Original Article

Phase 2 Study of RO4929097, a Gamma-Secretase Inhibitor, in Metastatic Melanoma: SWOG 0933

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BACKGROUND: Aberrant Notch activation confers a proliferative advantage to many human tumors, including melanoma. This phase 2 trial assessed the antitumor activity of RO4929097, a gamma-secretase inhibitor of Notch signaling, with respect to the progression-free and overall survival of patients with advanced melanoma. **METHODS:** Chemotherapy-naive patients with metastatic melanoma of cutaneous or unknown origin were treated orally with RO4929097 at a dose of 20 mg daily 3 consecutive days per week. A 2-step accrual design was used with an interim analysis of the first 32 patients and with continuation of enrollment if 4 or more of the 32 patients responded. **RESULTS:** Thirty-six patients from 23 institutions were enrolled; 32 patients were evaluable. RO4929097 was well tolerated, and most toxicities were grade 1 or 2. The most common toxicities were nausea (53%), fatigue (41%), and anemia (22%). There was 1 confirmed partial response lasting 7 months, and there were 8 patients with stable disease lasting at least through week 12, with 1 of these continuing for 31 months. The 6-month progression-free survival rate was 9% (95% confidence interval [CI], 2%-22%), and the 1-year overall survival rate was 50% (95% CI, 32%-66%). Peripheral blood T-cell assays showed no significant inhibition of the production of interleukin-2, a surrogate pharmacodynamic marker of Notch inhibition, and this suggested that the drug levels were insufficient to achieve Notch target inhibition. **CONCLUSIONS:** RO4929097 showed minimal clinical activity against metastatic melanoma in this phase 2 trial, possibly because of inadequate exposure to therapeutic drug levels. Although Notch inhibition remains a compelling target in melanoma, the results do not support further investigation of RO4929097 with this dose and schedule. ***Cancer* 2015;121:432-40.** © *2014 American Cancer Society.*

KEYWORDS: gamma-secretase inhibitor, metastatic melanoma, Notch, RO4929097.

INTRODUCTION

The Notch pathway is a highly conserved signaling cascade that plays an essential role in the normal development of a variety of human tissues through the regulation of gene expression that controls stem cell homeostasis and differentiation, cell survival, and apoptosis.[1] In oncogenesis, dysregulation of the Notch pathway confers to many human tumors a proliferative advantage, resistance to apoptosis, and the ability to maintain a stem cell–like phenotype.[2,3]

The role of aberrant Notch signaling in melanoma has garnered a great deal of interest in recent years. Melanoma is a particularly aggressive cancer with the ability to metastasize at a relatively small primary tumor size. Two well-established steps of melanoma invasion and metastasis include the loss of cell adhesion molecule E-cadherin and the acquisition of melanoma cell adhesion molecule.[4,5] Multiple groups have demonstrated that amplified Notch signaling contributes to melanoma growth in vitro and in vivo and promotes a more aggressive phenotype, at least in part by inhibiting E-cadherin expression and upregulating melanoma cell adhesion molecule.[6-9]

Notch signaling relies on the intramembrane cleavage of the Notch receptor by a gamma-secretase complex to release a Notch intracellular domain that translocates to the nucleus to activate the transcription of target genes, including hairy/enhancer of split related with YRPW motif protein 1 (Hey1) and hairy and enhancer of split 1 (Hes1), involved in cell fate

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Presented in part at the 48th Annual Meeting of the American Society of Clinical Oncology; June 4, 2012; Chicago, IL.

The authors thank Terri Li (Immunohistochemistry Core Facility, University of Chicago) for performing the Notch1 staining.

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DOI: 10.1002/cncr.29055, **Received:** May 15, 2014; **Revised:** July 12, 2014; **Accepted:** July 15, 2014, **Published online** September 23, 2014 in Wiley Online Library (wileyonlinelibrary.com)

determination, tissue differentiation, and vasculogenesis. The understanding of this pathway has fueled the investigation of gamma-secretase inhibitors (GSIs) as a therapeutic strategy for inhibiting Notch signaling in melanoma as well as other cancers.

In addition to a role for Notch signaling in melanoma progression, the Notch pathway has also been shown to be critical for normal T-cell development and function.[10,11] Therefore, because of the importance of T-cell immunity in the control of melanoma in particular, it is critical to assess the effects of these agents on T-cell function in patients as pharmacologic strategies for Notch inhibition in cancer therapy are developed. An analysis of the effects on T cells, with the production of interleukin-2 (IL-2), a potent T-cell growth factor, used as a measure of T-cell function, could also serve as an indirect pharmacodynamic biomarker of Notch inhibition.[12]

