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Negative prognostic implications of splenomegaly in nivolumab-treated advanced or recurrent pancreatic adenocarcinoma

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ABSTRACT

Immune checkpoint inhibitors have limited efficacy in the treatment of pancreatic ductal adenocarcinoma (PDAC). We investigated prognostic markers for nivolumab-based therapy in advanced or recurrent PDAC. Consecutive patients receiving nivolumab-based therapy at our institution between 2015 and 2020 were evaluated. Overall survival (OS) was analyzed through univariate and multivariate analyses. Spleen volume was estimated from the width, thickness, and length of the spleen. A total of 45 patients were identified. Biweekly nivolumab was administered as monotherapy (n = 5) or in combination with chemotherapy or targeted therapy (n = 40). Among 31 evaluable patients, the response and disease control rates were 7% and 36%, respectively. The baseline median spleen volume was 267 (110–674) mL. Patients with spleens ≥267 mL had significantly shorter median OS (1.9 months, 95% confidence interval [CI], 1.0–2.7) than did those with smaller spleens (8.2 months, 95% CI, 5.6–10.8; P = .003). In the multivariate analysis, spleen volume of <267 mL, ≤2 lines of prior chemotherapy, ECOG performance status of 0–2, add-on nivolumab with stable disease after prior therapy, concomitant or sequential cell therapy, high lymphocyte count, and total bilirubin <1 mg/dL were independent favorable prognostic factors for OS. In the control groups of patients receiving gemcitabine-based chemotherapy (n = 142) or FOLFIRINOX regimen (n = 24), spleen volume exhibited no prognostic significance. In heavily pretreated PDAC, a large spleen may predict poor OS following nivolumab-based immunotherapy. Studies with larger cohorts should confirm the prognostic value of spleen volume.

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Introduction

Pancreatic cancer is the seventh greatest cause of cancer death worldwide,[1] with 70% of patients receiving diagnoses in the advanced stage, without chance of being cured with current standard chemotherapy.[2,3] With curative resection, the recurrence rate is >60%, even with intensive adjuvant chemotherapy in carefully selected patients.[4] The refinement of surgical instruments and techniques has improved short-term surgical outcomes.[5] However, without modifiable factors other than chemotherapy, the original principles of tumor resection and the long-term survival outcomes have changed minimally.[6,7] Approximately 80% of patients with pancreatic cancer eventually face the dilemma of choosing between the poor efficacy and high toxicities of the current standard chemotherapy and a fragile body with comorbidities and cancer-associated complications.[2,3,8] Median overall survival (OS) of metastatic disease is within 1 year with standard chemotherapy, such as gemcitabine plus nab-paclitaxel or the FOLFIRINOX regimen.[2,3]

In the past decade, immune checkpoint inhibitors (ICIs) have revolutionized therapy for many cancer types, but not for pancreatic ductal adenocarcinoma (PDAC).[9] In patients with mismatch repair deficiency (MMRD), rarely detected in PDAC,[10] a high disease control rate (DCR) was observed.[11] In the absence of MMRD or a high tumor mutation burden (TMB), ICIs have demonstrated modest efficacy in patients who received prior chemotherapy for PDAC.[12–14] In first-line treatment settings, the addition of nivolumab or pembrolizumab to gemcitabine plus nab-paclitaxel has resulted in manageable toxicities.[15,16] However, other than the anticipated greater DCR and duration of response (DOR) than are achieved with chemotherapy alone,[3] no other benefits, such as in response rate (RR) or OS, have been observed.[15,16]

It remains a huge unmet need in the clinical application of ICIs in PDAC. To explore the efficacy and prognostic factors of programmed cell death 1 (PD-1)/programmed cell death ligand 1 (PD-L1) blockade for treatment of patients with advanced or recurrent PDAC, we conducted a retrospective analysis of patients who received nivolumab-based therapy with palliative intent.

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Methods and materials

Patient selection and response evaluation

This was a single-center retrospective study (REC. No. 201911042RINC) approved by the Research Ethics Committee of National Taiwan University Hospital (NTUH). Formal consent was waived for this type of study. In total, 45 consecutive patients from NTUH with advanced or recurrent PDAC diagnosed between June 2015 and May 2020 who received at least one dose of nivolumab-based therapy after first-line palliative chemotherapy were enrolled as the study group. Patients treated with other anti-PD-1/PD-L1 drugs (one with pembrolizumab and another with atezolizumab) were excluded. In addition, 142 patients with advanced or recurrent PDAC diagnosed between January 2016 and December 2017 who received at least one dose of gemcitabine-based palliative chemotherapy without an ICI were selected as the control group-1, and 24 patients with advanced or recurrent PDAC diagnosed between January 2016 and December 2020 who received first-line (modified) FOLFIRINOX without an ICI were selected as the control group-2 (Supplement Table S1). Either cytology or pathology was required to confirm the diagnoses in both groups. Complete medical records and imaging studies were required for the analyses.

Computed tomography (CT) or magnetic resonance imaging (MRI) was employed for response evaluation every 3 months. Evaluation by CT or MRI was performed ahead of schedule if deemed necessary because of disease progression. The revised Response Evaluation Criteria in Solid Tumors (version 1.1) were used for response evaluation.

Estimation of spleen volume

Spleen volume was estimated as previously described.[17] CT was used for the majority of patients, and MRI was used when CT scan was unavailable. In brief, the spleen volume was estimated using the following formula (method 1): spleen volume (mL) = 30 + 0.58 × W (maximal width of the spleen) × T (maximal thickness of the spleen) × L (length of the spleen). Width (W) was defined as the maximal diameter on any axial view, thickness (T) as the maximal distance between the inner and outer surfaces of the spleen on axial view and perpendicular to W, and length (L) as the sum of total slice thickness in consecutive axial views through the spleen. The other method (method 2) involved summing consecutive spleen areas and considering the slice thickness on axial views; this estimation was more elaborate and time consuming. The correlation of volume estimates using the 2 methods was high (Pearson r = .96) in the study group (Supplement Figure S1). We defined spleen volume for patients who received splenectomy as 0 mL. In the subsequent analyses, the median spleen volume was calculated after patients receiving splenectomy were excluded.

