

Phase I Study of LY2940680, a Smo Antagonist, in Patients with Advanced Cancer Including Treatment-Naïve and Previously Treated Basal Cell Carcinoma

Johanna Bendell¹, Valerie Andre², Alan Ho³, Ragini Kudchadkar⁴, Michael Migden⁵, Jeffrey Infante¹, Ramon V. Tiu⁶, Celine Pitou², Trevor Tucker⁷, Les Brail⁸, and Daniel Von Hoff^{9,10}



Abstract

Purpose: The purpose of this study was to determine a recommended phase II dose and schedule of LY2940680 (taladegib) for safe administration to patients with locally advanced/metastatic cancer.

Experimental Design: This was a phase I, multicenter, open-label study of oral LY2940680. The maximum tolerable dose (MTD) was determined using a 3+3 design, the dose was confirmed, and then treatment-naïve and previously hedgehog (Hh)-inhibitor-treated patients with basal cell carcinoma (BCC) were enrolled.

Results: Eighty-four patients were treated (dose escalation, $n = 25$; dose confirmation, $n = 19$; and BCC dose expansion, $n = 40$). Common treatment-emergent adverse events were dysgeusia [41 (48.8%)], fatigue [40 (47.6%)], nausea [38 (45.2%)], and muscle spasms [34 (40.5%)]. Four patients experienced events (3 were grade 3; 1 was grade 2) that were considered dose-limiting toxicities

(DLT). The MTD was determined to be 400 mg because of DLTs and dose reductions. Pharmacokinetic analyses showed no clear relationship between exposure and toxicity. Analysis of Gli1 mRNA from skin biopsies from unaffected areas suggested that all doses were biologically active [inhibition median of 92.3% (80.9% to 95.7%)]. All clinical responses (per RECIST 1.1) were in patients with BCC ($n = 47$); the overall and estimated response rate was 46.8% (95% confidence interval, 32.1%–61.9%). Responses were observed in patients previously treated with Hh therapy (11/31) and in Hh treatment-naïve (11/16) patients.

Conclusions: LY2940680 treatment resulted in an acceptable safety profile in patients with advanced/metastatic cancer. Clinical responses were observed in patients with locally advanced/metastatic BCC who were previously treated with Hh therapy and in Hh treatment-naïve patients. *Clin Cancer Res*; 24(9); 2082–91. ©2018 AACR.

Introduction

The hedgehog (Hh) protein family is critical for embryonic development, cell growth, and differentiation after embryogenesis (1–3). This pathway is responsible for regulating normal physiologic processes, whereas aberrant activation of Hh signaling pathway is implicated in a number of human cancers, including basal cell carcinoma (BCC), medulloblastoma, and others (1, 2, 4). Hh antagonists have been evaluated clinically for the treatment of locally advanced and metastatic BCC; however, treatment resistance and tolerability issues remain major concerns (5, 6). In a previous phase I study with an Hh inhibitor, clinical activity was observed

specifically in BCC tumors. Approximately two thirds of the patients had non-BCC solid tumors, and one third had BCCs. The best response among those patients with non-BCC tumors was stable disease in half of the patients (53%), whereas of the 28 patients who had BCCs and were naïve to Hh treatment, 2 achieved a complete response (CR) and 6 achieved a partial response (PR). All responses occurred in patients with locally advanced disease (7).

LY2940680 is a potent Hh pathway inhibitor that binds to the human Smo (hSmo) receptor and competitively inhibits binding of an hSmo agonist (8). The primary objective of this study was to evaluate the safety and tolerability of LY2940680 (taladegib) in patients with advanced cancer, using dose-escalation and dose-confirmation phases, followed by a dose-expansion phase in patients with advanced BCC. An integrated pharmacokinetic/pharmacodynamic (PK/PD) model, along with the preclinical toxicology data, was used to derive the dose and dosing frequency of LY2940680. The secondary objectives were to evaluate PK parameters of LY2940680 and LSN3185556, its major circulating and equipotent metabolite, to evaluate antitumor activity and to document the clinical benefit rate and duration of response in patients with locally advanced and metastatic BCC.

¹Sarah Cannon Research Institute/Tennessee Oncology, Nashville, Tennessee. ²Eli Lilly and Company, Erl Wood, United Kingdom. ³Memorial Sloan Kettering Cancer Center, New York, New York. ⁴Emory Winship Cancer Institute, Atlanta, Atlanta, GA. ⁵University of Texas, MD Anderson Cancer Center, Houston, Texas. ⁶Eli Lilly and Company, Indianapolis, Indiana. ⁷Ignity, Inc., San Diego, California. ⁸Infinity Pharmaceuticals, Cambridge, Massachusetts. ⁹Translational Genomics Research Institute, Phoenix, Arizona. ¹⁰HonorHealth Research Institute, Scottsdale, Arizona.

Corresponding Author: Johanna Bendell, Sarah Cannon Research Institute, 250 25th Avenue North, Suite 200, Nashville, TN 37203. Phone: 615-524-4125; Fax: 615-524-4625; E-mail: jibendell@tnonc.com

doi: 10.1158/1078-0432.CCR-17-0723

©2018 American Association for Cancer Research.

Materials and Methods

Design

This study was a multicenter, nonrandomized, open-label, dose-escalation, phase I clinical trial conducted at 12 investigational

Translational Relevance

The binding of Hh ligands to Patched (Ptch) alleviates Ptch-mediated suppression of Smoothed (Smo). This triggers a series of intracellular events, culminating in the stabilization of Gli zinc finger transcription factors and the expression of Gli-dependent genes, which are responsible for cell proliferation, cell survival, angiogenesis, and invasion. Abnormal activation of the Hh pathway has been linked to many different cancers, especially advanced/metastatic basal cell carcinoma. LY2940680 is a potent Hh pathway inhibitor that binds to the human Smo (hSmo) receptor and competitively inhibits binding of an hSmo agonist. In this phase I trial, LY2940680 was well tolerated in a variety of cancer types and showed clinical responses in patients with treatment-naïve and Hh-inhibitor-treated advanced/metastatic BCC. Results of the phase I trial led to the identification of the recommended phase II dose of LY2940680.

sites in the United States. This study had three phases. The first phase was a dose-escalation assessment in patients with advanced cancers. The MTD was determined using a 3 + 3 dose-escalation design. Dose cohorts were escalated from 50 to 600 mg once daily (QD) of oral treatment of LY2940680. Patients were instructed to only take 1 dose in the morning and to take each daily dose at least 20 hours after the last dose. The dose-escalation phase was followed by a dose-confirmation phase in patients who also had advanced cancers. Based on initial antitumor activity in several locally advanced and metastatic BCC patients, a disease-expansion cohort with locally advanced and metastatic BCC patients was added.

The dose escalation was guided primarily by safety during cycle 1, along with available PK/PD data as a secondary consideration, for all patients in the cohort. Dose escalation continued until the MTD was reached. The sponsor, in collaboration with the investigators, reviewed the patient safety data and the PK/PD data of the dose-escalation phase before determining which dose schedules were explored in the dose-confirmation and BCC expansion phases.