RO4929097 is a small-molecule inhibitor of gamma-secretase with high oral bioavailability and is a potent and selective inhibitor of gamma-secretase: it leads to the blockade of Notch signaling in tumor cells. A phase 1 dose-escalation study in 110 patients with refractory metastatic or locally advanced solid tumors demonstrated that RO4929097 was well tolerated, with the majority (95%) of toxicities being grade 1 or 2 fatigue and mucocutaneous effects.[13] Most toxicities, including all that were considered dose-limiting, were more common with a 7-days-on/14-days-off schedule versus a 3-days-on/4-days-off schedule for 2 of 3 weeks. Antitumor activity was seen in 26 of the 96 evaluable patients (27%), with 1 partial response from a colonic adeno/neuroendocrine tumor, 1 mixed response from an epithelioid sarcoma, and 1 minor response and 1 nearly complete positron emission tomography response from cutaneous metastases of melanoma; the other 22 patients had stable disease for at least 3 to 6 months (most frequently melanoma, sarcoma, and ovarian carcinoma). The clinical outcomes of this phase 1 trial provided the rationale for continuing the investigation of RO4929097 in patients with metastatic melanoma on the 3-days-on/4-days-off schedule with a 20-mg dose level, which demonstrated less auto-induction of drug metabolism and potential for drug-drug interactions than the other dose and schedule. We, therefore, conducted a phase 2 clinical trial of RO4929097 in 32 patients with metastatic melanoma. The study objectives were to assess the 6-month progression-free survival (PFS) and the 1-year overall survival (OS) in advanced treatment–naive melanoma patients with pooled data from a well-accepted advanced melanoma meta-analysis to set the levels of activity that would support further study of this regimen. We also wished to further assess the safety and tolerability of the regimen and to evaluate the effects of the study drug on T-cell function and Notch target genes.

MATERIALS AND METHODS

The trial was performed by the Southwest Oncology Group (SWOG), and the investigational agent was provided by the Cancer Therapy Evaluation Program of the National Cancer Institute under an agreement with Roche/Genentech (ClinicalTrials.gov identifier NCT01120275). All study subjects provided voluntary, written informed consent through a document approved by the institutions' human subject protection committee. The protocol and all amendments were also approved by SWOG and by the regulatory committees at the participating institutions.

Patient Selection

Eligible patients had histologically confirmed stage IV melanoma of cutaneous or unknown origin (ocular and mucosal origins excluded) with measurable disease as defined by Response Evaluation Criteria in Solid Tumors 1.1. Study subjects were not preselected for the expression of known oncogenic pathways or for any marker of Notch pathway activation; however, patients were required to have archival or fresh tissue available from before the study for laboratory correlates. Patients must have had no prior cytotoxic chemotherapy for stage IV disease (prior immunotherapy and adjuvant therapy were allowed) and no history of central nervous system metastasis. They were required to have a Zubrod performance status of 0 to 1 and adequate hematologic, hepatic, cardiac, and renal function with a leukocyte count $\geq 3000/\mu L$, an absolute neutrophil count $\geq 1500/\mu L$, a platelet count $\geq 100{,}000/\mu L$, a hemoglobin level ≥ 9 g/dL, a creatinine clearance ≥ 60 mL/min, a total bilirubin level $\leq$ the institutional upper limit of normal, aspartate aminotransferase and alanine aminotransferase levels ≤ 2.5 times the institutional upper limit of normal, and a QTcF ≤ 500 milliseconds. Women of childbearing potential were required to have a negative serum pregnancy test, and subjects of both sexes were required to practice adequate birth control during protocol participation.

Treatment and Monitoring

The study drug RO4929097 was given orally on an empty stomach at 20 mg daily on days 1 to 3, 8 to 10, and 15 to 17 of every 3-week cycle of therapy. This was the recommended phase 2 dose based on the phase 1 dose escalation study. Treatment was given on a continuous schedule

with dose adjustments and brief breaks from therapy specified in the protocol for treatment-related toxicities. Drug compliance was recorded by patients on an intake calendar that was submitted to the research team, along with all unused tablets, at each study visit. Before dispensing RO4929097, the investigator confirmed and documented the patient's use of 2 contraceptive methods and dates of negative pregnancy tests and confirmed the patient's understanding of the teratogenic potential of the study drug. Patients were removed from the study for disease progression, symptomatic deterioration, unacceptable toxicity, or a treatment delay >14 days for any reason or at the patient's request.

Additional patient consent (optional) was requested for fresh tumor samples before the study (if the patient had not already undergone a prestudy biopsy) and at week 3 of cycle 1. The tumor tissue could be obtained by surgical excision, surgical core biopsy, or computed tomography–guided core biopsy.

Patients were evaluated with a history, physical, laboratory analyses (complete blood count, metabolic panel, pregnancy test, and thyroid-stimulating hormone), electrocardiogram, toxicity assessment, and drug compliance assessment at least every 3 weeks at the beginning of each cycle. Imaging studies for disease assessment were performed before the study, at week 7, at week 13, and then as clinically indicated until progression. Specific guidance for dose modifications was provided for the management of hematologic toxicities, hypertension, electrolyte abnormalities, diarrhea, and other nonhematologic toxicities.