Statistical analysis

All statistical analyses were performed using SPSS (V.20.0, IBM, New York, USA). The data cutoff date was February 28, 2021. OS after nivolumab-based therapy was calculated from the initiation day of nivolumab-based regimens until the date of death or the last follow-up. Time to treatment failure (TTF) after nivolumab-based therapy was calculated from the initiation day of nivolumab-based therapy until the date of clinical or imaging-based progressive disease (PD) confirmation, treatment withdrawal due to toxicity, death, or the final follow-up. Fisher's exact test or the chi-square test was used to analyze the association between disease control status and clinical parameters. The Kaplan–Meier method was used to evaluate the survival data. A Cox proportional hazards regression model was used to compare OS in terms of clinical parameters. The parameters exhibiting significance (i.e., $P < .05$) in the univariate OS analyses with concomitant consideration of comprehensive literature data, mechanisms of action, and the study focuses were introduced into the multivariate analyses. The American Joint Committee on Cancer 8th Edition Cancer Staging System was used for staging. The significance level was set at $P < .05$.

Results

Demographics

All 45 patients in the study group were included in the analysis. Table 1 shows their baseline characteristics. The median age was 62 (46–81) years. Nearly half of the patients (n = 22) had good Eastern Cooperative Oncology Group (ECOG) performance status (PS) of 0–1. Among 20 patients with available microsatellite instability (MSI) data, one had germline *MSH6* mutation, with MSI-high tumor and high TMB. Among the study group, 12 (27%) patients had undergone curative surgery, and 8 had received adjuvant chemotherapy. The median recurrence-free survival of the 8 patients receiving adjuvant chemotherapy and of the 12 receiving curative surgery was 4.8 (95% confidence interval [CI], 4.0–5.7) and 3.3 (95% CI, 0–6.9) months, respectively. The majority of patients had been treated with gemcitabine-based (n = 43) or fluoropyrimidine-based (n = 42) chemotherapy. The median time from the initiation of palliative first-line chemotherapy to that of nivolumab-based therapy was 9.2 (95% CI, 6.7–11.8) months. The median number of previous lines of palliative treatment was 3 (1–7). Furthermore, 10 (22%) patients never achieved any disease control during any prior chemotherapy; 9 (20%) had been treated with radiotherapy for local control. Liver metastasis presented in 32 (71%) patients before nivolumab-based therapy. Small portions of patients were carriers of hepatitis B (n = 7) or C (n = 3). All patients carrying hepatitis B received antiviral prophylaxis. Of 43 patients with available data on C-reactive protein and albumin, 28 (65%) had a modified Glasgow prognostic score (mGPS) of 0.

In the control group-1 (Supplement Table S1), the median age was 64 years. The majority of the patients (n = 113) had good ECOG PS of 0–1 before first-line palliative chemotherapy. Among the control group-1, 43 patients had undergone surgery with curative intent. Second-line palliative chemotherapy had been administered to 105 (74%) patients. The median number of palliative chemotherapy lines was 2 (1–6). In the control group-2 (Supplement Table S1), the median age was 54 years. The majority of the patients (n = 21) had good ECOG

Table 1. Patient characteristics.

Characteristics		All patients n (%)	Patients without splenectomy n (%)	Patients with splenectomy n (%)	P*
N		45	39	6	
Age	median	62	61	65	0.467
	range	46–81	46–81	51–79	
Sex	male	31 (69)	27 (69)	4 (67)	1.000
	female	14 (31)	12 (31)	2 (33)	
Stage at diagnosis	I/II	10 (22)	7 (18)	3 (50)	0.240
	III	7 (16)	6 (15)	1 (17)	
	IV	28 (62)	26 (67)	2 (33)	
T at diagnosis	1–3	27 (60)	21 (52)	6 (100)	0.067
	4	18 (40)	18 (46)	0 (0)	
N at diagnosis	0	12 (27)	11 (28)	1 (17)	1.000
	1–2	33 (73)	28 (72)	5 (83)	
ECOG PS	0–2	29 (64)	25 (64)	4 (67)	1.000
	3-4	16 (36)	14 (36)	2 (33)	
Primary site in pancreas	head	29 (64)	26 (67)	3 (50)	0.302
	body	10 (22)	9 (23)	1 (17)	
	tail	6 (13)	4 (10)	2 (33)	
MSI	high	1 (2)	1 (3)	0 (0)	0.864
	stable	19 (42)	16 (41)	3 (50)	
	NA	25 (56)	22 (56)	3 (50)	
Curative surgery	Yes	12 (27)	8 (21)	4 (67)	0.035
	No	33 (73)	31 (79)	2 (33)	
Prior palliative chemotherapy	1–2	19 (42)	17 (44)	2 (33)	1.000
	>2	26 (58)	22 (56)	4 (67)	
Prior chemotherapy	Gem	43 (96)	37 (95)	6 (100)	1.000
	F	42 (93)	36 (92)	6 (100)	1.000
	Pt	33 (73)	29 (74)	4 (67)	0.650
	Nab	37 (82)	32 (82)	5 (83)	1.000
	Iri	27 (60)	24 (62)	3 (50)	0.670
Metastasis at nivolumab start	Liver	32 (71)	28 (72)	4 (67)	1.000
	Peritoneum	18 (40)	15 (39)	3 (50)	0.670
	Lung	14 (31)	10 (26)	4 (67)	0.065
	bone	5 (11)	3 (8)	2 (33)	0.125
mGPS	0	28 (65)	22 (59)	6 (100)	0.076
	1–2	15 (35)	15 (41)	0 (0)	
WBC	median	5970	5870	6730	0.300
(per mm^3)	range	2310–31860	2310–31860	4830–8440	
Neutrophil	median	3813	3891	3700	0.018
(per mm^3)	range	1441–29980	1441–29980	2400–4322	
Lymphocyte (per mm^3)	median	1320	1228	1709	0.257
	range	285–5471	285–5471	391–3283	
Hemoglobin	median	10.4	10.4	10.6	0.867
(g/dL)	range	7.6–14.4	7.6–14.4	8.8–12.5	
Platelet	median	227	226	255	0.504
($x10^3$per mm^3)	range	72–955	72–955	224–510	
ALP	median	103	91	123	0.017
(U/L)	range	25–1075	25–1075	53–152	
ALT	median	22	23	16	0.033
(U/L)	range	7–125	7–125	10–29	
Total bilirubin	median	0.5	0.5	0.5	0.054
(mg/dL)	range	0.1–18.6	0.1–18.6	0.4–1.1	
Creatinine (mg/dL)	median	0.7	0.7	0.8	0.357
	range	0.3–2.9	0.3–1.5	0.6–2.9	

