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Clinical Cancer Research

Phase I Dose-Escalation Trial of the Oral Investigational Hedgehog Signaling Pathway Inhibitor TAK-441 in Patients with Advanced Solid Tumors

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Abstract

Purpose: This first-in-human study assessed safety, tolerability, pharmacokinetics, pharmacodynamics, and preliminary clinical activity of single and multiple doses of TAK-441, an investigational inhibitor of the Hedgehog signaling pathway.

Experimental Design: Patients with advanced, solid tumors received daily oral TAK-441 (50–1,600 mg/day); daily dose was doubled in each subsequent cohort until the maximum tolerated/feasible dose (MTD/MFD) was reached. Blood was collected to evaluate TAK-441 plasma concentrations. Skin biopsies were obtained to evaluate suppression of the Hedgehog-regulated gene *Gli1*.

Results: Thirty-four patients were enrolled (median age 59). The most common diagnoses were colorectal cancer (26%), basal cell carcinoma (BCC, 21%), and pancreatic cancer (9%). The MFD of 1,600 mg/day (based on tablet size and strength) was considered the MTD. Dose-limiting toxicities included muscle spasms and fatigue. Grade ≥3 treatment-emergent adverse events, regardless of causality, occurred in 15 patients (44%), of which hyponatremia ($n = 4$) and fatigue ($n = 3$) were most common. Oral absorption was fairly rapid; median T_{max} was 2.0 to 4.0 hours after a single dose. Mean elimination half-life was 13.5 to 22.6 hours. Systemic exposure of TAK-441 based on the area under the plasma concentration–time curve was linear across the dose range. *Gli1* expression in skin biopsies was strongly inhibited at all dose levels. Best response was partial response (1 patient with BCC) and stable disease (7 patients with various solid tumors).

Conclusions: TAK-441 was generally well tolerated up to MFD of 1,600 mg/day, with preliminary antitumor activity. Further study of TAK-441 may be appropriate in populations selected for tumors with ligand-dependent or independent Hedgehog signaling. *Clin Cancer Res; 21(5); 1002–9. ©2014 AACR.*

Introduction

The Hedgehog (Hh) signaling pathway plays an important role in cell proliferation and control of survival signals in tumorigenesis and embryogenesis (1). Hedgehog ligands bind to the receptor protein Patched (Ptch). In the absence of Hh ligand, Ptch suppresses activation of the G-protein–coupled receptor, Smoothened (Smo; ref. 2). Upon binding of Hh ligands to Ptch, Smo is activated and upregulates glioma-associated oncogene homolog 1 (*Gli1*) transcriptional activity. Genes under the control of *Gli* regulate cellular proliferation and differentiation.

Dysregulation of the Hh pathway is a principal event in the carcinogenesis of multiple tumor types, including pancreatic cancer, prostate cancer, colon cancer, basal cell carcinoma (BCC), and medulloblastoma (3–5). Two separate mechanisms of aberrant Hh signaling have been identified in cancer cell lines: Hh ligand-dependent and Hh ligand-independent (Fig. 1). The ligand-independent mechanism involves inactivating mutations in Ptch that lead to unregulated Smo activation (Fig. 1A). Congenital mutations in Ptch are associated with the basal cell nevus syndrome (Gorlin syndrome), a disease characterized by the early development of BCC, odontogenic keratocysts, palmar and plantar pitting, and ectopic intracranial calcification. Somatic mutations in Ptch have been found in approximately one third of sporadic BCC, and further evidence suggests that Ptch inactivation, leading to increased transcription of *Gli1*-dependent genes, is a necessary step in BCC carcinogenesis (6, 7). Similar somatic mutations in Ptch have been described in medulloblastoma and rhabdomyosarcoma (8, 9).

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Note: Supplementary data for this article are available at Clinical Cancer Research Online (http://clincancerres.aacrjournals.org/).

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Translational Relevance

Signaling via the Hedgehog (Hh) pathway is critical for cell proliferation and survival during embryonic development. Activation of the Smoothened receptor (Smo), a key component of the Hh pathway, leads to activation of downstream effectors, including the transcriptional activator *Gli1*. Dysregulated Hh signaling has been implicated in carcinogenesis, and this pathway has emerged as a target for anticancer therapy. Inhibition of Smo has been studied in several tumor types, including basal cell carcinoma (BCC), in which *Gli1* is upregulated. TAK-441 is an investigational, orally available, small-molecule, high-affinity inhibitor of Smo that has demonstrated antitumor activity in preclinical models. In this first-in-human study of TAK-441, we observed strong inhibition of *Gli1* mRNA expression in skin at all dose levels regardless of the underlying cancer, and activity in BCC. Our results support Hh inhibition as a strategy for the treatment of BCC.