Dose-limiting toxicity

A dose-limiting toxicity (DLT) was defined as a nonhematologic toxicity \geq grade 3 possibly related to study drug that occurs within cycle 1. For the purpose of this study, the MTD was defined as the highest tested dose that has $<33\%$ probability of causing a DLT in cycle 1. Exceptions were made for nausea, vomiting, constipation, diarrhea, fatigue, or anorexia of ≤ 2 days with maximal supportive intervention or transient elevated levels of alanine aminotransferase/aspartate aminotransferase of ≤ 5 days, or thrombocytopenia of \geq grade 3 with bleeding. Hematological toxicities included grade 4 thrombocytopenia, grade 4 hematologic toxicity of >5 days, or any febrile neutropenia. Dose adjustments and delays were allowed after cycle 1 of treatment, as deemed appropriate by the investigator. Patients may have had more than one dose reduction; however, the lowest dose that may have been administered was 50 mg.

Patients

Eligible patients were ≥ 18 years of age and had histologic or cytologic evidence of cancer that was advanced and/or metastatic, including measurable or nonmeasurable disease as defined by the RECIST 1.1 (9). Patients eligible for the BCC expansion cohort must have had locally advanced or metastatic BCC supported by histologic or cytologic evidence and have been previously treated with an Hh/Smoothed (Smo) inhibitor other than LY2940680 (taladegib) or treatment-naïve (any treatment). In addition, these patients must have had an Eastern Cooperative Oncology Group (ECOG) performance status (PS) of ≤ 1 and discontinued previous treatments for cancer and recovered from the acute effects of therapy.

The sponsor, monitor, and investigators performed this study in compliance with the protocol, good clinical practice, and International Conference on Harmonisation guidelines, and applicable regulatory requirements. No study-specific procedures were performed until patients signed informed consent.

Safety evaluations

Safety monitoring included tests to permit initial characterization of the safety profile of LY2940680. Standard laboratory tests including chemistry, hematology, and urinalysis panels were performed. All adverse events (AE) were assessed by the Common Terminology Criteria for Adverse Events (CTCAE) Version 4.02 (10). Triplicate 12-lead electrocardiograms (ECG) were obtained during initial screening.

Efficacy evaluations

Tumor response was assessed using RECIST 1.1 guidelines and investigator-determined response (9). Responses for target lesions were categorized as *CR*, *PR*, *stable disease (SD)*, *progressive disease*, and *not evaluable or non-CR/nonprogressive disease* for patients with nontarget disease only. Responses for nontarget lesions were *absent*, *progressive disease*, *present*, and *not assessed*. Reported lesion data (target and nontarget) and investigator assessment of response (where available) were listed for all patients on therapy.

Objective response rate (ORR) was the proportion of patients who achieved a CR or PR out of all the patients who received at least 1 dose of study drug. Best response was determined from a sequence of responses assessed. Two objective status determinations of CR before progression were required for a best response of CR. Two determinations of PR or better before progression, but without qualifying for a CR, were required for a best response of PR. Best overall response was the best response recorded from the start of the treatment until disease progression/recurrence (taking as reference for progressive disease the smallest measurements recorded since the treatment started). The patient's best response assignment depended on the achievement of both measurement and confirmation criteria. If appropriate, the best overall tumor response was calculated using all available lesion measurement data to confirm investigator assessments. Clinical benefit rate was the proportion of patients who achieved a CR, PR, or SD out of all the patients who had at least one measurable lesion who received at least one dose of study drug.

Progression-free survival (PFS) time was measured from the start of treatment to the first date of progression of disease or of death from any cause, whichever occurred first. For each patient who was not known to have died or had disease progression as of the data inclusion cut-off date, PFS was censored at the date of last

objective progression-free disease assessment before the date of any subsequent systemic anticancer therapy.

Duration of response was calculated for ORR and clinical benefit, defined only for responders, and measured from the date of first evidence of a confirmed response to the date of first progression of disease or the date of death as a result of any cause, whichever was earlier. For each patient who was not known to have died or had disease progression as of the data inclusion cut-off date, duration of response was censored at the date of the last objective response assessment before the date of any subsequent systemic anticancer therapy.

To confirm objective responses, all lesions were radiologically assessed. The same radiologic method used for the initial response determination was repeated at least 4 weeks after the initial observation of an objective response using the sample method that was used at baseline. When a patient was discontinued from the study, repeat radiological assessments were omitted if clear clinical signs of progressive disease were present.

Pharmacokinetic and pharmacodynamic methods

Blood samples of approximately 4 mL were collected in ethylenediaminetetraacetic tubes for PK and PD analyses. Plasma concentrations of LY2940680 and LSN3185556 were assayed using a validated liquid chromatography/mass spectrometry method at Charles River Laboratories Preclinical Services Montreal.

Skin biopsy samples collected before and after LY2940680 treatment were analyzed for changes in gene expression using a reverse transcriptase PCR (RT-PCR) method at Asuragen. Of the Hh-regulated genes, Gli1 expression was selected for testing and analysis. Ribonucleic acid extraction, quantification, and quality assessment were performed according to Asuragen standard operating procedures. Applied Biosystems Life Technologies RT-PCR assays and reagents were used according to the manufacturer's instructions.

Statistical analysis

Data from all patients who received at least one dose of study therapy were included in summaries of safety and toxicity. As this was a dose-finding study, data were analyzed on a cohort-by-cohort basis throughout the study until an MTD was determined. Safety analyses included summaries of drug exposure, DLTs and DLT-equivalent toxicities for all patients on therapy, preexisting conditions, treatment-emergent AEs, discontinuations from the study because of AE or death, serious AEs (SAE), CTCAE grades for laboratory and nonlaboratory AEs, and concomitant medications. Vital signs, ECGs, chest x-ray, transfusion, and nutritional intake and consumption habits were listed for each patient on therapy. Antitumor activity was a secondary objective, and as such tumor response data were summarized and listed, and exploratory analyses were performed.

Tumor markers, disease progression data, and all tumor response data were listed for all patients on therapy. The clinical benefit rate, ORR, and time-to-event variables, including PFS and duration of response, were summarized by study phase and for all BCC patients. Change in tumor size was derived for all patients on therapy with measurable disease at baseline and at least 1 post-treatment assessment. The change in tumor size was summarized for all BCC patients using a waterfall plot at the time of the best response.

Pharmacokinetic analysis

The primary parameters for PK analysis were maximum plasma concentration (C_{max}), area under the plasma concentration–time curve from time zero to 24 hours (AUC_{0-24}), and the observed time of maximal concentration (t_{max}) of LY2940680 and LSN3185556. In addition, terminal elimination half-life ($t_{1/2}$), apparent volume of distribution (V_d/F), apparent clearance (CL/F), and other relevant parameters (e.g., the accumulation ratio) that could have been calculated by noncompartmental analyses from the data were reported. Dose proportionality was explored for LY2940680 and LSN3185556 using AUC_{0-24} and C_{max} values after multiple-dose administration of LY2940680.

Pharmacodynamic analysis

The *GLI1* expression was normalized by the housekeeping gene *RPLP0*, and percent inhibition was derived using the $2^{-\Delta\Delta C_T}$ method and results were summarized for all patients on therapy by dose level (11). Percentage inhibition of evaluable samples was summarized for each endpoint by sample, study part, and cohort. The minimal biologic effective dose (BED) was defined as the first dose level in which the inhibition of mGli1 was >50%.

