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Original Article

Incorporating nivolumab with preceded gemcitabine and S-1 chemotherapy for patients of metastatic pancreatic cancer: a pilot study

Shih-Hung Yang[1,2], Bang-Bin Chen[3], Jen-Chieh Lee[4], Yu-Ting Kuo[5], Sun-Hsin Kuo[1,2], Ann-Lii Cheng[1,2], Kun-Huei Yeh[1,2]

[1]Department of Oncology, National Taiwan University Hospital, Taipei, Taiwan; [2]Graduate Institute of Oncology, National Taiwan University College of Medicine, Taipei, Taiwan; [3]Department of Medical Imaging and Radiology, National Taiwan University Hospital, Taipei, Taiwan; [4]Department of Pathology, National Taiwan University Hospital, Taipei, Taiwan; [5]Division of Endoscopy, Department of Integrated Diagnostics and Therapeutics, National Taiwan University Hospital, Taipei, Taiwan

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Abstract: Pancreatic ductal adenocarcinoma (PDAC) rarely responds to immune checkpoint inhibitors. We conducted a pilot study to investigate chemotherapy followed by the addition of nivolumab in metastatic PDAC with limited tumor burden. A single cycle of gemcitabine (850 mg/m^2 on days 1 and 8) and S-1 (60-100 mg/day on days 1-12) was administered. Patients who had achieved control of carbohydrate antigen 19-9 (CA 19-9) (a decreased level of CA 19-9 or <10% increased level of CA 19-9 comparing to baseline) were provided adding-on nivolumab (3 mg/kg on days 1, 15, and 29) with the same doses of gemcitabine (on days 1, 8, 22, and 29) and S-1 (on days 1-12 and 22-33). The primary endpoint was response rate (RR). After enrolling seven patients, the study was terminated owing to slow recruitment. Five of the seven patients who completed one cycle of gemcitabine plus S-1 (GS) fulfilled the criteria for CA 19-9 and proceeded to receive nivolumab in addition to GS. One patient demonstrated a partial response, and the other four patients had stable disease (SD). The RR and disease control rate (DCR) for gemcitabine and S-1 plus nivolumab (GSN) were 20% and 100%, respectively. The median progression-free survival (PFS) was 6.3 (95% confidence interval [CI], 0-16.4) months. The median overall survival (OS) was 20.8 (95% CI, 16.4-25.2) months. Two patients who did not receive nivolumab continued the GS regimen; one SD and one progressive disease (PD) were observed with a PFS of 3.5 and 3.0 months, respectively. The most common adverse events (AEs) during the GS phase (n = 7) were grade 1-2 neutropenia (n = 5), skin rashes (n = 4), and fever (n = 3). During the nivolumab adding-on phase (n = 5), one grade 3 and one grade 4 neutropenia were observed. Grade 1-2 mucositis (n = 3) was the most common nonhematological AE. In conclusion, adding nivolumab to chemotherapy in patients who had achieved control of CA 19-9 in metastatic PDAC was feasible. (Registration at ClinicalTrials.gov: NCT04377048).

Keywords: Pancreatic cancer, adding-on nivolumab, gemcitabine, S-1, disease control

Introduction

Pancreatic cancer has been a growing disease burden worldwide with an increasing mortality rate in the recent two decades [1-3]. Globally, it has become the sixth leading cause of cancer-related deaths [4]. Moreover, a 64% increase in the death toll is expected in 2040 compared to that in 2020 [5]. Pancreatic ductal adenocarcinoma (PDAC) represents more than 90% of exocrine pancreatic cancers [6]. The median overall survival (OS) of unresectable, locally advanced PDAC is more than 1 year under chemotherapy with or without radiotherapy [7, 8]. However, more than half of patients succumb to this lethal disease within 1 year under combination chemotherapy, even those with good Eastern Cooperative Oncology Group (ECOG) performance status (PS), with distant metastasis [8-11].

S-1, a widely used oral chemotherapeutic agent for gastrointestinal (GI) cancers in Asian countries, consists of 5-FU prodrug tegafur, gimeracil, and oteracil potassium for modulating pharmacokinetics and reducing GI toxicities

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[12]. Also, for PDAC, S-1 demonstrated its single-agent activity in the GEST study [8]. With S-1 alone, the response rate (RR) was 21.0% and significantly higher than the RR (13.3%) of gemcitabine (P = 0.02) [8]. The disease control rate (DCR) of gemcitabine plus S-1 (GS) was 71.5% and significantly higher than the DCR (62.7%) of gemcitabine alone; the median progression-free survival (PFS) was also significantly longer with GS (5.7 months vs 4.1 months, P<0.001) [8]. Compared to gemcitabine plus nab-paclitaxel (GN) [9, 13], this GS regimen had a similar median OS not only in locally advanced disease (15.9 months) but also in metastatic disease (9.4 months) [8].

According to the phase I study (ClinicalTrials.gov identifier: NCT01946646), the tumor burden of metastatic PDAC is significantly associated with prognosis [14]. Following S-1-based concurrent chemoradiotherapy (CCRT) to control pancreatic tumor (local DCR = 100%), the median OS was significantly longer in patients with low tumor burden [14]. Low tumor burden is associated with better efficacy of immune checkpoint inhibitors (ICIs) in advanced non-small-cell lung cancer [15]. Based on these findings, we designed this phase II clinical trial and hypothesized that the greatest benefit from ICI for metastatic PDAC would be in the first-line setting in patients with relatively intact immune system, low tumor burden, and prerequisite disease control.

