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A Phase I Study of Dinaciclib in Combination With MK-2206 in Patients With Advanced Pancreatic Cancer

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The combination of drugs targeting Ral and PI3K/AKT signaling has antitumor efficacy in preclinical models of pancreatic cancer. We combined dinaciclib (small molecule cyclin dependent kinase inhibitor with MK-2206 (Akt inhibitor) in patients with previously treated/metastatic pancreatic cancer. Patients were treated with dinaciclib (6–12 mg/m^2 i.v.) and MK-2206 (60–135 mg p.o.) weekly. Tumor biopsies were performed to measure pAKT, pERK, and Ki67 at baseline and after one completed cycle (dose level 2 and beyond). Thirty-nine patients participated in the study. The maximum tolerated doses were dinaciclib 9 mg/m^2 and MK-2206 135 mg. Treatment-related grade 3 and 4 toxicities included neutropenia, lymphopenia, anemia, hyperglycemia, hyponatremia, and leukopenia. No objectives responses were observed. Four patients (10%) had stable disease as their best response. At the recommended dose, median survival was 2.2 months. Survival rates at 6 and 12 months were 11% and 5%, respectively. There was a nonsignificant reduction in pAKT composite scores between pretreatment and post-treatment biopsies (mean 0.76 vs. 0.63; $P = 0.635$). The combination of dinaciclib and MK-2206 was a safe regimen in patients with metastatic pancreatic cancer, although without clinical benefit, possibly due to not attaining biologically effective doses. Given the strong preclinical evidence of Ral and AKT inhibition, further studies with better tolerated agents should be considered.

Study Highlights

WHAT IS THE CURRENT KNOWLEDGE ON THE TOPIC?
☑ Ras signaling and the PI3K/AKT pathway are known to be commonly aberrant in pancreatic tumors. Preclinical studies showed that inhibiting these pathways with cyclin dependent kinase (CDK) and AKT inhibitors reduce cell proliferation and apoptosis.

WHAT QUESTION DID THIS STUDY ADDRESS?
☑ This study combined dinaciclib (CDK inhibitor) and MK-2206 (Akt inhibitor) together in a phase I study for patients with previously treated advanced pancreatic cancer.

WHAT DOES THIS STUDY ADD TO OUR KNOWLEDGE?
☑ This study showed that combining these agents can be done safely but that it was not possible to achieve biologically effective doses.

HOW MIGHT THIS CHANGE CLINICAL PHARMACOLOGY OR TRANSLATIONAL SCIENCE?
☑ Due to the clear preclinical rationale of combined CDK and Akt inhibition, targeting these pathways is still worth pursuing. However, alternative agents or combinations are required in future studies.

Pancreatic ductal adenocarcinoma (PDAC) continues to be a leading cause of cancer death in the United States, and is predicted to be the third most common cause of cancer death by the end of the next decade.[1] Although combination chemotherapy has provided modest benefit, the survival rates for metastatic pancreatic cancer remain dismal with median survivals still under 1 year in the metastatic setting.[2,3] With the improved understanding of the underlying molecular abnormalities in PDAC through broad scale tumor sequencing initiatives, such as The Cancer Genome Atlas, combination molecularly targeted therapy can now be used rationally to target specific driver signaling pathways in this deadly cancer.[4,5]

Mutations affecting the RAS pathway occur in > 90% of PDAC.[6] In addition, RAS signaling can be activated through dysregulation of upstream receptor tyrosine kinases. Despite being an obviously attractive target, RAS inhibition has proved to be difficult to inhibit

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pharmacologically.[7] RAS signaling results in the activation of three major downstream pathways: the RAF/MEK/ERK pathway, the PI3K/AKT pathway, and the Ral pathway.[8] These signaling pathways also display cross-talk resulting in positive and negative regulation of each other's signaling processes.[9]

The PI3K/AKT pathway is involved in the regulation of cell apoptosis and proliferation and dysregulations of this pathway are common in pancreatic cancer.[10] High levels of expression of phosphorylated AKT are associated with poor survival in patients with pancreatic cancer.[11] Inhibition of this pathway results in suppressed pancreatic cancer growth with associated increase in apoptotic markers in preclinical models.[12,13] The cyclin-dependent kinases (CDKs) are serine-threonine kinases that control cell cycle regulation and contribute to increased transcriptional regulation in cancer.[14,15] Specifically in PDAC, inactivating mutations of CDKN2A are found in over 80% of cases.[16] Moreover, CDK5 is an activator of Ral signaling, and inhibition of this protein results in inhibition of the RAS/Ral signaling cascade. Preclinical studies showed that the combined inhibition of CDK and PI3K/AKT pathways resulted in synergistic effects on the inhibition of proliferation and inducing apoptosis.[17] The combination regimen resulted in greater inhibition of p-Akt and p-Rb expression in tissues from patient-derived orthotopic and subcutaneous models.

Dinaciclib is a potent small molecule CDK inhibitor that has the greatest activity against CDK1, CDK2, CDK5, and CDK9. It inhibits DNA synthesis, reduces Rb protein phosphorylation, and has preclinical activity in multiple tumor models, including pancreatic cancer.[18] The recommended phase II dose is 12 mg/m^2.[19] MK-2206 is an allosteric inhibitor of AKT, which demonstrates inhibition of AKT and antiproliferative activity as a single agent and in combination with cytotoxic agents in preclinical models,[20–22] including primary patient xenografts of PDAC.[18] The recommended phase II dose of MK-2206 is 200 mg once weekly.[23]

Based on these preclinical data, we hypothesized that the combination of dinaciclib (CDK inhibitor) and MK-2206 (Akt inhibitor) would be tolerable and have clinical activity in patients with previously treated, unresectable/metastatic pancreatic cancer.