Statistical Methods

The primary objectives of this phase 2 trial were to assess 6-month PFS and 1-year OS with historical benchmarks established by a large meta-analysis of phase 2 cooperative group clinical trials by Korn et al.[14] Our objective was to distinguish between true 6-month PFS probabilities of $<15\%$ and $>30\%$ and true 1-year OS probabilities of $<35\%$ and $>50\%$. The results of this study would be considered evidence that this agent warranted further study if at least 17 of 72 eligible patients survived and were progression-free for at least 6 months or if 31 or more eligible patients survived at least 1 year. A 2-step accrual design was used, and this required an interim analysis of the first 32 patients evaluable for a response. The criterion for the continuation of enrollment to 72 patients would be the observation of 4 or more clinical responses in the first 32 patients or the observation that 9 or more of the first 32 patients evaluable for 6-month PFS were alive and progression-free at that milestone. An objective response was used in lieu of the primary study objectives of PFS and OS as a criterion for trial continuation because the prolonged time to reach the PFS and OS endpoints would have obviated their utility as interim checkpoints for this actively accruing trial. The secondary objectives were to investigate the relationship between the Notch activation status, the Notch target gene expression in the tumor, and the clinical outcome; to study the effects of the drug on T-cell function; to assess the objective response rate (ORR) and disease control rate (DCR), which was defined as the number of patients with a best response of stable disease or better 12 weeks after the initiation of therapy; and to assess toxicity. PFS and OS estimates were calculated with the Kaplan-Meier method.[15] Confidence intervals (CIs) for the medians were constructed with the method of Brookmeyer and Crowley,[16] and CIs for point estimates (eg, 6-month PFS) were calculated with a log-log transformation. Clopper-Pearson CIs were calculated for binary outcomes (eg, ORR). An exploratory analysis of the relationship between biomarker values and clinical outcomes was performed. The biomarker values were treated in 2 ways: first as continuous variables with a log transformation if the values were skewed and then by dichotomization at the observed median. Cox regression was used to analyze the relationship of the biomarker values with PFS and OS. Logistic regression was used to evaluate the relationships with ORR and DCR. Fisher's exact test was used to evaluate the dichotomized variables. To explore the relationship between the change in T-cell IL-2 production values from the baseline to week 3 and OS and PFS, a landmark analysis was performed, with OS and PFS beginning to be measured in week 3. All analyses were performed with SAS version 9.2.

Laboratory Correlates

The secondary objectives of this study included the evaluation of Notch1 by immunohistochemistry and real-time reverse transcription–polymerase chain reaction (RT-PCR) for Hey1 and Hes1 in pretreatment patient samples (and on-treatment samples, if available) and the exploration of potential indicators of Notch activity in on-treatment biopsies and their association with a clinical response to the study drug. Immunohistochemistry was performed on pretreatment paraffin tissue. The sections were prepared at SWOG and shipped to the University of Chicago for analysis. The slides were stained with antibodies specific for total Notch1 (#sc-6014, Santa Cruz Biotechnology) versus a secondary antibody alone. Hematoxylin and eosin staining was performed in parallel.

TABLE 1. Baseline Patient Characteristics (n = 32)

Patient Characteristic	Value
Age (y)	
Median	60.9
Minimum	32.8
Maximum	85.9
Sex, n (%)	
Male	22 (69)
Female	10 (31)
Performance status, n (%)	
0	24 (75)
1	8 (25)
Primary type, n (%)	
Cutaneous	22 (69)
Unknown primary	10 (31)
Site(s) of metastases, n (%)	
Bone	8 (25)
Liver	12 (38)
Lymph node, skin, soft tissue	17 (53)
Lung	17 (53)
Other nonvisceral	1 (3)
Other visceral	9 (28)
Elevated lactate dehydrogenase, n (%)	
No	19 (59)
Yes	13 (41)
Prior systemic therapy, n (%)	
No	23 (72)
Yes	9 (28)
Type of prior systemic therapy, n (%)	
Adjuvant interferon α	5 (16)
Interleukin-2	2 (6)
Other (denileukin diftitox, sargramostim)	2 (6)

Semiquantitative scoring was used to determine the immunohistochemistry results, which were manually evaluated and scored as negative, +, ++, or +++ by a pathologist. For quantitative RT-PCR, total RNA was extracted from formalin-fixed, paraffin-embedded tissue slides with an RNeasy FFPE kit (#74404, Qiagen) according to the manufacturer's procedure. Complementary DNAs were prepared with a high-capacity complementary DNA reverse transcription kit (#4368814, Applied Biosystems). Quantitative RT-PCR was performed with primer/probe sets specific for Hey1 (Hs01114113_m1, Applied Biosystems) and Hes1 (Hs00172878_m1, Applied Biosystems). Human β-actin (4352935E_3614263566, Life Technologies Corp) was used as an internal standard.