In other tumor types, upregulation of Hh ligands has been demonstrated to promote tumor development and growth. An autocrine pattern, in which tumor cells produce and respond to Hh ligands, has been observed in acute myeloid leukemia, *ALK*-positive anaplastic large cell lymphoma, and breast, pancreatic, lung, prostate, and gastrointestinal cancers (ref. 10; Fig. 1B). In addition, a paracrine pattern, in which Hh ligands secreted from tumor cells stimulate the stroma to secrete tumor growth factors, has been observed in chronic lymphocytic leukemia, plasma cell myeloma, pancreatic cancer, ovarian cancer, and colorectal cancer (refs. 10, 11; Fig. 1C).

On the basis of this evidence, Smo is a potential therapeutic target in a variety of tumor types. Inhibition of Smo has been studied across a wide array of *in vitro* tumor models, revealing antitumor activity in lung cancer cell lines, among others, in the absence of Ptch or Smo mutations (12). The Smo inhibitor vismodegib (Erivedge) is approved for the treatment of unresectable or metastatic BCC; however, vismodegib or other Hh inhibitors have not demonstrated clinical activity in solid tumors that are not driven by mutations in the Hh pathway (13, 14).

TAK-441 is an investigational, orally available, small-molecule inhibitor of Smo. In preclinical studies, the compound showed a 50% inhibitory concentration (IC_{50}) of Gli1 transcriptional activity of 4.4 nmol/L (15). TAK-441 is highly specific; assayed against a panel of 126 enzymes and transporters, 10 μmol/L TAK-441 demonstrated >50% inhibition of only the human phosphodiesterase type 4 inhibitor (PDE4) and the human dopamine transporter (Takeda Pharmaceuticals International Co., data on file). Strong antitumor activity was seen in a medulloblastoma murine allograft model harboring a Ptch1 mutation, and in pancreatic, ovarian, pancreas, and colon xenograft models, suggesting activity against both ligand-independent (mutationally driven) and ligand-dependent (via autocrine and paracrine signaling) mechanisms of Hh-driven cancer growth (16, 17). Preclinical toxicology evaluation demonstrated reversible bone marrow suppression, weight loss, gastrointestinal disturbances, and blood chemistry imbalances. Preclinical studies in rats and dogs revealed locomotor effects including gait abnormalities and tremors at doses that exceeded those predicted to be required for efficacy. In murine models of castration-resistant prostate cancer, TAK-441 was shown to abrogate Hh ligand paracrine signaling and inhibit tumor progression (18). Here we report the first study of TAK-441 in human cancer patients (NCT01204073).

Materials and Methods

Patients

Eligible patients were 18 years of age or older with a diagnosis of advanced, histologically confirmed, solid tumors refractory to previous therapy. Patients were to have Eastern Cooperative Oncology Group (ECOG) performance status ≤2, and adequate hematologic, hepatic, and renal function [absolute neutrophil count ≥1,500 cells/mm^3; hemoglobin ≥8 g/dL; platelet count ≥100,000/mm^3; total bilirubin ≤1.5 × upper limit of normal (ULN); aspartate and alanine aminotransferases ≤3 × ULN; calculated creatinine clearance ≥30 mL/minute by the Cockcroft–Gault formula]. Female patients were required to be postmenopausal for at least 1 year before screening, to be surgically sterile, to simultaneously practice 2 effective methods of birth control, or to abstain from intercourse. Male patients, even if surgically sterile, were required to practice barrier contraception or to abstain from intercourse. Major exclusion criteria included female patients who were lactating or who had a positive serum pregnancy test, major surgery or infection ≤14 days before the first dose of study drug, diarrhea grade >1 according to National Cancer Institute Common Technology Criteria for Adverse Events (NCI CTCAE; version 4.02), systemic antineoplastic therapy or radiotherapy ≤21 days before the first dose of study drug, systemic treatment with known strong or moderate CYP3A or P-glycoprotein inhibitors or inducers, evidence of current uncontrolled cardiovascular conditions, rate-corrected QT (QTc) >470 msec

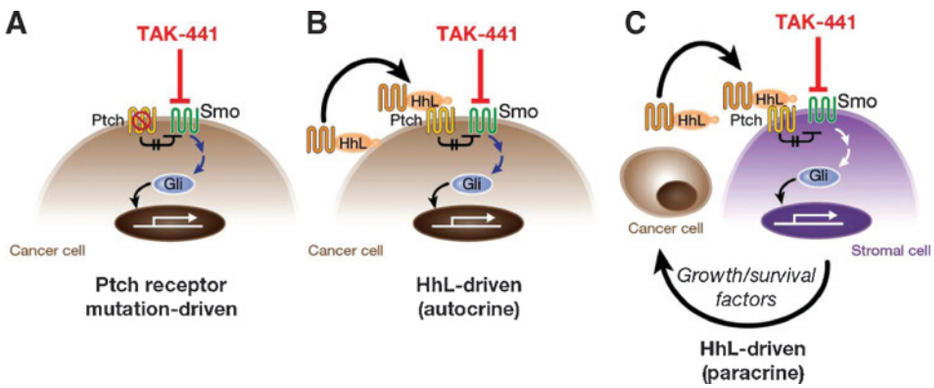

Figure 1.
Hedgehog (Hh) signaling in cancer: Ptch mutation-driven (A); HhL-driven (autocrine; B); HhL-driven (paracrine; C). HhL, Hh ligand; Ptch, Patched (protein); Smo, Smoothened receptor.

