Phase I Study of RO4929097, a Gamma Secretase Inhibitor of Notch Signaling, in Patients With Refractory Metastatic or Locally Advanced Solid Tumors

Anthony W. Tolcher, Wells A. Messersmith, Stanislaw M. Mikulski, Kyriakos P. Papadopoulos, Eunice L. Kwak, Darlene G. Gibbon, Amita Patnaik, Gerald S. Falchook, Arvind Dasari, Geoffrey I. Shapiro, John F. Boylan, Zhi-Xin Xu, Ka Wang, Astrid Koehler, James Song, Steven A. Middleton, Jonathan Deutsch, Mark DeMario, Razelle Kurzrock, and Jennifer J. Wheler

See accompanying editorial on page 2291 and articles on pages 2307 and 2418

Anthony W. Tolcher, Kyriakos P. Papadopoulos, and Amita Patnaik, South Texas Accelerated Research Therapeutics Center for Cancer Care, San Antonio; Gerald S. Falchook, Razelle Kurzrock, and Jennifer J. Wheler, MD, Anderson Cancer Center, Houston, TX; Wells A. Messersmith and Arvind Dasari, University of Colorado Cancer Center, Aurora, CO; Stanislaw M. Mikulski, John F. Boylan, Zhi-Xin Xu, Ka Wang, James Song, Steven A. Middleton, Jonathan Deutsch, and Mark DeMario, Hoffman-La Roche, Nutley; Darlene G. Gibbon, University of Medicine and Dentistry of New Jersey–Robert Wood Johnson Medical School, Piscataway, NJ; Eunice L. Kwak, Massachusetts General Hospital; Geoffrey I. Shapiro, Dana-Farber Cancer Institute, Boston, MA; and Astrid Koehler, Roche Diagnostics, Penzberg, Germany.

Submitted May 3, 2011; accepted January 30, 2012; published online ahead of print at www.jco.org on April 23, 2012.

Supported by Hoffmann-La Roche, which also provided support for third-party editing/writing assistance.

Presented in part as an oral presentation at the 46th Annual Meeting of the American Society of Clinical Oncology, Chicago, IL, June 4-8, 2010.

Authors' disclosures of potential conflicts of interest and author contributions are found at the end of this article.

Corresponding author: Anthony W. Tolcher, MD, FRCPC, FACP, South Texas Accelerated Research Therapeutics, 4383 Medical Dr, 4th Floor, San Antonio, TX 78229; e-mail: atolcher@start.stoh.com.

© 2012 by American Society of Clinical Oncology

0732-183X/12/3019-2348/\$20.00

DOI: 10.1200/JCO.2011.36.8282

ABSTRACT

Purpose

To determine the maximum-tolerated dose (MTD) and assess safety, pharmacokinetics, pharmacodynamics, and evidence of antitumor activity of RO4929097, a gamma secretase inhibitor of Notch signaling in patients with advanced solid malignancies.

Patients and Methods

Patients received escalating doses of RO4929097 orally on two schedules: (A) 3 consecutive days per week for 2 weeks every 3 weeks; (B) 7 consecutive days every 3 weeks. To assess reversible CYP3A4 autoinduction, the expanded part of the study tested three dosing schedules: (B) as above; modified A, 3 consecutive d/wk for 3 weeks; and (C) continuous daily dosing. Positron emission tomography scans with [¹⁸F]fluorodeoxyglucose (FDG-PET) were used to assess tumor metabolic effects.

Results

Patients on schedule A (n = 58), B (n = 47), and C (n = 5; expanded cohort) received 302 cycles of RO4929097. Common grade 1 to 2 toxicities were fatigue, thrombocytopenia, fever, rash, chills, and anorexia. Transient grade 3 hypophosphatemia (dose-limiting toxicity, one patient) and grade 3 pruritus (two patients) were observed at 27 mg and 60 mg, respectively; transient grade 3 asthenia was observed on schedule A at 80 mg (one patient). Tumor responses included one partial response in a patient with colorectal adenocarcinoma with neuroendocrine features, one mixed response (stable disease) in a patient with sarcoma, and one nearly complete FDG-PET response in a patient with melanoma. Effect on CYP3A4 induction was observed.

