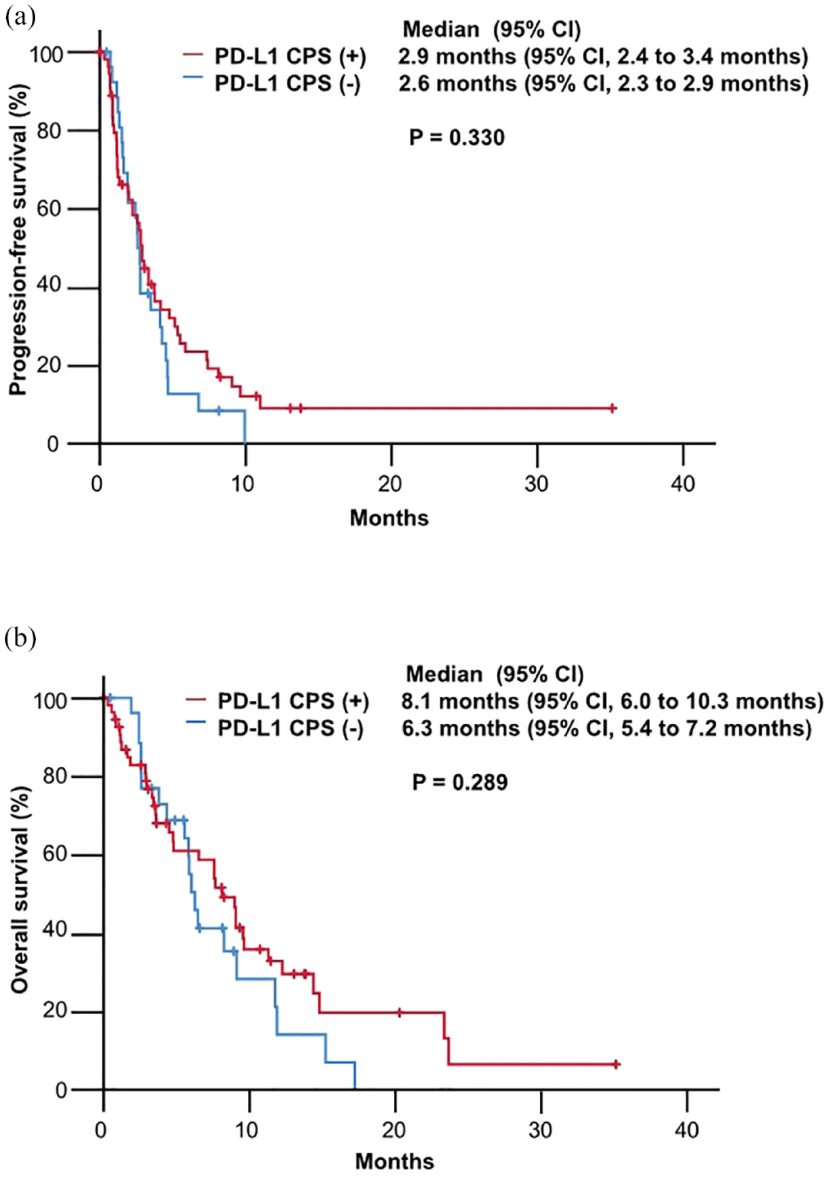

Figure 2. Kaplan–Meier curves of PFS (a) and OS (b) to ICIs according to PD-L1 expression.
ICIs, immune checkpoint inhibitors; OS, overall survival; PD-L1, programmed death-ligand 1; PFS, progression-free survival.

The role of PD-L1 as a biomarker of ICI sensitivity in advanced BTC is uncertain[17–19] because previous studies showed discordant findings.[10,12,20] In a subgroup analysis of the KEYNOTE-158 study with pembrolizumab, the incidence of PD-L1 expression in BTC was 58.7% (61/104); and the ORR was higher in patients with expression of PD-L1 compared to those without (6.6% *versus* 2.9%). However, median PFS and OS were shorter in patients with PD-L1 expression (PFS: 1.9 *versus* 2.1 months, OS: 7.2 *versus* 9.3 months).[10] In a phase II study with nivolumab, the incidence of PD-L1 expression was 42.8% (18/42), and a significantly prolonged median PFS was reported in patients with PD-L1 expression compared to those without (10.4 *versus* 2.3 months, $p < 0.001$).[12] The present study results are consistent with the finding in the KEYNOTE-158 study. In the present study, most patients were treated with the ICI pembrolizumab (90.4%). The expression of PD-L1 might be associated with the efficacy of

