

Cancer Therapy: Clinical

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A Phase I Study of an Agonist CD40 Monoclonal Antibody (CP-870,893) in Combination with Gemcitabine in Patients with Advanced Pancreatic Ductal AdenocarcinomaGregory L. Beatty^{1,2}, Drew A. Torigian^{1,3}, E. Gabriela Chiorean⁷, Babak Saboury³, Alex Brothers³, Abass Alavi³, Andrea B. Troxel^{1,4}, Weijing Sun⁶, Ursina R. Teitelbaum^{1,2}, Robert H. Vonderheide^{1,2,5}, and Peter J. O'Dwyer^{1,2}**Abstract**

Purpose: This phase I study investigated the maximum-tolerated dose (MTD), safety, pharmacodynamics, immunologic correlates, and antitumor activity of CP-870,893, an agonist CD40 antibody, when administered in combination with gemcitabine in patients with advanced pancreatic ductal adenocarcinoma (PDA).

Experimental Design: Twenty-two patients with chemotherapy-naïve advanced PDA were treated with 1,000 mg/m² gemcitabine once weekly for three weeks with infusion of CP-870,893 at 0.1 or 0.2 mg/kg on day three of each 28-day cycle.

Results: CP-870,893 was well-tolerated; one dose-limiting toxicity (grade 4, cerebrovascular accident) occurred at the 0.2 mg/kg dose level, which was estimated as the MTD. The most common adverse event was cytokine release syndrome (grade 1 to 2). CP-870,893 infusion triggered immune activation marked by an increase in inflammatory cytokines, an increase in B-cell expression of costimulatory molecules, and a transient depletion of B cells. Four patients achieved a partial response (PR). 2-[¹⁸F]fluoro-2-deoxy-D-glucose-positron emission tomography/computed tomography (FDG-PET/CT) showed more than 25% decrease in FDG uptake within primary pancreatic lesions in six of eight patients; however, responses observed in metastatic lesions were heterogeneous, with some lesions responding with complete loss of FDG uptake, whereas other lesions in the same patient failed to respond. Improved overall survival correlated with a decrease in FDG uptake in hepatic lesions ($R = -0.929$; $P = 0.007$).

Conclusions: CP-870,893 in combination with gemcitabine was well-tolerated and associated with antitumor activity in patients with PDA. Changes in FDG uptake detected on PET/CT imaging provide insight into therapeutic benefit. Phase II studies are warranted. *Clin Cancer Res*; 19(22); 6286–95. ©2013 AACR.

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G.L. Beatty and D.A. Torigian contributed equally to this work.

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This article presents original results of a phase I study of CP-870,893 with gemcitabine in patients with advanced pancreatic ductal adenocarcinoma.

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Introduction

Pancreatic ductal adenocarcinoma (PDA) is the fourth leading cause of cancer-related death in the United States, and is notoriously resistant to conventional forms of treatment including chemo- and radiotherapy (1, 2). This finding may be related to emerging data showing the importance of the tumor microenvironment in regulating therapeutic efficacy (3–6). In PDA, the tumor microenvironment is marked by poor vascularity, dense desmoplasia, and a leukocyte reaction dominated by macrophages (3, 7, 8). Strategies that target this desmoplastic reaction may be critical for improving therapeutic efficacy in PDA (3, 5).

Tumor-associated macrophages, among other hematopoietic and nonhematopoietic cells, can express the cell surface molecule CD40, a member of the TNF receptor superfamily (9). CD40 is a major determinant in the development of T-cell-dependent antitumor immunity through its ability to "license" antigen-presenting cells for tumor-specific T-cell priming and activation (10–15). In a

Translational Relevance

This phase I study investigates the tolerability and clinical impact of an agonist CD40 monoclonal antibody (CP-870,893) when administered in combination with gemcitabine for the treatment of patients with chemotherapy-naïve advanced pancreatic ductal adenocarcinoma (PDA). We show that combination of CP-870,893 with gemcitabine is well-tolerated and associated with preliminary evidence of efficacy. Treatment produced a systemic immune response that was marked by leukocyte trafficking, cytokine production, and cellular activation. Using novel analyses of metabolic imaging, we observed, in many patients, a cumulative decrease with each cycle of therapy in the metabolic activity of the primary pancreatic lesion. However, treatment responses of metastatic lesions were heterogeneous. These findings highlight the biologic heterogeneity of pancreatic carcinoma and indicate a role for metabolic imaging in understanding treatment responses.

preclinical model of PDA, we reported that systemic CD40 activation can also induce tumor regression that is independent of T cells, but requires macrophages that rapidly infiltrate tumor lesions, become tumoricidal, and attack the tumor microenvironment by facilitating stromal depletion (16).

CP-870,893 is a fully human and selective CD40 agonist monoclonal antibody (mAb) that, as a single agent, has shown promising clinical activity, particularly in patients with melanoma (17). Because preclinical data suggest that chemotherapy can synergize with CD40 agonists, we conducted a phase I study using CP-870,893 in combination with gemcitabine and observed major tumor regressions in some patients with advanced PDA (16). Here, we report a final analysis with biologic correlates of treatment with CP-870,893 in combination with gemcitabine in patients with advanced PDA. The primary objective was to define safety, tolerability, maximum-tolerated dose (MTD), and recommended phase II dose (RP2D) of CP-870,893 when given in combination with gemcitabine. Secondary objectives were to evaluate clinical activity as well as pharmacodynamics and immune modulation. In addition, we conducted novel analyses of metabolic imaging, and report biologically important heterogeneity in the treatment response of primary and metastatic lesions.