Conclusion

RO4929097 was well tolerated at 270 mg on schedule A and at 135 mg on schedule B; the safety of schedule C has not been fully evaluated. Further studies are warranted on the basis of a favorable safety profile and preliminary evidence of clinical antitumor activity.

J Clin Oncol 30:2348-2353. © 2012 by American Society of Clinical Oncology

INTRODUCTION

Uncontrolled growth in malignant cells shares characteristics with stem cells, including a major developmental signaling axis, the Notch signaling pathway.^{1,2} Notch, represented by four homologs in mammals (Notch1 to Notch4), is a cell surface protein receptor involved in transmitting growth signals. Cell membrane-bound ligands (Delta1, Delta3, Delta4 and Jagged1, Jagged2) on neighboring cells bind and activate the Notch receptor, inducing intramembrane cleavage by the gamma secretase complex at the intracellular domain. The gamma secretase-processed Notch be-

comes an active form called intracellular Notch, which activates genes that regulate cell fate through differentiation of progenitor cells during development and self-renewal of pluripotent stem cells. Increased Notch signaling promotes tumor cell proliferation by maintaining tumor cells in a stem-cell-like proliferative state. Inhibition of Notch signaling promotes differentiation of tumor cells and certain stem-cell populations in the GI tract, immune system, skin, and hair.¹⁻³

RO4929097 is a potent and selective gamma secretase inhibitor with a low nanomolar half maximal concentration (IC₅₀) in in vitro enzyme assays and cellular Notch reporter assays.⁴ In vivo, RO4929097

demonstrated antitumor activity in seven of eight animal models, was active when given intermittently or daily and, uniquely, its efficacy was maintained after dosing was stopped, with histologic analysis demonstrating a differentiated tumor phenotype characteristic of Notch inhibition.⁴ In preclinical toxicology studies, RO4929097 demonstrated toxicity within the GI tract, lymphoid system (particularly marginal zone B cells), peripheral blood leukocytes, and ovaries (data on file, Roche, Nutley, NJ).

In malignancies, Notch signaling inhibition may alter several cell fate decisions (cell growth, differentiation, and death), both directly during tumorigenesis and tumor progression and indirectly for endothelial and other tumor stromal cells. The pro-differentiation-like and antiangiogenic phenotypic changes observed with Notch signal inhibitors result in tumor growth inhibition, modulation/inhibition of tumorigenic ("cancer stem") cells, and a reduction in tumor vascularization, invasion, and metastatic characteristics in preclinical models.⁵⁻⁹

On the basis of its novel target (gamma secretase), its unique mechanism of action (Notch signal inhibition), preclinical evidence of antitumor activity, and its preclinical toxicology profile, RO4929097 entered phase I evaluation. The main objectives of this first-in-human safety and pharmacokinetic (PK) study of RO4929097 were to determine maximum-tolerated dose (MTD), toxicities, PK behavior, pharmacodynamic (PD) effects, and preliminary evidence of anticancer activity.

PATIENTS AND METHODS

Patient Selection

Eligible patients had pathologically confirmed solid tumors refractory to standard therapy or for which no standard therapy exists, age ≥ 18 years, life expectancy ≥ 12 weeks, Eastern Cooperative Oncology Group (ECOG) performance status 0 to 2, previous chemotherapy ≥ 4 weeks (6 weeks for prior mitomycin or nitrosourea), hemoglobin ≥ 9 g/dL, absolute neutrophil count (ANC) $\geq 1,500/\mu\text{L}$, platelets $\geq 100,000/\mu\text{L}$, creatinine $\leq 1.5 \times$ upper limit of normal (ULN), bilirubin $\leq 1.5 \times$ ULN, AST and ALT $\leq 2.5 \times$ ULN, absence of pregnancy, hemoglobin A_{1C} less than 8%, fasting glucose less than 160 mg/dL, and no coexisting severe medical conditions.

Dose Escalation

In the dose-escalation part of the study, the schedule A cohort received RO4929097 for 3 consecutive days with 4 days rest for the first 2 weeks, followed by a third week off treatment. In the schedule B cohort, RO4929097 was administered for 7 consecutive days followed by 14 days off treatment during each 21-day cycle (Fig 1).