Results

Patient characteristics

From 30 September 2010 through 5 January 2015, 84 patients were enrolled and treated in the QD dose-escalation ($n = 25$), dose-confirmation ($n = 19$), and BCC expansion phases ($n = 40$). Of the 98 patients who entered in the study, 14 failed screening criteria (Fig. 1). The data cut-off date occurred on 5 January 2015, and the database was locked on 27 March 2015. The median age of patients was 62.5 years (range, 29–89). The majority of patients were white (94.0%) and male (72.6%). Across treatment groups, the median body weight was 80.3 kg (range, 42.9–151.4).

At study entry, most patients (66.7%) had an ECOG PS of 0, and 33.3% had an ECOG PS of 1. The initial pathologic diagnosis was BCC for 47 patients (56.0%) and colon adenocarcinoma for 15 (17.9%) patients. All other initial diagnoses were reported in less than 10% of patients. Based on the available disease stage, tumor, lymph node, and metastases information, the 47 BCC patients were classified as follows: 18 patients had metastatic disease, 21 patients had locally advanced disease, and 8 patients could not be classified based on currently available data.

Adverse events

The most common study drug-related treatment-emergent AEs were dysgeusia [$n = 41$ (48.8%)], fatigue [40 (47.6%)], nausea [38 (45.2%)], and muscle spasms [34 (40.5%); Table 1], and these were deemed to be study-related. The most common reasons for treatment discontinuation were disease progression ($n = 43$), AE ($n = 10$), and withdrawal by patient ($n = 10$; Fig. 1). Of the 10 patients who discontinued treatment because of AEs, 2 patients had AEs that were considered SAEs.

Thirty-eight SAEs were reported in the clinical database for 22 patients. Of these, eight events were considered possibly study drug related by the investigator, including two events of hyponatremia, one event of nausea, one event of vomiting, one event of clostridium difficile infection, one event of colitis, one event of muscle spasms, and one event of syncope. The two SAEs

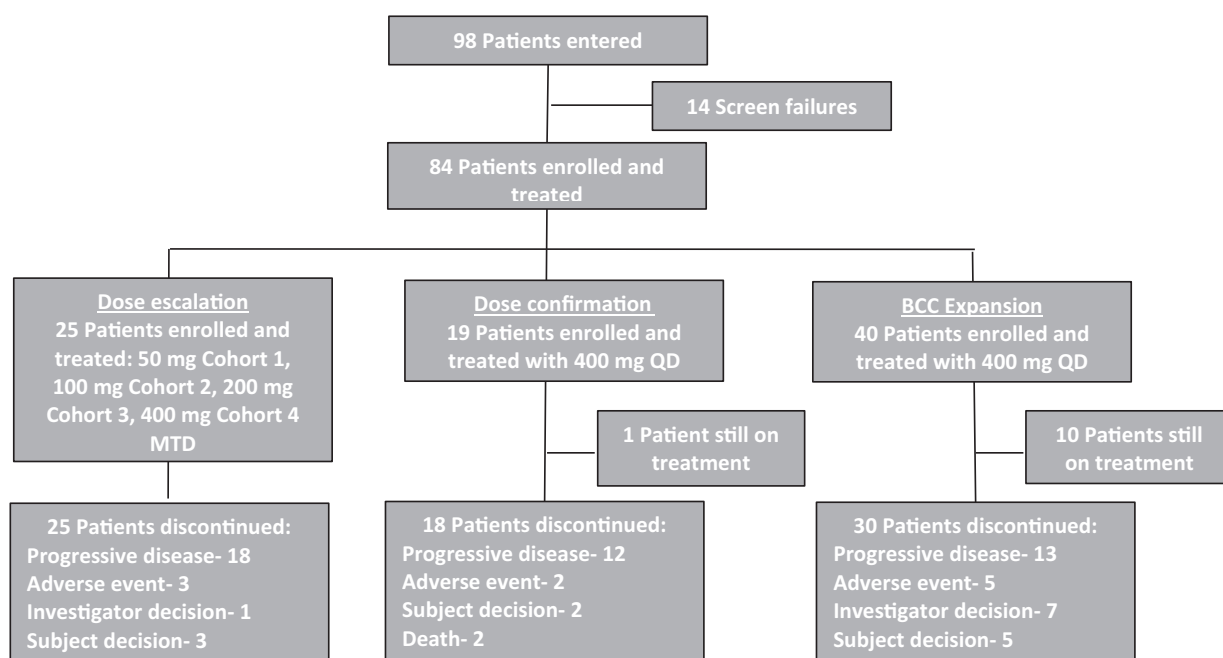


Figure 1.
Study disposition.

considered unrelated to study drug included grade 4 gastrointestinal hemorrhage and grade 4 sepsis.

A total of 9 (9.2%) patients died during this study; 6 of the reported deaths occurred within 30 days of the last dose of treatment. The primary cause of death for 8 patients was disease progression ($n = 8$) and not related to treatment. Although the primary cause of death for 1 patient was unknown, the patient had not recovered from grade 3 pneumonia at the time of death. Study drug had not changed, and the investigator stated that the pneumonia and death were not related to study drug or protocol procedures.

Laboratory results

There were no clinically relevant differences between dose groups in the incidence of patients who had abnormal laboratory values. In addition to other standard laboratory tests,

creatinine kinase levels were monitored; 16 patients had increases in creatine kinase levels (14 patients grade 1/2; 2 patients grade 3). Although a small subset of patients with elevated creatine kinase levels experienced muscle cramping or spasm, no association between the 2 AEs could be established. Sixteen patients experienced grade 1/2 elevated creatine levels.

Exposure to study drug and dose reductions

In the dose-escalation phase, 25 patients were exposed to study drug with doses ranging from 50 to 600 mg QD on a 28-day cycle; the median number of cycles received was 2 (range, 1–24 cycles). In the dose-confirmation phase, 19 patients were evaluated at the 400 mg QD dose of study drug. Overall, the median number of cycles received in this part of the study was 2 (range, 1–31). Four (21.1%) patients received more than two cycles of treatment. Dose reductions were

Table 1. Study-related treatment-emergent AEs occurring in $\geq 10\%$ of patients on LY2940680

<i>N</i> = 84	Grades 1-2	Grade 3	Grades 4-5	Total
Dysgeusia	39 (46.4%)	2 (2.4%)	0	41 (48.8%)
Fatigue	37 (44.0%)	3 (3.6%)	0	40 (47.6%)
Nausea	36 (42.9%)	2 (2.4%)	0	38 (45.2%)
Muscle spasms	30 (35.7%)	4 (4.8%)	0	34 (40.5%)
Decreased appetite	30 (35.7%)	0	0	30 (35.7%)
Alopecia	28 (33.3%)	0	0	28 (33.3%)
Vomiting	25 (29.7%)	2 (2.4%)	0	27 (32.1%)
Weight decreased	22 (26.2%)	1 (1.2%)	0	23 (27.4%)
Myalgia	14 (16.7%)	2 (2.4%)	0	16 (19.0%)
Diarrhea	12 (14.3%)	2 (2.4%)	0	14 (16.7%)
Rash ^a	13 (15.5%)	1 (1.2%)	0	14 (16.7%)
Constipation	10 (11.9%)	0	0	10 (11.9%)
Asthenia	8 (9.5%)	2 (2.4%)	0	10 (11.9%)
Dizziness	8 (9.5%)	1 (1.2%)	0	9 (10.7%)