Materials and methods

Eligibility

The key inclusion criteria were as follows: (1) histologically or cytologically confirmed, newly diagnosed PDAC; (2) limited tumor burden with the following definition: (2-1) ≤10 liver and ≤10 lung identifiable (≥0.5 cm) metastatic lesions with the diameter between 0.5 and 3.0 cm in single lesions and the sum of diameters of all identifiable lesions ≤10 cm; (2-2) metastatic sites other than the liver and lung with ≤3 cm in diameter in single lesions and the sum of diameters of all identifiable lesions ≤5 cm; (2-3) asymptomatic non-measurable lesions, such as ascites; (2-4) pancreatic tumors ≤5 cm in diameter; (3) no previous radiotherapy, local therapy, systemic therapy, and surgery for PDAC; (4) at least one measurable lesion; (5) age between 20 and 80 years; (6) ECOG PS of 0 or 1; (7) adequate organ function indicated by white blood cell (WBC) count ≥3,500/mm^3, absolute neutrophil count ≥2,000/mm^3, hemoglobin level ≥10.0 g/dL, platelet count ≥ 100,000/mm^3, total bilirubin level ≤1.5-fold the upper limit of normal value (ULN) and ≤1.5 mg/dL, liver transaminases ≤2.5-fold the ULN, prothrombin time ≤1.5-fold the ULN, activated partial thromboplastin time ≤1.5-fold the ULN, creatinine ≤1.2 mg/dL, and creatinine clearance ≥60 mL/min; (8) baseline CA 19-9> the ULN (37 U/mL) but <90% of upper limit of detection; and (9) ability to take S-1 orally. The key exclusion criteria were as follows: (1) significant lung fibrosis or interstitial pneumonitis; (2) diarrhea ≥ grade 2 by Common Terminology Criteria for Adverse Events (CTCAE) v.5.0; (3) systemic infection requiring treatment; (4) significant comorbidities; (5) autoimmune disease; (6) organ allograft or allogeneic bone marrow transplantation; (7) systemic corticosteroids or immunosuppressants; (8) history of testing positive for human immunodeficiency virus or known acquired immunodeficiency syndrome; (9) significant ascites or pleural effusion requiring treatment; (10) central nervous system metastasis; (11) prior or concurrent malignancies within the last 3 years; and (12) pregnant women, nursing mothers, or positive pregnancy tests.

Comparing to the initial protocol, several important amendments had been made for improving recruitment: (1) specification of number and size of metastatic lesions in organs; (2) extension of the upper limit of age; (3) removal of the exclusion of carriers with hepatitis B virus (HBV) or hepatitis C virus (HCV) due to regular use of antiviral agents for HBV prophylaxis and low probability of HCV reactivation; (4) removal of the restriction from blood transfusion, and (5) allowance of COVID-19 vaccination for the pandemic outbreak.

Written informed consent from all participants had been obtained before trial initiation. This study adhered to the Declaration of Helsinki, was approved by the Research Ethical Committee (REC) of the National Taiwan University Hospital (202001045MIPA) and registered at ClinicalTrials.gov (NCT04377048).

Design and treatment

This was a single-institution pilot study using Simon's two-stage optimal design. The first

stage required at least 4 responders out of 15 participants to proceed to the second stage. The maximal number in total would be 27 patients with the assumption of an improvement of RR from 25% to 45% under $\alpha = 0.1$ and $\beta = 0.2$. For further development of the nivolumab plus gemcitabine and S-1 (GSN) regimen, 9 responders out of 27 patients would be required.

The study consisted of two phases. In the GS phase, one cycle of GS was administered, and patients who achieved control of CA 19-9 (marker response-1), which indicates a decreased level of CA 19-9 or <10% increased level of CA 19-9 compared to baseline, entered the nivolumab adding-on phase. Otherwise, patients who did not fulfill the CA 19-9 criteria, had evident clinical progression, or with intolerance to GS were excluded from the study treatment and received survival follow-up. We assumed that approximately 70% of the initially enrolled patients would proceed to the nivolumab adding-on phase; therefore, 21 and 38 evaluable patients would be recruited in the first stage and both of first and second stages, respectively.

In the GS phase, gemcitabine was administered at 850 mg/m^2 on days 1 and 8. S-1 was administered with two divided doses on day 1 to day 12 according to body surface area (BSA): 60 mg (BSA <1.25 m^2), 80 mg (BSA ≥1.25 m^2 to <1.5 m^2), and 100 mg per day (BSA ≥1.5 m^2). The cycle length of the GS phase was 21 days. In the nivolumab adding-on phase, nivolumab was administered at 3 mg/kg every 2 weeks on days 1, 15, and 29. The dose of GS was followed and adjusted according to adverse events (AEs) during the GS phase; however, the dosing schedule was shifted to days 1, 8, 22, and 29 for gemcitabine, and days 1-12 and 22-33 for S-1. The nivolumab dosing schedule was adjusted according to the GS dose delay. The cycle length of the nivolumab adding-on phase was 42 days. Treatment was administered until disease progression, intolerable toxicity, or the discontinuation criteria were met.