The starting dose level for schedules A and B was 3 mg per day, based on the MTD identified from 13-week three-dimensional Good Laboratory Practice toxicology studies in the rodent, standard conversion factors, and a 10-fold safety margin.¹⁰ The dose was escalated by 100% until grade ≥ 2 drug-related toxicity occurred. In both schedules, the dose was escalated by 50% if grade 2 drug-related toxicity occurred at the previous level and by less than 50% for grade ≥ 3 toxicity. If one patient experienced a dose-limiting toxicity (DLT) during cycle 1, the cohort was expanded to six patients. MTD was defined as the highest dose at which less than 33% of the patients experienced a DLT. Toxicity was graded according to the National Cancer Institute Common Toxicity Criteria (NCI-CTC), Version 3.0. The protocol design included dose escalation in the absence of predefined DLTs.

A dose-expansion phase was initiated to assess the decrease in RO4929097 exposure after repeated dosing because of reversible CYP3A4 autoinduction at daily doses above 24 mg in schedule A and above 18 mg in schedule B. In the dose-expansion phase, doses were 24 mg daily for both original and modified schedule A and 10 mg daily for schedule C. Drug-drug

Fig 1. Original study design with changes to show end of study treatments at END CYCLE 2 (ie, day 42), indicated by the addition of arrows for continuous dosing from day 1 to 42 in schedule C.

interaction with midazolam was investigated in this portion, and further safety data were collected.

Definition of DLT

DLT was assessed during the first 21-day treatment cycle and defined as a treatment-related event meeting prespecified criteria: nonhematologic grade ≥ 3 adverse events (AEs) or laboratory findings, except nausea, vomiting, and/or diarrhea, which were considered DLTs only if they reached grade ≥ 3 severity despite adequate supportive care measures; grade ≥ 3 hypophosphatemia, hypomagnesemia, and hypocalcemia were considered DLTs only if they were clinically symptomatic or were accompanied by acute ECG changes. An ECG was required if any of these grade ≥ 3 electrolyte abnormalities was observed: grade 4 neutropenia lasting ≥ 7 days, febrile neutropenia (ANC $< 1.0 \times 10^9/\text{L}$ and fever $\geq 38.5^\circ\text{C}$), documented infection with ANC less than $1.0 \times 10^9/\text{L}$, grade 4 thrombocytopenia (ie, $< 25.0 \times 10^9/\text{L}$), any thrombocytopenia requiring platelet transfusions, or cycle 2 day 1 treatment delayed for more than 14 days.

Follow-Up Assessments

Radiologic assessment of disease status was repeated every 6 weeks, and tumor response was assessed by Response Evaluation Criteria in Solid Tumors (RECIST; version 1.0). All patients who received at least one dose of RO4929097 were evaluable for safety. For each schedule, cohorts of at least three patients were treated.

The following analyses were performed: standard safety assessments according to NCI Common Terminology Criteria for Adverse Events (NCI-CTCAE); PK sampling during cycle 1 (first and last dosing days) and day 1 of cycle 2; CYP3A4 induction was evaluated with all available midazolam data from patients in the expanded cohorts before and after study drug treatment; amyloid-beta 40 (A β -40) plasma levels by enzyme-linked immunosorbent assay; Hes-1 and *c-myc* expression levels by quantitative reverse transcriptase polymerase chain reaction in hair follicles and peripheral blood mononuclear cells (as surrogate tissues); microvessel density and additional biomarkers in available tumor tissues before and after RO4929097 treatment; soluble biomarkers related to angiogenesis and selected cytokines, and peripheral blood T-cell subsets; tumor assessments (by RECIST) every 6 weeks by computed tomography (CT) or magnetic resonance imaging; and positron emission tomography (PET) -CT evaluations at baseline and at the end of dosing in cycles 1 and 2.

Samples for CBCs and chemistry were collected weekly on days 1, 8, 15, and 22 and thereafter on every cycle. ECG monitoring was performed for all patients before and after RO4929097 dosing (Data Supplement).