Materials and Methods

Patients

Twenty-two patients with chemotherapy-naïve advanced PDA were enrolled between June 28, 2008 and March 19, 2010 at the Abramson Cancer Center, University of Pennsylvania (Philadelphia, PA) and the Indiana University Simon Cancer Center (Indianapolis, IN).

Inclusion criteria were age >18 years, Eastern Cooperative Oncology Group performance status of 0 to 1, and

adequate end organ function. Exclusion criteria included previous systemic treatment for pancreatic cancer or radiotherapy within 4 weeks before randomization, autoimmune disorder, coagulopathy, major illness, and pregnancy or lactation.

Written informed consent was required. The study was approved by local Institutional Review Boards.

Study design and treatment plan

This was an open-label, phase I dose-escalation study. Primary objective was to determine safety, tolerability, MTD, and RP2D of CP-870,893 when given in combination with gemcitabine. Secondary objectives were to characterize pharmacodynamics, immune modulation, and preliminary evidence of efficacy including response rate, overall survival (OS) and progression-free survival (PFS).

Patients received 1,000 mg/m² gemcitabine on days 1, 8, and 15 of each 28-day cycle (Fig. 1). On the basis of preclinical data showing synergy when CD40 agonists are delivered 48 hours after gemcitabine, CP-870,893 was administered once during each treatment cycle on day 3 (18, 19). To determine the MTD of CP-870,893, two dose cohorts (0.1 and 0.2 mg/kg) of at least three patients were enrolled on the basis of single-agent safety data (17, 20). To further evaluate safety and efficacy, an expansion cohort of at least 12 patients was planned. Safety assessments included incidence of treatment-related adverse events, according to the National Cancer Institute Common Terminology Criteria of Adverse Events version 3.0, and incidence of patients experiencing dose modifications and/or premature discontinuation of study drug. Dose-limiting toxicity (DLT) was defined during cycle 1 of treatment as treatment-related nonhematologic grade 3 to 4 adverse events despite optimal supportive care; grade 3 or 4 neutropenia or grade 3 febrile neutropenia; grade 4 thrombocytopenia or grade 3 thrombocytopenia associated with bleeding; grade 4 lymphopenia if complicated by infection, or any other grade 3 hematologic adverse event. MTD was estimated as the highest dose level at which fewer than 2 of 6 patients experienced DLT. Patients continued treatment until disease progression or unacceptable toxicity. CP-870,893 was supplied by the Pfizer Corporation (17, 21).

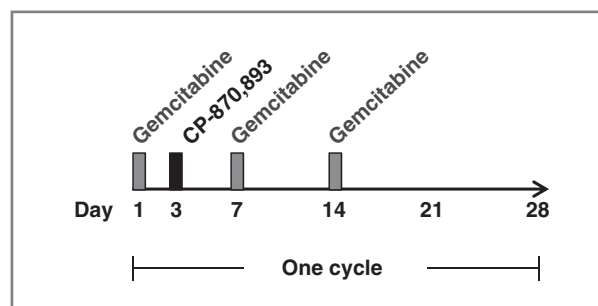


Figure 1. Treatment schema. Gemcitabine (1,000 mg/m²) was infused on days 1, 8, and 15 of each 28-day cycle, with CP-870,893 administered once during each treatment cycle on day 3.

Pharmacodynamic studies

Serum and peripheral blood for assessment of cytokines and B lymphocyte activation were obtained at baseline and at defined times during cycle 1 after infusion of gemcitabine and CP-870,893. Serum cytokine levels of interleukin (IL)-6, IL-8, and IL-10 were determined by ELISA. Peripheral blood CD19⁺ B lymphocytes were evaluated by flow cytometry, and molecules of equivalent soluble fluorochrome (MESF) were calculated for HLA-DR (MHC class II) and CD86 (costimulatory molecule).

Tumor response

All patients who received at least one dose of CP-870,893 were evaluated for toxicity and efficacy. Tumor response was assessed by computed tomography (CT) or MRI at baseline and every 8 weeks according to Response Evaluation Criteria in Solid Tumors (RECIST) version 1.0.

Positron emission tomography data acquisition and analysis

Metabolic activity was assessed in patients enrolled in the MTD expansion cohort by determining 2-[¹⁸F]fluoro-2-deoxy-D-glucose (FDG) uptake within tumor lesions seen on positron emission tomography/computed tomography (PET/CT) imaging at baseline, 2, 8, and then every 8 weeks while on treatment. FDG-PET/CT was conducted after patients had fasted for ≥ 6 hours and plasma glucose levels were determined to be less than 200 mg/dL. Approximately, 15 mCi of FDG were administered intravenously, and images were acquired approximately 60 minutes later from skull base to mid-thighs. Analysis was conducted using the ROVER software package (ABX, GmbH), providing a semiautomated approach to delineate FDG-avid lesions and to estimate metabolically active volume (MAV) and mean standardized uptake value (SUV_{mean}) for individual lesions from PET datasets as previously described (22). For each tumor lesion, mean metabolic volumetric product (MVP_{mean}) was determined by multiplying metabolism by volume (i.e., $MVP_{mean} = SUV_{mean} \times MAV$). Total MVP_{mean} , a quantitative measure of global disease burden, was determined by summing MVP_{mean} of all lesions. Total hepatic lesion MVP_{mean} , a measure of hepatic disease burden, was determined by summing MVP_{mean} of hepatic lesions only. Image assessment also included measuring the maximum SUV (SUV_{max}) for each of five target lesions and determining the sum of SUV_{max} for all lesions.