^aIncluding rash maculopapular, dermatitis acneiform, and urticarial.

observed for these 4 patients. In the BCC expansion phase, 40 patients were evaluated at the 400 mg QD dose of study drug; the median number of cycles received was 8 (range, 1–22). Out of the 40 patients in the BCC expansion phase, 16 patients experienced at least 1 dose reduction while on study: 2 patients had 3 reductions, 4 patients had 2 reductions, and 10 patients had 1 reduction. The majority of reported dose reductions were attributed to AEs (86.2%). The most commonly reported AEs (more than 2 occurrences) resulting in dose reductions included fatigue ($n = 8$), muscle spasms ($n = 8$), decreased appetite ($n = 3$), dysgeusia ($n = 3$), nausea ($n = 3$), and weight decreased ($n = 3$).

DLTs and dose reductions

DLTs were noted in 4 patients during the study (3 in the dose-escalation phase and 1 in the dose-confirmation phase). The first DLT observed at the 100-mg QD dose level (day 22) was grade 3 hyponatremia resulting in hospitalization; it was determined by the investigator to be possibly related to LY2940680. This event was considered by the investigator to be possibly related to the study drug and not related to a protocol procedure. The second DLT, observed at the 600-mg QD dose level, was grade 3 maculopapular rash. This patient had a history of allergic reactions to medications, including anaphylaxis with oxaliplatin, severe cetuximab rash, drug eruption desquamating drug rash, and drug hypersensitivity to sulfa and nitrofurantoin. The third DLT, also observed at the 600-mg QD dose level, was determined by the investigator to meet the criteria for a DLT based on multiple grade 2 toxicities (confusion, nausea, and dehydration) and grade 1 anorexia.

Three patients had dose reductions at 600 mg. For the patient who experienced grade 3 fatigue and vomiting in cycle 3, the fatigue determined the DLT and the vomiting caused the dose reduction. Other dose reductions occurred for 1 patient experiencing a DLT in cycle 1 and for another as a precautionary measure because of the other patients experiencing toxicities. As a result, it was concluded that the MTD had been exceeded at the 600 mg dose level and 400 mg was determined to be the MTD. During the dose-confirmation phase, 1 patient experienced a DLT of grade 3 vomiting at the 400-mg QD dose level, which was not considered drug-related.

Pharmacokinetics

After single- and multiple-dose administrations, LY2940680 levels reached C_{max} after approximately 3 hours. The geometric mean value for the CL/F was 8.2 L/hour [70% coefficient of variation (CV)], indicating that LY2940680 was slowly eliminated from the plasma. The geometric mean value for the Vd/F was 235 L (63% CV). The accumulation ratio of LY2940680 based on AUC_{0-24} was approximately 2 between days 1 and 15 (range, 0.7–4.8; 39% CV). After multiple-dose administrations across all doses, the geometric mean of LY2940680 $t_{1/2}$ was estimated to be approximately 19 hours (range, 5–170; 73% CV) with the limitation of the 24-hour sample schedule (Fig. 2A and B). From the every-other-cycle predose PK collection, the predose value from cycle 1 day 15 did not increase in subsequent cycles (Fig. 2C). The LSN3185556 PK profile was incompletely captured following daily administration of LY2940680. The accumulation ratio of LSN3185556 based on AUC_{0-24} was approximately 3.4 (range, 1.5–8.0; 43% CV). The exposure ratio between metabolite and parent was calculated

from the AUC_{0-24} ; it was approximately 2.5 on day 15 (range, 0.8–5.4; 35% CV).

From a toxicity versus exposure relationship perspective, daily administration of dose levels above 400 mg QD was associated with several cases of DLTs, as well as 1 DLT at 100 mg. Some of the toxicities observed in patients appeared after cycle 1, when the steady state for both LY2940680 and LSN3185556 had already been reached. LSN3185556 concentrations were higher than LY2940680 concentrations on day 15. The accumulation after QD dosing also was higher for LSN3185556 compared with LY2940680.

Pharmacodynamics

The PD effect for LY2940680 was assessed by the relative decrease in *GLI1* expression in healthy skin following 15 and 30 days of exposure to LY2940680. PD assessment was conducted on three occasions: predose, cycle 1 day 15, and cycle 2 day 1. In the dose-escalation phase, two samples were collected 2 hours after dose administration and associated with maximal concentrations. In the dose-confirmation and BCC expansion phases, the second sample was collected 24 hours after dose administration of cycle 2 on day 1 and was associated with minimal concentrations. During the dose escalation, a high level of inhibition was observed at all dose levels tested. Given that the minimal BED was defined as the first dose level in which the inhibition of mGli1 was >50%, it was concluded that LY2940680 was pharmacologically active at all dose levels tested. In normal skin, the median values of mGli1 inhibition in normal skin were consistently high across all doses tested. Overall, Gli1 inhibition was 92.3% (interquartile range: 80.9%–95.7%; Fig. 3). There was no correlation with the % mGli1 inhibition between those that responded versus those that did not. Because of outliers in the data, the error bars were wider at the 400 mg dose; these data affected the variability but not the median inhibition.

A limited number of archival and fresh tumor samples from a selected number of patients with BCC were tested for Smo molecular alterations. At least one Smo mutation was present in 5 of 6 new biopsy samples tested. Of these Smo mutations, *V321M* and *D473H*, both known vismodegib-resistance variants, were each detected in 2 separate BCC patients. The remaining three Smo variants observed are of unknown function relative to prior Hh treatment. In addition to the small number of tested new tumor samples, matched archival samples for comparison were not available, and the somatic origin of the mutations was not confirmed.

Tumor response

Of the 84 patients on therapy, 26 (30.9%) patients had a best response of SD, 24 (28.6%) patients had a best response of progressive disease, and 22 (26.2%) patients had a response [16 (19.0%) confirmed PR; 5 (5.9%) confirmed CR; 1 (1.2%) unconfirmed PR]. A total of 11 patients were not assessed. All patients who experienced a clinical response (PR or CR) per RECIST 1.1 had a diagnosis of BCC.