Evaluation

Baseline computed tomography (CT) of the chest, abdomen, and pelvis was performed within 2 weeks before study treatment. The first CT evaluation was performed during the 10th week after initiation of the GS phase. Further CT evaluations were performed every 6 weeks. Responses were confirmed by repeating the CT assessment within 4 weeks. AEs were assessed using the CTCAE v 5.0, according to the study phase and the most severe grade.

Next-generation sequencing

This study employed targeted exon sequencing using the ACTOnco®+ cancer gene panel. A minimum mean depth of 500x and base coverage of 100x for over 85% of targets were obtained. Hematoxylin and eosin stained, formalin-fixed, paraffin-embedded tumor tissue sections were evaluated for tissue adequacy, and 5-20 unstained sections with a total area of ≥125 mm^2 were used for DNA extraction. In cases with <30% tumor purity, macrodissection was performed to enrich the tumor content. The tumor mutation burden (TMB) was estimated using sequenced regions with the threshold of ≥7.5 Muts/Mb as "TMB-high". Microsatellite instability status was determined by a machine learning prediction algorithm using >400 genomic loci with changes in repeat numbers from a pooled microsatellite-stable baseline as features.

Statistical analysis

The primary endpoint was the RR of the GSN regimen. The secondary endpoints included the safety profiles of the GSN regimen, such as AE, severe adverse events (SAE), death, DCR, duration of response (DoR), PFS, and OS. The DCR was defined as the sum of the complete response (CR), partial response (PR), and stable disease (SD) rates. DoR was defined as the time from the initiation of the GS phase to disease progression, death, or last follow-up in patients who achieved CR or PR. The dose intensity (DI) for each drug was calculated as the actual cumulative dose divided by the scheduled cumulative dose during the entire treatment course. Marker response-1 was defined as previously described. Marker response-2 was defined as at least a 50% decrease in the CA 19-9 level compared to that before entering the nivolumab adding-on phase. OS and PFS were evaluated by Kaplan-Meier analysis. OS was calculated from the initiation of the GS phase to the day of death from any cause or the last follow-up. PFS was calculated from the

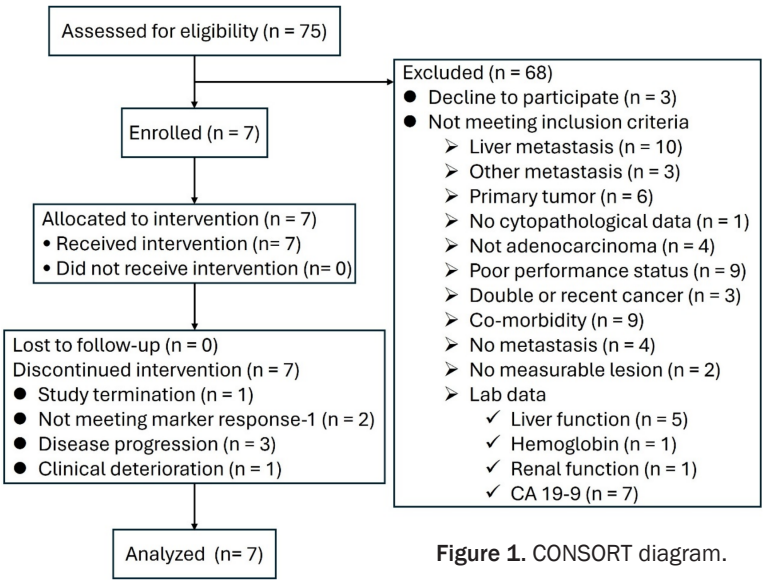

Figure 1. CONSORT diagram.

initiation of the GS phase to disease progression, death from any cause, or the last follow-up.

Results

The study initiated the screening of candidates since December 1st, 2020. Several amendments of the protocol had been performed due to stringent enrollment criteria in the original design. The most common reasons for ineligibility included large hepatic tumor number, size, or poor liver function. From November 2021 to December 2022, seven patients were enrolled in the GS phase (**Figure 1**), and five of them proceeded to the nivolumab adding-on phase. Considering the development policy of nivolumab and slow recruitment, the study was prematurely terminated to recruit additional patients on December 31st, 2022, and then the study treatment was continued in patients who remained in disease control. All patients stopped the study treatment and survival follow-up by January 2024. The clinical characteristics, treatment course, and patient outcomes are summarized in **Table 1**.

All patients completed the GS phase without a dose reduction. However, four patients had a dose delay at C1D8 in the GS phase.

Regarding the nivolumab adding-on phase, the dosing times in each patient were 5-48 for GS and 3-36 for nivolumab. Dose delay occurred in 35 of 112 (range, 2-21 in each patient) for dosing of GS and 19 of 83 (range, 0-10 in each patient), including one omitted dose, for nivolumab dosing. One level of dose reduction of gemcitabine from 850 mg/m^2 to 700 mg/m^2 had been administered to case 1 since C7D29 of GSN. No two levels of dose reduction occurred. The DI was 0.73, 0.76, and 0.73 for gemcitabine, S-1, and nivolumab, respectively.