Statistical analysis

Statistical analyses were conducted using Stata 10.0 for Windows (StataCorp). PFS was defined as time from start of study treatment to disease progression or patient death, whichever occurred first. OS was defined as time from start of study treatment to patient's death. PFS and OS were analyzed using Kaplan–Meier methods. The Spearman rank test was used to estimate correlations between changes in biomarkers relative to baseline and OS. For analysis of change from baseline of pharmacodynamic end points, a repeated measures ANOVA was conducted. All analyses

were conducted as per protocol on patients receiving at least one dose of CP-870,893.

Results

Patient characteristics

Twenty-two patients with advanced chemotherapy-naïve PDA were enrolled (Table 1). Ninety percent ($n = 20$) of patients had metastatic disease. One patient in the MTD expansion cohort received a single dose of gemcitabine and withdrew from study before receiving CP-870,893 due to acute cholangitis related to tumor progression. The remaining patients ($n = 21$) received gemcitabine (1,000 mg/m²) administered on days 1, 8, and 15 with infusion of CP-870,893 at either 0.1 or 0.2 mg/kg on day 3 of a 28-day cycle (Fig. 1).

Table 1. Baseline patient demographics and clinical characteristics

Characteristic	CP-870,893 Dose cohort		
	0.1 mg/kg (<i>n</i> = 3) <i>n</i> (%)	0.2 mg/kg (<i>n</i> = 6) <i>n</i> (%)	MTD expansion 0.2 mg/kg (<i>n</i> = 13) <i>n</i> (%)
Age, y			
Median	59		59
Range	57–72		40–81
Sex			
Male	2 (67)	3 (50)	9 (69)
Female	1 (33)	3 (50)	4 (31)
Race/ethnicity			
White	0 (0)	5 (83)	11 (84)
Black	3 (100)	1 (17)	1 (8)
Asian	0 (0)	0 (0)	1 (8)
ECOG PS			
0	2 (67)	5 (83)	2 (15)
1	1 (33)	1 (17)	11 (85)
Extent of disease			
Locally advanced	0 (0)	0 (0)	2 (15)
Metastatic	3 (100)	6 (100)	11 (85)
Location of primary			
Head	2 (67)	2 (33)	4 (31)
Body or tail	1 (33)	2 (33)	9 (69)
Not reported	0 (0)	2 (33)	0 (0)
Site of metastases			
Liver	3 (100)	5 (83)	10 (77)
Lung	0 (0)	2 (33)	0 (0)
Peritoneal	0 (0)	0 (0)	3 (23)
Skeleton	0 (0)	2 (33)	0 (0)
Other	0 (0)	0 (0)	1 (8)
Prior radiotherapies			
No	3 (100)	6 (100)	11 (84)
Yes	0 (0)	0 (0)	1 (8)
Not reported	0 (0)	0 (0)	1 (8)

Safety results, DLTs, and MTD

None of the 3 patients in cohort 1 (0.1 mg/kg) or 6 patients in cohort 2 (0.2 mg/kg) experienced DLT. Among 12 evaluable patients in the expansion cohort (0.2 mg/kg), 1 patient experienced DLT due to grade 4 cerebrovascular accident on day 3 of cycle 1 after receiving CP-870,893, and was withdrawn from study. As this adverse event occurred within several hours after receiving the first dose of CP-870,893, it was considered possibly related to treatment. Clinical evaluation of the patient showed paroxysmal atrial fibrillation on telemetry, and MRI brain imaging revealed evidence of bilateral frontal and parietal region gray-white matter junction lesions consistent with both acute and chronic embolic infarcts. Together, these findings suggested a chronic pathologic process that may have been provoked by treatment. MTD and RP2D for CP-870,893, when combined with 1,000 mg/m² gemcitabine in patients with PDA, were estimated at 0.2 mg/kg per clinical protocol.

Adverse events are summarized in Table 2. Cytokine release syndrome (CRS) was the most common adverse event; it occurred within minutes to several hours after CP-870,893 infusion, and most commonly manifested as chills and rigors. Rigors were managed with meperidine and routinely resolved within 1 hour. Prophylactic use of ibu-

profen (400 mg) before CP-870,893 infusion was provided to lower CRS grade and incidence. One patient treated in cohort 1 experienced a grade 3 CRS after CP-870,893 infusion during cycle 9. Symptoms resolved with diuretic therapy, and the patient continued on study without dose modification. However, after 10 cycles of therapy in the absence of disease progression, the patient was diagnosed with glomerulonephritis related to gemcitabine and was withdrawn from the study. A second patient developed grade 3 gastrointestinal bleed related to pancreatic tumor invasion of the stomach on day 24 of cycle 1, and was eventually withdrawn from study due to development of deep vein thrombosis during hospitalization.

Treatment exposure

The median number of cycles administered was 4 (range, 1–12). Gemcitabine dose was reduced by 25% for thrombocytopenia or neutropenia, and was required in 55% of patients with a median of one dose reduction (range, 0–5) per patient. No dose reductions in CP-870,893 were required. Discontinuation of therapy was due to progressive disease ($n = 14$; 66%), unacceptable toxicity without progressive disease ($n = 1$; 5%), patient discretion ($n = 4$; 19%), or adverse event ($n = 2$; 10%).