The effect of the study drug on T-cell function was also investigated through the evaluation of IL-2 production via pretreatment and on-treatment patient peripheral blood mononuclear cells (PBMCs) stimulated with the superantigen staphylococcal enterotoxin A (SEA). This assay also served as a pharmacodynamic biomarker. Cryopreserved PBMCs were thawed, counted, and resuspended at 1×10^6/mL in Iscove's modified Dulbecco's medium and then seeded at 100,000 PBMCs per well in a 96-well flat-bottom tissue culture plate. Cells were treated with the medium alone, the superantigen SEA at 100 ng/mL, or SEA (100 ng/mL) plus a nonclinical GSI (InSolution γ-Secretase Inhibitor X, #565771, Calbiochem; 10 μM) for 24 hours. IL-2 levels in the supernatant were measured with an enzyme-linked immunosorbent assay.

RESULTS

Patient Characteristics

Thirty-six patients from 23 SWOG institutions were registered for the first stage of this study between January 2011 and November 2011. The study was then closed after not enough activity was shown to warrant opening the second stage of accrual. Three patients were ineligible: 2 had no measurable disease at the baseline per the Response Evaluation Criteria in Solid Tumors, and 1 had inadequate renal function. In addition, 1 eligible patient, after giving initial consent, refused the protocol treatment. For the 32 evaluable patients, the median age was 60.9 years (range, 32.8–85.9 years), 69% were male, and 41% had a serum lactate dehydrogenase level greater than the institutional upper limit of normal. The sites of metastases were node/soft tissue/skin (53%), lungs (53%), liver (38%), and bone (25%). Nine patients (28%) received prior systemic therapies, including adjuvant interferon α (16%), IL-2 (6%), denileukin diftitox (3%), and sargramostim (3%). Mutational testing was not required for enrollment, and the BRAF mutation status of the study patients was not recorded. The patient characteristics are listed in Table 1.

Toxicities

The majority of the toxicities attributed to RO4929097 were grade 1 or 2. The most common adverse events (across all grades) were nausea (53%), fatigue (41%), anemia (22%), anorexia (19%), headache (13%), constipation (13%), and diarrhea (13%). Six patients experienced grade 3 events (see Table 2). There were no grade 4 or 5 toxicities.

Clinical Responses

A 2-stage accrual design was applied as detailed previously, and after the accrual of the first 32 evaluable patients, the clinical outcomes did not meet the criteria for the accrual of additional patients. There was 1 confirmed partial response lasting 7 months (ORR, 3%; 95% CI, 0%-16%); this patient was taken off the protocol therapy for progression at 10 months but remained alive at 28+ months after protocol entry. Eight additional patients had stable disease lasting at least through the

TABLE 2. Toxicities of Therapy

Adverse Event	Any Grade, n (%)	Grade 3, n (%)
Nausea	17 (53)	1 (3)
Fatigue	13 (41)	1 (3)
Anemia	7 (22)	1 (3)
Anorexia	6 (19)	0 (0)
Diarrhea	4 (13)	0 (0)
Constipation	4 (13)	0 (0)
Headache	4 (13)	0 (0)
Hypophosphatemia	3 (9)	2 (6)
Vomiting	3 (9)	0 (0)
Hyponatremia	3 (9)	0 (0)
Dysgeusia	3 (9)	0 (0)
Transaminase elevation	2 (6)	1 (3)
Pain in extremity	2 (6)	1 (3)
Abdominal pain	2 (6)	1 (3)
Decreased lymphocyte count	2 (6)	1 (3)
QTc prolongation	2 (6)	1 (3)
Small intestinal obstruction	1 (3)	1 (3)
Stroke	1 (3)	1 (3)
Any adverse event	30 (94)	6 (19)

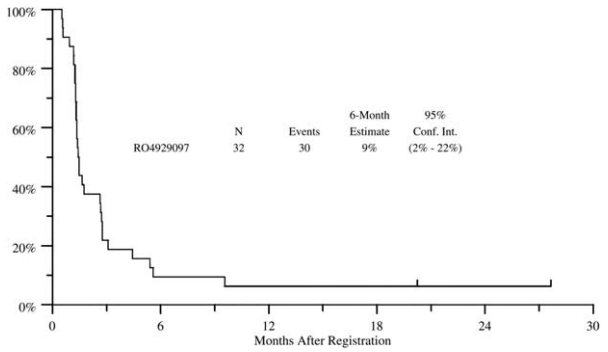

Figure 1. Progression-free survival.