*P: difference among parameters (with vs without splenectomy); P of the laboratory data (t test).

ALP, alkaline phosphatase; ALT, alanine aminotransferase; CIK, cytokine-induced killer; ECOG PS, Eastern Cooperative Oncology Group performance status; G-CSF, granulocyte colony stimulating factor; Gem, gemcitabine; F, 5-FU/5-FU analog; Iri, irinotecan; mGPS, modified Glasgow prognostic score; MSI, microsatellite instability; NA, not available; Nab, nab-paclitaxel; Pt, platinum; WBC, white blood cell.

PS of 0–1 before first-line FOLFIRINOX, and 5 patients had undergone surgery with curative intent. A half of them had a locally-advanced disease before first-line FOLFIRINOX.

Dosing and efficacy of nivolumab-based regimens

Various regimens of either single-agent nivolumab (n = 5) or a combination of nivolumab and chemotherapy had been used (Table 2). A biweekly schedule was chosen. Only 4 patients had been treated with more than one regimen of nivolumab-based combination therapy. The median number of doses was 3 (1–22). The median dose was 2.5 (0.25–3) mg/kg. Moreover, 40 (89%) and 27 (60%) patients were treated with doses of ≥2 and ≥2.5 mg/kg, respectively. Granulocyte colony-stimulating factor had been used in 25 (56%) patients, mainly for prophylaxis or rescue of chemotherapy-associated neutropenia. Furthermore, 8 patients had been treated with cytokine-induced killer (CIK) cell therapy, and 6 of them had received CIK cell therapy after several doses (5–14) of nivolumab-based combination chemotherapy.

Nivolumab monotherapy was administered to 5 patients. One patient, with a microsatellite-stable tumor, achieved partial response (PR) with a long DOR (>35.2 months). In this

Table 2. Nivolumab-based regimens and efficacy.

Regimen	N	Dose (mg/kg)	CR/PR/SD	PD	RD (mo)	TTF (mo)	OS (mo)	AO (N)	CIK (N)	Refractory (N)	Prior
Nivolumab	5	2.3 (2.0–2.7)	0/1/0	2	35.2+	2.0 (0–4.3)	3.6 (0–9.3)	NA	0	3	3 (1–5)
Nivolumab + Gemcitabine	1	2.3	0/0/0	1	NA	Combination 1.4	1.6	0	0	0	6
2-combo chemotherapy	17	2.5 (0.25–3.0)	0/1/6	6	2.0	2.5 (0.7–4.3)	5.8 (0.5–11.2)	8	3	1	3 (1–5)
3-combo chemotherapy	20	2.5 (0.3–3.0)	1/0/7	6	15.8+	2.3 (1.3–3.2)	6.8 (0–15.1)	12	5	5	2 (1–7)
Targeted therapy	2	3.0	NA	NA	NA	NA	0.7, 2.3	0	0	1	3

AO, add-on nivolumab to disease control under prior therapy; CIK, cytokine-induced killer cell therapy; CR, complete response; NA, not analyzed; OS, overall survival (median & 95% CI; month); PD, progressive disease; PR, partial response; Prior, prior palliative treatment regimens (median & range); RD, response duration (month); Refractory, no stable disease or response under prior treatment; SD, stable disease; TTF, time-to-treatment failure (median & 95% CI; month).

patient, the baseline expression of PD-L1 was 0% (Figure 1(a)) with scant infiltration of T cells in the neoplastic ducts (Figure 1(b,d)). One patient received nivolumab plus gemcitabine, and PD was documented after 3 doses. In total, 17 patients were treated with nivolumab plus 2 chemotherapy agents: 9 with gemcitabine plus nab-paclitaxel, 6 with (nanoliposomal) irinotecan plus a 5-FU or 5-FU analog, 1 with gemcitabine plus oxaliplatin, and 1 with gemcitabine plus S-1. One patient exhibited PR after initial stable disease (SD) with second-line gemcitabine/nab-paclitaxel. However, the DOR was only 2 months. Among patients who were treated with add-on nivolumab under SD with concomitant chemotherapy, 6 (gemcitabine-based regimens in 4, nanoliposomal irinotecan plus 5-FU in 2) achieved continued SD after the addition of nivolumab. Two patients were treated with nivolumab plus regorafenib, but both showed clinical progression shortly after initiation of therapy, without response evaluation.