Of the 47 BCC patients, 22 (46.8%) patients had a best overall response of CR or PR, 5 of which were confirmed CR, whereas 16 were confirmed PR and 1 was unconfirmed PR. Twenty-one (44.7%) patients had a best response of SD; 1 patient had a best response of non-CR nonprogressive disease based on nontarget lesions; 1 patient had a best response of progressive disease; and 2 patients were not assessed. The estimated response rate was

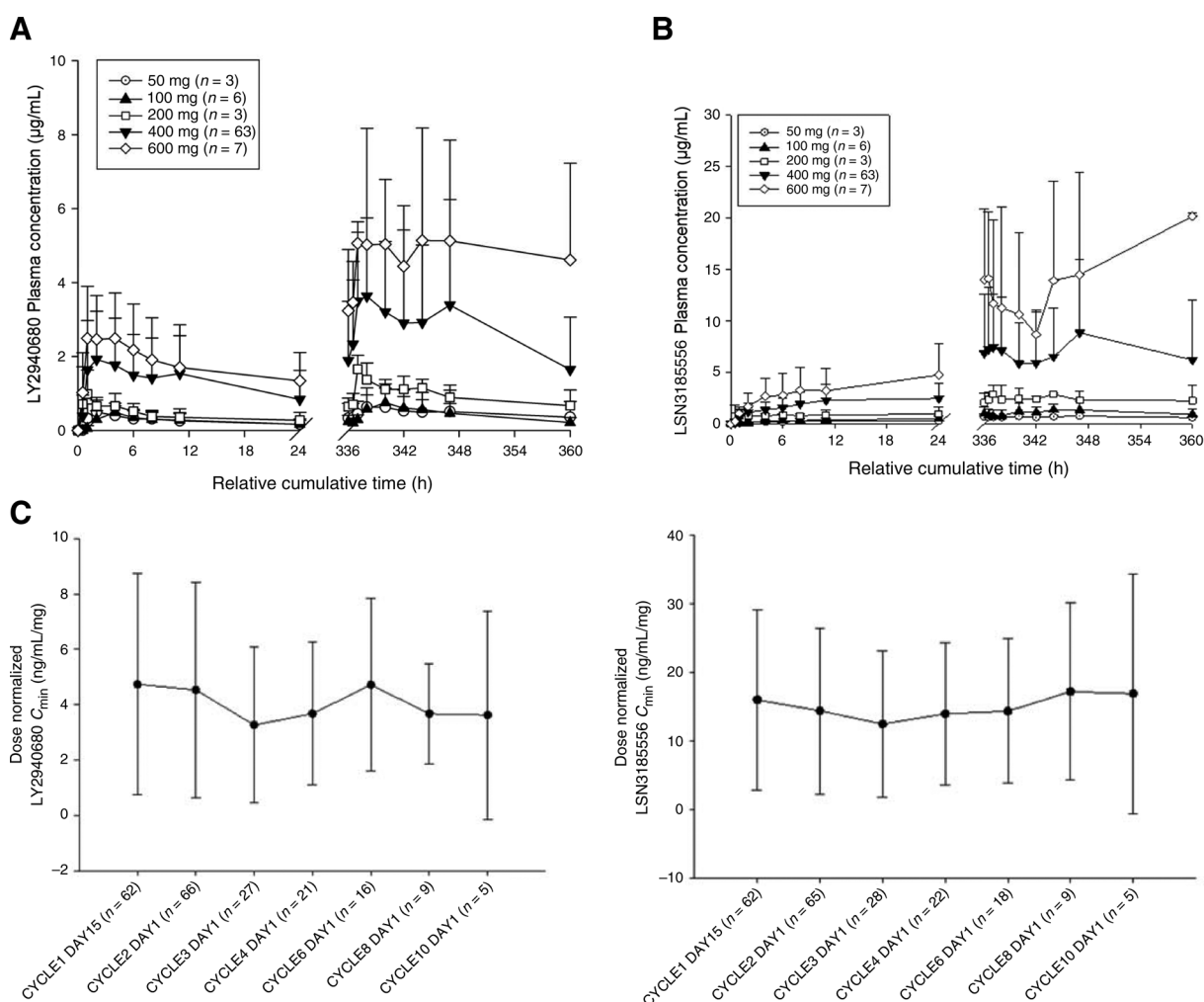


Figure 2. Mean and standard deviation of plasma concentration of (A) LY2940680 and (B) LSN3185556.

46.8% [95% confidence interval (CI), 32.1%–61.9%]. Two patients with Hh-inhibitor treatment-naïve BCC who were treated below the MTD during dose escalation achieved a PR. The first patient with locally advanced BCC was treated at 50 mg QD and had a sustained response lasting for 8.3 months. The second patient with metastatic BCC was initially treated at 200 mg QD and treated for 16 months. The patient received 16 cycles of study drug and had 2 dose reductions; the dose was reduced to 100 mg because of grade 2 nausea and tetany during cycle 5, and then further reduced to 50 mg because of grade 1 decreased appetite during cycle 9. The patient had sustained PR for 10.2 months.

Of the 40 BCC patients treated at the 400 mg QD dose level of study drug, the median number of cycles received was 8 (range, 1–22). Sixteen of the 40 patients had at least 1 dose reduction while on study: 2 patients had 3 reductions, 4 patients had 2 reductions, and 10 patients had 1 reduction. The majority of dose reductions were attributed to AEs (86.2%). The most commonly reported AEs (more than 2 occurrences) resulting in dose reductions included fatigue ($n = 8$), muscle spasms ($n = 8$),

decreased appetite ($n = 3$), dysgeusia ($n = 3$), nausea ($n = 3$), and weight decreased ($n = 3$; Fig. 4).

Of these 16 BCC patients requiring dose reductions, 9 (56.3%) patients had a best overall response of CR or PR, 3 of which were confirmed CR and 6 of which were confirmed PR. Seven (43.8%) patients had a best response of SD.

The change in tumor size for BCC patients who had measurable disease ($n = 43$) is shown in Fig. 5A. A total of 4 patients are not represented. Measurements were not available for 2 patients in the dose-confirmation phase who had only nontarget lesions at baseline and 2 patients from the BCC expansion phase who only had baseline data available. The majority of patients had a decrease in their tumor burden with the disease control rate of 93.6% (95% CI, 82.5%–98.7%). In the BCC expansion phase, 31 patients had received prior Hh therapy, including vismodegib and sonidegib, and 16 patients were Hh therapy-naïve. Responses were observed both in patients who had not previously received Hh therapy (11/16; 68.8%) and those who had received prior Hh therapy (11/32; 35.5%).

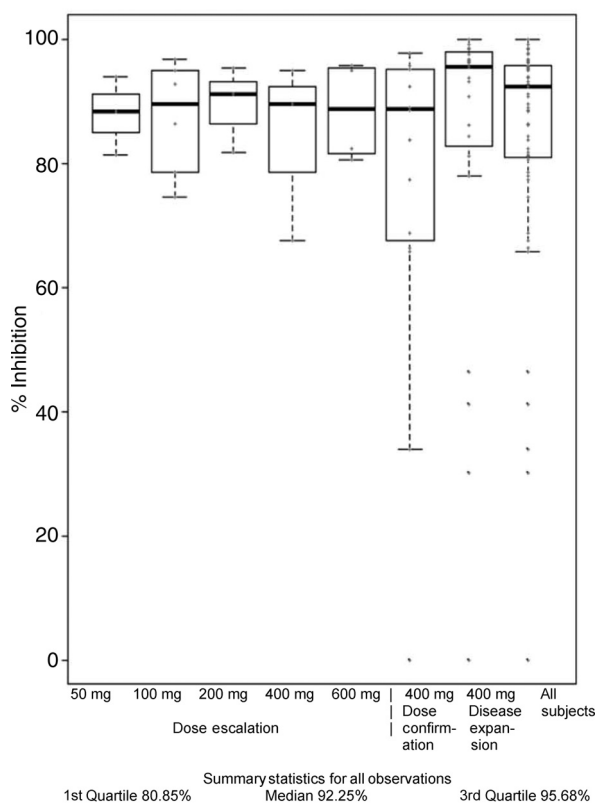


Figure 3. Gli1 inhibition (%) in skin sample during cycle 1 (day 15) across dose levels.