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on a 12-lead electrocardiograph (ECG), or central nervous system metastasis [unless the patient's neurologic status was stable without the use of corticosteroids and the patient had no neurologic dysfunction that would confound evaluation of neurologic adverse events (AE)]. The study was conducted in accordance with good clinical practice and the ethical principles founded in the Declaration of Helsinki and was authorized by the institutional review boards at the clinical sites. All patients provided signed informed consent before initiation of any study procedures.

Study design

This was a phase I, multicenter, dose-escalation study of TAK-441 in patients with advanced cancer (NCT01204073). The primary objectives of the study were to determine the safety profile, maximum tolerated dose (MTD), and recommended phase II dose of TAK-441; the maximum feasible dose (MFD) of TAK-441, based on tablet size and strength, was considered to be 1,600 mg daily. Secondary objectives included determination of the single- and multiple-dose pharmacokinetic profiles and pharmacodynamic effects of TAK-441 on the expression of *Gli1* in skin and tumors. TAK-441 was administered orally at doses of 50, 100, 200, 400, 800, and 1,600 mg. The starting dose was selected on the basis of preclinical findings suggesting that 50 mg daily would be pharmacologically active with an acceptable safety profile. Maximal inhibition of pancreatic xenograft tumors in mice was achieved at a dose of 10 mg/kg (17), which is equivalent to 30 mg/m^2 and corresponds to a human dose of 50 mg/day (based on a human body surface area of 1.7 m^2). On day 1 of cycle 1, patients received a single dose of TAK-441, followed by a 1-week washout period to assess the single-dose pharmacokinetic profile; dosing resumed on day 8 and continued daily until day 28. The duration of subsequent cycles was 21 days with daily continuous dosing. A series of dose-escalation cohorts was used to establish the MTD and inform the recommended phase II dose; 3 to 6 patients were enrolled at each dose level, according to a standard 3 + 3 design. The starting dose of TAK-441 was 50 mg daily, and was doubled in each successive cohort until a patient experienced a dose-limiting toxicity (DLT) during cycle 1 or any 2 patients experienced drug-related AEs of grade $\geq$2 during cycle 1. The MTD was defined as the highest dose at which <33% of patients experienced DLTs during cycle 1. A DLT was defined as any grade 4 neutropenia or grade 3 neutropenia with fever and/or infection, grade 4 thrombocytopenia or grade 3 thrombocytopenia with clinically significant bleeding, any grade $\geq$3 nonhematologic toxicity (other than fatigue <1 week in duration or nausea, vomiting, and diarrhea that could be controlled with standard supporting care), QTc prolongation (>500 msec assessed by a qualified reader and confirmed on a repeat electrocardiogram), or any TAK-441–related toxicity resulting in a treatment delay >14 days.

Clinical and safety assessments

Safety was assessed by physical examination, vital signs, electrocardiogram changes, laboratory evaluations, and occurrence of AEs. Adverse events were evaluated at each study visit and were graded using NCI CTCAE version 4.02. Disease assessments (computed tomography, magnetic resonance imaging, X-ray, and/or bone scans) were performed at baseline and during cycles 2, 4, and every fourth cycle thereafter according to Response Evaluation Criteria in Solid Tumors (RECIST) version 1.1 (19).

Pharmacokinetic assessments and data analyses

During cycle 1, blood samples for single-dose pharmacokinetic analysis were collected on day 1 starting before treatment, and at 0.5, 1, 2, 3, 4, 5, 8, 24, 48, 96, and 168 hours after dosing. Blood samples for pharmacokinetic analyses following multiple doses of TAK-441 were collected on day 22 of cycle 1 according to the same schedule up to 24 hours after dosing.

The concentration of TAK-441 in plasma was measured by a validated high-performance liquid chromatography/tandem mass spectrometry method with a dynamic range of 1 to 2,000 ng/mL. Pharmacokinetic parameters were calculated from concentration–time data using standard noncompartmental methods with WinNonlin software (Version 6.2, Pharsight Corporation). Single-dose pharmacokinetic parameters included the maximum plasma concentration (C_{max}), the time of first occurrence of C_{max} (T_{max}), area under the plasma concentration–time curve (AUC) from time 0 to the time of the last measurable concentration of TAK-441 (AUC_{0-last}), AUC from time 0 extrapolated to infinity ($AUC_{0-\infty}$), terminal disposition phase half-life ($t_{1/2}$), apparent clearance (CL/F), and volume of distribution (Vz/F). The pharmacokinetic parameters that were evaluated following multiple doses of TAK-441 included C_{max}, T_{max}, AUC_{0-last}, $AUC_{0-\infty}$, AUC over the dosing interval ($AUC_{0-\tau}$), accumulation ratio (R_{ac}), $t_{1/2}$, CL/F, and Vz/F. Dose proportionality with single and multiple dosing was assessed by examining the relationship between dose and C_{max} or AUC using linear regression analysis.