Table 2. Summary of the most commonly reported adverse events by grade

Adverse events	CP-870,893 dose cohort					
	0.1 mg/kg ($n = 3$)		0.2 mg/kg ($n = 6$)		MTD expansion 0.2 mg/kg ($n = 13$)	
	Grade 1 or 2 n (%)	Grade 3 or 4 n (%)	Grade 1 or 2 n (%)	Grade 3 or 4 n (%)	Grade 1 or 2 n (%)	Grade 3 or 4 n (%)
Clinical events						
CRS	2 (67)	1 (33)	6 (100)	0 (0)	11 (85)	0 (0)
Fatigue	3 (100)	0 (0)	5 (83)	0 (0)	11 (85)	0 (0)
Nausea	3 (100)	0 (0)	5 (83)	0 (0)	10 (77)	0 (0)
Vomiting	1 (33)	0 (0)	3 (50)	0 (0)	6 (46)	0 (0)
Pyrexia	1 (33)	0 (0)	1 (17)	0 (0)	6 (46)	0 (0)
Peripheral edema	3 (100)	0 (0)	2 (33)	0 (0)	3 (23)	0 (0)
Constipation	1 (33)	0 (0)	3 (50)	0 (0)	6 (46)	0 (0)
Cerebrovascular accident	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	1 (8)
Hematologic events						
Anemia	3 (100)	0 (0)	4 (67)	1 (17)	10 (77)	2 (15)
Lymphopenia	1 (33)	1 (33)	4 (67)	0 (0)	7 (54)	4 (31)
Neutropenia	2 (67)	0 (0)	1 (17)	3 (50)	3 (23)	2 (15)
Thrombocytopenia	2 (67)	0 (0)	3 (50)	0 (0)	3 (23)	0 (0)
Nonhematologic events						
ALT	2 (67)	0 (0)	4 (67)	0 (0)	8 (62)	0 (0)
AST	2 (67)	0 (0)	3 (50)	0 (0)	11 (85)	0 (0)
Alkaline Phosphatase	2 (67)	0 (0)	5 (83)	1 (17)	8 (62)	2 (15)
Total bilirubin	2 (67)	1 (33)	2 (33)	1 (17)	5 (38)	1 (8)
Hyperglycemia	2 (67)	1 (33)	2 (33)	4 (67)	11 (85)	1 (8)
Hypocalcemia	2 (67)	0 (0)	1 (17)	0 (0)	10 (77)	0 (0)
Hyponatremia	2 (67)	0 (0)	3 (50)	0 (0)	2 (15)	2 (15)

Immune modulation

Rapid decreases in absolute monocyte count (AMC) and absolute lymphocyte count (ALC) were observed in peripheral blood 1 day after gemcitabine administration (Supplementary Fig. S1A). Further decreases in AMC and ALC were observed after CP-870,893 infusion, with a nadir on day 4. Both AMC and ALC recovered by day 8 to near baseline levels. Decreases in ALC were associated with a rapid decline in absolute levels of CD19⁺ B lymphocytes seen within 2 hours of CP-870,893 infusion (Supplementary Fig. S1B). Cellular recovery of peripheral blood CD19⁺ B lymphocytes to near baseline levels was seen by day 8. A rapid increase in B-cell expression of costimulatory molecules CD86 and HLA-DR was also observed within 24 to 48 hours of CP-870,893 treatment, a pattern similar to findings previously reported for CP-870,893 when used as a single agent (17). In addition, inflammatory cytokines IL-6, -8, and -10 transiently increased in serum of patients within 24 hours after CP-870,893, but not gemcitabine, treatment (Supplementary Fig. S1C). These findings are consistent with preclinical data showing that CD40 agonists induce immune activation and trafficking of leukocytes to lymphoid organs (16). No relationship was found between peak cytokine levels and development or grade of CRS.

FDG-PET response

Ten patients in the MTD expansion cohort were evaluated with the European Organisation for Research and Treatment of Cancer (EORTC) criteria for partial metabolic response (PMR; >25% reduction in SUV_{max}) detected by FDG-PET/CT (23). Eight of 10 patients completed at least two cycles of therapy, and two achieved a PMR. To further quantify metabolic responses, we determined the percentage reduction in the sum of SUV_{max} for up to five predefined lesions (up to two per organ) with the most intense FDG uptake (24). On average, the sum of SUV_{max} was reduced by 29% in target lesions after two cycles of therapy, with 6 of 8 patients achieving >25% reduction.

Because preclinical data show the capacity of CD40 agonists to target the microenvironment of primary pancreatic tumors (16), we examined metabolic responses specifically within the primary lesion. After two cycles of therapy, an average reduction of 26.9% in SUV_{max} was observed in pancreatic lesions, and all patients showed some reduction. To quantify this metabolic response, changes in primary tumor MVP_{mean} were determined. A decrease in MVP_{mean} was observed in 6 of 8 patients after two cycles of therapy, with changes seen as early as day 15 (Fig. 2A and B). Further reductions in MVP_{mean} were also observed with subsequent cycles of therapy. However, after six cycles of therapy, 3 patients, despite achieving more than 50% reduction in MVP_{mean} of the primary lesion, were withdrawn from study due to progressive disease of metastatic lesions. This suggested heterogeneity of the tumor response to therapy, which prompted investigation of metastatic lesions.