METHODS

Study design

This study was approved by the National Cancer Institute Cancer Therapy Evaluation Program (NCI CTEP) and run through the NCI-sponsored phase I clinical trials network. The primary objective of this multi-institutional phase I study was to determine the safety, tolerability, and maximum tolerated dose (MTD) of the combination of MK-2206 and dinaciclib in patients with advanced pancreatic cancer. Secondary objectives included assessment of the antitumor efficacy and characterizing the pharmacokinetic (PK) profile of the combination of MK-2206 and dinaciclib. Pharmacodynamic effects on the Ras/MEK/ERK, PI3K/Akt, and the Ral/CDK pathways were included as exploratory objectives.

Eligibility criteria

Eligible patients had histologically confirmed unresectable or metastatic pancreatic cancer. Patients had adequate organ function, Eastern Cooperative Oncology Group (ECOG) performance status ≤ 1, and at least one lesion amenable to biopsy. Exclusion criteria included receiving any medications/substances that were strong inhibitors or inducers, sensitive substrates, or substrates with a narrow therapeutic index of CYP3A4 or P-glycoprotein. The protocol was approved by the institutional review boards of all participating institutions, and written informed consent was obtained from all patients before performing study-related procedures in accordance with federal and institutional guidelines.

Drug administration and dose escalation procedures

Patients received MK-2206 orally once weekly and dinaciclib intravenously (infused over 2 hours) once weekly for 3 weeks over 28-day cycles. MK-2206 and dinaciclib were escalated sequentially in the dose escalation cohort (**Table 1**). Dose levels enrolled 3 patients per cohort with escalation performed according to a standard 3 + 3 design.[24] Once the MTD was determined, the dose level was expanded and patients were randomized to either arm A: dinaciclib given first for 1 week followed by the combination of dinaciclib/MK-2206, or arm B: MK-2206 given first for 1 week followed by the combination.

Dose limiting toxicities (DLTs) were defined as any grade 4 toxicity, any grade 3 toxicity with the exception of nausea, vomiting, or diarrhea that improved to grade ≤ 2 within 3 days of receiving maximal medical support, and any grade 3-electrolyte abnormality that did not correct to grade ≤ 2 within 48 hours. Asymptomatic lymphopenia of any grade was not regarded as a DLT.

Clinical evaluation and safety assessment

Patients underwent a history and physical examination, vital signs, performance status assessment, echocardiogram and blood work at baseline. Baseline radiographic evaluation was performed with computed tomography with contrast) within 28 days of starting treatment. While receiving study treatment, patients were evaluated weekly with brief history and physical examinations, evaluation of any adverse events, vital signs, complete blood count, and serum chemistry. Adverse events assessment was performed weekly and graded according to the NCI Common Terminology Criteria of Adverse Events, version 4.0. Disease response was assessed every 8 weeks by computed tomography imaging using Response Evaluation Criteria in Solid Tumors version 1.1.

Correlative methods

Tumor biopsies were performed in patients who received dose level 2 and beyond, at baseline and at the beginning of the second cycle. The effects of dinaciclib and MK-2206 on the expression of pAKT, pERK, and Ki-67 were compared on pretreatment and post-treatment biopsy samples using immunohistochemistry. Expression was scored quantitatively by determining percentage staining of Ki-67 and pERK. For pAKT, stain intensity was scored 0–3 and normalized by the percentage area involvement resulting in a final composite score (0–3).

Table 1 Dose levels of dinaciclib and MK-2206

Dose level	Dinaciclib i.v. (weekly dose × 3)	MK-2206 p.o. (weekly dose continuous)	Number of participants (all arms)
1	6 mg/m^2	60 mg	3
2	9 mg/m^2	90 mg	4
2.5	9 mg/m^2	135 mg	24
3	12 mg/m^2	90 mg	8

One cycle is 28 days of treatment.

PK analysis

Dinaciclib and MK-2206 PK analyses were performed in patients enrolled in the dose escalation cohort at dose levels 2.5 and 3 and in all patients enrolled to the dose expansion cohort. For patients in the escalation cohort, a full dinaciclib PK profile was obtained from immediately prior to the end of infusion on cycle 1 day 1 through 8 hours. A full MK-2206 PK profile was obtained from first dose on cycle 1 day 1 through 96 hours, and trough levels were collected on cycle 1 day 8 and cycle 1 day 15. For the expansion cohort, PK was collected when both drugs were administered alone (cycle 1) or in combination (cycle 2) with the same time points being collected. However, in arm A, only dinaciclib samples were collected during cycle 1, and, in arm B, only MK-2206 samples were collected during cycle 1.

Plasma levels of total dinaciclib and MK-2206 were determined using validated liquid-chromatography tandem mass spectrometry methods (**Supplementary Methods**).

Dinaciclib and MK-2206 concentrations were analyzed using Phoenix WinNonlin version 7.0 (Certara LP, Princeton, NJ) and by using standard noncompartmental PK methods.[25] See **Supplementary Methods** for further details.

Statistical analysis

Adverse events were summarized using descriptive statistics. The event time distribution for overall survival (OS) was estimated with the method of Kaplan and Meier.[26] The proportion of patients with clinical responses are reported with exact 95% binomial confidence intervals. The median ages of each cohort were compared using the Wilcoxon test for continuous variables.

For correlative data, comparisons in immunohistochemical percentages were analyzed with paired *t*-tests. For PK, values were summarized using descriptive statistics. The Kruskal-Wallis analysis of variance or Mann–Whitney *U* test were used to compare medians across groups with respect to drug exposure, response, and toxicity when samples were independent. The Wilcoxon signed-rank test was used were samples were paired. All reported *P* values are two-sided with the significance level set at 0.05. Statistical analyses were done using JMP statistical discovery software version 7 (SAS Institute, Cary, NC) and GraphPad Prism version 7.02.