PK

In the dose-escalation part of the study, blood samples for RO4929097 were collected serially for PK analysis following the daily doses at day 1 for schedules A and B, on day 10 for schedule A and day 7 for schedule B, and then

on day 1 of cycle 2 for both schedules. During the dose-expansion phase, serial blood sampling was obtained on day 1 of cycles 1 and 2 (schedules A and C), on day 10 for patients on original schedule A, or on day 17 for patients on modified schedule A. The daily dose of RO4929097 was 24 mg for both original and modified schedule A and 10 mg for schedule C. On the basis of PK findings related to RO4929097 from schedules A and B in the dose-escalation phase of the study and on results from PK modeling and simulation efforts, these dose levels tested in the expanded phase were expected to minimize CYP3A4 autoinduction.

Drug-drug interactions between RO4929097 and the CYP3A4 substrate midazolam were also investigated in this phase, and further safety data were collected. Midazolam was administered as a single 3- or 4-mg dose, and blood samples were collected at baseline (before RO4929097 treatment) for schedules A and C, on day 10 or 17 for schedule A, and on day 22 for schedule C. Plasma concentrations of RO4929097 and midazolam were measured with validated methods.

PK/PD Correlations

PK/PD correlations were evaluated for three PD markers: A β -40 protein and vascular endothelial growth factor receptor 2 (VEGFR-2) protein in blood plasma and Hes-1 mRNA expression in hair follicles. For each PD marker, PK/PD relationships were explored by plotting percent change (or change) from baseline versus time-matched PK concentration level (or area under the concentration $\times$ time curve from 0 to 24 hours [AUC₀₋₂₄]). Assuming a linear correlation between PK and PD parameters, Pearson correlation coefficients and descriptive *P* values were calculated.

RESULTS

Patients

In all, 110 patients were recruited. Of these, 58 were treated with 3 to 270 mg in schedule A, 47 were treated with 3 to 135 mg in schedule B, and five were treated with 10 mg per day continuously in schedule C. Patients' baseline characteristics are listed in Table 1.

Table 1. Patient Characteristics

Characteristic	No.	%
Total No. of patients	110	
Males		43
Females		57
Age, years		
Median	60	
Range	26-87	
ECOG performance status		
0	31	
1	77	
2	2	
Tumor type		
Melanoma	24	
Colorectal	12	
Sarcoma	12	
Breast	10	
Ovarian	9	
Neuroendocrine	7	
Hormone-refractory prostate	7	
Other	29	
No. of prior regimens		
Median	3	
Range	0-12	

Abbreviation: ECOG, Eastern Cooperative Oncology Group.

Dose Escalation

In schedule A, dose escalation continued to 80 mg when grade 3 asthenia was observed, at which point the cohort was increased to six patients. Following no further DLTs, dose escalation continued to 270 mg without the MTD being defined. In schedule B, dose escalation continued to 27 mg when grade 3 asymptomatic transient hypophosphatemia was observed in two of the six patients during cycle 1. These AEs were reversible and not considered clinically significant, so the protocol was amended to continue dose escalation with careful monitoring of plasma and urine phosphate concentrations. One further DLT (grade 3 pruritus) occurred in one of the six patients at 60 mg, and dose escalation continued to 135 mg again without determining the MTD.

Dose escalation was halted at 270 mg for schedule A and at 135 mg for schedule B because of PK evidence of CYP3A4 autoinduction with a decline in plasma concentrations with continued dosing. Plasma concentrations in both schedules exceeded that which predicted antitumor activity in xenograft models (equivalent to a 6-mg dose level; Fig 2). All schedule C patients were treated at 10 mg per day continuously.

Fig 2. Pharmacokinetics shown as area under the concentration versus time curve from time 0 to 24 hours (AUC₀₋₂₄), with annotated preclinical efficacious levels of exposure. The mean $\pm$ standard deviation RO4929097 AUC₀₋₂₄ (ng-h/mL) is shown for each dosing cohort in (A) schedule A and (B) schedule B on day 1 of cycle 1 (C1D1), on day 10 of cycle 1 (C1D10) or day 7 of cycle 1 (C1D7), and on day 1 of cycle 2 (C2D1) in the dose-escalation phase of the study. Efficacy exposure levels observed in nude mouse models with (solid line) 10 mg/kg (1,758 ng-h/mL) and (dotted line) 30 mg/kg (5,280 ng-h/mL) daily dosing. Schedule A: RO4929097 for 3 days on/4 days off during weeks 1 and 2 every 3 weeks for the first two cycles, followed by a third week off treatment, then 3 days on/4 days off during subsequent cycles; schedule B: RO4929097 on 7 consecutive days every 3 weeks.

