

# A Phase I Study of ABC294640, a First-in-Class Sphingosine Kinase-2 Inhibitor, in Patients with Advanced Solid Tumors



Carolyn D. Britten<sup>1</sup>, Elizabeth Garrett-Mayer<sup>2</sup>, Steven H. Chin<sup>1</sup>, Keisuke Shirai<sup>1</sup>, Besim Ogretmen<sup>3</sup>, Tricia A. Bentz<sup>4</sup>, Alan Brisendine<sup>4</sup>, Kate Anderton<sup>4</sup>, Susan L. Cusack<sup>5</sup>, Lynn W. Maines<sup>5</sup>, Yan Zhuang<sup>5</sup>, Charles D. Smith<sup>5</sup>, and Melanie B. Thomas<sup>1</sup>

## Abstract

**Purpose:** Sphingosine kinases (SK1 and SK2) regulate tumor growth by generating the mitogenic and proinflammatory lipid sphingosine 1-phosphate (S1P). This phase I study investigated the safety, pharmacokinetics, pharmacodynamics, and antitumor activity of ABC294640, a first-in-class orally available inhibitor of SK2.

**Experimental Design:** Escalating doses of ABC294640 were administered orally to patients with advanced solid tumors in sequential cohorts at the following dose levels: 250 mg qd, 250 mg bid, 500 mg bid, and 750 mg bid, continuously in cycles of 28 days. Serial blood samples were obtained to measure ABC294640 concentrations and sphingolipid profiles.

**Results:** Twenty-two patients were enrolled, and 21 received ABC294640. The most common drug-related toxicities were

nausea, vomiting, and fatigue. Among the 4 patients at 750 mg bid, one had dose-limiting grade 3 nausea and vomiting, and 2 were unable to complete cycle 1 due to diverse drug-related toxicities. The 500 mg bid dose level was established as the recommended phase II dose. ABC294640 administration resulted in decreases in S1P levels over the first 12 hours, with return to baseline at 24 hours. The best response was a partial response in a patient with cholangiocarcinoma at 250 mg qd, and stable disease was observed in 6 patients with various solid tumors across dose levels.

**Conclusions:** At 500 mg bid, ABC294640 is well tolerated and achieves biologically relevant plasma concentrations. Changes in plasma sphingolipid levels may provide a useful pharmacodynamic biomarker for ABC294640. *Clin Cancer Res*; 23(16): 4642–50. ©2017 AACR.

## Introduction

Sphingolipids are bioactive lipids that act intracellularly as second messengers and extracellularly through cell surface G-protein-coupled sphingosine 1-phosphate (S1P) receptors to regulate proliferation, apoptosis, inflammation, and angiogenesis (1–3). In response to a variety of proliferative or inflammatory stimuli, sphingomyelin is hydrolyzed to produce ceramides that induce apoptosis in tumor cells (Fig. 1). Ceramides are further

hydrolyzed to produce sphingosine, which is phosphorylated by sphingosine kinases (SK1 and SK2) to produce S1P, which is mitogenic and proinflammatory. A critical balance of these lipids, that is, a ceramide: S1P rheostat, determines the fate of tumor cells (4, 5). Specifically, upon stimulation, tumor cells increase S1P levels with concomitant reduction of ceramide levels, thereby promoting survival and proliferation. In contrast, accumulation of ceramide through inhibition of its metabolism to S1P leads to tumor cell apoptosis.

Mammals encode two SK isozymes (SK1 and SK2) that have different subcellular localizations, functions, and pharmacology (3, 6, 7). For example, overexpression of SK1 is oncogenic (8, 9), while transfection with SK2 was originally reported to inhibit cell growth and to induce apoptosis (10). However, these effects of SK2 were not dependent on its catalytic activity, suggesting that the antiproliferative effect may be mediated by its BH3 domain (10). We used RNAi to examine the individual roles of SK1 and SK2 in regulating sphingolipid levels and tumor cell proliferation and demonstrated that cell proliferation and migration were suppressed more by downregulation of SK2 than SK1 (11). Furthermore, exogenous S1P and overexpression of SK1 did not rescue the cells from the antiproliferative effects of the SK2 inhibition. Therefore, we have focused on SK2 as a target for new anticancer drugs.

Despite intensive research on sphingolipid signaling, there are relatively few inhibitors of SKs (2, 7, 12, 13). Commonly used lipid analogues, such as N,N-dimethylsphingosine and dihydro-sphingosine, lack selectivity for SK (14, 15) and are ineffective *in*

<sup>1</sup>Division of Hematology/Oncology, Department of Medicine, Medical University of South Carolina, Charleston, South Carolina. <sup>2</sup>Department of Population Sciences, Medical University of South Carolina, Charleston, South Carolina. <sup>3</sup>Department of Biochemistry & Molecular Biology, Medical University of South Carolina, Charleston, South Carolina. <sup>4</sup>Hollings Cancer Center, Medical University of South Carolina, Charleston, South Carolina. <sup>5</sup>Apogee Biotechnology Corporation, Hummelstown, Pennsylvania.