RESULTS

Patients and treatment

Forty patients were enrolled in the study between March 12, 2013, and June 14, 2016. One patient withdrew consent; this patient did not receive any study drug. Of the 39 patients who received study drugs, the median age was 63 years; the median age in expansion cohort B (68 years) was not statistically significantly different than expansion cohort A (58 years). Patients were heavily pretreated for a PDAC cohort with a median number of three prior therapies (range 1–7). The baseline characteristics and demographics are further summarized in **Table 2**.

Dose escalation phase

Twenty-one patients were evaluable for toxicity. There were 3, 4, 7, and 7 patients treated at dose levels 1 (dinaciclib 6 mg weekly and MK-2206 90 mg weekly), 2 (dinaciclib 9 mg weekly and MK-2206 90 mg weekly), 2.5 (dinaciclib 9 mg weekly and MK-2206 135 mg weekly), and 3 (dinaciclib 12 mg weekly and MK-2206 90 mg weekly), respectively. There was one protocol-defined DLT in the dose escalation. One patient in dose level 3 had grade 3 neutropenia lasting > 7 days. One additional patient was assigned to each of dose levels 2 and 3 due to one patient in each level developing symptomatic clinical progression where they each received < 1 month of treatment. In dose level 3, 4 of 6 evaluable patients had dose holds on or before cycle 2 day 1. The study team, in consultation with CTEP sponsors, therefore, amended the protocol to enroll dose level 2.5 of dinaciclib 9 mg weekly and MK-2206 135 mg weekly (not specified in the original protocol) in an attempt to define a more tolerable dose level.

Four of 7 patients on dose level 2.5 (dose escalation) were able to receive all planned doses of study therapy in cycle 1, with 1 patient at this level who only received 1 dose due to leukopenia. Accordingly, the dose level chosen for the expansion cohorts was level 2.5 (dinaciclib 9 mg weekly and MK-2206 135 mg weekly). Of note, 1 patient in dose level 2.5 (dose escalation) died due to a serious adverse event.

Safety

Thirty-nine patients total were evaluable for toxicity (**Table 3**). The most common treatment-related adverse events (all grades, all cycles) experienced by patients in all dose levels were nausea (63%), fatigue (61%), vomiting (50%), lymphopenia (45%), diarrhea (45%), and leukopenia (42%). Grade 3 or 4 adverse events included neutropenia (29%), lymphopenia (21%), anemia (18%), hyperglycemia (16%), hyponatremia (16%), and leukopenia (13%). There was one DLT due to grade 3 neutropenia lasting beyond 7 days (dose level 3). One patient (dose level 2.5) died from a serious adverse event having completed one cycle. This patient developed hemoptysis resulting in cardiopulmonary arrest (no thrombocytopenia at that time). Subsequent bronchoscopic examination revealed extensive tumor necrosis without mucosal bleeding not attributed to study agents and the patient subsequently died due to respiratory failure.

Six patients (15%) required dose modifications (2 patients at dose level 2, 3 patients at dose level 2.5, and 1 patient at dose level 3). The reasons for dose modification did not meet the definition of DLT and doses were modified according to a dose reduction table, which was prespecified in the trial protocol.

Clinical activity

Thirty-nine patients were considered evaluable for disease response measurement (completed at least one cycle of

Table 2 Baseline characteristics of patients

Characteristics	Dose escalation (*n* = 21)	A: Dinaciclib-combination (*n* = 11)	B: MK-2206-combination (*n* = 7)	All arms (*n* = 39)
Dose level				
1	3 (14%)	0	0	3 (8%)
2	4 (19%)	0	0	4 (10%)
2.5	7 (33%)	11 (100%)	7 (100%)	25 (64%)
3	7 (33%)	0	0	7 (18%)
Age (median)	64 (51, 70)[a]	58 (58, 63.5)	68 (64, 69.5)	63 (55, 69)
Sex				
Male	13 (62%)	7 (64%)	4 (57%)	24 (61%)
Female	8 (38%)	4 (36%)	3 (43%)	15 (39%)
Race				
Asian	4 (19%)	2 (18%)	0	6 (15%)
African American	1 (5%)	3 (27%)	1 (14%)	5 (13%)
White	16 (76%)	6 (55%)	6 (86%)	28 (72%)
ECOG				
0	8 (38%)	2 (18%)	2 (29%)	12 (31%)
1	13 (62%)	9 (82%)	5 (71%)	27 (69%)
Prior treatment (median)	3 (3, 4)	3 (2.5, 3.5)	3 (3, 4)	3 (3, 4)
Prior RT (yes)	14 (67%)	2 (18%)	1 (14%)	17 (44%)
Prior resection (yes)	11 (52%)	3 (27%)	3 (43%)	16 (41%)
Prior adjuvant chemotherapy	5 (24%)	2 (18%)	1 (14%)	8 (21%)
Prior 5-FU	13 (62%)	6 (55%)	3 (43%)	22 (56%)
Prior gemcitabine	17 (81%)	10 (91%)	7 (100%)	34 (87%)

[a]Represents median (lower quartile, upper quartile).

treatment). Twenty-five of these patients were evaluable for response by Response Evaluation Criteria in Solid Tumors version 1.1. No objective responses were observed. Four patients (10%) had stable disease as their best response (**Table 4**). There were no significant differences between the expansion arms.

Combining dose escalation and expansion cohort patients treated at the recommended dose (dose level 2.5), there were 25 patients with a median OS of 2.2 months (**Figure 1**). Survival rates at 6, 10, and 12 months for this group were 11%, 5%, and 5%, respectively. Focusing on the expansion cohorts, median OS for the 18 patients treated on the expansion cohorts in this study was 2.9 months (**Figure 2**). Survival rates at 6, 10, and 12 months were 12%, 6%, and 6%, respectively. There were no differences in the survival rates between both expansion cohort arms.