Table 2. Common Treatment-Related Adverse Events in Dose Schedules ($\geq 10\%$ in any schedule)

Adverse Event	Schedule A (n = 58)						Schedule B (n = 47)						Schedule C (n = 5)					
	Grade 1		Grade 2		Grade 3		Grade 1		Grade 2		Grade 3		Grade 1		Grade 2		Grade 3	
	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
Diarrhea	6	10	2	3	0		6	13	2	4	1	2	0		0		0	
Skin (erythema, rash, pruritus)	3	5	1	2	0		6	13	2	4	3	6	1	20	0		0	
Fatigue	4	7	6	10	0		2	4	6	13	0		0		0		0	
Headache	5	9	1	2	0		2	4	0		0		0		0		0	
Hypophosphatemia	0		1	2	2	3	0		8	17	3	6	0		0		0	
Nausea	8	14	3	5	0		11	23	3	6	0		0		0		0	
Vomiting	3	5	0		0		4	9	4	9	0		0		0		0	

NOTE. There were no grade 4 or 5 RO4929097-related adverse events.

Tolerability

Common toxicities potentially related to treatment were nausea, vomiting, diarrhea, fatigue, hypophosphatemia, and an eczematoid skin rash on the face and brow; these were generally mild and are summarized in Table 2. Five patients experienced grade 3 hypophosphatemia over multiple cycles, although no clinical sequelae were observed. No correlations were observed between hypophosphatemia in serum and increased urine phosphate excretion among more than 40 patients who had both urine and blood electrolyte assessments.

ECG data are available for all 110 patients. Three patients were identified with signal ECGs ($QT_c > 500$ ms or change from baseline > 60 ms), but these patients had abnormal ECGs before drug administration, including right bundle branch block (two patients) and paced rhythm (one patient). Two patients with AEs reported QT prolongation less than 500 ms and less than 60 ms relative to baseline. Both patients were in schedule A: one in the 54-mg dose cohort and the other in the 120-mg cohort.

PK

Following oral administration, RO4929097 exposure increased with higher doses on day 1 for schedules A and B (Data Supplement). However, after repeated dosing at doses above 24 mg, a dose-dependent decrease in exposure was observed consistent with autoinduction of CYP3A4. Following a 10- or 14-day washout in schedules A and B, respectively, PK exposures on day 1 of cycle 2 were consistent with day 1 of cycle 1, indicating recovery of CYP3A4 to baseline levels. Importantly, as shown in Figure 2, single and repeated dosing exposure reached and exceeded the effective (ie, inhibiting tumor growth by $> 70\%$) level of ≥ 6 mg, including in dose cohorts that demonstrated autoinduction.

The effect of RO4929097 on CYP3A4 induction was further assessed by comparing the plasma concentrations of midazolam in patients before and after receiving RO4929097 treatment. For schedule A, after the last dose of RO4929097 (24 mg), midazolam exposure decreased compared with baseline levels (Appendix Fig A1, online only). Consistent with these changes, the exposure ratio of 1-hydroxymidazolam (the CYP3A4-associated metabolite of midazolam) versus midazolam increased after RO4929097 treatment (Appendix Fig A2, online only). Decreased midazolam exposures and increased ratios of metabolite parent drug confirmed CYP3A4 induction and provided the rationale for halting further dose escalation.

PD Analysis and PK/PD Correlations

Weak to moderate PK/PD correlations were observed for A β -40 protein in plasma, VEGFR-2 protein in plasma, and Hes-1 mRNA expression in hair follicles (Fig 3 and Appendix Figs A3 and A4, online only). Higher plasma RO4929097 concentrations resulted in decreased levels of A β -40 versus baseline ($r^2 = -0.36$; $P < .001$; Fig 3). VEGFR-2 levels increased with RO4929097 exposure as determined by AUC_{0-24} ($r^2 = 0.25$; $P = .001$). Expression of the Notch target gene Hes-1 decreased with increasing RO4929097 plasma concentration ($r^2 = 0.29$; $P < .001$).