The marker response-1 was achieved in five of seven (71%) patients. Of the five patients who entered the nivolumab adding-on phase, one PR and four SD were observed. The RR and DCR rates of the GSN regimen were 20% and 100%, respectively. The marker response-2 was achieved in three of five (60%) patients in the nivolumab adding-on phase. The median PFS of the GSN regimen was 6.3 (95% confidence interval [CI], 0-16.4) months. Considering the resection of case 1 and interruption of the original GSN administration without PD, the median PFS was 6.3 (95% CI, 0-13.0). The median OS of the GSN regimen was 20.8 (95% CI, 16.4-25.2) months. The two patients who did not enter the nivolumab adding-on phase continued the original GS regimen covered by the National Health Insurance. One SD and one PD were observed with a PFS of 3.5 and 3.0 months, respectively. The median OS of all enrolled patients was 20.8 (95% CI, 16.2-25.4) months.

Treatment-related AEs stratified by phase are summarized in **Table 2**. Overall, the study treatment was well-tolerated. During the GS phase, the most common AEs were grade 1-2 neutropenia (n = 5), skin rash (n = 4), and fever (n = 3). During the nivolumab adding-on phase, the most common AE was hematological, with one grade 3 and one grade 4 neutropenia. Among non-hematological AE, grade 1-2 mucositis (n = 3) was the most common. One patient developed grade 2 hypothyroidism after C3D15 of the GSN regimen, presenting with muscle weakness, exertional dyspnea, cough, and weight gain. Laboratory findings demonstrated undetectable free T4 but high thyroid stimulat-

Table 1. Baseline characteristics and outcomes of enrolled patients

Case No.	Age	Sex	Staging	Metastatic site	ECOG PS	CA 19-9 (U/mL)		Genetic alterations	TMB (/Mb)	MSI	GSN dosing	Best response of GSN	PFS (mo)	OS (mo)	Status
						Before GS	Before GSN								
1	62	M	T3N0	liver	0	11,600	7,661	*KRAS* G12A, *ARID1A* Q802*	NA	NA	C12D29	PR	25.5	25.5	Alive
2	64	F	T4N1	distant lymph nodes	1	230	138	*KRAS* G12R, *TP53* P190L, *CDK6* (6), *AKT2* (9)	<1	MSS	C10D22	SD	15.6	21.2	Dead
3	61	M	T4N1	peritoneum	1	958	1,126	NA	NA	NA	None	NA	3.0	8.9	Dead
4	68	M	T4N0	liver	1	105	11	*KRAS* G12R, *TP53* C275Y, *CDKN2A* P821fs, *TGFBR2* Q511*	1.3	MSS	C2D1	SD	4.6	20.8	Dead
5	52	M	T3N2	lung, peritoneum	1	3,300	7,374	*KRAS* G12D, *TP53* R196*	NA	NA	None	NA	3.5	17.4	Dead
6	56	F	T4N1	peritoneum	1	380	275	*KRAS* G12D, *TP53* R213L, PRKCI (34)	<1	MSS	C3D15	SD	5.3	16.7	Alive
7	73	F	T4N0	distant lymph nodes, peritoneum	1	8,177	2,606	*KRAS* G12V, *TP53* R175H	<1	MSS	C3D15	SD	6.3	11.5	Dead

Abbreviations: ECOG PS, Eastern Cooperative Oncology Group performance status; GS, gemcitabine plus S-1; GSN, gemcitabine plus S-1 with adding-on nivolumab; MSI, microsatellite instability; MSS, microsatellite instability-stable; NA, not analyzed; OS, overall survival; PFS, progression-free survival; PR, partial response; SD, stable disease; TMB, tumor mutation burden.

Table 2. Treatment-related adverse events

Phase	GS			Nivolumab adding-on		
N	7			5		
Adverse events	All grade	Grade 3	Grade 4	All grade	Grade 3	Grade 4
Leucopenia	6	0	0	4	0	0
Anemia	0	0	0	3	0	0
Thrombocytopenia	0	0	0	1	0	0
Neutropenia	5	0	0	5	1	1
Vomiting	2	0	0	0	0	0
Diarrhea	0	0	0	1	0	0
Constipation	1	0	0	0	0	0
Nausea	1	0	0	1	0	0
Anorexia	1	0	0	2	0	0
Abdominal pain	0	0	0	1	0	0
Mucositis	1	0	0	3	0	0
Fever	3	0	0	1	0	0
Rash	4	0	0	2	0	0
Skin pigmentation	0	0	0	1	0	0
Pruritus	1	0	0	1	0	0
Hypothyroidism	0	0	0	1	0	0
Adrenal insufficiency	0	0	0	1	0	0

Abbreviation: GS, gemcitabine plus S-1.