Correlative results

Pretreatment biopsies were obtained in 36 patients (92%). Post-treatment biopsies were available for 12 subjects (31%). There was a nonsignificant reduction in pAKT composite scores between pretreatment and post-treatment biopsies (mean 0.76 vs. 0.63; $P = 0.635$; **Figure S1**). There were similar levels of Ki-67 index expression between pretreatment and post-treatment biopsies (median 27.5% vs. 30%; $P = 0.077$; **Figure S2a**). There were also similar levels of expression of total pERK between pretreatment and post-treatment biopsies (median 9% vs. 10%; $P = 0.139$; **Figure S2b**).

Pharmacokinetics

Dinaciclib and MK-2206 data were available for all 14 patients enrolled in the escalation cohort at dose levels 2.5 and 3, and for all 18 patients enrolled into the expansion cohort (**Table 5**). There was no statistical difference between dose-normalized exposure and cohorts or between single and multiple doses of dinaciclib and MK-2206. There was a statistically significant association between dinaciclib exposure (maximum concentration (C_{max})) and race, with African Americans and Asians having lower exposure than white patients ($P = 0.04$). There were no associations between exposure and other demographic variables.

Pharmacodynamics

There was a statistically significant association between MK-2206 total exposure (area under the curve to infinity (AUC_{inf})) and occurrence of vomiting during any cycle ($P = 0.04$). Otherwise, there were no significant associations between dinaciclib or MK-2206 exposure and any grade toxicities during cycle 1 or any cycle. There were no associations between dinaciclib or MK-2206 exposure and response.

DISCUSSION

Despite evidence of the survival benefit for first-line and second-line chemotherapy for patients with metastatic pancreatic cancer, there are limited therapeutic options for those with chemorefractory disease.[27–30] Given the high prevalence of RAS and CDK pathway mutations

Table 3 Adverse events at least possibly attributable to therapy (all cycles)

	Level 1 *n* (%) *N* = 3			Level 2 *n* (%) *N* = 4			Level 2.5 *n* (%) *N* = 24			Level 3 *n* (%) *N* = 8			All dose levels *N* = 39		
Event	**Grades 1/2**	**Grades 3/4**	**All grades**	**Grades 1/2**	**Grades 3/4**	**All grades**	**Grades 1/2**	**Grades 3/4**	**All grades**	**Grades 1/2**	**Grades 3/4**	**All grades**	**Grades 1/2**	**Grades 3/4**	**All grades**
Anemia	1 (33)	0	1 (33)	1 (25)	0	1 (25)	5 (21)	5 (21)	10 (42)	0	2 (33)	2 (33)	7 (18)	7 (18)	14 (37)
Lymphopenia	3 (100)	0	3 (100)	1 (25)	1 (25)	2 (50)	5 (21)	5 (21)	10 (42)	0	2 (33)	2 (33)	9 (24)	8 (21)	17 (45)
Neutropenia	0	0	0	0	1 (25)	1 (25)	2 (8)	7 (29)	9 (38)	0	3 (50)	3 (50)	2 (5)	11 (29)	13 (34)
Thrombocytopenia	1 (33)	0	1 (33)	2 (50)	0	2 (50)	8 (33)	0	8 (33)	1 (17)	0	1 (17)	12 (32)	0	12 (32)
Leukopenia	1 (33)	0	1 (33)	0	1 (25)	1 (25)	10 (42)	2 (8)	12 (50)	0	2 (33)	2 (33)	11 (29)	5 (13)	16 (42)
QTC elevation	0	0	0	0	0	0	1 (4)	0	1 (4)	1 (17)	0	1 (17)	2 (5)	0	2 (5)
Hypotension	0	0	0	0	0	0	2 (8)	0	2 (8)	0	0	0	2 (5)	0	2 (5)
Chills	0	0	0	0	0	0	1 (4)	0	1 (4)	0	0	0	1 (3)	0	1 (3)
Fatigue	0	0	0	3 (75)	0	3 (75)	15 (63)	2 (8)	17 (71)	3 (50)	0	3 (50)	21 (55)	2 (5)	23 (61)
Fever	0	0	0	0	0	0	3 (13)	0	3 (13)	1 (17)	0	1 (17)	4 (11)	0	4 (11)
Malaise	0	0	0	1 (25)	0	1 (25)	0	0	0	0	0	0	1 (3)	0	1 (3)
Weight loss	1 (33)	0	1 (33)	1 (25)	0	1 (25)	4 (17)	0	4 (17)	1 (17)	0	1 (17)	7 (18)	0	7 (18)
Alopecia	0	0	0	0	0	0	0	0	0	1 (17)	0	1 (17)	1 (3)	0	1 (3)
Cold sore	0	0	0	0	0	0	1 (4)	0	1 (4)	0	0	0	1 (3)	0	1 (3)
Dry skin	0	0	0	0	0	0	1 (4)	0	1 (4)	0	0	0	1 (3)	0	1 (3)
Erythema	0	0	0	0	0	0	0	0	0	1 (17)	0	1 (17)	1 (3)	0	1 (3)
Follicular rash	0	0	0	0	0	0	1 (4)	0	1 (4)	0	0	0	1 (3)	0	1 (3)
Maculopapular rash	0	0	0	0	0	0	2 (8)	1 (4)	3 (13)	0	0	0	2 (5)	1 (3)	3 (8)
Mucosal infection	0	0	0	0	0	0	1 (4)	0	1 (4)	0	0	0	1 (3)	0	1 (3)
Rash (acneiform)	0	0	0	0	0	0	0	1 (4)	1 (4)	0	0	0	0	1 (3)	1 (3)
Urticaria	0	0	0	0	0	0	1 (4)	0	1 (4)	0	0	0	1 (3)	0	1 (3)
Anorexia	2 (67)	0	2 (67)	0	0	0	7 (29)	0	7 (29)	1 (17)	0	1 (17)	10 (26)	0	10 (26)
Bloating	0	0	0	1 (25)	0	1 (25)	0	0	0	0	0	0	1 (3)	0	1 (3)
Blood bilirubin increased	0	0	0	0	0	0	1 (4)	2 (8)	3 (13)	0	0	0	1 (3)	2 (5)	3 (8)
Constipation	1 (33)	0	1 (33)	0	0	0	6 (25)	0	6 (25)	0	0	0	7 (18)	0	7 (18)
Dehydration	0	0	0	0	0	0	2 (8)	0	2 (8)	0	0	0	2 (5)	0	2 (5)
Diarrhea	2 (67)	0	2 (67)	1 (25)	0	1 (25)	8 (33)	2 (8)	10 (42)	4 (67)	0	4 (67)	15 (39)	2 (5)	17(45)
Dry mouth	0	0	0	0	0	0	1 (4)	0	1 (4)	0	0	0	1 (3)	0	1 (3)
Mucositis	1 (33)	0	1 (33)	0	0	0	1 (4)	0	1 (4)	2 (33)	0	2 (33)	4 (11)	0	4 (11)
Nausea	1 (33)	0	1 (33)	3 (75)	0	3 (75)	16 (67)	0	16 (67)	4 (67)	0	4 (67)	24 (63)	0	24 (63)
Pain (abdominal)	1 (33)	0	1 (33)	1 (25)	0	1 (25)	1 (4)	0	1 (4)	2 (33)	0	2 (33)	5 (13)	0	5 (13)
Taste alteration	0	0	0	1 (25)	0	1 (25)	0	0	0	0	0	0	1 (3)	0	1 (3)
Vomiting	1 (33)	0	1 (33)	2 (50)	1 (25)	3 (75)	10 (42)	0	10 (42)	5 (83)	0	5 (83)	18 (47)	1 (3)	19 (50)
Infection (site unknown)	0	0	0	0	0	0	0	1 (4)	1 (4)	0	0	0	0	1 (3)	1 (3)

