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A Phase II Study of the Gamma Secretase Inhibitor RO4929097 in Patients with Previously Treated Metastatic Pancreatic Adenocarcinoma

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Abstract

Purpose—The notch pathway is overexpressed in pancreatic adenocarcinoma. RO4929097, an oral inhibitor of the γ-secretase enzyme has been safely given as a single agent in patients with advanced solid tumors. We aimed to evaluate the efficacy of RO4929097 in patients with pancreatic adenocarcinoma (PDA).

Methods—A two-stage, single-arm Phase II trial was conducted in patients with previously treated metastatic PDA. RO4929097 was administered at a dose of 20 mg daily on days 1-3, 8-10 and 15-17 of 21-day cycles. The primary endpoint was survival at 6 months. Secondary endpoints included overall survival (OS), response rate, toxicities, pharmacokinetic and pharmacodynamic analyses.

Results—Eighteen patients were recruited, 17 in the first stage and one in the 2nd stage. It was decided to stop further enrollment after RO4929097 was discontinued by the sponsor and was no longer a development candidate. Three (25%) of 12 evaluable patients achieved stable disease. The six-month survival rate was 27.8% (95 % CI 9.7–53.5). The median OS was 4.1 months (95 % CI 2.7–5.8 months) and median progression-free survival was 1.5 months (95 % CI 1.3–1.6 months). Pharmacokinetic properties of RO4929097 in patients (n=5) with PDA was similar to that previously reported in other patient populations. There was a trend towards a decrease in HeyL (p = 0.08) gene expression in three patients following study drug administration.

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Conclusions—RO4929097 was well-tolerated in patients with previously treated PDA. Development of RO4929097 has been discontinued, but development of other notch-targeting agents in pancreatic cancer is continuing.

Keywords

Pancreatic cancer; notch signaling; gamma secretase inhibitor; pharmacokinetics

INTRODUCTION

Pancreatic cancer is a devastating disease despite intensive research over the past decades. Most patients will present with advanced metastatic disease at diagnosis with a dismal survival of 6–11 months [1-3]. Current knowledge about the molecular mechanisms of cancer-related pathways involved in cellular signaling, cell cycle regulation, cell death, angiogenesis, adhesion, motility and invasion are yielding emerging therapies directed at specific components of these pathways.

The Notch pathway is important in development and regeneration of pancreatic tissue [4] and is activated in both preclinical models and human pancreatic carcinoma promoting progression of pancreatic intra epithelial neoplasia into pancreatic adenocarcinoma[5-8]. It is represented by a group of four cell surface receptors (Notch 1 – 4) which are activated by ligands on neighboring cells (Delta 1, 3, 4 and Jagged 1, 2). Binding of Notch ligand to its receptor activates the pathway through a cascade of proteolytic cleavages, mediated by γ-secretase (presenilin) producing an activated form, ICN (Intra Cellular Notch). ICN is then translocated to the nucleus to be part of a large transcription complex that regulates several genes with key roles in proliferation and differentiation of cells. Additionally, elevated Notch activity maintains pleuripotent cells in a stem cell state thereby promoting tumor proliferation while inhibition of this pathway leads to differentiation of tumor cells. As a result, one attractive target to suppress this pathway is the γ-secretase enzyme which is a key player in intramembrane processing to produce the active form, ICN.

RO4929097 is a potent and selective inhibitor of the γ-secretase enzyme that has demonstrated anti-tumor activity in in vitro and in vivo studies. In genetically modified and orthotopic animal models of pancreatic cancer, γ-secretase enzyme inhibition retarded tumor progression [8], resulted in apoptosis of pancreatic cell lines [9-12] and inhibited tumor progression in xenografts [8]. Clinically, RO4929097 was shown to be safe with few serious adverse effects in early phase clinical trials in patients with advanced solid tumors [13-17]. In this study, we aimed to evaluate the efficacy of RO4929097 in patients with previously treated metastatic pancreatic adenocarcinoma.