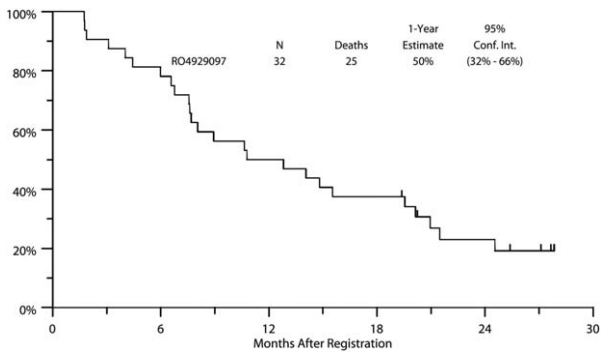

Figure 2. Overall survival.

week 12 assessment as their best response to therapy. Among these patients with stable disease, 1 patient with BRAF wild-type melanoma remained on the protocol treatment for 31 months before stopping because of disease progression. This patient had not received any additional therapy other than the study drug. This patient and the 1 patient with a partial response both received adjuvant interferon α as their only prior systemic treatment.

The DCR at 12 weeks was 31% (95% CI, 16%-50%). The median PFS was 1.5 months (95% CI, 1.3-2.6 months), and the median OS was 13 months (95% CI, 8-20 months). With the model proposed by Korn et al,[14] predicted values for 6-month PFS and 1-year OS were calculated on the basis of the observed distributions of sex, performance status, and visceral metastases. A 1-sided exact binomial test was used to test the hypothesis that the observed 6-month PFS, 1-year OS, or both were superior to these predicted values. The 6-month PFS was 9% (95% CI, 2%-22%), which was not superior to the predicted value of 17% ($P = .91$). The 1-year OS was 50% (95% CI, 32%-66%), which was not superior to the predicted value of 44% ($P = .32$). The Kaplan-Meier estimates for PFS and OS are shown in Figures 1 and 2, respectively.

Analysis of Peripheral T-Cell Function

Notch pathway inhibition has been shown to inhibit T-cell function in vitro,[17] and Notch signaling is required for T-cell development.[18,19] Therefore, we analyzed the effects of RO4929097 administration on the activation of peripheral T cells to assess both the potential impact on immune function and the potential as a pharmacodynamic biomarker for drug effect. Cryopreserved PBMC preparations were available from 23 patients. The production of IL-2 was evaluated in response to the polyclonal T-cell stimulus provided by SEA before the study and during week 3 on treatment. However, no significant difference was observed with this ex vivo assay. In contrast, all patients showed inhibition of T-cell cytokine production when a nonclinical GSI was included during the in vitro stimulation (Fig. 3). Therefore, these data suggest that the Notch pathway was likely not adequately inhibited in week 3 of the administration of RO4929097 in treated patients. We investigated in a preliminary manner the relationship between clinical outcomes and baseline IL-2 levels as well as the change in IL-2 levels at week 3 in 20 eligible patients. There was no significant association found between baseline IL-2 levels and PFS ($P = .21$), OS ($P = .58$), ORR ($P = .74$), or DCR. ($P = .51$) or between the change at week 3 and PFS ($P = .48$), OS ($P = .88$), ORR ($P = .23$), or DCR ($P = .64$). However, the 1 patient who achieved a partial response on the study drug (patient 229180 in Fig. 3) also experienced a 65% drop in IL-2 production, whereas the median change in IL-2 production for all study subjects was 0%.

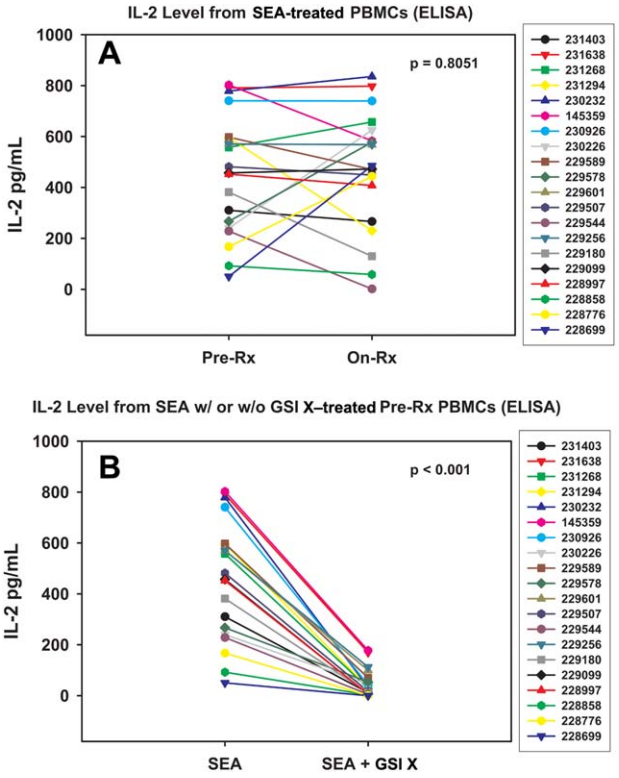

Figure 3. T-cell functional studies. (A) SEA-stimulated IL-2 production by peripheral blood lymphocytes and (B) control inhibition with a nonclinical gamma-secretase inhibitor. ELISA indicates enzyme-linked immunosorbent assay; GSI, gamma-secretase inhibitor; IL-2, interleukin-2; PBMC, peripheral blood mononuclear cell; Rx, treatment; SEA, staphylococcal enterotoxin A.