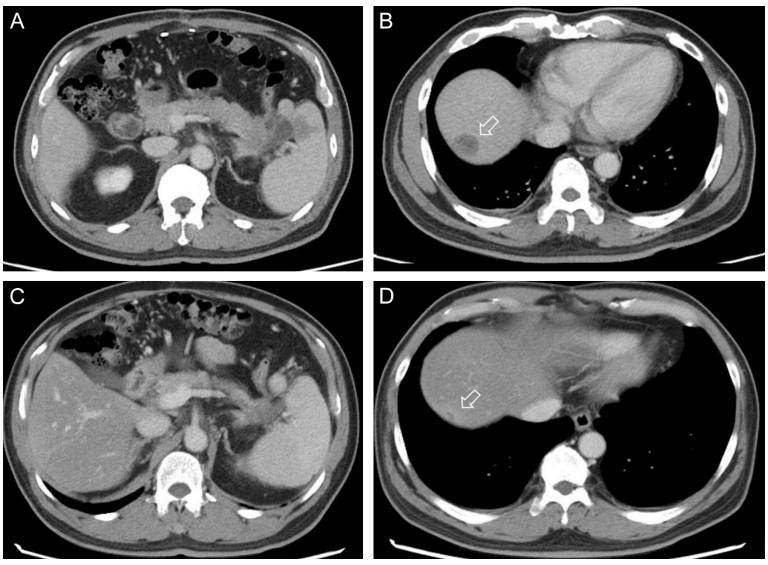

Figure 2. Pancreatic tail tumor (A) and liver metastasis (B) before study treatment. Pancreatic tail tumor (C) and liver metastasis (D) before surgery. Arrow: liver metastatic lesion.

ing hormone (75 μIU/mL) and creatine kinase levels (589 U/L). A small amount of pericardial effusion was observed on CT. After thyroid hormone replacement, the symptoms and pericardial effusion resolved completely with the continuation of GSN.

Of the three patients who had PD after the GSN regimen, all received (nanoliposomal) irinotecan/fluoropyrimidine-based regimens as second-line therapy. The patient who had been withdrawn from the GSN regimen because of a prolonged dose delay continued to receive GS alone and then nanoliposomal irinotecan plus 5-FU and leucovorin (nal-IRI/FL) after PD from GS. Two patients did not enter the nivolumab adding-on phase: one received nal-IRI/FL, and the other received gemcitabine, oxaliplatin, S-1, and leucovorin after PD from GS.

The DoR of the responder (case 1) was at least 25.5 months. Initially, a pancreatic tail tumor (**Figure 2A**) with a metastatic liver lesion (**Figure 2B**) was identified. The patient underwent distal pancreatectomy and splenectomy because of tumor shrinkage (**Figure 2C**) after C7D15 dosing of the GSN regimen. The liver metastatic

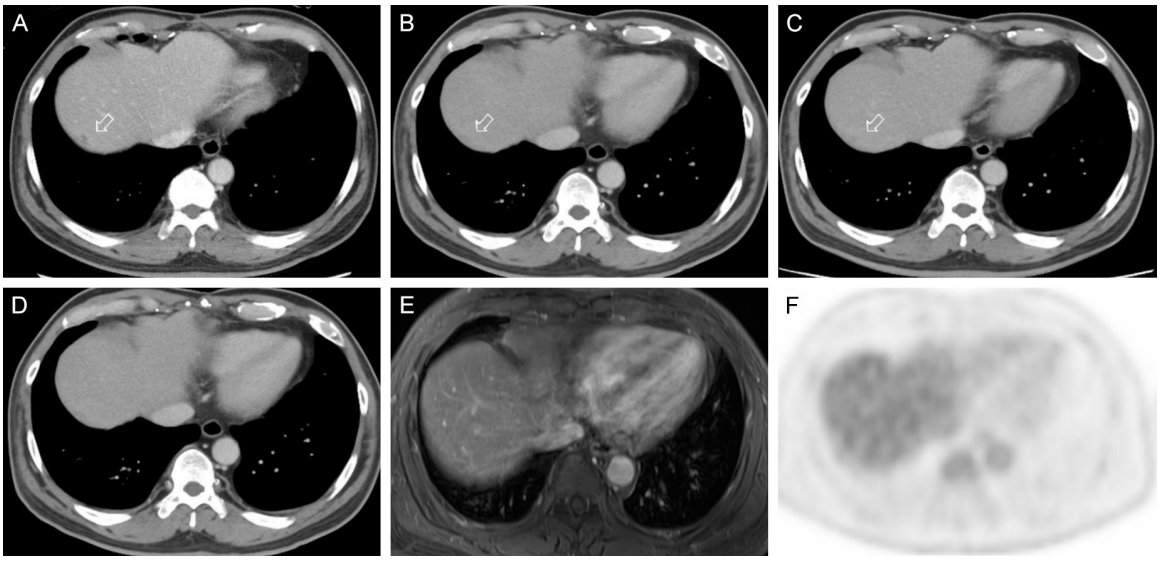

Figure 3. Evolutionary changes in liver metastasis at (A) 3, (B) 6, (C) 9, and (D) 12 months after pancreatic tumor resection. Magnetic resonance imaging (E) and positron emission tomography (F) performed 12 months after pancreatic tumor resection also demonstrate complete resolution of the liver metastasis. Arrow: liver metastatic lesion.