Nivolumab plus triplet chemotherapy regimens were administered to 20 patients, 2 of whom also received sequential radiotherapy for the primary tumor. One patient, who received nivolumab plus nab-paclitaxel, oxaliplatin, and S-1 achieved a long duration of complete response (>15.8 months). Seven

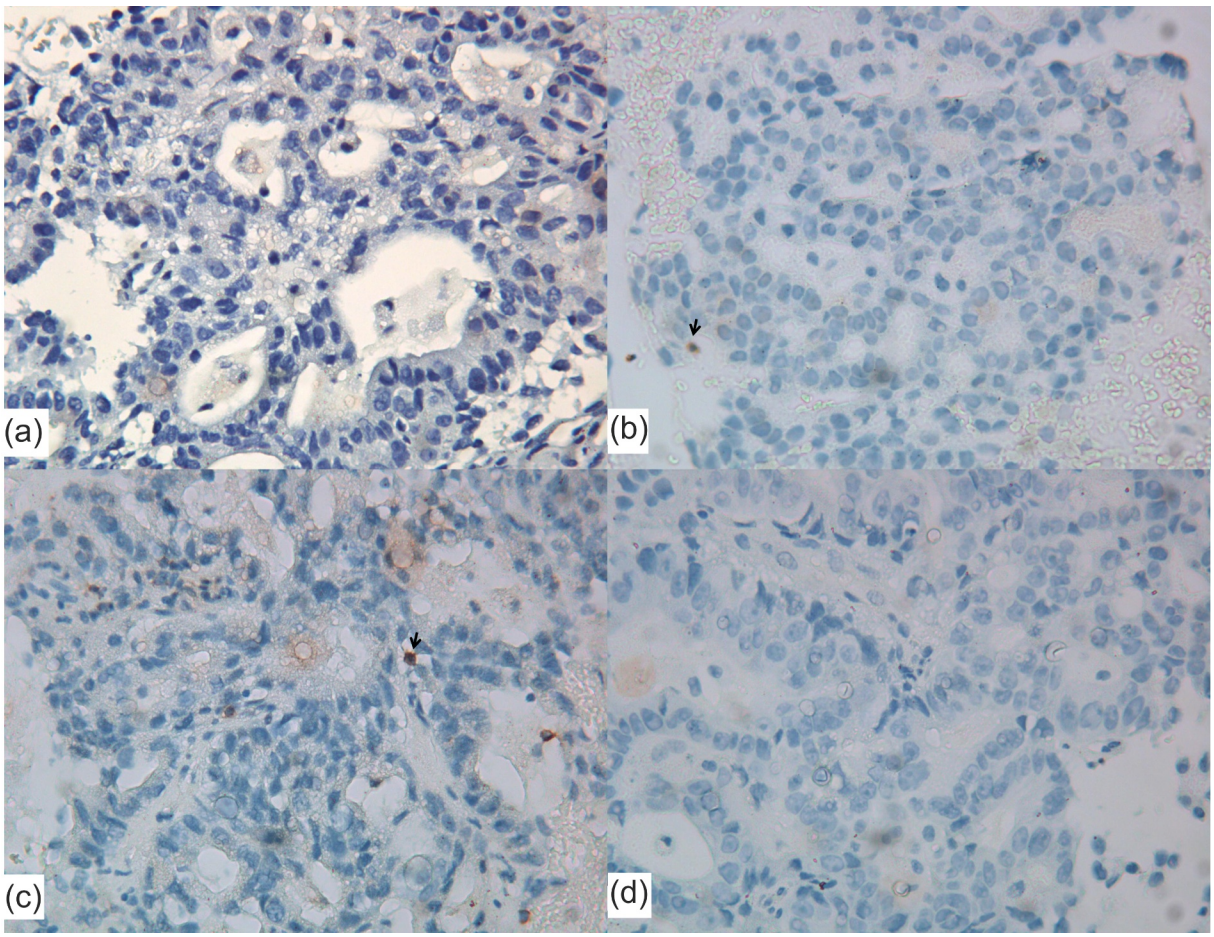

Figure 1. The baseline expression of (a) PD-L1 (22C3) and infiltration of (b) $CD3^+$ (arrow), (c) $CD4^+$ (arrow), and (d) $CD8^+$ T cells in the neoplastic ducts in the patient with a partial response to nivolumab monotherapy.

patients with SD who were treated with add-on nivolumab and concomitant chemotherapy (gemcitabine/oxaliplatin/S-1 in 3, nab-paclitaxel/oxaliplatin/S-1 in 2, gemcitabine/nab-paclitaxel /S-1 in 1, nab-paclitaxel/irinotecan/5-FU in 1) achieved continued SD after the addition of nivolumab.

Overall, the RR was 7% (n = 3), and the DCR was 36% (n = 16). Among nivolumab-based therapy, add-on nivolumab for disease control after prior therapy, concomitant or sequential CIK cell therapy, and undergoing ≤2 lines of prior palliative chemotherapy were significantly associated with disease control (Table 3).

Spleen volume and prognostic analyses

In the study group, the median OS after first-line palliative chemotherapy for the whole group (n = 45) and for patients with initially unresectable disease (n = 33) was 17.8 (95% CI, 14.8–20.8) and 15.7 (95% CI, 7.9–23.6) months, respectively. The median OS after nivolumab-based therapy for the whole group and for patients with initially unresectable disease was 5.5 (95% CI, 2.8–8.2) and 5.5 (95% CI, 2.6–8.4) months, respectively. In the control group-1, the median OS after first-line (n = 142) and second-line (n = 105) palliative chemotherapy was 9.4 (95% CI, 7.7–11.2) and 6.2 (95% CI, 5.1–7.3) months, respectively.

The median time from the available imaging measurement of spleen volume until the initiation of nivolumab-based therapy was 27 (1–100) days. In the study group, the median spleen volume was 199 (82–478) mL before first-line palliative chemotherapy and 267 (110–674) mL before initiation of nivolumab-based therapy. Patients with a spleen of median or greater volume (i.e., ≥267 mL) had significantly shorter median OS (1.9 months, 95% CI, 1.0–2.7) after nivolumab-based treatment than did those with a spleen smaller than the median (8.2 months, 95% CI, 5.6–10.8; P = .003, Figure 2(a)). The negative impact of spleen volume on OS was similar in patients not receiving splenectomy (not reached vs. 1.9 months, P = .006) or whatever the prior use of platinum (platinum used, 6.8 vs. 1.9 months; platinum not used, not reached vs. 1.7 months; P = .022). By the data cutoff date, 37 patients had treatment failure: 22 had imaging-confirmed PD, 9 died, 4 had clinical progression, and 2 experienced treatment-emergent adverse events. TTF was significantly shorter in patients with a larger spleen. The median TTF after nivolumab-based therapy was 3.0 (95% CI, 2.1–3.8) months in patients with spleens smaller than the median and 1.3 (95% CI, 1.0–1.7) months in those with spleens of at least the median volume (P = .027, Figure 2(b)). However, no prognostic value of spleen volume before first-line palliative chemotherapy was found within the study group. With a cutoff of median spleen volume (199 mL), the median OS after first-line palliative chemotherapy was 20.4 (95% CI, 15.1–25.6) and 14.9 (95% CI, 11.5–18.2) months in patients with spleen volumes below and at or above the cutoff, respectively (P = .137). No prognostic value of spleen volume was found with patients who underwent splenectomy excluded (P = .226).