Duration of response/progression-free survival

For all patients who had a documented clinical response (21 confirmed and 1 unconfirmed), the duration of response was assessed from the date of the initial response to progression. Out of the 22 responders, 13 patients did not have progression and were censored at the time of the last progression-free assessment. Overall, the median duration of response was 10.2 months (95% CI, 5.55–not evaluable). The upper limit of the 95% CI could not be estimated because of the high censoring rate (59.1%) and the small sample size. Kaplan–Meier estimates of duration of response for BCC patients are shown in Fig. 5B. The confirmed median duration of response for BCC patients was 10.2 months. Of the 13 patients who did not have progression and were censored at the time of the last progression-free assessment, 6 patients were responders [CR ($n = 4$) or PR ($n = 2$)] and still on treatment at the time of data cut-off.

In the dose-escalation and BCC expansion phases where patients with advanced cancers of multiple histologies were enrolled, the estimated median PFS was 1.8 months (95% CI, 0.95–2.10) and 1.7 months (95% CI, 0.95–1.87), respectively. In the BCC expansion phase ($n = 40$), the estimated median PFS was 12.0 months (95% CI, 7.62–17.54). A total of 11 patients remained on treatment at the time of data cut-off, with best responses of CR ($n = 4$), PR ($n = 2$), and SD ($n = 5$).

Discussion

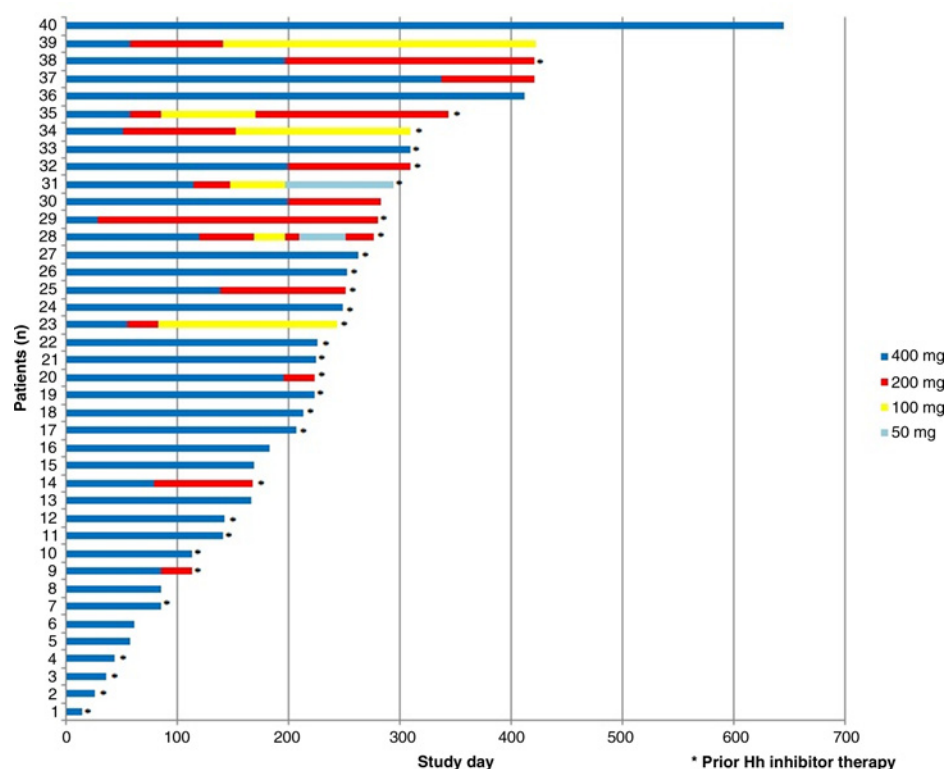
Smoothed, a distant relative of G protein–coupled receptors, is a key regulator of Hh signaling. The binding of Hh ligands to

Patched (Ptch) alleviates Ptch-mediated suppression of Smoothed (12, 13). Although the precise mechanism by which it regulates Hh signaling is not clear, it is an attractive target to inhibit aberrant Hh signaling in cancer. The dose range of 50 to 1,000 mg of LY2940680 taken orally was selected for the dose-escalation phase of the study based on PK/PD and preclinical toxicology data. The treatment was well tolerated from the lowest starting dose of 50 mg up to the 400-mg dose, which was selected as the MTD, across different cancer subtypes, including advanced and metastatic BCC that was treatment naïve and treated previously with an Hh inhibitor. Among the patients with advanced and metastatic BCC, no difference was detected in the tolerability or in the dose reduction incidence between patients with and without prior Hh-inhibitor exposure. The most frequent AEs experienced by patients treated with LY2940680 were similar to those reported in other Hh-inhibitor studies, including dysgeusia, fatigue, nausea, and muscle spasms (14, 15). We also observed rash in 14 patients treated with taladegib; the AE rash was not reported with other Smo antagonists (16, 17). Morphologic description of the rash was available for only a few patients. The majority of the episodes observed were grade 1 except one case of grade 3 maculopapular rash, which was one of the DLTs. Biopsies were not obtained from the rash to further clarify its morphology or etiopathogenesis. Thirty-eight SAEs were reported for 22 patients, 8 of which were considered possibly drug-related by the investigator. Eight of the 9 deaths reported during the study were attributed to disease progression, whereas for 1 patient, the primary cause of death was unknown but not considered related to study drug. The most common reasons for treatment discontinuation were disease progression and patient decision to withdraw. Of the 10 patients who discontinued treatment because of AEs, 2 patients had AEs that were considered SAEs and included grade 4 gastrointestinal hemorrhage and grade 4 sepsis. The MTD identified for LY2940680 was 400 mg orally QD. The study design included a dose reduction table, which was implemented to obtain consistency in dose reduction decisions. The rationale included efficacy versus risk of AEs, where 400 mg was chosen as the starting point, with the option of reducing the dose if AEs occurred.

The clinical activity of a Smo antagonist in BCC was reported in the phase I study of vismodegib (14). Similar observations were noted with another Smo antagonist, sonidegib (15). Both agents appear to be active in Hh-inhibitor treatment–naïve patients with locally advanced BCC and metastatic BCC. Clinical responses and SD in Hh-inhibitor treatment–naïve patients with locally advanced BCC and metastatic BCC were also observed with LY2940680, but a discerning feature is the clinical response to LY2940680 in patients with locally advanced BCC and metastatic BCC who received prior treatment with other Hh inhibitors. Unfortunately, based on the available data collected in this study, it was not possible to distinguish between the patients who were intolerant to prior Hh therapy, those who had lack of response to initial treatment with a SMO inhibitor (refractory disease), and those who had disease progression after initially responding to a prior Smo inhibitor (relapsed disease). The patients who had progression may have developed treatment resistance to Smo antagonists. At least one Smo mutation was present in 5 of 6 new biopsy samples tested.