None of the baseline values of biomarkers were found to be predictive of response to RO4929097. Exploratory analyses indicated that patients with at least two cycles of confirmed stable disease (SD) had a smaller percentage increase of interleukin-6 (IL-6) on day 2 of cycle 1 and day 2 of cycle 2 versus patients with early progression (Fig 4). The trend was more obvious in patients who had at least four cycles of SD. Results from additional assessments for biomarkers of Notch pathway modulation, including the analysis of a limited number of paired biopsies, are summarized in the Data Supplement.

Fig 3. Relationship between RO4929097 plasma concentration and amyloid beta-40 (A β -40; a product of the proteolytic cleavage of amyloid precursor protein by gamma secretase) percent change from baseline. Solid blue circles indicate individual patient data; red line indicates linear regression. Blood plasma samples were collected at baseline and then serially for assessments of A β -40 by using an enzyme-linked immunosorbent assay (Amyloid β 40 ELISA high sensitive; The Genetics Company, Basel, Switzerland).

Fig 4. Percent change for interleukin-6 from baseline in patients with and without clinical benefit. Solid blue circles indicate individual patient data. Blood plasma samples were collected at baseline and then serially (schedule A: cycle 1 day 2, cycle 1 day 10, and cycle 2 day 2; schedule B: cycle 1 day 2, cycle 1 day 7, and cycle 2 day 2). Analysis was performed by using multianalyte profiling technology at Rules-Based Medicine (Austin, TX). 0C represents patients without stable disease, 2C represents patients with two cycles of confirmed stable disease, and 4C represents patients with at least four cycles of confirmed stable disease. Schedule A: RO4929097 for 3 days on/4 days off during weeks 1 and 2 every 3 weeks for the first two cycles, followed by a third week off treatment, then 3 days on/4 days off during subsequent cycles; schedule B: RO4929097 on 7 consecutive days every 3 weeks. H, hours.

Antitumor Activity

RO4929097 showed evidence of antitumor activity (Data Supplement). One patient with colorectal adenocarcinoma with neuroendocrine features achieved a partial response on schedule B at 40 mg and continued treatment for 10 cycles (Appendix Fig A5, online only). One patient with epithelioid sarcoma and pulmonary and soft tissue metastases had a mixed response (overall, approximately 12% tumor reduction) and continued on schedule B at 6 mg for six cycles. One patient with in-transit metastatic melanoma had a near 100% positron emission tomography with [^{18}F]fluorodeoxyglucose (FDG-PET) response (Appendix Fig A6, online only) with a clinical flattening of in-transit lesions and continued on schedule B for a total of 16 cycles. Another patient with widespread cutaneous melanoma had a minor response (27% reduction by RECIST) and received a total of six cycles on schedule B (at 27 mg). Prolonged SD was achieved by 12 (25%) of 48 evaluable patients who continued treatment on schedule A for at least four cycles (3 months) and four (8%) of 48 who continued for at least eight cycles (6 months). For schedule B, 14 (32%) of 44 evaluable patients who continued treatment for at least four cycles (3 months) and four (9%) of 44 who continued for at least eight cycles (6 months) had SD, respectively. Melanoma, sarcoma, and ovarian carcinoma were the tumor types most frequently observed in patients with prolonged SD (Data Supplement).

DISCUSSION

This phase I clinical trial evaluated RO4929097, a potent, selective, small molecule gamma secretase inhibitor with unique preclinical activity and safety profile.⁴ Intermittent schedules were selected on the basis of the schedule-specific preclinical GI toxicity observed in animals related to Notch pathway inhibition–mediated increased formation of intestinal goblet cells. Further support for the intermittent schedules came from preclinical human tumor xenograft models that

demonstrated sustained tumor growth inhibition over time despite cessation of dosing.⁴

RO4929097 was well tolerated with skin, GI events, and fatigue being the most common toxicities at least possibly related to treatment. Most (95%) of the treatment-related toxicities were grade 1 or 2 severity, and no grade 4 toxicities were observed. Discontinuations for treatment-related AEs occurred rarely (2%), and dose adjustments in any cycle beyond cycle 1 were uncommon (11%). Although four of 96 patients developed DLTs during cycle 1, this did not preclude continued dose escalation nor could an MTD be determined for either schedule A or schedule B.