In the univariate analysis (Table 3), spleen smaller than the median volume (267 mL), no prior irinotecan use, ≤2 lines of prior palliative chemotherapy, ECOG PS of 0–2, no liver metastasis, add-on nivolumab under SD with prior therapy, nivolumab dose of ≥2.5 mg/kg, concomitant or sequential CIK cell therapy, high lymphocyte count (≥1,300/mm^3), high hemoglobin level (≥10 g/dL), low mGPS (mGPS = 0), normal ALP level (<100 U/L), and normal total bilirubin level (<1 mg/dL) were significant and favorable prognostic factors for OS after nivolumab-based therapy. In the multivariate analysis (Table 3), spleen smaller than the median volume (267 mL), ≤2 lines of prior palliative chemotherapy, ECOG PS of 0–2, add-on nivolumab under SD with prior therapy, concomitant or sequential CIK cell therapy, high lymphocyte count, and normal total bilirubin level were independent and favorable prognostic factors for OS. The association between patient characteristics and spleen volume was demonstrated in Supplement Table S2. Liver-related parameters, including liver metastasis and levels of ALP, ALT, and total bilirubin, were not associated with spleen volume (Supplement Table S2) and prior use of platinum (P = .088 for ALP, P = .655 for ALT, and P = .407 for total bilirubin).

To reduce the heterogeneity in the prognostic analyses, further univariate, multivariate, and subgroup analyses were performed. After excluding the patients with *MSH6* mutation (n = 1, nivolumab with 3-combo chemotherapy), nivolumab with targeted therapy (n = 2), and nivolumab with gemcitabine (n = 1), the univariate and multivariate analyses were performed for patients with nivolumab monotherapy, nivolumab with 2-combo chemotherapy or 3-combo chemotherapy (n = 41). As the Supplement Table S3 and Table 3 showed, the results of multivariate analyses were remarkably similar. Comparing the overall results (Table 3) and the subgroup analyses (Supplement Table S4) for nivolumab with 2-combo chemotherapy (n = 17) or 3-combo chemotherapy (n = 19), the trends for OS among parameters were also similar.

In the control group-1, the median spleen volume was 197 (75–726) mL before first-line palliative chemotherapy. Before second-line chemotherapy, the median spleen volume was 200 (90–881) mL. With the median spleen volume (197 mL) as the cutoff, the median OS after first-line palliative chemotherapy was 12.7 (95% CI, 10.0–15.4) and 7.4 (95% CI, 5.4–9.5) months in patients with spleens smaller than and at or larger than the cutoff, respectively (P = .012). However, the prognostic value of spleen volume for OS after first-line palliative chemotherapy was not significant after adjustments for other significant prognostic factors (Supplement Table S5). With the cutoff of median spleen volume (200 mL), the median OS after second-line palliative chemotherapy was 6.4 (95% CI, 4.8–8.0) months in patients with spleens smaller than the median and 5.4 (95% CI, 4.0–6.9) months in patients with spleens of median volume or greater (P = .100). After exclusion of patients who underwent splenectomy, median OS after first-line palliative chemotherapy (n = 123) was 11.5 (95% CI, 8.6–14.4) and 7.4 (95% CI, 5.4–9.5) months in patients with spleens volumes smaller than the median and median volume or greater, respectively (P = .109). Median OS after second-line palliative chemotherapy (n = 82) was 6.2 (95% CI, 5.2–7.2) months in patients with spleens smaller than the median and 5.4 (95% CI, 4.0–6.9) months in patients with spleens of median volume or greater (P = .434).

Table 3. Prognosis analyses and association of characteristics and disease control.