Sonidegib was not efficacious in investigational study of advanced BCC patients who were previously treated with vismodegib (18). The underlying mechanism of this activity is not clear

Figure 4.
Treatment duration by patient and dose,
BCC expansion.



but may be partly related to the high level of mGli1 inhibition which was observed at all dose levels tested. Given that the minimal BED was defined as the first dose level at which the inhibition of mGli1 in normal skin was >50%, it was concluded that taladegib was pharmacologically active at all dose levels tested. Conversely, this may also explain the clinical efficacy in BCC patients treated with even the lowest LY2940680 dose.

The *in vitro* protein binding of taladegib and LSN3185556 was investigated in human plasma. The mean percentages bound were 94.4% for taladegib and 98.2% for LSN3185556. In addition, the *in vitro* binding of taladegib was investigated with human serum albumin and human α -1-acid glycoprotein. Human serum albumin was shown to be the predominant plasma-binding protein for taladegib: no apparent concentration-dependent binding to human serum albumin over the dose range of 1 to 10 μ mol/L was observed. This could be seen as an advantage for taladegib over vismodegib, which experienced saturable binding to α -1-acid glycoprotein (19), and sonidegib, which is highly bound (over 99%) to plasma protein (20).

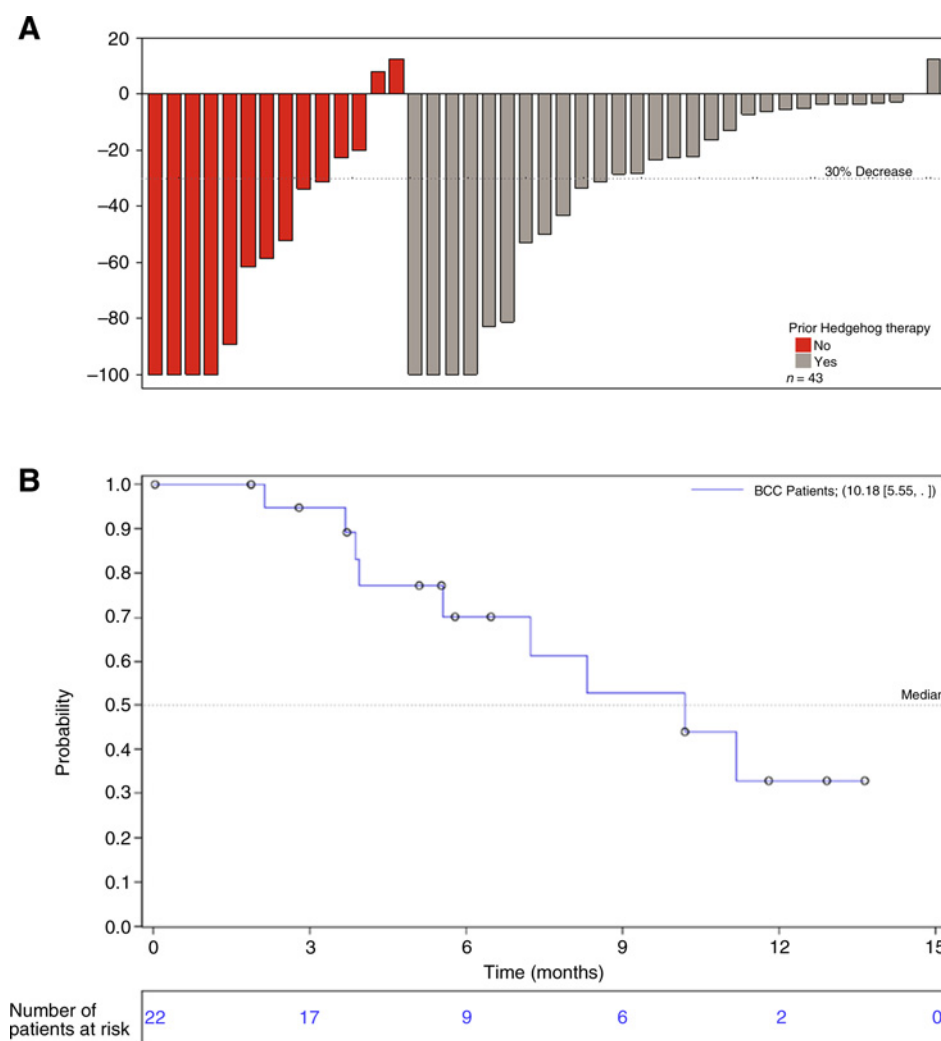
In considering these data in context, a few limitations need to be weighed. The sample size was small; therefore, a formal phase II study to investigate the clinical efficacy in treatment-naïve patients and patients who did not respond to previous Hh-inhibitor treatment is needed. Patients with BCC and potentially other tumor subtypes where aberrant Hh signaling is evident may be of interest. The PK and PD assessments were secondary endpoints in this study. After multiple doses, LY2940680 and LSN3185556 C_{max} and AUC_{0-24} increased with dose and did not suggest any large deviation from dose proportionality. This appears to be an advantage over both vismodegib and sonidegib, which displayed nonlinear PK: sonidegib at doses above 400 mg and vismodegib at all doses

tested in the first human dose trial (19–22). The data suggest steady state being attained for both entities on cycle 2, day 1 and PK variability being approximately 70% on exposure. *In vitro* data suggest that both LY2940680 and LSN3185556 are 100% metabolized by CYP3A4. Given the patient population, with its concomitant medications and varying stages of disease, this could explain at least some of the PK variability. Pharmacokinetic/PD analysis using observed combined exposure of the parent drug, and active metabolite and Gli1 inhibition in normal skin, demonstrates maximal inhibition of the target at all doses.

From a toxicity versus exposure relationship perspective, daily administration of dose levels above 400 mg QD was associated with several cases of DLTs, as well as 1 DLT of grade 3 hyponatremia at 100 mg. Some of the toxicities observed in patients appeared after cycle 1, when both LY2940680 and LSN3185556 were not increasing; therefore, for some patients, it appears that there is a delay in the onset of AEs. The relationship between safety and exposure at the individual level (all patients with LY2940680-related AEs as grade 3–4 during cycle 1) is not completely clear.

Conclusions

LY2940680 treatment resulted in an acceptable safety profile in patients with advanced cancer, including locally advanced and metastatic BCC. Clinical responses were observed in locally advanced and metastatic BCC patients naïve to Hh-inhibitor treatment and also in those who previously received treatment with other Hh-inhibitor agents. Integration of data from this study, along with the preclinical toxicology data, will contribute to the establishment of the dose and dosing frequency of

**Figure 5.**

Percent change in tumor size at best response for individual BCC patients with measurable disease (A). Kaplan-Meier estimates of duration of response in locally advanced and metastatic patients with basal cell carcinoma (B).

LY2940680 for the treatment of BCC. This phase I study supports further clinical development of LY2940680 in advanced BCC, including those who are treatment naïve and those who previously received prior Hh-inhibitor therapy.

Disclosure of Potential Conflicts of Interest

A. Ho is a consultant/advisory board member for Novartis, Omniprex America LLC, and Sun Pharmaceuticals. M. Migden is a consultant/advisory board member for Eli Lilly & Company, Genentech, and Novartis. J. Infante is an employee of Janssen Pharmaceuticals. L. Brail is a consultant/advisory board member for Ignyta. D. Von Hoff reports receiving commercial research grants from Eli Lilly. No potential conflicts of interest were disclosed by the other authors.