PATIENTS AND METHODS

We conducted a two-center clinical trial sponsored by U.S. National Cancer Institute/Cancer Therapy Evaluation Program (clinicaltrials.gov identifier NCT01232829). The protocol was approved at the Institutional Review Boards of the participating institutions, and written informed consent was obtained for all patients prior to performing study-related procedures

in accordance with federal and institutional guidelines. Patients were enrolled at the University of Colorado Cancer Center and Johns Hopkins Hospital.

This was an open-label, phase II study of the oral administration of RO4929097. Subjects were eligible if they were at least 18 years old, had a Karnofsky Performance Status (KPS) of ≥70, at least one previous chemotherapy for metastatic disease, histologically or cytologically confirmed metastatic adenocarcinoma of the pancreas, and measurable disease by Response Evaluation Criteria in Solid Tumor (RECIST) v1.1. Patients with islet cell neoplasms and patients with locally advanced disease were excluded. All patients had adequate hematologic, hepatic, and renal function (including absolute neutrophil $\geq 1.5\times10^9$/L, hemoglobin ≥9 g/dL, and bilirubin ≤ upper limit of normal [ULN], AST(SGOT)/ALT(SGPT) <2.5 X institutional ULN). Pregnant and nursing women were excluded due to potential for teratogenic or abortifacient effects.

Study Drug Administration and Evaluations

Eligible patients were treated with oral RO4929097 at a dose of 20 mg daily on days 1–3, 8–10 and 15–17. Cycles were repeated every three weeks until evidence of disease progression, unacceptable adverse event, withdrawal of consent, or an intercurrent illness precluding further study drug administration. Dose reductions (to 10mg or 5 mg dose levels) for RO4929097 were planned if there were severe grade > 3 adverse events related to study drug. Tumor assessments were obtained by CT scans at baseline, after cycle 2 and every three cycles thereafter. Response was assessed using standard RECIST 1.1 criteria [18] .

Correlative Studies

: A subset of patients underwent serial tumor biopsies for correlative studies including serial tumor biopsies and blood samples for pharmacodynamic (PD) and pharmacokinetic (PK) studies. Tumor biopsies were performed at baseline (within 2 weeks of the first study drug administration) and on Cycle 1 Day 17. Two-four core biopsies using an 18–22 gauge needle were obtained. The initial core biopsy was placed into a container with RNAlater and stored after 30–60 minutes at 4°C. The second biopsy was stored in formalin at room temperature. All biopsies were stained with H&E to assess adequacy. Analyses were performed at Johns Hopkins University and the University of Colorado.

Pharmacokinetic Analysis

Blood (5 mL) was collected for pharmacokinetic studies before and at 1, 2, 5, 7, and 24 hours after oral administration of RO4929097 on Cycle 1 Day 1 and at the time of biopsy (e.g., Cycle 1 Day 17). Samples were collected in heparinized tubes, processed by centrifugation, and the resultant plasma was stored at −70°C until analysis. RO4929097 concentrations were determined in plasma samples by validated high pressure liquid chromatography with mass spectrometry detection (LC/MS/MS), with a lower limit of quantitation of 1 ng/ml [19]. Individual RO4929097 plasma concentration–time data were analyzed by noncompartmental methods using Phoenix WinNonlin version 6.3 (Pharsight, a Certara company, Cary NC).

Pharmacodynamic analysis

RT-PCR: Total RNA was extracted from tumor samples preserved in RNAlater using the RNeasy Mini kit (Qiagen). cDNA was synthesized using the Applied Biosystems high capacity cDNA reverse transcription kit, following the manufacturer's instructions. Validated and pre-designed primer/probes for HeyL and Hes-1 and housekeeping gene(s) were purchased from Applied Biosystems. Samples were amplified using the ABI Step One Plus RT-PCR system. Relative expression of the mRNA analyzed was estimated using the formula: $2^{-\Delta CT}$, where #CT = CT (mRNA) − CT (Housekeeper). **ELISA:** Blood samples for plasma stromal cell derived factor-1 (SDF-1), vascular endothelial growth factor (VEGF), interleukin 6 (IL-6) and interleukin 8 (IL-8) were collected immediately prior to dosing on C1D1, C1D17, C2D1, and end of study. Concentrations of SDF-1, VEGF, IL-6, and IL-8 were determined in undiluted plasma samples by ELISA, per the manufacturer's instructions (R&D Systems). ELISA plates were read at 450 nm on a Synergy 2 plate reader (Biotek).