Pharmacodynamic assessments and analyses

Skin punch biopsies were obtained at baseline and on day 22 of cycle 1. The clinical assessment of pharmacodynamic effect was evaluated by quantitative reverse transcription PCR assessment of *Gli1* mRNA expression along with the control genes *B2M*, *POLR2A*, and *RPLP0*. The control genes were chosen to cover the low to high range of baseline expression in normal skin. The *Gli1* assay, using the ABI assay ID Hs01110776_g1, was validated according to Takeda standard operating procedures. Precision was established using *in vitro* transcribed RNA standards demonstrating linearity in RNA detection over 7 orders of magnitude. Normal healthy volunteer skin samples were assessed to establish normal parameters for viability of the measured transcripts and control genes. *Gli1* expression was normalized to the average of the control gene expression, and the difference (% inhibition) between drug-treated samples on day 22 and baseline samples was calculated as the pharmacodynamic effect.

Statistical analysis

Descriptive statistical analyses were conducted for patient demographic and baseline characteristics; summary statistics [mean, median, maximum, minimum, standard deviation, and 95% confidence interval (CI)] were used to evaluate safety and efficacy. Plasma pharmacokinetic parameters were summarized descriptively.

Results

Patient characteristics

Thirty-four patients were enrolled, and all received at least 1 dose of TAK-441 (Table 1). Median age was 59 years, and 18 patients (53%) were women. The most common primary malignancies were colorectal cancer (26%), BCC (21%), and pancreatic cancer (9%). Most patients (82%) had received prior systemic antineoplastic therapy.

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Table 1. Patient demographics

	Total ($N = 34$)
Median age, y (range)	59 (28–82)
Female, *n* (%)	18 (53)
White, *n* (%)	31 (91)
ECOG PS, *n* (%)	
0	8 (24)
1	26 (76)
Primary tumor type, *n* (%)	
Basal cell carcinoma	7 (21)
Colon cancer	6 (18)
Colorectal cancer	3 (9)
Pancreatic cancer	3 (9)
Ovarian cancer	2 (6)
Non–small cell lung cancer	3 (9)
Other[a]	10 (29)
Prior therapy, *n* (%)	
Systemic therapy	28 (82)
Radiotherapy	14 (41)
Surgery	32 (94)

Abbreviation: ECOG PS, Eastern Cooperative Oncology Group performance status.

[a]Other primary malignancies included anal cancer, breast cancer, gastric cancer, head and neck cancer, liver cancer, neuroendocrine pancreatic cancer, prostate cancer, rectal cancer, squamous cell carcinoma of the head and neck, and uterine leiomyosarcoma.

Dose escalation and safety

Three patients were enrolled in the initial dosing cohort of 50 mg daily. Dose levels were escalated in 5 additional cohorts: 100 mg daily ($n = 5$), 200 mg daily ($n = 6$), 400 mg daily ($n = 6$), 800 mg daily ($n = 4$), and 1,600 mg daily ($n = 9$). One of 9 patients at the highest dose level experienced DLTs of grade 3 fatigue and grade 3 muscle spasms during cycle 1. On the basis of tablet size and strength, the MFD of TAK-441 was determined to be 1,600 mg daily. Following the dose-escalation phase, 1 additional patient was enrolled at the 800 mg dose level.

Seven patients (21%) discontinued treatment because of AEs: 2 patients in the 50 mg cohort (1 grade 4 respiratory distress and 1 fatal cerebral hemorrhage); 2 patients in the 400 mg cohort (1 grade 2 increase in aspartate aminotransferase and alanine aminotransferase, 1 grade 2 groin pain); and 3 patients in the 1,600 mg cohort (1 grade 3 hepatic failure, 1 grade 3 epistaxis, and 1 grade 3 muscle spasm). The muscle spasms were considered by the investigator to be related to TAK-441, and the cerebral hemorrhage was considered to be possibly related. All other AEs were not considered to be study drug related; the grade 3 hepatic failure was attributed to disease progression.