lesion was persistently identified before surgery (**Figure 2D**); however, the liver lesion was not resected because of local adhesions. Pathological examination of the resected specimen revealed ypT2N1 with one regional lymph node metastasis and a tumor regression score of 2 for the pancreatic tumor. After discussion with the study team, the GSN regimen was resumed 6 weeks postoperatively. The residual liver metastatic lesions resolved gradually and (**Figure 3A-C**) completely disappeared (**Figure 3D**) after C12D1 dosing of the GSN regimen. Magnetic resonance imaging (**Figure 3E**) and positron emission tomography (**Figure 3F**) also demonstrated CR. Additionally, lymphocytosis and neutropenia developed postoperatively. Granulocyte colony-stimulating factor was administered frequently to support further dosing with GSN. The flow cytometry of peripheral blood revealed T cells (45.7%) [$CD8^+$ T cells (52.6%), $CD4^+$ T cells (42.4%), $\gamma\delta^+$ T cells (1.6%)], B cells (3.64%), and NK cells (13%) without clonal lymphocytes. The two-year support of nivolumab was terminated after C12D29 of the GSN. The clinical course of case 1 is summarized in **Figure 4**. Before treatment, immunohistochemical staining of the pancreatic tumor revealed abundant $CD4^+$ T cells (**Figure 5A**) and rare or absent $CD8^+$ T cells (**Figure 5B**) and $FoxP3^+$ T cells (**Figure 5C**) in the tumor microenvironment (TME). By contrast, after C7D15 dosing of the GSN regimen, abundant $CD8^+$ T cell infiltration was observed in the TME (**Figure 5E**) without evident changes in the number of $CD4^+$ T cells (**Figure 5D**) or $FoxP3^+$ T cells (**Figure 5F**).

Discussion

For the first time in a prospective study, we demonstrated the potential benefits of adding-on nivolumab in metastatic PDAC. The marker response-1 (71%) in the initial GS phase was in line with our proposal (70%). The RR was 20% in our present study, and it was numerically lower compared to the overall RR (29.3%) of the GS arm in the GEST study and the pooled RR (28.5%) in patients with metastatic PDAC of the GEST, JACCRO PC-01, and GEMSAP studies [8, 16]. However, the DCR (100%) in patients achieving marker response-1 with the GSN regimen in our study was significantly higher than the DCR of the GEST study (71.5%) or the pooled DCR (70.0%) of metastatic PDAC [8, 16]. Not only the doses but also the DIs of gemcitabine and S-1 were lower in our present study than in those in the GEST and GEMSAP studies [8, 17]. In the GEST study, the median DI of gemcitabine and S-1 was 83.3% and 87.4%, respectively, in the GS regimen [8]. However, the grade 3 or worse hematological toxicity, such as neutropenia, was still very high (62.2%) [8]. Therefore, according to the median DI of the GEST study, 85% of the gemcitabine dose and 12-days but not 14-days dosing of

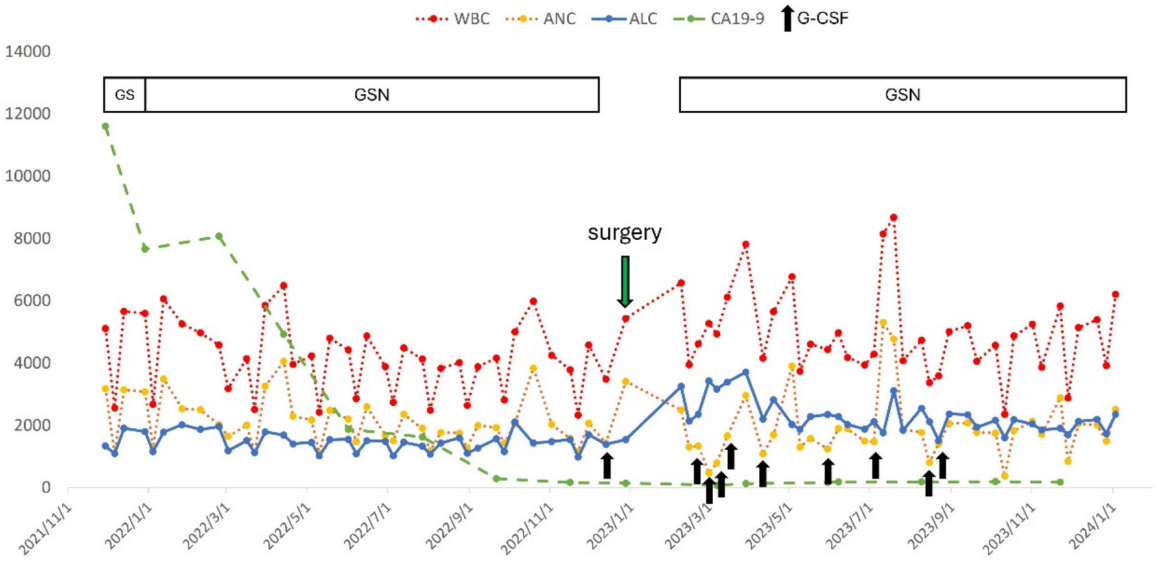

Figure 4. Clinical course of case 1 during the study treatment. ALC, absolute lymphocyte count [/μL]; ANC, absolute neutrophil count [/μL]; CA 19-9, carbohydrate antigen 19-9 (U/mL); G-CSF, granulocyte colony-stimulating factor; GS, gemcitabine plus S-1; GSN, gemcitabine and S-1 plus nivolumab; WBC, white blood cell count [/μL].