Parameter Value	Univariate analysis	Multivariate analysis	Disease control (CR/PR/SD vs PD)			
	OR (95% CI) P1*	OR (95% CI) P2*	Value	+	-	P3**
Age (years)	0.77 (0.36–1.65)	-	> 62	7	8	0.594
≥ 62 vs < 62	0.501		≤ 62	9	7	
Initial stage	0.72 (0.32–1.61)	-	I–III	5	8	0.213
I–III vs IV	0.426		IV	11	7	
Initial T	0.71 (0.33–1.52)	-	1–3	11	8	0.379
1–3 vs 4	0.373		4	5	7	
Initial N	0.38 (0.13–1.11)	-	0	7	2	0.113
0 vs 1–2	0.076		1–2	9	13	
Initial primary site	0.98 (0.45–2.15)	-	head	10	10	0.809
head vs others	0.957		others	6	5	
Curative surgery	0.97 (0.42–2.21)	-	No	13	9	0.252
no vs yes	0.933		Yes	3	6	
Splenic size at nivolumab	0.32 (0.15–0.70)	0.19 (0.05–0.66)	< 267	12	8	0.273
< 267 ml vs ≥ 267 ml	0.004	0.009	≥ 267	4	7	
Prior platinum	0.33 (0.10–1.09)	-	No	6	4	0.704
no vs yes	0.068		Yes	10	11	
Prior irinotecan	0.21 (0.08–0.52)	-	No	10	5	0.104
no vs yes	0.001		Yes	6	10	
Prior nab-paclitaxel	0.53 (0.18–1.53)	-	No	5	1	0.172
no vs yes	0.239		Yes	11	14	
Prior chemotherapy	0.33 (0.14–0.79)	0.12 (0.03–0.43)	≤ 2	11	4	0.032
≤ 2 lines vs > 2 lines	0.013	0.001	> 2	5	11	
Prior best response	0.50 (0.21–1.19)	-	Yes	15	11	0.172
CR/PR/SD vs PD	0.116		No	1	4	
ECOG PS at nivolumab	0.15 (0.07–0.34)	0.10 (0.03–0.36)	< 3	14	11	0.394
0–2 vs ≥ 3	<0.001	<0.001	≥ 3	2	4	
Liver mets at nivolumab	0.36 (0.14–0.95)	-	No	7	4	0.458
no vs yes	0.039		Yes	9	11	
Peritoneal mets at nivolumab	0.53 (0.25–1.14)	-	No	12	9	0.458
no vs yes	0.102		Yes	4	6	
Lung mets at nivolumab	0.52 (0.23–1.14)	-	No	14	11	0.394
no vs yes	0.101		Yes	2	4	
Nivolumab regimen	1.60 (0.55–4.65)	-	Mono	1	2	0.600
mono vs combo	0.386		Combo	15	13	
Nivolumab timing	0.20 (0.08–0.48)	0.32 (0.11–0.91)	Add-on	15	3	<0.001
add-on vs others	<0.001	0.032	Others	1	12	
Nivolumab dose (mg/kg)	0.46 (0.21–0.99)	-	≥ 2.5	12	9	0.458
≥ 2.5 vs < 2.5	0.048		< 2.5	4	6	
G-CSF use	1.74 (0.81–3.73)	-	No	4	8	0.149
no vs yes	0.153		Yes	12	7	
CIK cell therapy	0.06 (0.01–0.48)	0.09 (0.01–0.82)	Yes	7	1	0.037
yes vs no	0.008	0.033	No	9	14	
mGPS	0.20 (0.09–0.43)	-	0	13	11	0.685
0 vs 1–2	<0.001		1–2	3	4	
Laboratory data						
WBC (/mm^3)	0.89 (0.42–1.90)	-	< 6,000	9	9	0.833
< 6,000 vs ≥ 6,000	0.762		≥ 6,000	7	6	
Neutrophil (/mm^3),	0.46 (0.21–1.01)	-	< 3,800	10	8	0.605
<3,800 vs ≥ 3,800	0.052		≥ 3,800	6	7	
Lymphocyte (/mm^3)	0.23 (0.10–0.51)	0.15 (0.05–0.43)	< 1,300	3	8	0.066
≥ 1,300 vs < 1,300	<0.001	<0.001	≥ 1,300	13	7	
Hemoglobin (g/dL)	0.34 (0.16–0.74)	-	< 10	3	5	0.433
≥ 10 v < 10	0.006		≥ 10	13	10	
Platelet (x10^3/mm^3)	0.72 (0.33–1.56)	-	< 230	9	6	0.366
≥ 230 vs < 230	0.404		≥ 230	7	9	
ALP (U/L)	0.34 (0.15–0.78)	-	< 100	10	7	0.376
< 100 vs ≥ 100	0.011		≥ 100	6	8	
ALT (U/L)	0.49 (0.20–1.16)	-	< 50	14	13	1.000
< 50 vs ≥ 50	0.104		≥ 50	2	2	
Total bilirubin (mg/dL)	0.36 (0.16–0.82)	0.18 (0.06–0.59)	< 1	14	14	1.000
<1 vs ≥ 1	0.016	0.004	≥ 1	2	1	

*P1 and P2: difference among parameters (Cox regression model); **P3: difference among parameters (Fisher's exact test).

ALP, alkaline phosphatase; ALT, alanine aminotransferase; CIK, cytokine-induced killer; CR, complete response; ECOG PS, Eastern Cooperative Oncology Group performance status; G-CSF, granulocyte colony stimulating factor; mGPS, modified Glasgow prognostic score; PD, progressive disease; PR, partial response; SD, stable disease; WBC, white blood cell.

Because the median number of lines of palliative chemotherapy before nivolumab-based therapy was 3 in the study group, patients (n = 25) who had received 4 or more lines of palliative chemotherapy in the control group-1 were selected for comparison (Supplement Table S1). The median spleen volume before fourth-line therapy was 242 (112–526) mL. Median OS after fourth-line palliative chemotherapy was 3.5 (95% CI, 2.6–4.4) months in patients with spleens

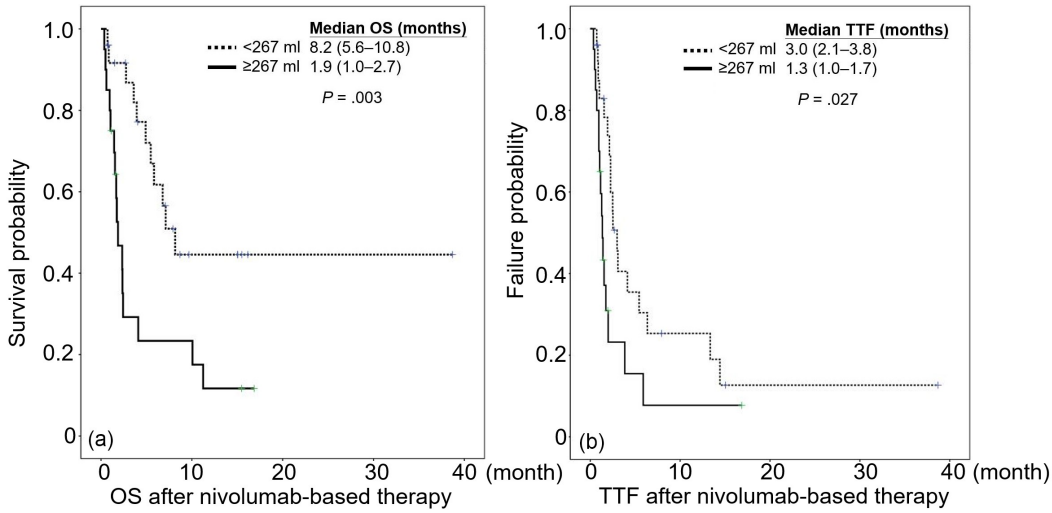

Figure 2. (a) Overall survival (OS) and (b) time to treatment failure (TTF) after nivolumab-based therapy.