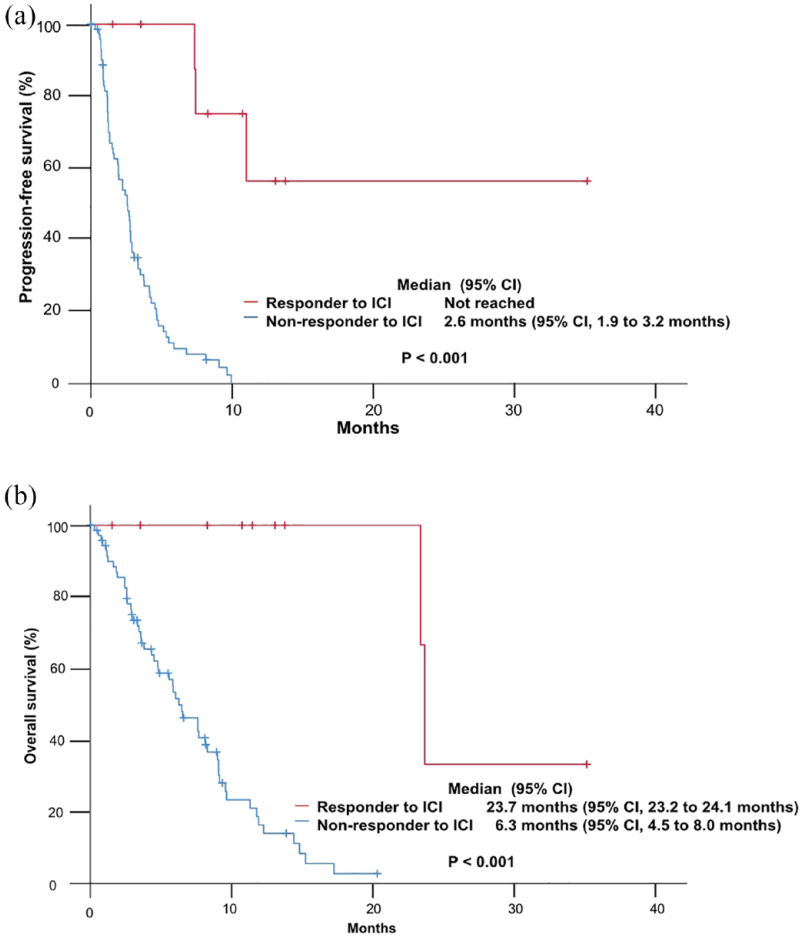

Figure 3. Kaplan–Meier curves of (a) and OS (b) to ICIs according to responders and non-responders.
ICIs, immune checkpoint inhibitors; OS, overall survival; PFS, progression-free survival.

Table 3. Univariate analysis of PFS and OS.

Variables	PFS		OS	
	HR (95% CI)	*p* Value	HR (95% CI)	*p* Value
Age		0.707		0.622
<65 years	1		1	
⩾65 years	0.91 (0.57–1.47)		0.87 (0.51–1.50)	
Sex		0.622		0.524
Male	1		1	
Female	0.88 (0.54–1.45)		0.83 (0.48–1.46)	
Tumor site				
Intrahepatic	1	0.648	1	0.200
Extrahepatic	0.45 (0.48–1.39)	0.451	0.68 (0.36–1.26)	0.215

(Continued)

Table 3. (Continued)