Endpoints and statistical methods

This was a two-stage phase II study designed to assess the 6-month survival rate, calculated as the percentage of evaluable patients alive at least 6-months post-registration in patients with previously treated pancreatic cancer treated with RO4929097. On the basis of previous phase II trials of targeted agents in this patient population, the expected 6-month survival was estimated at approximately 15%. The original study design was a two-stage MiniMax design with an interim analysis that was used to test whether there was sufficient evidence to determine that the 6-month survival rate was at least 35% (i.e., clinically promising) versus at most 15% (i.e., clinically inactive). All patients meeting the eligibility criteria and who received treatment were considered evaluable for the primary endpoint.

The initial stage had a planned accrual of 17 patients for the interim analysis. If at least 3 of these 17 evaluable patients lived for 6 months or more, the study would continue to a full accrual of 32 patients. Otherwise, the study would be closed early due to a lack of sufficient activity. If the study continued to full accrual, 8 or more of the 32 evaluable patients would need to live at least 6 months for the treatment to be considered promising for further study. The study design had a 91% probability of concluding that the regimen is promising if the true success rate is 35%, using a 9% level of significance when the true success rate is 15%.

Correlative (PK and PD) studies were planned such that most of the patients in the 2nd stage of the trial (n=1 of the planned 15) would undergo pre- and post-treatment tumor biopsies and PK studies. Patients who were compliant and with complete pharmacokinetic sampling were considered evaluable for pharmacokinetic analysis and were included in the descriptive statistics. Pharmacodynamic studies were assessed using descriptive statistics. No formal pharmacokinetic-pharmacodynamic assessment occurred due to the small number of patients with matched pair data.

Secondary endpoints included tumor response rate, adverse events, progression-free survival, and overall survival. Adverse events were assessed using the National Cancer Institute Common Terminology Criteria for Adverse Events (NCI CTAE) version 3.0 and

summarized in a tabular manner as the maximum grade for a given type of event for each patient. All grade 3+ adverse events are reported. Kaplan–Meier methodology was used to describe the distributions of progression-free survival and overall survival. Best response was assessed by the investigators using the Response Evaluation Criteria in Solid tumors (RECIST) guidelines (version 1.1) [18]. Survival was measured from time of registration on protocol until death from any cause. Progression free survival was measured from the time of registration on protocol until progression or death, whichever occurred first.

RESULTS

From December 2010 to May 2012, eighteen patients were enrolled. The median age was 67 years (range 46–80) and the median ECOG performance status was 1. Baseline patients' characteristics are presented in Table 1. All patients had received at least one line of prior systemic chemotherapy for metastatic pancreatic cancer. The median number of prior treatments was 2 (range 1–4). Seven patients had previous surgery with curative intention and 9 patients had previously received radiation. Three patients received previously immunotherapy. A total of 4 patients (22%) received the protocol regimen as second-line treatment; the other 14 patients (78 %) received the protocol regimen as third-line treatment or beyond.

Dose Intensity

All patients have ended treatment. A median of two cycles of therapy was given (range: 1–6). The most common reasons for ending treatment consisted of disease progression: n= 13 (72%); adverse events: n = 1 (6%) (confusion); intercurrent illness that precluded further treatment administration n = 3 (17%); (ascites/pain/renal dysfunction); and patient choice: 1 (6%). No dose reductions of RO4929097 were required.

Adverse Events

Common toxicities potentially related to treatment were lymphopenia, hyperglycemia, hypophosphatemia, dysgeusia and fatigue; these were generally mild (Table 2). One patient developed grade 4 confusion that was deemed related to study drug and was removed from protocol. There were no deaths related to study drug.