Notch Gene Correlates in Tumor Tissue

Fresh pretreatment and 3-week on-treatment tumor biopsies for gene expression profiling were obtained from 1 patient. When the known Notch target genes Hey1 and Hes1 were interrogated, no decrease was seen at the on-treatment time point (Fig. 4). Although these data were obtained from only 1 patient, they are consistent with the peripheral blood T-cell surrogate tissue analysis and suggest that stable inhibition of the Notch pathway might not have been achieved in tumor tissue.

Archived formalin-fixed, paraffin-embedded tumor tissue was available from 12 eligible patients and was analyzed for baseline parameters indicative of Notch pathway activation. All samples showed some degree of staining for Notch1 by immunohistochemistry (Fig. 5), and this suggested the availability of Notch for engagement. To further explore whether the Notch pathway was activated, quantitative RT-PCR was performed for the expression of the Notch target genes Hes1 and Hey1. The signal for Hes1 was more robust and was chosen as the reference for

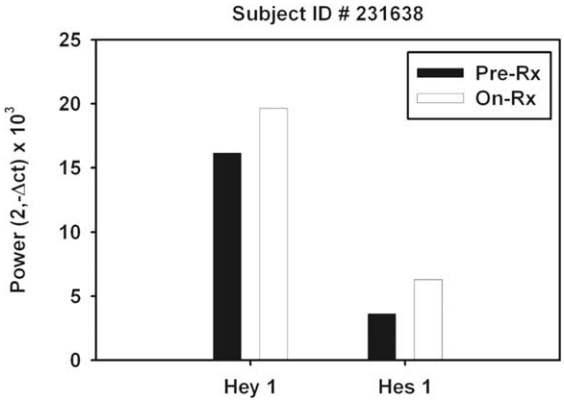

Figure 4. Pretreatment and on-treatment tumor tissue from 1 patient showed a lack of inhibition of Notch target genes Hey1 and Hes1 according to real-time reverse transcription–polymerase chain reaction. Hes1 indicates hairy and enhancer of split 1; Hey1, hairy/enhancer of split related with YRPW motif protein 1; Rx, treatment.

activation. All samples showed detectable expression of Hes1 messenger RNA above the background at the baseline; most of these showed expression of Hey1 as well (Fig. 6). Hey1 expression alone was detected in 1 additional sample. These results confirm that the Notch pathway is indeed activated in a major fraction of melanoma patients. When the patient samples were divided into high and low expression of Hes1 and Hey1 according to whether the expression was above or below the median for all the samples, there was no significant association found between high expression of Hes1 and PFS ($P = .83$), OS ($P = .70$), ORR ($P = 1.00$), or DCR ($P = .55$) or between high expression of Hey1 and PFS ($P = .48$), OS ($P = .59$), ORR ($P = .45$), or DCR ($P = 1.00$). However, the sample size was small and was not powered to evaluate a correlation between target gene expression and clinical outcomes. Because tumors in our study were not required to be molecularly characterized, we were unable to study a potential relationship between Notch1 activation and the presence of other oncogenic or related pathways such as BRAF activation.

DISCUSSION

This phase 2 clinical trial evaluated the safety, antitumor activity, and laboratory correlates of the GSI RO4929097 in patients with stage IV cutaneous melanoma. Although well-tolerated, RO4929097 demonstrated minimal activity with the recommended phase 2 dose and schedule in these molecularly unselected patients with advanced melanoma. One of the known downstream effects of Notch inhibition is the impairment of T-cell function, which is