smaller than the median and 4.2 (95% CI, 0–8.7) months in patients with spleens of median volume or greater (P = .912). After patients who underwent splenectomy were excluded, no value of spleen volume was found for predicting OS (P = .744)

In the control group-2 (Supplement Table S1), the median spleen volume was 282 (170–356) mL before first-line FOLFIRINOX. With the median spleen volume (282 mL) as the cutoff, the median OS after first-line FOLFIRINOX was 11.2 (95% CI, 0–25.1) and 15.1 (95% CI, 6.2–24.1) months in patients with spleens smaller than and at or larger than the cutoff, respectively (P = .821). Alternatively, with the cutoff of 340 mL, 5 (21%) patients had splenomegaly (≥340 ml). The median OS after first-line FOLFIRINOX was not reached and 15.1 (95% CI, 4.4–25.9) months in patients with and without splenomegaly, respectively (P = .914).

Evolution of spleen volume under nivolumab-based therapy

In the 27 patients receiving response evaluations and not undergoing splenectomy, the evolution of spleen volume was evaluated. After stratification of these patients into 3 subgroups based on the first evaluation, none of the 3 (0%) patients exhibiting treatment response (Figure 3(a)) and 2 of the 12 (17%) patients with SD (Figure 3(b)) had spleen of >1.1 times the volume at baseline. However, 5 of 12 (42%) patients with PD (Figure 3(c)) had spleen of >1.1 times the volume at baseline. However, the disease control status was not associated with splenic vein invasion before or after nivolumab-based therapy (Table 4).

Discussion

Splenomegaly is common in PDAC, and it may be attributed to right-side portal hypertension due to portal vein thrombosis or invasion or to massive liver metastases and left-side portal hypertension caused by splenic vein compression or invasion by the pancreatic body or tail tumor.[18] However, splenomegaly was not associated with pancreatic body-tail tumor or liver metastasis in our study group. During the evolution of spleen volume after the initial diagnosis, disparate changes in tumor status may have differing effects on spleen volume before nivolumab-based therapy.

A previous study had demonstrated the negative prognostic impact of baseline splenomegaly in advanced PDAC patients treated with first-line FOLFIRINOX.[19] By contrast, no significant difference in OS was found in our control group-2. The heterogeneity in the patient population, such as the high percentage of locally-advanced disease in our control group-2, may partially explain the discrepancy. Although splenomegaly had been also reported to be associated with prolonged exposure of oxaliplatin and hepatic injury,[20] especially in colorectal cancer and gastric cancer, prior use of platinum was not associated with spleen

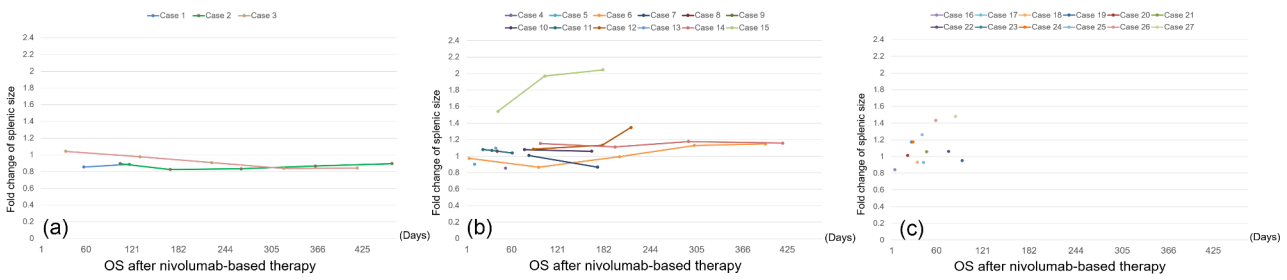

Figure 3. Evolution of spleen volume with nivolumab-based therapy for patients with (a) complete or partial response, (b) stable disease, and (c) progressive disease.

volume before nivolumab-based treatment (Supplement Table S2) and not associated with OS after nivolumab-based treatment (Table 3) in our study. In addition, the negative prognostic impact of splenomegaly on OS existed whatever the prior use of platinum. The difference in the cause of splenomegaly among different cancer types and relatively shorter duration of exposure to platinum in advanced PDAC comparing to colorectal cancer and gastric cancer may explain the discrepancy. A previous study had demonstrated the negative prognostic impact of liver dysfunction after ICI treatment.[21] However, liver dysfunction was rarely observed after nivolumab-based therapy in our patient population. The short duration of nivolumab use (median cycle = 3) and combination of chemotherapy (n = 38) in the majority patients may preclude or reduce the occurrence of ICI-induced, immune-related, liver-associated adverse events.

Traditionally, PDAC is considered an immunologically cold tumor with the hallmarks of a highly immunosuppressive tumor microenvironment (TME). One mechanism of immune escape in PDAC is the PD-1/PD-L1 axis. Tumor-infiltrating $CD8^+$ and $CD4^+$ T cells are usually scarce in the neoplastic ducts and TME in patients with various levels of PD-1 expression.[22–24] In addition, $CD8^+PD\text{-}1^+$ tumor-infiltrating lymphocytes (TILs) frequently coexpress lymphocyte-activation gene 3,[22] indicating functional exhaustion. The ligand PD-L1 is expressed not only in cancer cells and $CD4^+FoxP3^+$ regulatory T (Treg) cells[22,25] but also in myeloid cells, such as tumor-associated macrophages (TAMs) and myeloid-derived suppressor cells (MDSCs).[22,26] Although multidirectional PD-1/PD-L1 signaling among cancer cells and immune cells in the TME represents a functionally important pathway of tumor growth in animal models,[25,27,28] the clinical benefits of blocking the signaling pathway are limited in PDAC without a high TMB or high MSI.[13–16] In the study group, only one of the 20 patients with MSI data was MSI-high; this fact may partially explain the poor efficacy of nivolumab-based therapy in our study. Somatic mutations and the TMB in PDAC are low compared with those in lung cancer or melanoma.[29,30] In addition, $CD4^+$ or $CD8^+$ TILs reactive to neoantigens are rarely detected in PDAC.[31]