The most common metastatic site in this study was the liver (Table 1). For this reason, we evaluated the impact

of therapy on metabolic responses of hepatic lesions only. Eight of 10 patients had hepatic lesions; 6 of these patients completed at least two cycles of therapy. Four patients achieved more than 25% reduction in total hepatic lesion MVP_{mean}; this metabolic response, however, was sometimes transient (Fig. 2C). Moreover, metabolic changes in hepatic lesions were found to be heterogeneous (Fig. 2D). For patient 10031016, a marked metabolic response was observed in both the primary and hepatic lesions (Fig. 2). After four cycles of therapy, we biopsied the responding hepatic lesion. We previously reported histologic analysis of this lesion, which showed an impressive immune infiltrate that was dominated by macrophages with an absence of viable tumor cells (16). Thus, a metabolic response correlated with a pathologic response for this patient. To understand the heterogeneity of metabolic responses observed with treatment, we quantified responses of all identifiable lesions after two cycles of therapy (Fig. 3A). This analysis revealed that metabolic responses within individual patients varied considerably from complete metabolic response (CMR) to progression, sometimes with development of new lesions. This treatment response heterogeneity was particularly pronounced in the liver (Fig. 3B). Taken together, these data show that FDG-PET/CT-based treatment responses were heterogeneous in many patients.

Efficacy, pharmacodynamic and immunologic correlative results

Survival. Median PFS was 5.2 months [95% confidence interval (CI), 1.9–7.4 months]; median OS was 8.4 months (95% CI, 5.3–11.8 months); and 1-year OS was 28.6% (Fig. 4A).

Response rate. Overall response rate (ORR) based on RECIST 1.0 was 19%, with 4 patients achieving a partial response (PR). Stable disease was seen in 11 patients. In addition, 1 patient withdrawn after one dose of CP-870,893 due to DLT restarted gemcitabine alone and achieved a PR off-study, as previously reported (16).

Correlative studies. Exploratory analyses were conducted to correlate changes in immune activation, tumor biomarkers, tumor response, and metabolic response with OS. Treatment-related increases in cytokine levels (i.e., IL-6, -8, and -10) during cycle 1 were not associated with OS (Supplementary Table S1). CA19-9 levels were available for 12 patients, with 9 patients showing decreases after cycle 1 that correlated with improved OS (Fig. 4B). Response rate determined by RECIST 1.0 after cycle 2 was also associated with improved OS (Fig. 4C). In contrast, metabolic responses using EORTC criteria or by evaluating SUV_{max} for up to five predefined lesions did not correlate with OS (Supplementary Table S1).

Because metabolic responses to therapy were heterogeneous, we examined the association between OS and changes in metabolic activity observed within the liver and pancreas separately. Changes in total MVP_{mean} as well as SUV_{max} and MVP_{mean} of primary pancreatic lesions were not associated with OS. In contrast, a decrease in total