Variables	PFS		OS	
	HR (95% CI)	*p* Value	HR (95% CI)	*p* Value
Gallbladder	0.60 (0.26–1.43)	0.249	0.35 (0.11–1.14)	0.081
Ampulla of Vater	0.78 (0.19–3.23)	0.730	0.39 (0.05–2.84)	0.350
Pathological type, *n* (%)		0.806		0.19
Poorly	1		1	
Well/moderate	0.94 (0.56–1.56)		0.69 (0.39–1.20)	
Disease status		0.550		0.141
Metastasis	1		1	
Locally advanced	1.27 (0.58–2.78)		0.42 (0.13–1.34)	
No. of metastatic sites		0.076		0.009
⩽2	1		1	
2<	1.65 (0.95–2.86)		2.18 (1.22–3.90)	
Prior curative surgery		0.600		0.228
No	1		1	
Yes	1.14 (0.70–1.84)		0.71 (0.41–1.24)	
Prior systemic therapy		0.198		0.271
1	1		1	
⩾2	0.69 (0.39–1.22)		0.70 (0.37–1.32)	
PD-L1 expression		0.334		0.292
Negative	1		1	
Positive	0.78 (0.47–1.29)		0.74 (0.42–1.30)	

CI, confidence interval; HR, hazard ratio; OS, overall survival; PD-L1, programmed death-ligand 1; PFS, progression-free survival.

pembrolizumab in BTC patients. However, the ORR was low, and the response to pembrolizumab was not of significant duration. These factors might have affected the result that expression of PD-L1 was not a prognostic marker for PFS to pembrolizumab and OS.

GP are currently used as the standard first-line therapy in advanced BTCs. We additionally evaluated the efficacy of and survival after ICI therapy according to response to a prior potentially DNA-damaging GP regimen. Platinum-based chemotherapy evoked modulation of the immune system, enhancement of the effector immune response through modulation of PD-L1 expression, and increased ICI efficacy.[21,22] However, in the present analysis, there were no significant differences in ORR, PFS, and OS