(Continues)

Table 3 (Continued)

Event	Level 1 *n* (%) *N* = 3			Level 2 *n* (%) *N* = 4			Level 2.5 *n* (%) *N* = 24			Level 3 *n* (%) *N* = 8			All dose levels *N* = 39		
	Grades 1/2	Grades 3/4	All grades	Grades 1/2	Grades 3/4	All grades	Grades 1/2	Grades 3/4	All grades	Grades 1/2	Grades 3/4	All grades	Grades 1/2	Grades 3/4	All grades
Oral thrush	1 (33)	0	1 (33)	0	0	0	0	0	0	0	0	0	1 (3)	0	1 (3)
Sepsis	0	0	0	0	0	0	0	0	0	0	0	0	0	1 (3)	1 (3)
Alk phos increased	1 (33)	1 (33)	2 (67)	1 (25)	0	1 (25)	9 (38)	0	9 (38)	2 (33)	0	2 (33)	13 (34)	1 (3)	14 (37)
ALT increased	2 (67)	0	2 (67)	0	0	0	4 (17)	0	4 (17)	2 (33)	0	2 (33)	8 (21)	0	8 (21)
AST increased	2 (67)	0	2 (67)	0	0	0	5 (21)	0	5 (21)	1 (17)	0	1 (17)	8 (21)	0	8 (21)
Creatinine increased	0	0	0	0	0	0	2 (8)	0	2 (8)	0	0	0	2 (5)	0	2 (5)
Hyperglycemia	2 (67)	1 (33)	3 (100)	2 (50)	1 (25)	3 (75)	4 (17)	3 (13)	7 (29)	0	1 (17)	1 (17)	8 (21)	6 (16)	14 (37)
Hyperkalemia	0	0	0	0	0	0	0	2 (8)	2 (8)	0	0	0	0	2 (5)	2 (5)
Hyperphosphatemia	0	0	0	0	0	0	1 (4)	0	1 (4)	0	0	0	1 (3)	0	1 (3)
Hyperuricemia	0	0	0	0	0	0	2 (8)	0	2 (8)	0	0	0	2 (5)	0	2 (5)
Hypoalbumenia	1 (33)	0	1 (33)	1 (25)	0	1 (25)	6 (25)	0	6 (25)	1 (17)	0	1 (17)	9 (24)	0	9 (24)
Hypocalcemia	1 (33)	0	1 (33)	0	0	0	4 (17)	1 (4)	5 (21)	1 (17)	0	1 (17)	6 (16)	1 (3)	7 (18)
Hypoglycemia	1 (33)	0	1 (33)	0	0	0	0	0	0	0	0	0	1 (3)	0	1 (3)
Hypokalemia	2 (67)	0	2 (67)	0	0	0	4 (17)	1 (4)	5 (21)	0	0	0	7 (18)	1 (3)	8 (21)
Hyponatremia	1 (25)	0	1 (25)	0	0	0	4 (17)	5 (21)	9 (38)	0	1 (17)	1 (17)	5 (13)	6 (16)	11 (29)
Hypophosphatemia	1 (33)	0	1 (33)	1 (25)	1 (25)	2 (50)	7 (29)	1 (4)	8 (33)	2 (33)	0	2 (33)	11 (29)	2 (5)	13 (34)
Generalized muscle weakness	0	0	0	0	0	0	0	0	0	0	0	0	1 (3)	0	1 (3)
Muscle cramping	0	0	0	0	0	0	1 (4)	0	1 (4)	0	0	0	1 (3)	0	1 (3)
Muscle weakness lower limbs	0	0	0	0	0	0	1 (4)	0	1 (4)	0	0	0	1 (3)	0	1 (3)
Dizziness	0	0	0	0	0	0	2 (8)	0	2 (8)	0	0	0	2 (5)	0	2 (5)
Headache	0	0	0	0	0	0	1 (4)	0	1 (4)	0	0	0	1 (3)	0	1 (3)
Memory impairment	0	0	0	0	0	0	1 (4)	0	1 (4)	0	0	0	1 (3)	0	1 (3)
Peripheral sensory neuropathy	0	0	0	0	0	0	1 (4)	0	1 (4)	0	0	0	1 (3)	0	1 (3)
Pain (back)	1 (33)	0	1 (33)	0	0	0	0	0	0	0	0	0	1 (3)	0	1 (3)
Dyspnea	1 (33)	0	1 (33)	0	0	0	2 (8)	1 (4)	3 (13)	0	0	0	3 (8)	1 (3)	4 (11)
Pneumonitis (pneumonia)	1 (33)	0	1 (33)	0	0	0	0	0	0	0	0	0	1 (3)	0	1 (3)
Abdominal cramping	0	0	0	0	0	0	0	0	0	1 (17)	0	1 (17)	1 (3)	0	1 (3)
Acute kidney injury	0	0	0	0	0	0	1 (4)	0	1 (4)	0	0	0	1 (3)	0	1 (3)
Dysuria	0	0	0	0	0	0	1 (4)	0	1 (4)	1 (17)	0	1 (17)	2 (5)	0	2 (5)
Hematuria	0	0	0	0	0	0	1 (4)	0	1 (4)	0	0	0	1 (3)	0	1 (3)