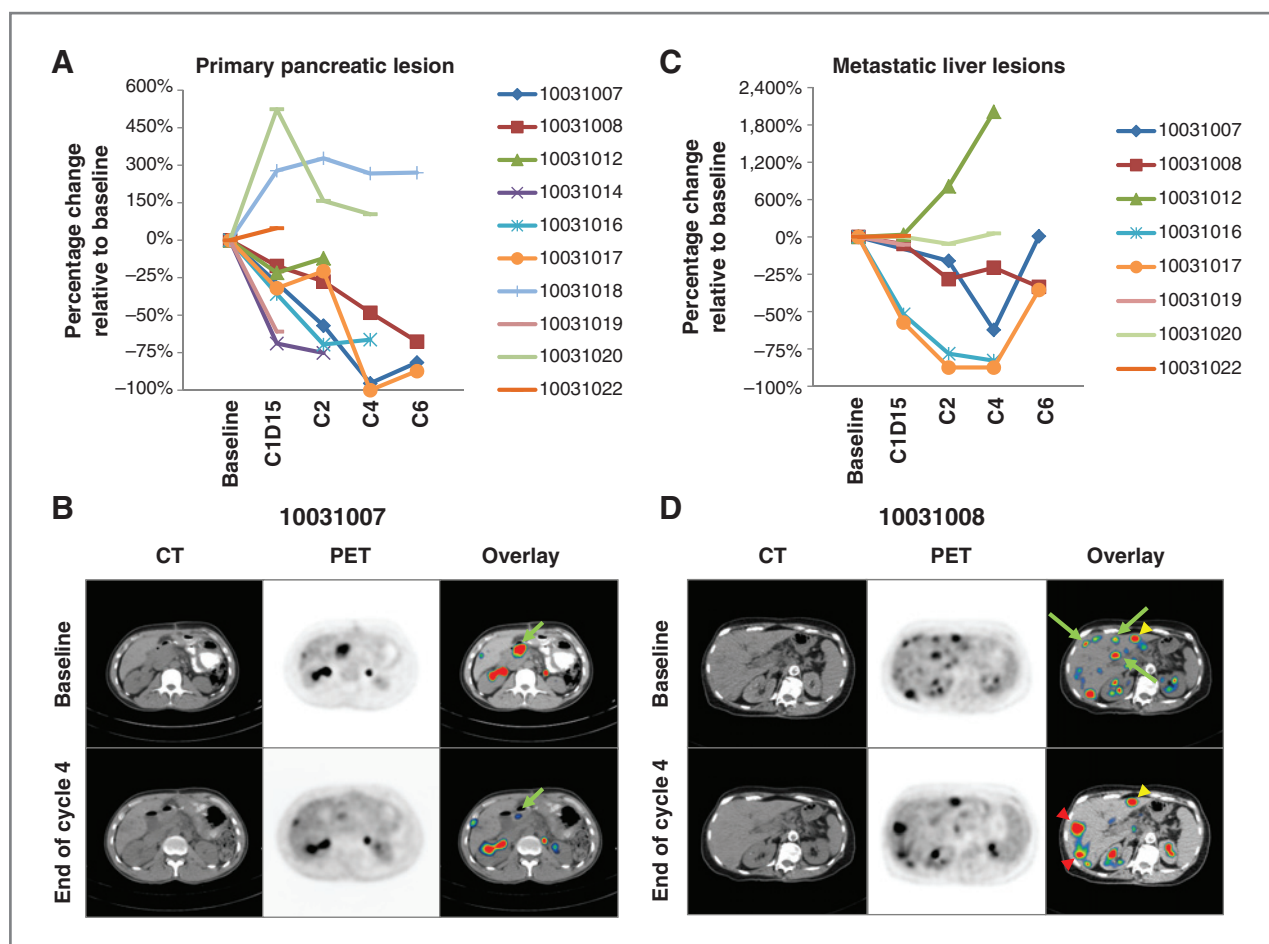


Figure 2. FDG-PET/CT imaging of (A and B) primary pancreatic tumors and (C and D) metastatic hepatic lesions. A and C, change relative to baseline in MVP_{mean} as a measure of metabolic activity. B and D, CT, FDG-PET, and fused PET/CT images (baseline vs. end of cycle 4) showing (B), a responding primary pancreatic lesion (green arrow) and (D) a mixed response in liver with representative new or progressive lesions (red arrowheads), stable lesions (yellow arrowheads), and responding lesions (green arrows). Normal FDG excretion in kidneys and ureters is also seen.

hepatic lesion MVP_{mean} after cycle 2 was associated with improved OS (Supplementary Table S1). Furthermore, analysis of 7 patients with hepatic lesions who underwent FDG-PET/CT after 2 weeks of therapy revealed a decrease in total hepatic lesion MVP_{mean} at this early time point that was also strongly correlated with OS (Fig. 4D). Patients with a metabolic response in the liver at 2 weeks also achieved a PR by RECIST 1.0.

Discussion

This investigation evaluated the safety and biologic impact of combining a fully human agonist CD40 mAb (CP-870,893) with gemcitabine for treatment of patients with advanced PDA. Combination treatment with CP-870,893 and full-dose gemcitabine was well-tolerated and associated with objective tumor responses (19%) in patients with advanced PDA. In addition, FDG-PET/CT imaging revealed therapy-induced metabolic responses in both primary and metastatic lesions. These responses, however, were heterogeneous, with some lesions responding

and others progressing during therapy. All patients receiving CP-870,893 in combination with gemcitabine responded with immune activation (i.e., increases in serum cytokines and/or upregulation of costimulatory molecules on B lymphocytes) that was transient. However, we determined that changes in serum cytokines and peripheral lymphocyte cell populations did not correlate as biomarkers of tumor response or survival parameters. In contrast, in an exploratory analysis we found that improved OS correlated with a decrease in FDG uptake in hepatic lesions.

Strategies that target the stromal microenvironment may improve outcomes for patients with PDA. Preclinical studies have suggested that the mechanism of action of CP-870,893 when used in PDA is dependent on peripheral blood monocytes which are induced to rapidly leave circulation, infiltrate tumor lesions, and orchestrate degradation of the stromal microenvironment (16). Consistent with this, we observed a rapid decrease in monocyte levels in patients after CP-870,893 infusion; however, because