All patients experienced at least 1 AE, and most AEs were of mild to moderate severity. The most common treatment-emergent AEs were dysgeusia (47%), fatigue (47%), nausea (47%), and muscle spasm (44%; Table 2). Fifteen patients (44%) experienced grade ≥3 AEs. The most common grade ≥3 AEs were hyponatremia (12%) and fatigue (9%) (Table 2). Twelve patients (35%) experienced at least 1 serious AE. The most frequent serious AEs were gastrointestinal disorders (4 patients, 12%), neoplasms (progression of underlying disease: 4 patients, 12%), and hepatobiliary disorders (3 patients, 9%). Twenty-five patients (74%) experienced AEs that were considered to be drug related; the most common were dysgeusia (47%), muscle spasms (41%), and nausea/vomiting (29%). Most drug-related AEs were grade 1 or 2. Five deaths occurred on-study (Supplementary Table S1); 3 deaths were attributed to progressive disease in patients with gastric cancer, pancreatic adenocarcinoma, and breast cancer, and one was attributed to respiratory distress in a patient with progressive non–small cell lung cancer. One death resulting from cerebral hemorrhage in a patient with pancreatic cancer was assessed by the investigator as possibly study drug-related.

Pharmacokinetics

After oral administration, absorption of TAK-441 was fairly rapid, and peak plasma concentration was achieved 2.0 to 4.0 hours after a single dose (Table 3). After attaining C_{max}, mean TAK-441 concentrations on day 1 declined in a multiexponential fashion whereby plasma concentrations were generally quantifiable up to 96 hours postdose for doses of 50 to 400 mg, and up to 168 hours postdose for doses of 800 and 1,600 mg. Mean plasma elimination $t_{1/2}$ ranged from 13.5 to 22.6 hours across the dose range. Consistent with this $t_{1/2}$, the observed accumulation ratio (day 22 $AUC_{0-\tau}$/day 1 $AUC_{0-\tau}$) was approximately 1.5 to 2 with

Table 2. Common adverse events (>10% of patients)

	TAK-441 dose													
	50 mg ($n = 3$)		100 mg ($n = 5$)		200 mg ($n = 6$)		400 mg ($n = 6$)		800 mg ($n = 5$)		1,600 mg ($n = 9$)		Total ($N = 34$)	
AE, *n* (%)	All grades	Grade ≥3	All grades	Grade ≥3	All grades	Grade ≥3	All grades	Grade ≥3	All grades	Grade ≥3	All grades	Grade ≥3	All grades	Grade ≥3
Dysgeusia	1 (33)	0	4 (80)	0	0	0	4 (67)	0	2 (40)	0	5 (56)	0	16 (47)	0
Fatigue	1 (33)	0	1 (20)	0	4 (67)	1 (17)	3 (50)	0	2 (40)	0	5 (56)	2 (22)	16 (47)	3 (9)
Nausea	2 (67)	0	2 (40)	0	4 (67)	0	2 (33)	0	3 (60)	0	3 (33)	1 (11)	16 (47)	1 (3)
Muscle spasms	1 (33)	0	4 (80)	0	3 (50)	0	3 (50)	0	1 (20)	0	3 (33)	1 (11)	15 (44)	1 (3)
Constipation	1 (33)	0	1 (20)	0	2 (33)	0	2 (33)	0	1 (20)	0	3 (33)	1 (11)	10 (29)	1 (3)
Alopecia	0	0	2 (40)	0	1 (17)	0	2 (33)	0	1 (20)	0	3 (33)	0	9 (26)	0
Decreased appetite	1 (33)	0	1 (20)	0	2 (33)	0	0	0	2 (40)	0	3 (33)	0	9 (26)	0
Diarrhea	1 (33)	0	2 (40)	0	0	0	2 (33)	0	2 (40)	0	1 (11)	0	8 (24)	0
Vomiting	0	0	0	0	2 (33)	0	2 (33)	0	2 (40)	0	2 (22)	1 (11)	8 (24)	1 (3)
Chills	0	0	2 (40)	0	1 (17)	1 (17)	1 (17)	0	1 (20)	0	0	0	5 (15)	1 (3)
Headache	0	0	1 (20)	0	2 (33)	0	1 (17)	0	0	0	1 (11)	0	5 (15)	0
Peripheral edema	0	0	2 (40)	0	0	0	2 (33)	0	0	0	1 (11)	0	5 (15)	0
Weight loss	0	0	0	0	1 (17)	0	1 (17)	0	2 (40)	0	1 (11)	0	5 (15)	0
Anemia	0	0	0	0	1 (17)	1 (17)	2 (33)	0	0	0	1 (11)	0	4 (12)	1 (3)
Cough	0	0	1 (20)	0	0	0	1 (17)	0	0	0	2 (22)	0	4 (12)	0
Hyponatremia	1 (33)	1 (33)	0	0	1 (17)	1 (17)	1 (17)	1 (17)	1 (20)	1 (20)	0	0	4 (12)	4 (12)
Pyrexia	0	0	2 (40)	0	1 (17)	0	1 (17)	0	0	0	0	0	4 (12)	0

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Table 3. Pharmacokinetic parameters of TAK-441