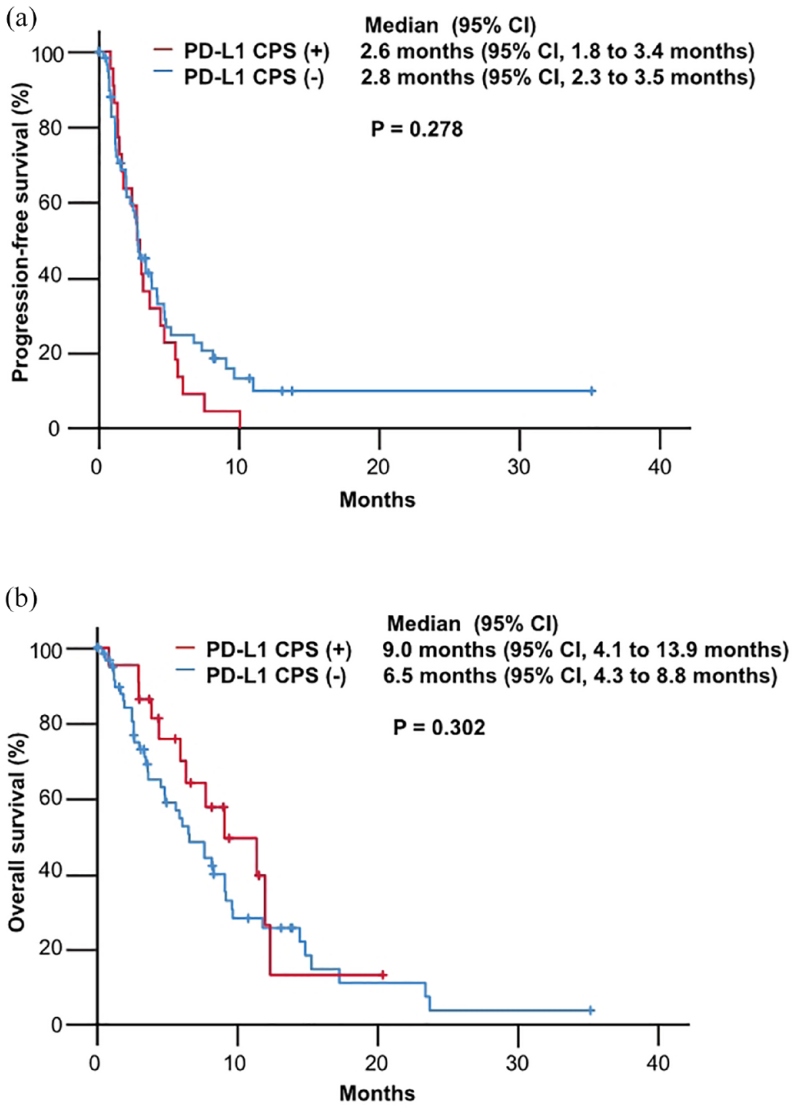

Figure 4. Kaplan–Meier curves of PFS (a) and OS (b) to ICIs according to responders and non-responders to GP.
GP, gemcitabine plus cisplatin; ICIs, immune checkpoint inhibitors; OS, overall survival; PFS, progression-free survival.
ICIs, immune checkpoint inhibitors; ORR, objective response rate; PD-L1, programmed death-ligand 1.

according to the use of ICIs and response to a prior GP regimen ($p = 0.654$, $p = 0.278$, and $p = 0.302$, respectively).

Among 83 advanced BTC patients who received ICIs, we retrospectively additionally reviewed 28 patients who have been examined with next-generation sequencing with the TruSight™ Oncology 500 assay (Illumina Inc., San Diego, CA, USA) for identification of reliable other biomarkers of response to ICI therapy and molecular profiles. The most common alterations were KRAS mutation (35.7%, 10/28), HER2 aberration (14.3%, 4/28), and FGFR fusion (10.7%, 3/28). However, any patient did not receive targeted therapies. In our study populations, none of the patients showed MSI-high, which was defined as more than 20 unstable MS loci. However, we found that four patients (14.3%, 4/28) were categorized as tumors with TMB-high, which was defined as more than 10 mutations per Mb (≥10 Mut/Mb). Of four patients with TMB-high, all patients (100%) had PD-L1 CPS positivity, and three patients (75%) achieved tumor response to ICIs. Recently, several retrospective studies have investigated the role of PD-L1 expression, TMB, and MSI-H in advanced BTC patients. One study

reported TMB-high was associated with high PD-L1 CPS and MSI score correlated with PD-L1 CPS score in 73 advanced BTC patients.[23] Also, another study showed that TMB is associated with responses to ICIs in advanced BTC patients, but that study included only five patients with TMB-high.[24] These results have consistently shown our results. Based on these results, a single biomarker was not sufficient to select patients likely to benefit from immunotherapy. Further studies are needed to evaluate single or combination biomarker research, such as PD-L1, MSI, tumor microenvironment, and other predictors.

This research has several limitations. This study was retrospective in nature and had a small sample size. The patients were substantially heterogeneous. Thus, our findings should be interpreted with caution.

Conclusion

The present analysis suggests that the expression of PD-L1 alone is not sufficient as a novel marker to select advanced BTC patients who might benefit from ICIs. Further comprehensive BTC biomarker studies to pembrolizumab and/or nivolumab therapy are needed.

Declarations

Ethics approval and consent to participate

This study was approved by the Institutional Review Board (IRB No. 2021-11-038) of Samsung Medical Center and individual consent for this analysis was waived. This study was also conducted in accordance with the ethical principles of the Declaration of Helsinki and the Korea Good Clinical Practice guidelines.

Consent for publication

Not applicable.

Author contribution(s)

Hongsik Kim: Conceptualization; Data curation; Formal analysis; Investigation; Methodology; Visualization; Writing – original draft; Writing – review & editing.

Ryul Kim: Investigation; Writing – review & editing.

Hyunji Jo: Investigation; Writing – review & editing.

Hye Ryeon Kim: Investigation; Writing – review & editing.

Joohyun Hong: Investigation; Writing – review & editing.

Sang Yun Ha: Investigation; Writing – review & editing.

Joon Oh Park: Investigation; Writing – review & editing.

Seung Tae Kim: Conceptualization; Data curation; Formal analysis; Investigation; Methodology; Writing – review & editing.

Acknowledgements

A grant of the Korea Health Technology R&D Project through the Korea Health Industry Development Institute (KHIDI) and the Ministry of Health & Welfare, Republic of Korea (grant number: HR20C0025) supported this research.

Funding

The authors received no financial support for the research, authorship, and/or publication of this article.

Competing interests

The authors declare that there is no conflict of interest.

Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

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Supplemental material

Supplemental material for this article is available online.

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