Table 4 Summary of clinical activity in dose escalation and expansion cohorts

	N	Dose escalation *N* = 21	A: Dinaciclib/combination *N* = 11	B: MK-2206/combination *N* = 7
Best response	39			
Stable disease		3 (14%)	1 (9%)	0 (0%)
Progressive disease		15 (71%)	9 (81%)	7 (100%)
N/A		3 (14%)	1 (9%)	0 (0%)
RECIST sum[a]	25	161 (99, 182.5)	172 (112, 200)	219 (167.5, 275)
New lesion	39	8 (38%)	2 (18%)	0 (0%)
Dose reduction Dinaciclib	39	3 (14%)	2 (18%)	0 (0%)
Dose reduction MK-2206	39	1 (5%)	0 (0%)	1 (14%)
Dose limiting toxicity	39	1 (5%)	0 (0%)	0 (0%)

N/A, not available; RECIST, Response Evaluation Criteria in Solid Tumors.
[a]Values represent the median (lower quartile, upper quartile).

in pancreatic cancer, these are obvious therapeutic targets.[10,14,15] Based on preclinical studies showing synergistic effects of combined CDK5 and PI3K/Akt pathway inhibition,[17] we hypothesized that the combination of dinaciclib (CDK inhibitor) and MK-2206 (Akt inhibitor) would be tolerable and demonstrate clinical activity in patients with PDAC who had progressed on at least one line of systemic therapy.

The results from this clinical study highlight two significant challenges in the development of novel therapeutic strategies that focus on downstream KRAS signaling in PDAC. First, there are known drug-induced toxicity hurdles with AKT/PI3K inhibitors that thwart dose optimization of these classes of agents. Second, the rapidly progressive course of the disease that compromises our ability to translate even strong preclinical data into effective therapy. Both of these difficulties presented themselves during the dose-finding component of this study. In the dose-finding cohort of the study, 52.3% (*n* = 11) of patients did not receive at least 1 of 3 doses of study therapy, 24% (*n* = 5) due to drug-induced toxicity and 29% (*n* = 6) due to disease complications or progression. These realities interfered with the ability to test the hypothesis that inhibition of KRAS-activation through downstream signaling of Ral (using dinaciclib to inhibit CDK5 thus Ral) and AKT (MK-2206) had antitumor effects in this population. Post-treatment vs. pretreatment tumor biopsies showed no significant reduction in pAKT nor pERK at the doses tested. Unsurprisingly, with these pharmacodynamics measures, there were no objective responses, with only four patients (10%) who had stable disease. The lack of clinical responses could also potentially be explained by our heavily pretreated population (median 3 prior treatment regimens).

In a prior study of 100 patients with resected pancreatic cancer, pAkt expression levels (determined by immunohistochemistry) were only prognostic and predictive of lower rates of progression-free survival and OS in those determined to have high levels of pAkt expression.[31] Our cohort of patients had variable pAkt expression levels in the pretreatment biopsy specimens with median composite scores 0.625 (range 0–3). It is plausible that MK-2206 inhibition may