RO4929097 exposure decreased after repeated dosing due to reversible CYP3A4 autoinduction at daily doses above 24 mg in schedule A and above 18 mg in schedule B. These findings are consistent with the findings from in vitro testing and were confirmed by the midazolam drug-drug interaction component of the study. The rationale for discontinuing dose escalation was based on the favorable safety profile of RO4929097 and because plasma concentrations were achieved that exceeded efficacious exposure levels established in animal xenograft studies at human doses ≥ 6 mg. Efficacious levels were attained even in dose cohorts that demonstrated an impact on RO4929097 exposure from CYP3A4 autoinduction. With 10-mg daily dosing in schedule C, the effect of autoinduction became negligible. However, a decrease in midazolam exposure after repeated dosing of RO4929097 indicated that the potential drug-drug interaction liability still exists in schedule C. The effects of RO4929097 autoinduction and drug-drug interaction potential could limit clinical utility by limiting the ability to combine RO4929097 with anticancer agents metabolized hepatically (eg, paclitaxel).

Weak to moderate PK/PD correlations were observed for A β -40 protein and VEGFR-2 protein in plasma, and Hes-1 mRNA expression in hair follicles, consistent with expected RO4929097 PD effects (Data Supplement). Although none of the baseline values of analyzed biomarkers could be correlated with response to RO4929097, exploratory analyses indicated differences in IL-6 plasma changes in patients with and without clinical benefit on day 1 of cycle 1 and day 2 of cycle 2. IL-6 levels increased to a lesser degree in patients with a longer duration of SD versus those with early PD, potentially suggesting that IL-6 is involved in tumor escape mechanisms.

Encouraging evidence of antitumor activity was observed. This includes one RECIST partial response in a patient with a colorectal adenocarcinoma with neuroendocrine features and progressive disease following four prior treatment regimens. A mixed response was observed in one patient with sarcoma, and prolonged SD was observed in several other patients. Activity in melanoma was also observed, including a minor response in one patient and a durable FDG-PET/CT response in one patient. Prolonged SD among other patients with melanoma, sarcoma, and ovarian carcinoma was observed.

In conclusion, RO4929097 was well tolerated when administered by using both intermittent and continuous dosing schedules. Several Cancer Therapy Evaluation Program (CTEP)-sponsored phase I and II studies are being conducted with RO4929097 alone and in combination. Most of these studies use the three-on/four-off schedule at a starting dose of 20 mg, which is a dose expected to not result in significant autoinduction and potential drug-drug interaction in this schedule. Recruitment on most of these studies is ongoing and results are awaited with interest.

AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

Although all authors completed the disclosure declaration, the following author(s) indicated a financial or other interest that is relevant to the subject matter under consideration in this article. Certain relationships marked with a "U" are those for which no compensation was received; those relationships marked with a "C" were compensated. For a detailed description of the disclosure categories, or for more information about ASCO's conflict of interest policy, please refer to the Author Disclosure Declaration and the Disclosures of Potential Conflicts of Interest section in Information for Contributors.

Employment or Leadership Position: Stanislaw M. Mikulski, Hoffmann-La Roche (C); John F. Boylan, Hoffmann-La Roche (C); Zhi-Xin Xu, Hoffmann-La Roche (C); Ka Wang, Hoffmann-La Roche (C); Astrid Koehler, Roche Diagnostics (C); James Song, Hoffmann-La Roche (C); Steven A. Middleton, Hoffmann-La Roche (C); Jonathan Deutsch, Hoffmann-La Roche (C); Mark DeMario, Hoffmann-La Roche (C) **Consultant or Advisory Role:** None **Stock Ownership:** Stanislaw M. Mikulski, Hoffmann-La Roche; John F. Boylan, Hoffmann-La Roche; Zhi-Xin Xu, Hoffmann-La Roche; Ka Wang, Hoffmann-La Roche; Astrid Koehler, Hoffmann-La Roche AG; Steven A. Middleton, Hoffmann-La Roche; Jonathan Deutsch, Hoffmann-La Roche; Mark DeMario, Hoffmann-La Roche **Honoraria:** None **Research Funding:** Anthony W. Tolcher, Hoffmann-La Roche; Wells A. Messersmith, Hoffmann-La