Dosing cohort	Single dose (Day 1)					Repeated dosing (Day 22)				
	C_{max}[a], μg/mL	T_{max}[b], h	$AUC_{0-\infty}$[a], μg*h/mL	CL/F[a], L/h	$t_{1/2}$[a], h	C_{max}[a], μg/mL	T_{max}[b], h	$AUC_{0-\tau}$[a], μg*h/mL	CL/F[a], L/h	R_{ac}[a]
50 mg	0.59 ± 0.28	3.0 (2.0, 4.0)	5.51 ± 0.32	9.1 ± 0.5	13.5 ± 0.8	0.73 ± 0.14	3.0 (1.1, 3.0)	8.68 ± 2.86	6.1 ± 2.0	1.15 ± 0.40
100 mg	0.93 ± 0.24	2.0 (1.0, 3.2)	18.35 ± 9.62	7.0 ± 3.9	16.4 ± 2.3	2.03 ± 1.33	2.5 (2.0, 3.1)	23.11 ± 14.91	6.3 ± 4.6	2.18 ± 0.68
200 mg	2.05 ± 1.30	4.0 (1.9, 7.5)	26.30 ± 16.82	9.9 ± 5.3	17.1 ± 9.5	2.79 ± 1.49	2.8 (2.0, 4.0)	31.35 ± 14.73	7.8 ± 4.3	1.52 ± 0.68
400 mg	2.81 ± 0.56	3.5 (2.3, 7.2)	59.22 ± 26.73	8.2 ± 4.1	13.8 ± 2.5	4.37 ± 1.24	3.5 (2.0, 7.1)	49.17 ± 13.16	8.5 ± 2.3	1.67 ± 0.33
800 mg	4.98 ± 0.83	3.0 (0.5, 3.9)	125.55 ± 30.29	6.6 ± 1.3	20.9 ± 8.3	8.87 ± 0.95	2.5 (1.0, 5.2)	127.33 ± 23.59	6.4 ± 1.2	2.29 ± 1.11
1,600 mg	9.07 ± 1.90	3.0 (1.0, 7.9)	199.88 ± 58.11	8.7 ± 2.9	22.6 ± 15.7	12.57 ± 2.19	3.0 (1.0, 7.5)	183.00 ± 60.11	9.7 ± 3.6	1.47 ± 0.16

Abbreviations: $AUC_{0-\infty}$, area under the concentration–time curve from time 0 extrapolated to infinity; $AUC_{0-\tau}$, area under the concentration–time curve from time 0 until the end of the dosing interval; CL/F, clearance; C_{max}, maximum concentration; R_{ac}, accumulation ratio ($AUC_{0-\tau}$ on day 22/$AUC_{0-\tau}$ on day 1); $t_{1/2}$, half-life; T_{max}, time to maximum concentration.

[a]Mean ± standard deviation.

[b]Median (minimum, maximum).

once-daily dosing. Plasma concentration increased with dose following single or multiple dosing (Fig. 2; Table 3). Linear regression analysis revealed a linear relationship between systemic exposure and dose. With single dosing, the estimated slope of the regression line for dose and $AUC_{0-\infty}$ was 0.98 (95% CI, 0.83–1.12; $P = 0.731$). With multiple dosing, the estimated slope of the regression line for dose and $AUC_{0-\tau}$ was 0.86 (95% CI, 0.69–1.03; $P = 0.096$). C_{max} increased in a slightly less than dose proportional manner; estimated slope of the regression line for dose and C_{max} was 0.80 (95% CI, 0.71–0.89; $P < 0.001$) for single dosing and 0.80 (95% CI, 0.66–0.93; $P = 0.005$) for multiple dosing. Interindividual variability in TAK-441 systemic exposure after single- and multiple-dose administration was moderate, as indicated by coefficients of variation ranging from 10.8% to 65.9% for