Efficacy

The primary endpoint of the study was 6-month survival. Eighteen patients were evaluable for the survival endpoint (Table 3) and all patients have died at the time of this report. For the first stage of the study (n=17), four patients (24%) survived at least 6 months (95% confidence interval 7-50%), which exceeded the boundary (three patients) needed to continue the trial to full accrual at stage 2. One additional patient was accrued during stage 2 for a total of 18 patients in the study. It was decided to stop further enrollment in the study after RO4929097 was discontinued by the sponsor and was no longer a development candidate. Including all patients treated per protocol (n = 18), five patients survived at least six months (28%). The median survival (Table 3) was 4.1 months (95% CI 2.7–5.8 months), and the median progression-free survival (Table 3) was 1.5 months (95% CI 1.3–1.6 months). Of the 18 patients enrolled, 12 patients were evaluable for response based on

protocol criteria; 3 patients (25% of evaluable; 17% of total enrolled) had stable disease at best response. There were no complete or partial responses.

PK and PD Analysis

The study was planned such that PK/PD studies were optional in the first stage (n=4 of 17) and required in the 2nd stage (n=1 of planned 15), after the study drug had shown some evidence of efficacy in the patient population studied. As the study terminated early, complete RO4929097 pharmacokinetics were obtained on five subjects. The concentration-time profile and trend towards auto-induction was consistent with previous reports. After a single dose on Cycle 1 Day 1, the average C_{max} was 598.0±263.1 ng/mL which occurred at 5.0 hours (median; range: 2.0–6.9 hours). While on Cycle 1 Day 16, the average C_{max} was 516.4±304.0 ng/mL which occurred at 4.8 hours (median; range: 1.3–21.4 hours). The average total exposure ($AUC_{0\text{-}24h}$) was 10201±4277 ng*h/mL on Day 1 and decreased to 8187±5064 ng*h/mL on Day 16, which is suggestive of auto-induction.

The effects of RO4929097 on gene expression of the Notch target genes Hes-1 and HeyL were evaluated in pre and post (17 days) treatment biopsies. A trend towards a decrease in HeyL ($p = 0.08$) was seen in 3 patients following treatment (Figure 1). No difference in gene expression of Hes-1 was seen with treatment (data not shown). In addition, no significant differences in plasma concentrations of SDF-1, VEGF, IL-6, and IL-8 were seen with RO4929097 treatment (supplemental figure 1). Due to the small number of patients with matched pharmacokinetic/pharmacodynamic data, correlations were not performed.

DISCUSSION

This study, sponsored by the U.S. National Cancer Institute/Cancer Therapy Evaluation Program, was conducted to explore the efficacy of RO4929097 in second- and third-line metastatic pancreatic cancer. The Notch pathway has been shown to be important in pancreatic cancer initiation, progression and maintenance [6, 12]. Authors within our group have previously shown over-expression of the Notch receptors 1, 2 and 4 in 40%, 20% and 25% respectively within a panel of 20 human pancreatic cancer cell lines. Ligand-dependent activation of the Notch pathway, through overexpression of JAG2 and DLL4 ligands, resulted in over-expression of Notch-target genes Hes-1 (80%) and Hey2 (65%). Furthermore, overexpression of a constitutively active Notch intracytoplasmic domain in a pancreatic cell line resulted in enhanced growth potential, whereas using a γ-secretase inhibitor or siRNA to inhibit the pathway diminished the malignant phenotype [12].

Previously, RO4929097 was evaluated in a phase I clinical trial (n=110) to assess the safety, pharmacokinetics and pharmacodynamics of intermittent or continuous doses in patients with refractory metastatic or locally advanced solid tumors. Intermittent schedules were explored based on preclinical gastrointestinal toxicity in animals. RO4929097 was well-tolerated at 270 mg on a 3-days on / 4 days off schedule[16]; however, reversible CYP3A4 autoinduction resulting in decreased exposure at daily doses above 24 mg were noted and several other doses/schedules were explored including continuous daily dosing at 10 mg. Toxicities were generally mild and included nausea/vomiting, diarrhea, fatigue, hypophosphatemia, and rash. There was one PR in a patient with colorectal cancer and a CR

via PET scan in a melanoma patient. Unfortunately, only weak PK/PD correlations were seen. A dose/schedule of 20 mg using a 3 on/4 off schedule was chosen for further development based on a lack of autoinduction and avoidance of potential drug/drug interactions.