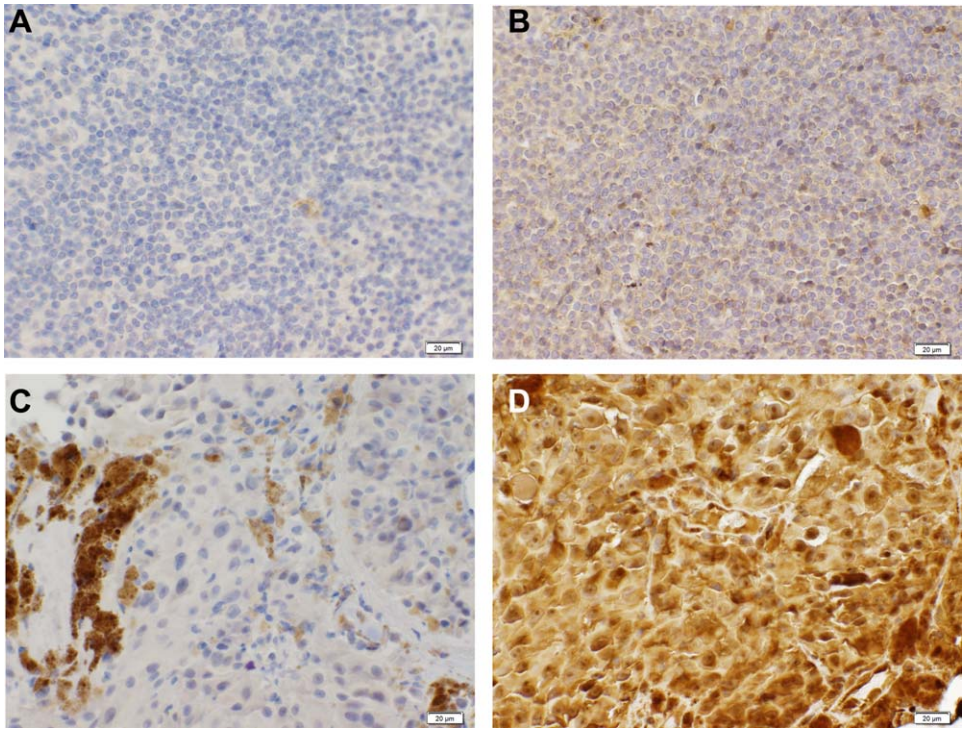

Figure 5. Notch1 expression was seen via immunohistochemistry in all 12 available patient tumor samples (7 had moderate expression, and 5 had strong expression). Two representative patient samples are shown here of (B) moderate Notch1 expression and (D) strong Notch1 expression, along with their IgG controls (A) and (C), respectively.

perhaps best demonstrated by the ability of Notch inhibitors to block graft-versus-host disease in animal models.[20,21] The absence of T-cell functional impairment, as measured by changes in IL-2 production, in most patients after treatment with the study drug, in addition to the lack of downregulation of Notch target genes Hey1 and Hes1 after 3 weeks on the study drug in 1 patient, suggests that sustained target inhibition may not have been achieved in most subjects. A definitive analysis would require serial biopsies from a greater number of patients. Although a firm conclusion cannot be drawn from a single data point, it is of interest that the only patient who experienced a partial response to the study drug also demonstrated a 65% drop in IL-2 production, whereas the median change in IL-2 production of the study patients overall was 0%; this suggests the possibility that more effective Notch inhibition may have been achieved in this patient.

One possible explanation for why responses to RO4929097 were higher in the phase 1 trial may be that the more intense dosing schedules investigated in that study, while causing more toxicity, may have also achieved better target inhibition. All 4 patients who experienced tumor regression in the phase 1 study had been treated on the 7-days-on/14-days-off schedule, and the best responder, who had an objective partial response in that trial, was treated at a dose of 40 mg, whereas a 20-mg dose and a 3-days-on/4-days-off schedule were used in the current study. The reported stable disease rate was also slightly higher (32%) on the 7-days-on/14-days-off schedule versus the 3-days-on/4-days-off schedule (25%). A phase 2 study of RO4929097 in metastatic colorectal cancer treated with at least 2 prior lines of systemic chemotherapy also used the 20-mg dose on a 3-days-on/4-days-off schedule and observed no objective responses among 33 evaluable patients despite immunohistochemistry evidence for the expression of Notch receptor, intracellular Notch, and transcriptional target HES1 in the majority of patient tumors.[22] However, repeated dosing higher than 20 mg on a 3-days-on/4-days-off schedule also led to significant cytochrome P450 3A4 auto-induction in the phase 1 trial, and this poses an additional pharmacokinetic ceiling further limiting the narrow therapeutic index of this drug. Notably, in T-cell acute lymphoblastic leukemia, a tumor with a greater than 50% incidence of activating mutations in Notch1, the use of GSIs also led to disappointing clinical results, largely because of an unfavorable therapeutic index.[23] Thus, although we did not perform pharmacokinetic analyses in this study, we believe that the use of the recommended phase 2 dose