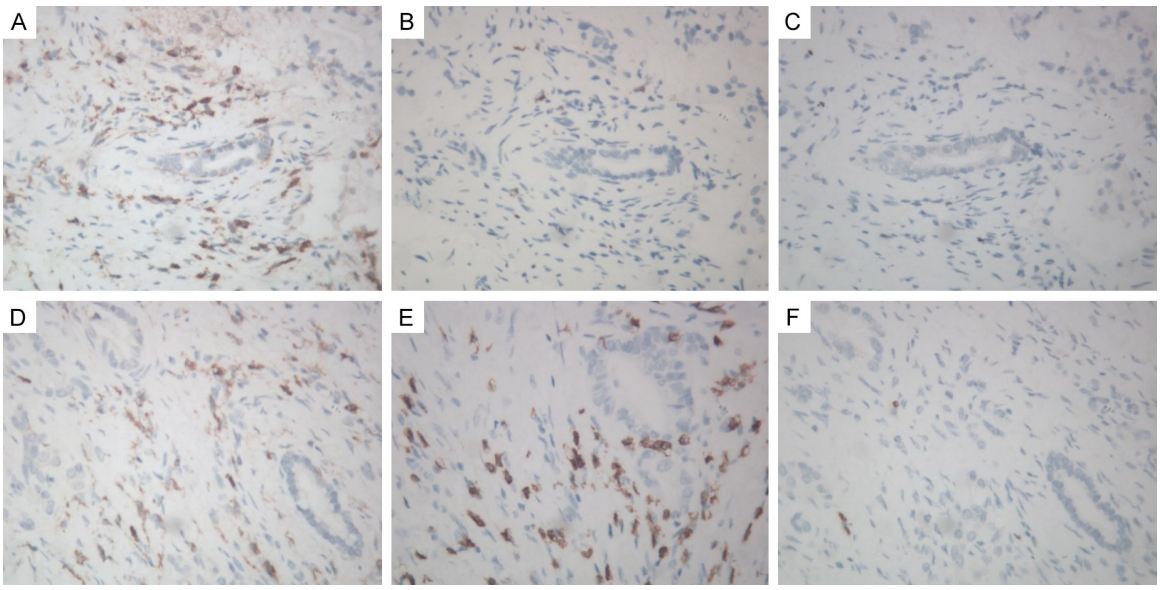

Figure 5. Immunohistochemical (IHC) staining of (A) $CD4^+$ T cells, (B) $CD8^+$ T cells, and (C) $FoxP3^+$ T cells in the pancreatic tumor bed before treatment and (D) $CD4^+$ T cells, (E) $CD8^+$ T cells, and (F) $FoxP3^+$ T cells in the pancreatic tumor bed after C7D15 dosing of the GSN regimen. The method of IHC staining was as previously described [18].

S-1 per 21 days in the original GS regimen were arbitrarily chosen. This difference in the dosing including individual drug dose, interval, and cycle length of the GS regimen may partially explain the lower RR in our present study. Actually, the pooled RR of the GS regimen in patients with either locally-advanced (RR 29.6%) or metastatic disease (RR 28.5%) was quite similar [16]. The median PFS (6.3 months) and median OS (20.8 months) of the GSN regimen in our present study were better than the pooled data (median PFS, 5.36 months; median, OS 9.43 months) with the GS regimen in metastatic PDAC from the three studies [16]. In addition, the median OS of our present study enrolling highly selected patients with limited tumor burden was even longer than that of the patients with low tumor burden and local DCR

of 100% in our previous CCRT trial (median OS, 15.1 months) [14]. The incidence of ≥ grade 3 neutropenia (40%) in our present study was lower than the pooled incidence (55.8%) of the three trials with the GS regimen, although this was similar to that of the MPACT trial (38%) with the GN regimen [9, 16]. However, the DCR (48%) of the GN regimen of the MPACT study was lower than that of the GSN regimen in our present study [9].

Recently, our study group has demonstrated the significant survival benefits of adding-on nivolumab in advanced PDAC [18]. In the retrospective study, the patients who received adding-on nivolumab after achieving disease control with chemotherapy had significantly better median time to treatment failure (TTF) and OS than those who received additional nivolumab therapy irrespective of the status of disease control with chemotherapy [18]. Moreover, adding nivolumab to chemotherapy also demonstrated significantly better median TTF and OS in patients who had achieved disease control under chemotherapy [18]. Although the method representing disease control before adding-on nivolumab is different between our present study and the previous one (imaging versus biochemical, i.e., CA 19-9 in marker response-1) [18], the concept of these two studies is the same. In fact, the dynamic change in CA 19-9 has been recognized as an early indicator of response to gemcitabine-based therapy and was associated with prognosis in advanced PDAC [19]. Obtaining a CA 19-9 response as early as completing one cycle of GS in our present study was not only more cost-effective than imaging evaluation but also matched the following imaging-documented disease control of the nivolumab adding-on phase. Dynamic change of circulating tumor DNA is also useful to predict the response to ICI in advanced cancers; however, the methods and thresholds of detection in different cancer types need to be standardized and validated [20, 21]. In addition, the cost of serial monitoring is of concern.