Our study showed that RO4929097 was well-tolerated in the $2^{nd}/3^{rd}$ line pancreatic cancer population. RO49290977 was well tolerated with minimal adverse events. Most of the treatment-related toxicities were grade 1 or 2 severity and there was only one grade 4 adverse event (confusion). There were no treatment-related deaths.

The study met criteria to proceed to full accrual (n=32) after the interim analysis of stage 1 (n=17), but development of the agent was discontinued. Although we were not able to complete full accrual of the second stage we did see some early signs of activity via disease stabilization. The lack of RECIST responses seems to be in accordance with preclinical studies that suggested these agents have a cytostatic effect rather than cell death or a decrease in tumor volume when used as monotherapy. Previous *in vitro* studies demonstrated that RO4929097 treatment resulted in a cytostatic effect, and when combined with gemcitabine in a mouse xenograft model of pancreatic cancer led to prolonged survival despite the lack of decrease in tumor volume. We also hypothesized that as preclinical studies showed effect in the pleuripotent stem cells which compose a small percentage of tumor bulk, changes in tumor volume may not occur but efficacy may be based on disease stabilization.

The median survival in our study was 4.1 months and the median progression-free survival was 1.5 months. It is important to mention that this trial included a population of heavily pretreated patients, since a majority of patients (78%) received RO4929097 as third-line therapy or beyond. At present, there is a lack of studies to define survival in PDA patients receiving therapy in this setting. With the recent approval of newer combinational regimens for patients with PDA (FOLFIRINOX and Gem/Abraxane), more therapeutic options are available for patients with this disease and clinical trials now on will be enrolling patients in the third-line setting or beyond. Our study may provide survival information in this setting.

Our original design included serial tumor biopsies during the second stage of the study to explore biological correlates in determining response to RO4929097 and for pharmacokinetic and pharmacodynamic studies. As the trial was terminated earlier we were unable to obtain all planned samples but we were able to explore the effects of RO4929097 on gene expression of the Notch target genes Hes-1 and HeyL in a limited subset of patients using pre and post (17 days) treatment biopsies. There was a trend towards a decrease in HeyL ($p = 0.08$) in three evaluable patients following treatment. We did not see significant differences in plasma concentrations of SDF-1, VEGF, IL-6, and IL-8. Due to the small number of patients with matched pharmacokinetic/pharmacodynamic data, correlations with survival or other clinical endpoints were not performed. Previous studies using gamma-secretase inhibitors who were able to assess these biomarkers did not see any correlation with response. The concentration-time profile and trend towards auto-induction was consistent with previous reports [13, 15-17].

In conclusion, RO4929097 was well-tolerated in patients with previously treated pancreatic adenocarcinoma. We successfully completed the first stage of the study. This preliminary evidence warrants further clinical investigation of these agents in patients with pancreatic cancer. However, given the absence of tumor response and the limited activity seen using gamma-secretase inhibitors as monotherapy in other malignancies, it may be interesting to test these small molecules in combination with cytotoxic chemotherapy. Larger trials of notch pathway inhibitors are underway in patients with pancreatic cancer and may provide more definitive evidence of its anticancer activity in this patient population.