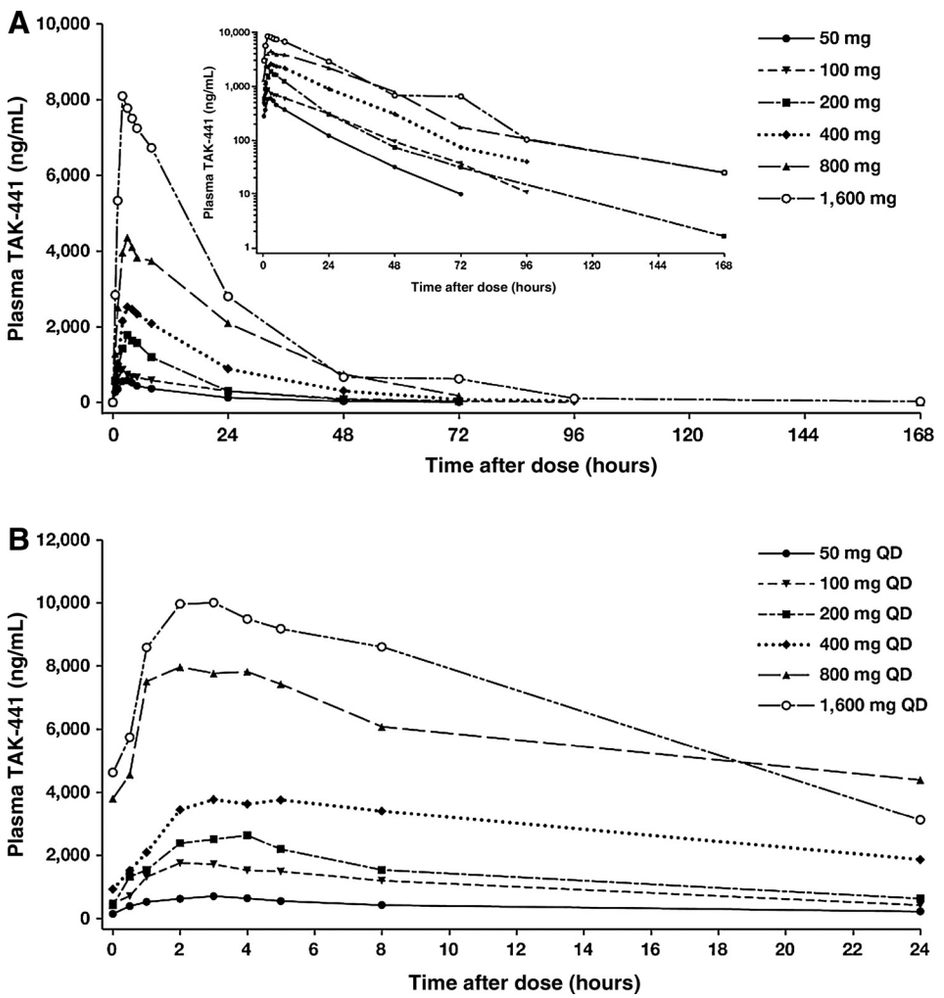

Figure 2.
Mean plasma concentration–time profiles of TAK-441. A and B, overlays of the mean concentration–time profiles from patients in the dose-escalation cohorts (50 mg daily to 1,600 mg daily) measured on day 1 (A) and on day 22 (B). A (inset), concentration–time profiles on day 1 plotted on a logarithmic scale. QD, daily.

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Table 4. Inhibition of skin *Gli1* mRNA (pharmacodynamic-evaluable population)

Dosing cohort (*n*)	Mean inhibition of *Gli1* mRNA relative to control genes[a], % (SD)	Mean inhibition of *Gli1* mRNA relative to *Smo*, % (SD)
50 mg (1)	94.2 (NA)	94.5 (NA)
100 mg (4)	92.7 (4.1)	92.8 (5.1)
200 mg (4)	92.6 (5.0)	92.2 (5.6)
400 mg (4)	94.5 (2.7)	93.5 (3.5)
800 mg (4)	92.8 (2.2)	91.9 (2.4)
1,600 mg (4)	87.3 (18.0)	89.9 (13.6)
Total (21)	92.1 (7.9)	92.2 (6.4)

Abbreviations: *Gli1*, glioma-associated oncogene homolog 1; mRNA, messenger ribonucleic acid; SD, standard deviation, *Smo*, Smoothened.
[a]Control genes were *B2M*, *POLR2A*, and *RPLP0*.

C_{max} and from 5.8% to 64.5% for $AUC_{0-\infty}$ and $AUC_{0-\tau}$ across doses.

Pharmacodynamics

Among the 21 pharmacodynamic-evaluable patients, the overall mean decrease in *Gli1* mRNA expression was >90%. Strong inhibition of *Gli1* mRNA in skin was apparent at all dose levels, including the lowest dose of 50 mg daily (Table 4).

Efficacy

Twenty-eight patients were evaluable for tumor response to TAK-441. One patient with BCC in the 400 mg cohort experienced a confirmed partial response (PR) that was durable throughout the study (24 cycles). Stable disease (SD) was observed in 7 patients (25%) and was sustained for ≥4 cycles in 4 patients (14%). Among the 7 patients whose best response was SD, 4 had BCC (1 patient each in the 200, 400, 800, and 1,600 mg cohorts), 1 had pancreatic cancer (50 mg cohort), 1 had neuroendocrine cancer (400 mg cohort), and 1 had colorectal cancer (1,600 mg cohort).