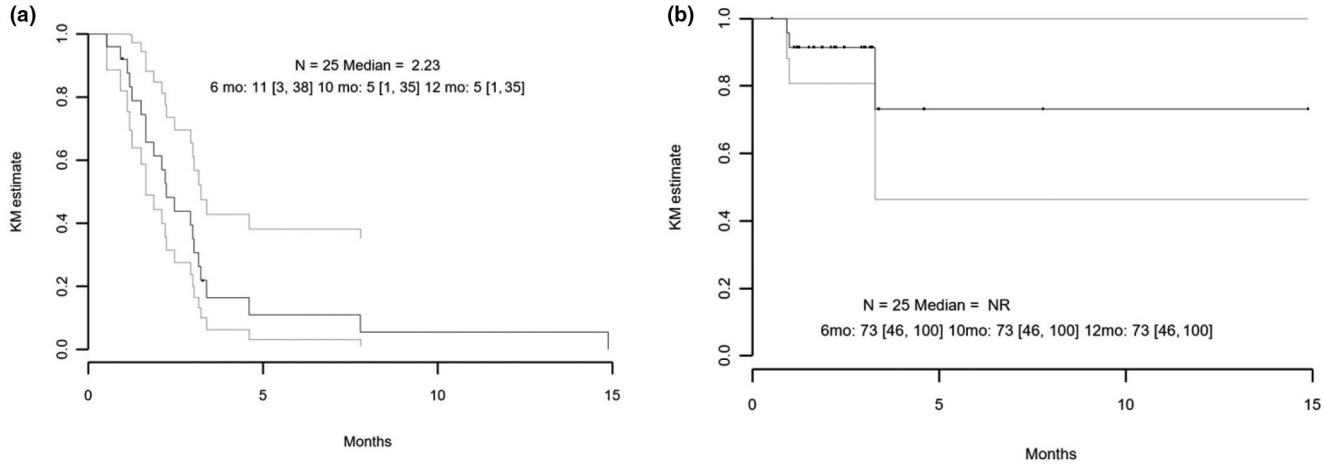

Figure 1 Survival outcomes of recommended phase II dose (dose level 2.5). (**a**) Combining patients from dose escalation and expansion cohorts at dose level 2.5, median overall survival was 2.2 months. (**a**) Shows 6, 10, and 12-month survival rates for this group (11%, 5%, and 5%, respectively). (**b**) Shows the censoring distribution where deaths are censored so that study follow-up is calculated. This shows that the follow-up for all patients treated at the recommended phase II dose was 73% completed for 14 months. The percentage censored for overall survival was 12%.

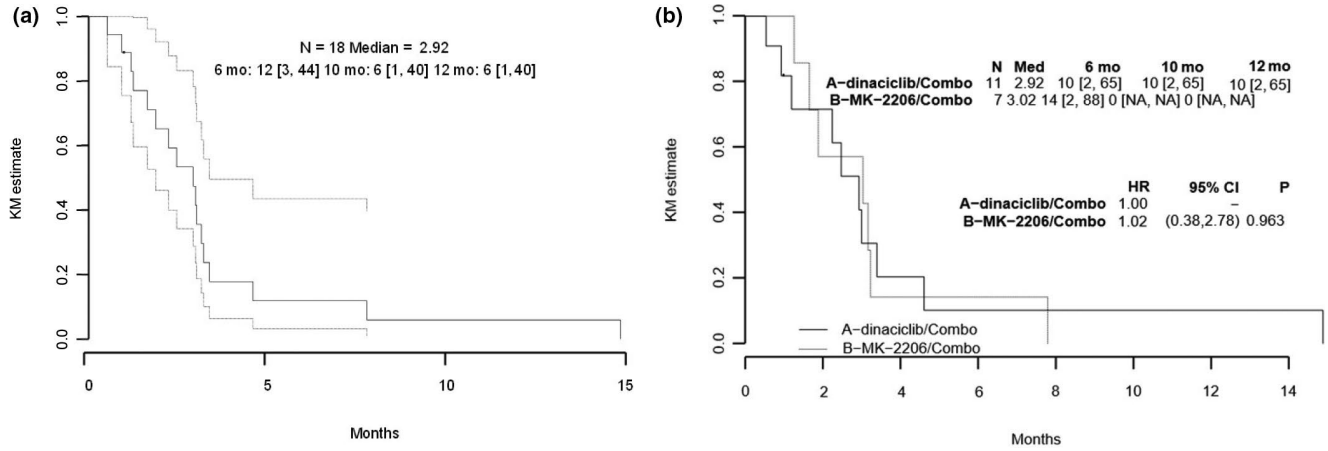

Figure 2 Overall survival of dose expansion cohorts. Patients in the two expansion cohorts were treated at the recommended dose of dinaciclib 9 mg/m^2 and MK-2206 135 mg weekly. (**a**) The median overall survival for the expansion cohorts is 2.9 months. Survival rates at 6, 10, and 12 months were 12%, 6%, and 6%, respectively. (**b**) Separate survival curves for each arm **a, b** with similar survival rates in both arms.

have greater inhibitory effects in a more selected population with high pAkt expression. Yap *et al*. demonstrated the pharmacodynamic effects of MK-2206 in a phase I study with significant reduction of pSer473 Akt expression in tumor biopsies at the lowest dose level tested (60 mg once weekly).[23] We were unable to demonstrate changes in cell proliferation (Ki67) or pERK expression levels but our analysis was limited due to small numbers of patients who had post-treatment biopsies.

Due to the high rate of missed doses in the highest dose level (dose level 3) during the dose escalation cohort (maximum dose dinaciclib 12 mg/m^2 and MK-2206 90 mg weekly), we chose dose level 2.5 for the expansion cohort (dinaciclib 9 mg/m^2 and MK-2206 90 mg weekly). Both dinaciclib and MK-2206 exposures were consistent with what has been reported in earlier studies.[19,23] Co-administration did not alter their exposures. There were no differences between dose-normalized exposure and cohorts, or between single and multiple doses of dinaciclib and MK-2206. There was a significant association between dinaciclib exposure (C_{max}) and race—African Americans and Asians had lower exposure than white patients. We hypothesize this could be due to differences in CYP3A4/5 variants as dinaciclib is a CYP3A4 substrate.[32,33] This has not been reported in earlier studies.