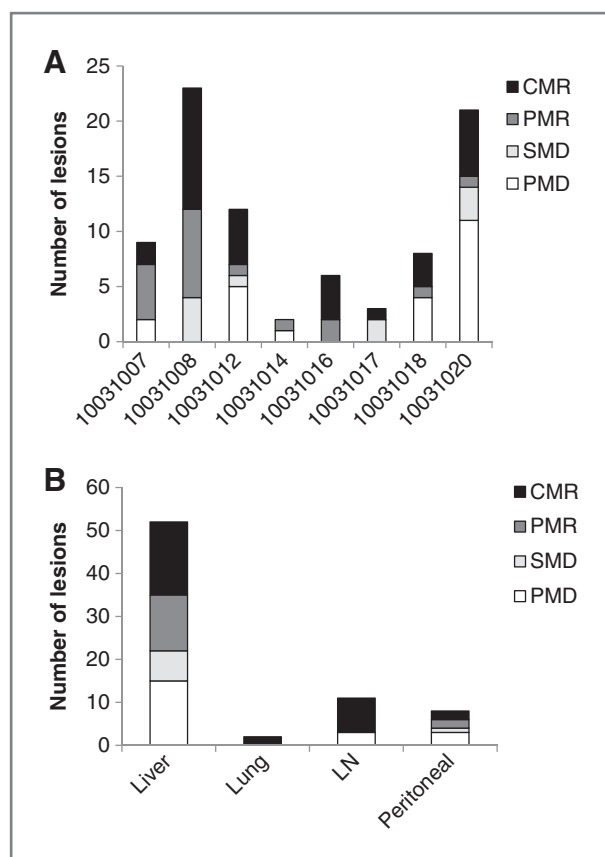


Figure 3. The response of individual lesions measured by changes in lesional MVP_{mean} from baseline to end of cycle 2, shown for: A, each patient, and B, for each metastatic site (all patient lesions pooled) including liver, lung, lymph node (LN), and peritoneal cavity. Changes in MVP_{mean} were classified as CMR indicating disappearance of lesion; PMR indicating $\geq 25\%$ reduction in MVP_{mean} ; progressive metabolic disease (PMD) indicating $>25\%$ increase in MVP_{mean} , or new lesion; and stable metabolic disease (SMD) indicating insufficient decrease to qualify for PMR and insufficient increase to qualify for PMD.

gemcitabine also produced a decrease in peripheral blood monocytes, we speculate that the full antitumor activity of an agonist CD40 antibody in patients may have been hindered in the current study by myelosuppression from repeated administration of gemcitabine. In this regard, because agonist CD40 antibodies can rapidly deplete the stroma that is a critical barrier to drug delivery, we propose that chemotherapy administration after, rather than before, the infusion of CD40 agonists would represent a novel approach to enhance efficacy while eliminating potential deleterious effects of chemotherapy on CP-870,893 activity. Moreover, this treatment schedule would permit the use of functional imaging approaches such as FDG-PET/CT conducted 1 day after administration of CP-870,893 to investigate the impact of delivering an agonist CD40 antibody on the biology of primary and metastatic tumor lesions. To understand the impact of therapy on tumor-associated stroma, tissue biopsies could also be conducted before and after delivery of CP-870,893 or, alternatively, CP-870,893 could be administered to patients with resectable PDA in the

neoadjuvant setting before surgical resection. It is expected that these study approaches would provide further insight into the biologic impact of CP-870,893 on PDA tumor biology.

In this phase I study, we administered CP-870,893 2 days after gemcitabine chemotherapy based on preclinical data showing the effectiveness of this treatment schedule in producing robust T-cell dependent antitumor immunity (18). Treatment with CP-870,893 was administered monthly to allow sufficient time for resolution of immune activation and to coincide with the schedule of gemcitabine chemotherapy. In addition, this timeframe was selected on the basis of previous investigation showing deleterious effects on T cells when CP-870,893 was administered more frequently (i.e., weekly dosing) to patients with advanced cancer (20). However, in our studies of this treatment schedule, we have found that antitumor T-cell immunity is not induced when a CD40 agonist is administered 2 days after gemcitabine chemotherapy in a clinically relevant mouse model of PDA (16). This discrepancy between our findings and previously reported work may be related to immune suppression imposed by PDA, which may inhibit the development of productive antitumor T-cell immunity. Consistent with our preclinical findings, tumor biopsies from patients with PDA treated in this phase I study revealed an absence of tumor-infiltrating lymphocytes and an abundance of macrophages, which we have previously reported are required for the antitumor effects observed with CD40 agonists (16). Although lymphocytes do not seem to be involved in antitumor immunity induced with CD40 agonists in PDA, we speculate that chronic immune activation reported with weekly dosing of CP-870,893 could be detrimental as inflammation can also be a key driver of tumorigenesis. Nonetheless, shorter dosing intervals that allow for sufficient time for resolution of immune activation (e.g., every 3 weeks) could be explored in subsequent clinical studies. Moreover, future combinations of CP-870,893 with chemotherapy should include more efficacious chemotherapeutic regimens such as FOLFIRINOX and gemcitabine/nab-paclitaxel (5, 25).

To understand the impact of therapy on tumor biology, we incorporated FDG-PET/CT imaging into this study. FDG-PET/CT has been used to assess responses to chemotherapies and targeted therapies in various solid malignancies including PDA (5, 26, 27). However, its use in predicting responsiveness to immunotherapy in solid malignancies has not been well explored. SUV_{max} is the most common semiquantitative approach applied to PET image analysis; however, SUV_{max} only reflects a small fraction of the metabolic activity in any given lesion. For this reason, we calculated MVP_{mean} of individual lesions as an overall measure of metabolic activity (22) and showed the ability of FDG-PET/CT, obtained as early as 2 weeks after therapy, to detect metabolic responses in primary and metastatic lesions. In our investigation for imaging biomarkers, we found a lack of statistically