Discussion

Aberrant Hh signaling has been implicated in a variety of malignancies, including BCC. We report a first-in-human, dose-escalation trial of TAK-441, an orally available inhibitor of Smo, a key component of the Hh pathway, in patients with advanced solid tumors. The drug was well tolerated, and the MTD was not reached. Most AEs were mild to moderate in severity and did not appear to be dose related. The most common treatment-emergent AEs were dysgeusia, fatigue, nausea, and muscle spasms. Grade ≥3 AEs were infrequent, and those observed in >1 patient were limited to fatigue and hyponatremia. Five patients died during the study. Four of the deaths were attributed to progression of the patient's underlying disease and were not considered to be related to study treatment. The fifth on-study death was the result of a cerebral hemorrhage in a patient receiving 50 mg TAK-441 daily (the lowest dose that was evaluated). The investigator considered that event to be possibly related to TAK-441, but also considered the patient's diagnosis of pancreatic cancer to be a potential cause for the event; the definitive cause of the cerebral hemorrhage was unknown. The observed safety profile of TAK-441 was similar to that reported for the Hh inhibitors vismodegib and IPI-926, suggesting that AEs such as dysgeusia, fatigue, and muscle spasms may be class-effect toxicities (20–22). Of note, little is known regarding the pathophysiology of the muscle spasms, although locomotor effects including gait imbalance and tremors were noted in rat and dog toxicology studies. No associated creatine phosphokinase elevations or other laboratory evidence of myositis were observed in this study (data not shown) or have been reported elsewhere.

The single- and multiple-dose pharmacokinetics of TAK-441 were linear over the dose range of 50 to 1,600 mg daily. The median $t_{1/2}$ of 12.9 to 18.3 hours following a single oral dose is similar to that of IPI-926 (16.1–19.3 hours; ref. 22) and shorter than that of vismodegib (>7 days; ref. 23). Pharmacodynamic responses to TAK-441 were evaluated by analysis of skin biopsies. Expression of *Gli1* mRNA in skin was strongly inhibited at all dose levels and was reduced >90% relative to control genes or to *Smo*. This result compares favorably with the pharmacodynamic effects reported with other Hh inhibitors. Downregulation of skin *Gli1* mRNA was observed in 25 of 34 patients (74%) receiving vismodegib (21), and in 48 of 65 patients (74%) receiving IPI-926 (22).

Despite excellent target inhibition by TAK-441, only modest antitumor activity was observed in this broad population of patients with solid tumors. Best response was a confirmed PR in 1 patient and SD in 7 patients. The confirmed PR was observed in the 400 mg cohort, and SD was observed in all but the 100 mg dosing cohort. However, activity was observed in the 7 BCC patients, with 5 (71%) experiencing PR or SD. These results are similar to those from single-agent trials with other Hh inhibitors (21–23), and suggest that Smo activity is important in BCC, whereas ligand-dependent Hh signaling may play a lesser role in other tumor types. A phase I trial of vismodegib in patients with advanced solid tumors reported that clinical responses were only observed in patients with BCC and medulloblastoma (21, 24). In that trial, 33 of 68 enrolled patients had BCC, and the objective response rate in those patients was 58%. Therefore, further study of TAK-441 may be appropriate in populations selected for ligand-dependent or ligand-independent reliance on Hh signaling, particularly in patients with Ptch-driven tumors such as BCC.

Another role for Hh inhibitors, such as TAK-441, may be as part of combination therapy with other targeted agents, particularly in tumors wherein the Hh pathway is not the principal oncogenic driver, but may contribute to oncogenesis or interact with other pathways. For example, Hh signaling appears to modulate the response to EGFR signaling in head and neck squamous cell carcinoma (25); dual inhibition of both the Hh and EGFR pathways is therefore an intriguing strategy. In addition, evidence of cross-talk between the Hh and PI3K-mTOR pathways suggests that combined inhibition may be of value; a clinical trial evaluating the Hh inhibitor sonidegib plus the PI3K inhibitor buparlisib has been initiated (26, 27).

Increased Hh expression in cancer stem cells (CSC) has been reported for a number of tumor types, and inhibition of Hh signaling is being explored as a method to overcome CSC-driven resistance to systemic therapy (27, 28). In a mouse model of pancreatic adenocarcinoma, IPI-926 led to increased intratumoral delivery of gemcitabine, and significantly improved survival (29). However, this combination did not show clinical activity; a phase II trial of IPI-926 plus gemcitabine in patients with metastatic prostate cancer was stopped early because of lack of efficacy (30). Additional trials are ongoing to evaluate CSC-directed rational combinations of Hh inhibitors with other agents including temozolomide, the BCR-ABL inhibitor dasatinib, and the notch inhibitor RO4929097 (27).

In conclusion, TAK-441 appears to be a potent inhibitor of the Hh signaling pathway and was generally well tolerated up to the

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maximum feasible dose. It has modest single-agent anticancer activity and may be appropriate for evaluation in combination therapy for tumors driven by Hh signaling.

Disclosure of Potential Conflicts of Interest

S. Gail Eckhardt reports receiving a commercial research grant from and is a consultant/advisory board member for Takeda. A. Parikh holds ownership interest (including patents) in Takeda. J. Partyka is an employee of and has ownership interest in Puma Biotechnology, Inc. E. Gangolli is an employee of AstraZeneca and holds ownership interest (including patents) in Novartis. No potential conflicts of interest were disclosed by the other authors.

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