Prominent infiltration of immunosuppressive myeloid cells, including TAMs and MDSCs, is a common feature of the TME in PDAC.[32–34] The spleen is considered a potential reservoir for these myeloid cells as well as bone marrow.[32–35] In mice with preinvasive to invasive lesions, MDSCs accumulated in the spleen.[32] Extramedullary hematopoiesis in the spleen modulated by tumor-derived granulocyte-macrophage colony-stimulating factor developed in the tumor-bearing KPC mouse model with associated splenomegaly; neutralization of this factor in the TME inhibited tumor growth and MDSC infiltration.[33] Moreover, MDSCs from the TME and spleen induced immunosuppression of $CD8^+$ T cells in the Panc 02 mouse model.[33] In patients with malignancies such as PDAC, MDSCs have also been observed to accumulate in the spleen and to inhibit T-cell proliferation.[36,37] The immunosuppressive functions of myeloid cells may be partial within the spleen but full upon their recruitment to the TME.[33,38] In the $KRAS^{G12D}$/*P53*-null (KP) mouse lung cancer model, splenic macrophages contributed to TAMs in the TME.[39] More importantly, CCR2 knockdown in splenic monocytes reduced TAM recruitment to the TME and slowed tumor progression.[39] Human PDAC also expressed CCL2 with $CCR2^+$ TAMs in the TME, and increased infiltration of $CD4^+$ and $CD8^+$ TILs was noted after CCR2 inhibition in another animal model.[40] Blockade of the CCL2/CCR2 axis abrogated splenic recruitment of myeloid hematopoietic progenitors and synergistically increased the efficacy of anti-PD-L1 in the murine (Hepa) hepatoma model.[35] However, blockade of the PD-1/PD-L1 axis without modulation of TAMs or MDSCs was ineffective for tumor control in the KPC mouse model.[41]

In our study group, high lymphocyte count was an independent favorable prognostic factor for OS. Lymphopenia was significantly associated with splenomegaly and marginally associated with PD after nivolumab-based therapy. Lymphocyte count was associated with spleen size. In patients with pancreatic neoplasms who received distal pancreatectomy and splenectomy, peripheral blood lymphocyte count recovered gradually and even exceeded the preoperative level 2 weeks after the operation.[42] By contrast, patients with preserved spleens had lymphocyte counts comparable to the baseline.[42] Splenomegaly and lymphopenia may be directly or indirectly implicated in the efficacy of nivolumab. First, skewed expansion of myeloid cells in the spleen or TME may suppress T-cell proliferation or induce lymphopenia through multiple pathways in addition to the PD-1/PD-L1 axis.[22,34] The interaction between the Fc portion of nivolumab and the FcR on the abundant M2-like TAMs in the TME of PDAC may activate these immunosuppressive cells.[43] Second, the entrapment of nivolumab by immune cells in a large spleen should not be neglected. The accumulation of pembrolizumab – another anti-PD-1 antibody – in normal lymphoid organs, including the spleen, was implicated in the low tumor distribution observed in an animal model.[44] Furthermore, in patients with lung cancer, prominent accumulation of nivolumab in the spleen was observed through radionucleotide imaging.[45] Third, the number of $CD4^+FoxP3^+$ Treg cells increases in PDAC.[22] In the context of lymphopenia, with its intrinsically scant effector T cells, in the TME, anti-PD-1 treatment may actually augment the immunosuppression mediated by PD-1-expressing $CD4^+FoxP3^+$ Treg cells and overwhelm the functions of the effector T cells.[46]

Strategies can potentially counteract the detrimental effects associated with splenomegaly and improve the efficacy of PD-1/PD-L1 blockade in PDAC. However, without reliable biomarkers for efficacy prediction, an adequate dosage and early intervention may be the most crucial identifiable and reliable factors for maximizing the clinical benefits. In fact, a nivolumab dose of ≥2.5 mg/kg, ≤2 lines of prior chemotherapy, ECOG PS of 0–2, and add-on nivolumab with disease

Table 4. Association of splenic vein invasion and disease control.

	Splenic vein invasion	Disease control		P
		+	-	
N		14	11	
Before nivolumab	+	5	3	1.000
	-	9	7	
After nivolumab*	+	4	5	0.403
	-	10	5	

*First CT evaluation after nivolumab-based treatment.

control were good prognostic factors for OS in our study group. Based on the data of this retrospective study and clinical trials in ICIs for PDAC,[15,16] we have designed a prospective clinical trial (NCT04377048) to explore the concept. In addition, CIK cell therapy was an independent good prognostic factor for OS and associated with disease control. CIK cells may overcome the immunosuppression induced by myeloid cells in the spleen and TME and enhance the efficacy of PD-1/PD-L1 blockade.[47] A higher DCR was demonstrated after S-1 and concomitant cell therapy than after either therapy alone in advanced PDAC.[48] Combination of anti-PD-1/PD-L1 and other agents, such as CCR2 inhibitors, renin–angiotensin system inhibitors, or CD40 agonists, for reversing immunosuppression or inhibiting the mobilization of TAMs and MDSCs may be worthy of further exploration.[49–51]

This study has several limitations, including small sample drawn from a single center, heterogeneous population and regimens, nonfixed nivolumab doses and schedule, and inherent biases of a retrospective study. Pathological examination data for the spleen and tumor immediately before nivolumab-based therapy were not obtained for analysis. In addition, the heterogeneity of the control group, which rendered challenging the matching between the control and heavily pretreated study groups, may have generated additional biases.

In conclusion, this study elucidates the prognostic implications of splenomegaly with nivolumab-based therapy in advanced or recurrent PDAC. Clinical trials and basic studies are required to confirm the insights of this hypothesis-generating study to continue the battle against PDAC.

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Disclosure statement

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