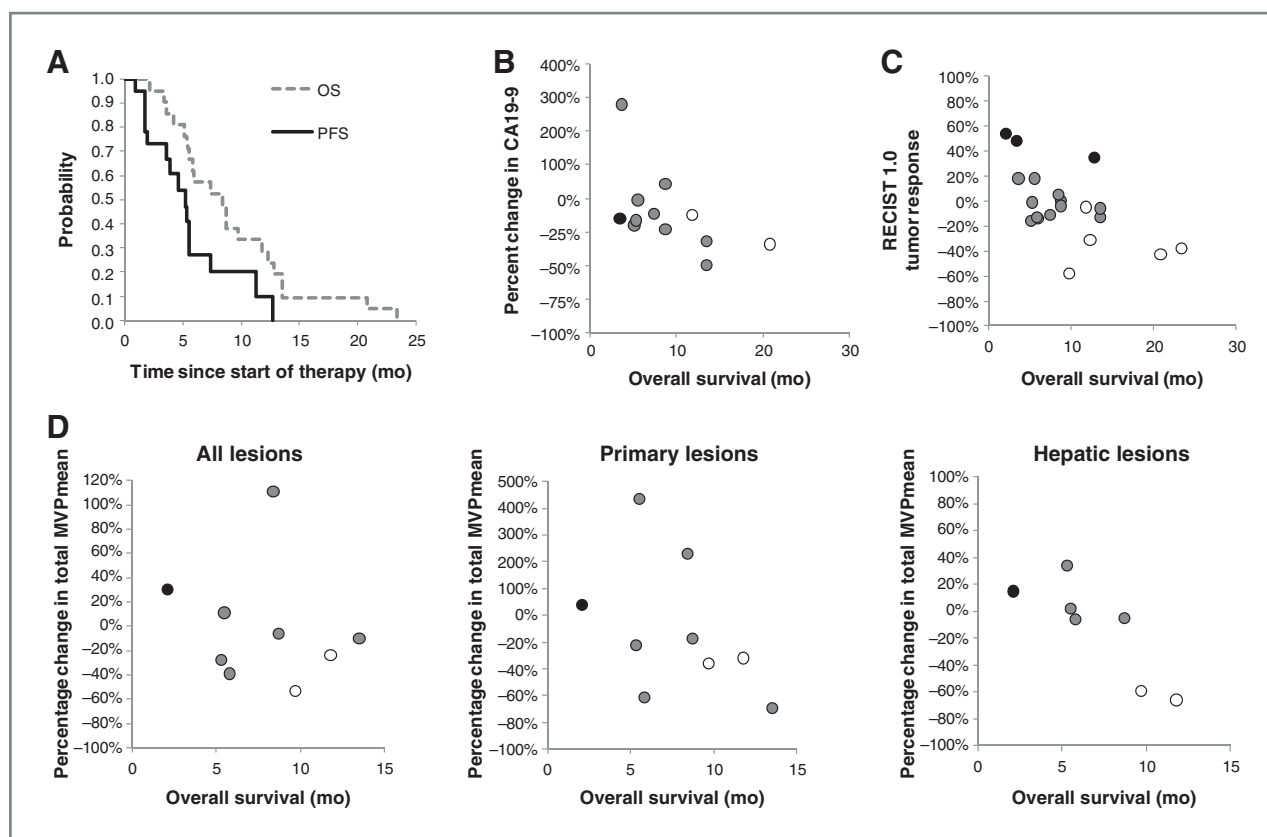


Figure 4. Biologic correlates for OS after treatment with CP-870,893 in combination with gemcitabine. A, median OS and PFS for patients ($n = 21$) receiving at least one dose of CP-870,893. Relationship between (B) percentage change in CA19-9 at end of one cycle of therapy compared with baseline ($R = -0.579$; $P = 0.049$), (C) OS and tumor response determined by RECIST 1.0 at completion of two cycles of therapy ($R = -0.652$; $P = 0.002$), and (D) percentage change in total MVP_{mean} for all lesions, primary pancreatic lesion, and all hepatic lesions after 2 weeks of therapy compared with baseline ($R = -0.929$; $P = 0.007$). Best overall tumor response measured by RECIST 1.0 is indicated in (B), (C), and (D) using open circles (PR), gray circles (stable disease), and black circles (progressive disease).

significant associations between OS and changes in many FDG-PET imaging correlates, including EORTC criteria. This finding may reflect the small sample size of this study. However, consistent with the heterogeneity in metabolic responses that we observed between primary and metastatic lesions, we found that changes in hepatic lesion MVP_{mean} determined by FDG-PET/CT strongly correlated with OS. Although decreases in primary lesion MVP_{mean} were not statistically significant, a trend toward improved OS was observed and may have been appreciated with a larger sample size.

Metabolic responses were commonly seen within primary lesions with each cycle of therapy. However, the metabolic response of hepatic lesions was heterogeneous with some lesions showing no response to therapy, even in patients where other lesions responded with complete loss of metabolic activity. This disparity in response observed between primary and metastatic lesions can be observed by FDG-PET imaging in some patients receiving standard chemotherapy alone (28–30). Here, we have used FDG-PET/CT imaging to show and quantify heterogeneity in metabolic responses observed with therapy in

PDA. Although the role of CP-870,893 in contributing to response heterogeneity seen between primary and metastatic lesions cannot be determined from our analyses, the disparate responses observed suggest differences in tumor biology such that metastasis biology may be a unique and major site of resistance for both chemotherapy- and immunotherapy-based approaches.

Although the results from this phase I study are encouraging, our sample size was small and, thus, we are unable to determine the clinical benefit achieved with the addition of CP-870,893 to gemcitabine chemotherapy. As a result, further investigation with randomized clinical studies will be necessary to determine a role for CP-870,893 in the treatment of PDA. Nonetheless, our study suggests the value of incorporating early FDG-PET/CT imaging to evaluate benefit from therapeutic approaches in PDA and for understanding heterogeneity in treatment responses.

Disclosure of Potential Conflicts of Interest

R.H. Vonderheide has other commercial research support and P. O'Dwyer has commercial research grant from Pfizer. No potential conflicts of interest were disclosed by the other authors.

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A Phase I Study of an Agonist CD40 Monoclonal Antibody (CP-870,893) in Combination with Gemcitabine in Patients with Advanced Pancreatic Ductal Adenocarcinoma

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