Authors' Contributions

Conception and design: V. Andre, J. Infante, C. Pitou, T. Tucker, L. Brail, D. Von Hoff

Development of methodology: V. Andre, J. Infante, C. Pitou, L. Brail

Acquisition of data (provided animals, acquired and managed patients, provided facilities, etc.): J. Bendell, A. Ho, R. Kudchadkar, M. Migden, J. Infante, T. Tucker, L. Brail, D. Von Hoff

Analysis and interpretation of data (e.g., statistical analysis, biostatistics, computational analysis): V. Andre, A. Ho, R. Kudchadkar, M. Migden, J. Infante, R.V. Tiu, C. Pitou, T. Tucker, L. Brail, D. Von Hoff

Writing, review, and/or revision of the manuscript: J. Bendell, V. Andre, A. Ho, R. Kudchadkar, M. Migden, J. Infante, R.V. Tiu, C. Pitou, T. Tucker, L. Brail, D. Von Hoff

Administrative, technical, or material support (i.e., reporting or organizing data, constructing databases): C. Pitou, T. Tucker

Study supervision: A. Ho, J. Infante, R.V. Tiu, T. Tucker, L. Brail, D. Von Hoff

Acknowledgments

The author would like to acknowledge the contribution of Shawn Estrem, PhD, for providing input on the molecular data and Laura Bean Warner, of INC Research, for her medical writing assistance. This research was funded in part through the NIH/NCI Cancer Center Support Grant P30 CA008748. The support was provided by Geoffrey Beene Cancer Research Center at Memorial Sloan Kettering Cancer Center. This study was also supported by Eli Lilly and Company.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked *advertisement* in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

Received March 13, 2017; revised June 29, 2017; accepted February 20, 2018; published first February 26, 2018.

References

- Jiang J, Hui CC. Hedgehog signaling in development and cancer. *Dev Cell* 2009;15:801–12.
- Robbins DJ, Liang Fei DL, Riobo NA. The Hedgehog signal transduction network. *Sci Signal* 2012;5:re6.
- Varjosalo M, Taipale J. Hedgehog: functions and mechanisms. *Genes Dev* 2008;22:2454–72.
- Kobune M, Iyama S, Kikuchi S, Horiguchi H, Sato T, Murase K, et al. Stromal cells expressing hedgehog-interacting protein regulate the proliferation of myeloid neoplasms. *Blood Cancer J* 2012;2:e87.
- Basset-Seguín N, Sharpe HJ, de Sauvage FJ. Efficacy of Hedgehog pathway inhibitors in basal cell carcinoma. *Mol Cancer Ther* 2015;14:633–41.
- Lear JT, Corner C, Dziejewski P, Fife K, Ross GL, Varma S, et al. Challenges and new horizons in the management of advanced basal cell carcinoma: a UK perspective. *Br J Cancer* 2014;111:1476–81.
- Jimeno A, Weiss GJ, Miller WH Jr, Gettinger S, Eigl BJ, Chang AL, et al. Phase I study of the hedgehog pathway inhibitor IPI-926 in adult patients with solid tumors. *Clin Cancer Res* 2013;19:2766–74.
- Bender MH, Hipskind PA, Capen AR, Cockman M, Credille KM, Gao H, et al. Identification and characterization of a novel smoothed antagonist for the treatment of cancer with deregulated hedgehog signaling. In: *Proceedings of the 102nd Annual Meeting of the American Association for Cancer Research*; 2011 Apr 2–6; Orlando, FL: AACR. Abstract nr 2819.
- Eisenhauer EA, Therasse P, Bogaerts J, Schwartz LH, Sargent D, Ford R, et al. New response evaluation criteria in solid tumours: revised RECIST guideline (version 1.1). *Eur J Cancer* 2009;45:228–47.
- [NCI] US Department of Health and Human Services/National Institutes of Health/National Cancer Institute. Common terminology criteria for adverse events, Version 4.02. 2009. Available from: https://www.eortc.be/services/doc/ctc/CTCAE_4.03_2010-06-14_QuickReference_5x7.pdf.
- Livak KJ, Schmittgen TD. Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T))Method. *Methods* 2001;25:402–8.
- Scales SJ, de Sauvage FJ. Mechanisms of hedgehog pathway activation in cancer and implications for therapy. *Trends Pharmacol Sci* 2009;30:303–12.
- Yauch RL, Dijkgraaf GJ, Alicke B, Januario T, Ahn CP, Holcomb T, et al. Smoothed mutation confers resistance to a Hedgehog pathway inhibitor in medulloblastoma. *Science* 2009;326:572–4.
- Sekulic A, Migden MR, Oro AE, Dirix L, Lewis KD, Hainsworth JD, et al. Efficacy and safety of vismodegib in advanced basal-cell carcinoma. *N Engl J Med* 2012;366:2171–9.
- Migden MR, Guminski A, Gutzmer R, Dirix L, Lewis KD, Combemale P, et al. Treatment with two different doses of sonidegib in patients with locally advanced or metastatic basal cell carcinoma (BOLT): a multicentre, randomised, double-blind phase 2 trial. *Lancet Oncol* 2015;16:716–28.
- Goel V, Hurh E, Stein A, Nedelman J, Zhou J, Chiparus O, et al. Population pharmacokinetics of sonidegib (LDE225), an oral inhibitor of hedgehog pathway signaling, in healthy subjects and in patients with advanced solid tumors. *Cancer Chemother Pharmacol* 2016;77:745–55.
- Von Hoff DD, LoRusso PM, Rudin CM, Reddy JC, Yauch RL, Tibes R, et al. Inhibition of the hedgehog pathway in advanced basal-cell carcinoma. *N Engl J Med* 2009;361:1164–72.
- Daniel C, Sarin KY, Oro AE, Chang AL. An investigator-initiated open-label trial of sonidegib in advanced basal cell carcinoma patients resistant to vismodegib. *Clin Cancer Res* 2016;22:1325–9.
- Rodon J, Tawbi HA, Thomas AL, Stoller RC, Turtschi CP, Baselga J, et al. A phase I, multicenter, open-label, first-in-human, dose-escalation study of the oral smoothed inhibitor Sonidegib (LDE225) in patients with advanced solid tumors. *Clin Cancer Res* 2014;20:1900–9.
- Lorusso PM, Jimeno A, Dy G, Adjei A, Berlin J, Leichman L, et al. Pharmacokinetic dose-scheduling study of hedgehog pathway inhibitor vismodegib (GDC-0449) in patients with locally advanced or metastatic solid tumors. *Clin Cancer Res* 2011;17:5774–82.
- Graham RA, Lum BL, Cheeti S, Jin JY, Jorga K, Von Hoff DD, et al. Pharmacokinetics of hedgehog pathway inhibitor vismodegib (GDC-0449) in patients with locally advanced or metastatic solid tumors: the role of alpha-1-acid glycoprotein binding. *Clin Cancer Res* 2011;17:2512–20.
- Pan S, Wu X, Jiang J, Gao W, Wan Y, Cheng D, et al. Discovery of NVP-LDE225, a potent and selective Smoothed antagonist. *ACS Med Chem Lett* 2010;1:130–4.