All the five patients entering the nivolumab adding-on phase had *KRAS* mutations, and four of them also had *TP53* mutations. However, none of their tumors were microsatellite instability-high or had high TMB, which are rarely observed in PDAC [22]. This may partially explain no additional benefit in RR after adding nivolumab to the GS regimen in our present study [8]. Similarly, the initial addition of nivolumab or pembrolizumab to the GN regimen also did not increase the RR [9, 23, 24]. However, the DCR of both studies was over 60%, outnumbering which was achieved with the GN regimen alone [9, 23, 24]. With our adding-on strategy in patients with limited tumor burden, the DCR of ICI plus chemotherapy improved further to 100%. Although a previous study has demonstrated the presence of rare neoantigen-reactive T cells in the TME of PDAC [25], ICI may leverage chemotherapy-induced antitumor immunity. Gemcitabine treatment increased the expressions of PD-L1, PD-L2, and major histocompatibility complex (MHC) class I in the neoplastic ducts of the KPC mouse model [26]. In the Panc02 mouse model, gemcitabine treatment increased the $CD4^+$ and $CD8a^+$ T cells in the TME [27]. 5-FU treatment upregulated the expression of MHC class I and NKG2D ligands in Panc02 cells [28]. Following response to chemotherapy in clinical studies, $CD4^+$ or $CD8^+$ T cells increased, whereas myeloid-derived suppressor cells decreased in the TME of PDAC [29, 30]. Indeed, a short-term induction chemotherapy can prime tumors for response to ICI as demonstrated in the TONIC trial for triple-negative breast cancer [31]. However, ICIs without chemotherapy may not be able to sustain the anticancer immunity and overcome the immunosuppressive TME derived from preceding chemotherapy in advanced PDAC [32, 33]. In our present study, potentially poor responders to the GS regimen with rapid progression were excluded from entering the nivolumab adding-on phase with the CA 19-9 criteria. This process may help select those who have the best chance of obtaining the weak beneficial effects of subsequent nivolumab treatment and may also partially explain the high DCR of the GSN regimen in our study.

One patient (case 1) had PR to the GSN regimen and underwent pancreatic tumor resection. After discussing with the sponsor and reporting to the REC, the surgery and followed by continuation of the GSN regimen due to no PD was planned in the patient's best interest. The residual tumor burden was very low after removal of the bulk pancreatic tumor with peripheral organ invasion. The liver lesion regressed gradually, accompanied by lymphocytosis of skewed $CD8^+$ T cells in the peripheral blood, while continuing the same GSN regimen

after removal of the pancreatic tumor. Concomitantly, the TME of the resected pancreatic tumor revealed abundant $CD8^+$ T-cell infiltration. The infiltration of $CD8^+$ T cell in the TME may imply the potential of chemoimmunotherapy-induced epitope spreading, which may be enhanced by surgical removal of the primary tumor and continuation of chemoimmunotherapy, and facilitating the regression of liver metastasis [34, 35]. With tumor load reduction to achieve a minimal residual disease, this case corroborates our previous case with long-term survival and demonstrates the potential of ICI in long-term maintenance of a minimal residual disease or even disease-free status in the neoadjuvant, adjuvant, and palliative setting in PDAC [36].

The genetic analysis of the patient (case 1) revealed *ARID1A* mutation. A recent study has reported that a significantly higher percentage of patients with basal-type PDAC harbored *ARID1A* mutations. Basal-type tumors had a significantly higher IFN-γ signature than classical type ones [37]. Pancreatic cancer with alterations of the switch/sucrose nonfermentable chromatin remodeling genes, such as *ARID1A* and *PBRM1*, is predictive of ICI response [18, 38]. Colorectal cancer with *ARID1A* mutations had more tumor-infiltrating lymphocytes in the TME compared to other cancer types [39]. The abundant $CD4^+$ T cells in the TME before treatment, a rare phenomenon in PDAC, in our case may corroborate previous studies [37, 39]. In addition, the spleen of our patient was also removed in conversion surgery. Splenomegaly was associated with negative prognostic impacts in patients with advanced PDAC treated with nivolumab [40]. Removal of the spleen in PDAC patients may potentially reduce immunosuppressive myeloid cells from the spleen and enhance the activation of T cells by nivolumab [41].

Our study has limitations. The sample size was small, and the study was prematurely terminated owing to slow recruitment, which precluded us from precisely estimating RR, the primary endpoint. The dose and schedule of the GS regimen in our study were modified for better safety profiles and were different from those used in the GEST study [8]. Before adding nivolumab, imaging-based documentation of disease control was not performed and replaced with the CA 19-9 criteria. The rationale for selecting patients based on early CA19-9 control and limited tumor burden as criteria for immunotherapy benefit requires further justification. More patient enrollment is required to confirm the feasibility and benefit of adding nivolumab to GS in metastatic PDAC with limited tumor burden.

In conclusion, to the best of our knowledge, our prospective study provides the first evidence of clinical benefits, such as high DCR, long PFS, and OS, with modest toxicities, of adding nivolumab to preceding chemotherapy in disease-controlled and metastatic PDAC with limited tumor burden. However, the optimal timing, selection criteria, and regimen for adding ICI to advanced PDAC require further exploration.

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Disclosure of conflict of interest

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Address correspondence to: Dr. Kun-Huei Yeh, Department of Oncology, National Taiwan University Hospital, No. 7, Chung-Shan South Road, Taipei 10002, Taiwan. Tel: +886-2-2312-3456 Ext. 266285; Fax: +886-2-2371-1174; E-mail: khyeh@ntu.edu.tw

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