Overall, combined dinaciclib and MK-2206 was tolerated as expected. The majority of grade 3 and 4 treatment-related toxicities were largely related to myelosuppression. There was one death due to a serious adverse event. These data are consistent with single agent MK-2206 administration in a small phase II study of patients with advanced biliary tract cancers and a phase II study in patients with relapsed or refractory lymphoma.[34,35] Similarly, dinaciclib had similar toxicity profiles in phase I studies in patients with advanced malignancies.[19,36]

Other studies have been reported assessing the value of targeting downstream KRAS signaling. Phase I studies assessing AKT and MEK inhibition in multiple tumor types also had shown poor tolerability without increased efficacy.[37–39] A phase I study of PI3K inhibition with MEK inhibition showed no activity in patients with PDAC.[40] In the largest study, Hochster *et al.* reported on a trial of MK-2206 + MEK inhibitor selumetinib vs. oxaliplatin and 5-fluorouracil chemotherapy and found no benefit to the targeted therapy.[41] Multiple studies have assessed vertical signaling inhibition with inhibiting receptor tyrosine kinases with MEK inhibition with minimal benefit.[42–50] Our study is limited by its small size and the fact that it is not a randomized controlled study. Its purpose was to determine the MTD in a population of patients with pretreated pancreatic cancer. Our study involved a molecularly unselected population, which may explain the absence of objective responses. Preclinical studies in ovarian cancer suggest a predictive role of cyclin E1 (*CCNE1*) amplification, a cell cycle regulator, in sensitizing tumor cells to CDK and Akt inhibition.[51] *CCNE1* amplification has been proposed as a candidate driver gene for periampullary pancreatic cancer based on transcriptomic data in this population.[52] We were unable to assess CCNE1 amplification due to tissue constraints.

In conclusion, our phase I study of combined dinaciclib and MK-2206 in patients with advanced pancreatic cancer determined that dinaciclib 9 mg/m^2 weekly and MK-2206 135 mg once weekly was a safe regimen but without clinical benefit, likely due to not attaining biologically active doses. Although this regimen is no longer being explored, the preclinical data that formed the basis of this bench-to-bedside clinical trial suggests that future studies with better tolerated agents targeting Ral and AKT signaling are worth consideration.

Table 5 Plasma pharmacokinetic parameters

Cohort	MK-2206 dose (mg)	Dinaciclib dose (mg/m^2)	C_{max} (ng/mL)	T_{max} (hour)	AUC_{INF} (ng*h/mL)	$T_{1/2}$ (h)	Cl (L/hour)	*V* (L)	$C_{ss,min}$
Dinaciclib alone									
Escalation, level 2.5	135	9	416.6 ± 115.1 (7)	1.92 (1.42–2.17; 7)	710.2 ± 177.5 (5)	1.53 ± 0.18 (5)	25.2 ± 8.0 (5)	39.5 ± 11.4 (5)	
Escalation, level 3	90	12	581.3 ± 200.9 (6)	1.95 (1.85–2.05; 6)	1,200.8 ± 656.9 (6)	1.49 ± 0.27 (6)	21.8 ± 9.1 (6)	34.8 ± 7.3 (6)	
Expansion, arm A	135	9	394.6 ± 140.3 (11)	1.92 (1.92–2.17; 11)	795.5 ± 309.7 (10)	1.69 ± 0.33 (10)	23.4 ± 10.4 (10)	43.2 ± 21.6 (10)	
Dinaciclib in combination									
Expansion, arm A	135	9	374.7 ± 114.8 (7)	1.92 (1.92–2.35; 7)	832.4 ± 409.5 (7)	1.52 ± 0.21 (7)	20.5 ± 5.5 (7)	37.0 ± 9.3 (7)	
Expansion, arm B	135	9	507.5 ± 180.3 (6)	2.03 (1.92–3.40; 6)	1,345.5 ± 782.2 (5)	1.57 ± 0.20 (5)	15.6 ± 7.0 (5)	28.7 ± 11.6 (5)	
MK-2206 alone									
Escalation, level 2.5	135	9	184.7 ± 79.7 (7)	4.50 (0.58–9.20; 7)	12,472 ± 5877 (7)	56.9 ± 9.7 (7)	30.4 ± 11.6 (7)	2,421 ± 809 (7)	20.4 ± 12.3 (7)
Escalation, level 3	90	12	113.1 ± 53.2 (7)	5.97 (1.95–48.02; 7)	9,148 ± 4,222 (4)	68.3 ± 16.6 (6)	27.6 ± 10.4 (4)	2,260 ± 577 (4)	21.6 ± 11.3 (6)
Expansion, arm B	135	9	260.7 ± 81.2 (7)	4 (2.17–25.17; 7)	13,390 ± 3725 (3)	78.6 ± 21.3 (6)	26.1 ± 7.5 (3)	2,231 ± 646 (3)	48.0 ± 22.7 (7)
MK-2206 in combination									
Expansion, arm A	135	9	214.9 ± 69.7 (7)	10.17 (6.07–23.00; 7)	15,682 ± 6590 (3)	69.7 ± 23.1 (5)	23.1 ± 11.1 (3)	1,741 ± 759 (3)	34.6 ± 18.5 (6)
Expansion, arm B	135	9	326.7 ± 213 (4)	5.13 (4.00–6.55; 4)	11,812 (1)	63.4 ± 19.6 (3)	29.0 (1)	2,698 (1)	NA
Expansion. arm B, dose reduced	90	9	97.6, 201.9 (2)	4.75, 6.80 (2)	6,239 (1)	64.5 (1)	36.0 (1)	3,352 (1)	NA

AUC_{INF}, area under the plasma concentration-time curve to infinity; Cl, systemic clearance; Cl/F apparent systemic clearance; C_{max}, peak plasma concentration; NA, not available; T_{max}, time to peak concentration; $t_{1/2}$, terminal half-life; *V*, volume of distribution; V/F, apparent volume of distribution.
Data are presented in the table as mean values ± SD (*n*). T_{max} is presented as median (range; *n*). If $n < 3$, the actual values are reported.

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