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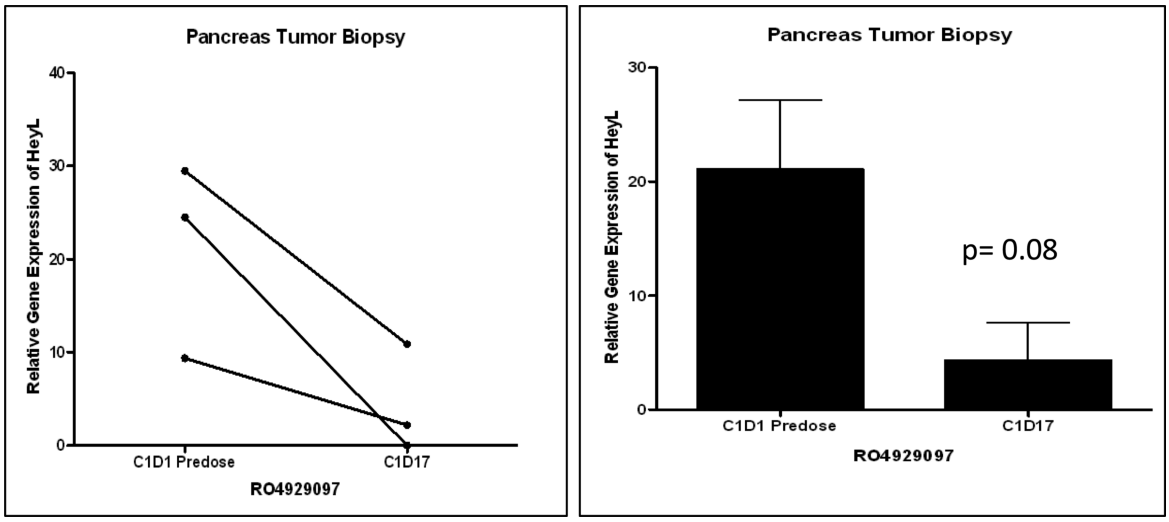

Figure 1.

Analysis of HeyL gene expression via PCR on matched pre- and post-treatment (day 17) tumor biopsies in subjects (n=3) treated with RO4929097, showing a trend of decreased expression (p=0.08)

Table 1

Baseline Patient Characteristics

Variable		Total Patients (N = 18)
Sex		
Male	N (%)	8 (44)
Female		10 (56)
Ethnicity/Race		
Hispanic	N (%)	2 (11)
NH White		15 (83)
NH Black		1 (6)
Age		
Years	Mean (SD)	65.0 (11.9)
	(Min, Max)	(46, 80)
	Med (Q_1, Q_3)	67 (58, 77)
ECOG Performance Status		
0	N (%)	4 (22)
1		13 (72)
2		1 (6)
Previous Chemotherapy		
Regimens	Mean (SD)	2.4 (1.1)
	(Min, Max)	(1, 5)
	Med (Q_1, Q_3)	2 (2, 3)
Previous Therapy		
Chemotherapy	N (%)	18 (100)
Radiotherapy		9 (50)
Surgery (Curative Intention)		7 (39)
Immunotherapy		3 (17)

Table 2

RO4929097-Related Adverse Events

	Grade				
Adverse Event	**1**	**2**	**3**	**4**	**Total**
Confusion				1	1
Hyperglycemia		2			2
Fatigue		2			2
Dysgeusia		1			1
Lymphocyte Count Decreased	2				2
Hypophosphatemia	1				1
Total	**3**	**5**	**0**	**1**	**9**

Table 3

Survival Measures

Outcome		Total Patients (N = 18)
Overall Survival		
Alive at 6 Months	N (%)	5 (28)
	Exact 95% CI	(10, 54)
Months-to-Death	Median (95% CI)	4.1 (2.7, 5.8)
	Mean (95% CI)	5.4 (3.5, 7.3)
Progression-Free Survival		
Non-Progression at 3 Months	N (%)	4 (22)
	Exact 95% CI	(6, 48)
Months-to-Progression	Median (95% CI)	1.5 (1.3, 1.6)
	Mean (95% CI)	1.9 (1.3, 2.5)

Table 4

Response to RO4929097 Treatment

Variable		Evaluable Patients (N = 12)
Response		
Stable Disease	N (%)	3 (25)
Progression		9 (75)

Note: 6 patients were not evaluable for response.