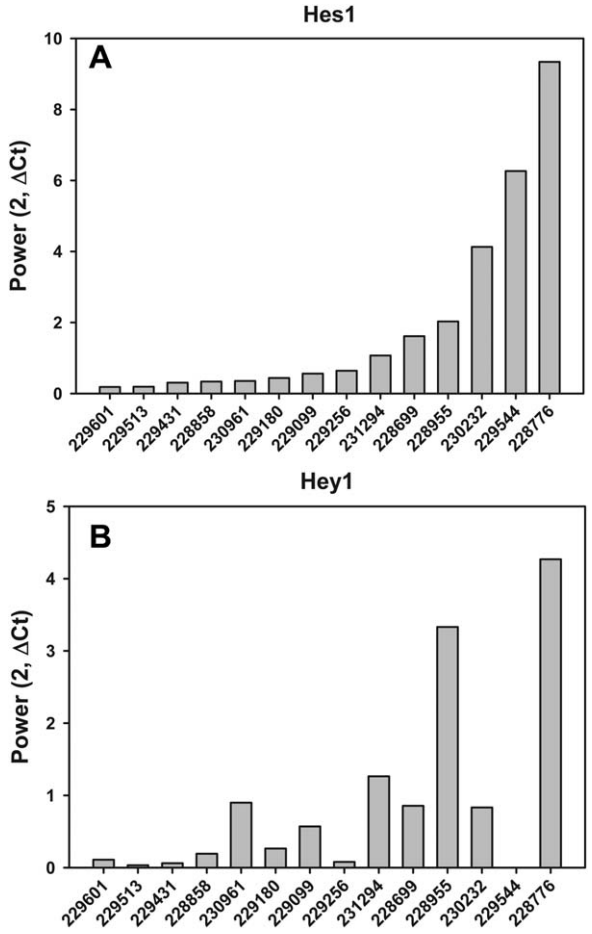

Figure 6. Notch1 activation was demonstrated by increased expression of (A) Hes1 and (B) Hey1 messenger RNA at the baseline. Hes1 indicates hairy and enhancer of split 1; Hey1, hairy/enhancer of split related with YRPW motif protein 1.

from phase 1 development could have underexposed patients to the drug and/or resulted in a gradual fall in peak and/or steady-state drug exposures.

It remains to be seen whether the degree and nature of T-cell impairment induced by optimal Notch inhibition will cause a clinical impact on the patient's endogenous antitumor T-cell responses to melanoma and how this would affect the timing of Notch therapy with respect to immune-based therapies in a patient's treatment course. The parallel development of both immunotherapies and targeted signal transduction inhibitors for melanoma, the latter of which could also adversely affect T-cell function, is a potential challenge that may require logical adjustments in scheduling to maximize therapeutic synergy in combination. The downstream effects on T-cell function by Notch inhibitors remain an important area of investigation in other tumor types also as the landscape of immunotherapy continues to unfold, and T-cell checkpoint blockade with anti–cytotoxic T lymphocyte antigen 4 and/or anti–programmed death 1 is rapidly becoming a mainstay of treatment for multiple cancers.

Because of the auto-induction of metabolism of RO4929097 and the gastrointestinal toxicities, which have limited higher dosing of this and other GSIs,[24-26] the use of GSIs for targeting the Notch pathway in tumors may not be the best direction for continued future drug development. However, our data confirming that the pathway is active in most melanomas, combined with laboratory data indicating that Notch inhibition has major antitumor activity against melanoma both in vitro and in vivo, suggest that alternative strategies for targeting the Notch pathway are warranted. A monoclonal antibody to a Notch ligand has been shown in preclinical studies to be a promising mechanism for achieving Notch inhibition,[20,27] and it is currently being studied in a phase 1 clinical trial (NCT01577745). As these and other more effective Notch inhibitors are studied as cancer therapeutics, a more detailed analysis of effects on T-cell subsets will be warranted. Aberrant Notch signaling is a critical pathway in tumorigenesis and remains a promising therapeutic target for cancer treatment; however, this phase 2 trial does not support the continued development of the GSI RO4929097 in unselected patients with metastatic melanoma.

FUNDING SUPPORT

This work was supported in part by Public Health Service Cooperative Agreements awarded by the National Cancer Institute and the US Department of Health and Human Services (CA3210, CA38926, CA27057, CA35261, CA20319, CA46282, CA67575, CA45808, CA35128, CA22433, CA95860, CA95860, CA35090, CA35178, CA52654, CA35176, CA128567, CA35421, CA13612, CA12644, CA35119, CA35431, CA68183 and CA11083).

CONFLICT OF INTEREST DISCLOSURES

Thomas F. Gajewski served on an advisory board for Roche/Genentech in 2013 for a drug that was not used in this study and received grants/personal fees. Vernon K. Sondak reports personal fees from Merck (speaker's bureau and consultant/advisory board), Amgen (consultant/advisory board), OncoSec (consultant/advisory board), MabVax (consultant/advisory board), Polynoma (consultant/advisory board), Bristol-Myers Squibb (data safety monitoring board), Glaxo Smith-Kline (data safety monitoring board), and Novartis (data safety monitoring board) outside the submitted work.

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