Roche; Kyriakos P. Papadopoulos, Hoffmann-La Roche; Eunice L. Kwak, Hoffmann-La Roche; Darlene G. Gibbon, Hoffmann-La Roche; Amita Patnaik, Hoffmann-La Roche; Razelle Kurzrock, Hoffmann-La Roche; Jennifer J. Wheler, Hoffmann-La Roche **Expert Testimony:** None **Other Remuneration:** None

AUTHOR CONTRIBUTIONS

Conception and design: Anthony W. Tolcher, Wells A. Messersmith, Stanislaw M. Mikulski, John F. Boylan, Steven A. Middleton, Mark DeMario, Razelle Kurzrock, Jennifer J. Wheler

Provision of study materials or patients: Anthony W. Tolcher, Wells A. Messersmith, Gerald S. Falchook, Geoffrey I. Shapiro, Jennifer J. Wheler

Collection and assembly of data: Anthony W. Tolcher, Wells A. Messersmith, Kyriakos P. Papadopoulos, Eunice L. Kwak, Darlene G. Gibbon, Amita Patnaik, Gerald S. Falchook, Arvind Dasari, Ka Wang, Astrid Koehler, Jonathan Deutsch, Mark DeMario, Razelle Kurzrock **Data analysis and interpretation:** Anthony W. Tolcher, Wells A. Messersmith, Stanislaw M. Mikulski, Gerald S. Falchook, Geoffrey I. Shapiro, John F. Boylan, Zhi-Xin Xu, Ka Wang, Astrid Koehler, James Song, Steven A. Middleton, Jonathan Deutsch, Mark DeMario, Razelle Kurzrock

Manuscript writing: All authors

Final approval of manuscript: All authors

REFERENCES

- Miele L, Miao H, Nickoloff BJ: NOTCH signaling as a novel cancer therapeutic target. *Curr Cancer Drug Targets* 6:313-323, 2006
- Bolós V, Grego-Bessa J, de la Pompa JL: Notch signaling in development and cancer. *Endocr Rev* 28:339-363, 2007
- Bray SJ: Notch signalling: A simple pathway becomes complex. *Nat Rev Mol Cell Biol* 7:678-689, 2006
- Luistro L, He W, Smith M, et al: Preclinical profile of a potent gamma-secretase inhibitor targeting notch signaling with in vivo efficacy and pharmacodynamic properties. *Cancer Res* 69:7672-7680, 2009
- Borggreve T, Oswald F: The Notch signaling pathway: Transcriptional regulation at Notch target genes. *Cell Mol Life Sci* 66:1631-1646, 2009
- D'Souza B, Miyamoto A, Weinmaster G: The many facets of Notch ligands. *Oncogene* 27:5148-5167, 2008
- Rizzo P, Osipo C, Foreman K, et al: Rationale targeting of Notch signaling in cancer. *Oncogene* 27:5124-5131, 2008
- Phng LK, Gerhardt H: Angiogenesis: A team effort coordinated by notch. *Dev Cell* 16:196-208, 2009
- Dufraigne J, Funahashi Y, Kitajewski J: Notch signaling regulates tumor angiogenesis by diverse mechanisms. *Oncogene* 27:5132-5137, 2008
- Center for Drug Evaluation and Research (CDER): Guidance for Industry: Estimating the Maximum Safe Starting Dose in Initial Clinical Trials for Therapeutics in Adult Healthy Volunteers. U.S. Department of Health and Human Services, Food and Drug Administration, July 2005

Acknowledgment

We thank Stacey Ukrainskyj for major contributions related to data retrieval and study monitoring. We also thank Annette Schell-Steven, MD, for conducting the amyloid-beta 40 (Aβ-40) analyses and Juliette Molnos, MD, for the real polymerase chain reaction reverse transcriptase polymerase chain reaction analyses. We acknowledge Amartya Basu, PhD, and Christoph Rutz, PhD, for their support of the pharmacodynamic marker assay implementation.