**Note:** C.D. Britten and M.B. Thomas contributed equally to this article.

Current address for M.B. Thomas: Department of Hematology-Oncology, Gibbs Cancer Center & Research Institute, Spartanburg, South Carolina; current address for S.H. Chin: Eli Lilly & Co., Lilly Corporate Center, Indianapolis, Indiana; and current address for K. Shirai: Hematology/Oncology Section, Dartmouth-Hitchcock Medical Center, 1 Medical Center Dr., Lebanon, New Hampshire.

**Corresponding Author:** Charles D. Smith, Apogee Biotechnology Corporation, 1214 Research Blvd, Suite 2014, Hummelstown, PA 17036. Phone/Fax: 717-531-4758; E-mail: cdsmith@apogee-biotech.com

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### Translational Relevance

Sphingolipid metabolism has been widely studied in cell culture and animal models because of its roles in regulating cancer proliferation, angiogenesis, and inflammation. However, translation of these basic research studies into clinical oncology uses has been extremely limited. We have developed ABC294640, the first-in-class clinical inhibitor of SK2, and herein report data from the first-in-human clinical trial of ABC294640. In addition to safety and efficacy findings, we provide the first data on sphingolipid profiles in humans treated with a sphingolipid metabolism-targeted drug. The results provide key information for further clinical testing of ABC294640, as well as proof of concept for monitoring plasma sphingolipid profiles in patients.

*in vivo* due to poor pharmacologic properties. The "first-generation" SK inhibitors that we described in 2003, particularly SKI-II (16), have also been widely used in nonclinical studies, although most investigators mistakenly refer to them as SK1 inhibitors when they are in fact more potent toward SK2 (17). More recently, several groups have described selective inhibitors of SK1 (7, 13); however, these SK1-selective inhibitors typically do not induce tumor cell death (18, 19). We have identified structurally novel "drug-like" inhibitors of SK1 and SK2 (16, 17, 20, 21), and ABC294640 was selected as the first compound in this program for clinical testing.

ABC294640 is an inhibitor of SK2 ( $K_i = 9 \mu\text{mol/L}$ ,  $3.4 \mu\text{g/mL}$ ) and is competitive with respect to sphingosine (17, 21), which greatly reduces its potential for off-target inhibition of protein kinases. ABC294640 depletes S1P and elevates ceramide in tumor cells, suppresses signaling through pERK and pAKT, and promotes autophagy and/or apoptosis in tumor cells (17, 21–26). ABC294640 downregulates the expression of c-Myc in a variety of tumor cell lines (25, 27–30) and also reduces androgen receptor expression in prostate cancer cells (25, 27). ABC294640 was also recently shown to inhibit dihydroceramide desaturase, which accounts for the marked increases in dihydroceramides in cells treated with the drug (21, 27). The antitumor activity of ABC294640 has been demonstrated by us and others in a variety of mouse models (21, 24, 25, 27, 28, 31–37). Single-agent

antitumor activity is typically apparent with doses of ABC294640 of 25 mg/kg/day or greater, and the response is associated with accumulation of the drug in the tumors, depletion of tumoral S1P levels, and induction of apoptosis (21). As a pharmacodynamic marker, ABC294640 reduces plasma S1P levels up to 50% at therapeutically efficacious drug doses (34). Preclinical studies demonstrated a good oral bioavailability and safety profile for ABC294640, with no hematologic or major organ toxicity (21). On the basis of its strong preclinical profile and its novel mechanism of action, this first-in-human phase I trial with ABC294640 was undertaken to assess the drug's safety, and to identify the MTD and the dose-limiting toxicities (DLT) using a continuous daily dosing regimen (ClinicalTrials.gov registry identifier NCT01488513). Secondary objectives were to characterize the plasma exposure of ABC294640, to determine its effects on the plasma sphingolipid profile, and to seek preliminary evidence of anticancer activity.

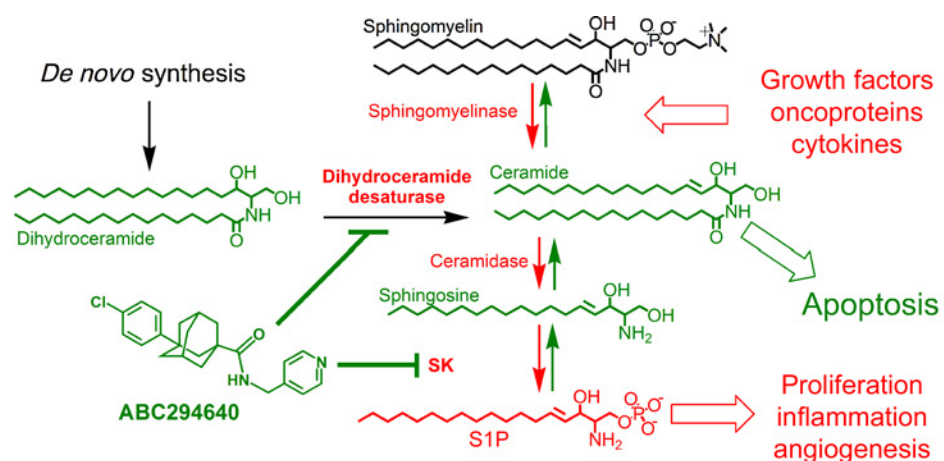
## Materials and Methods

### Study design and patient population

This first-in-human, phase I trial employed a standard 3 + 3 method, as allowed by the FDA under IND 109662. Sequential cohorts of 3 to 6 patients were assigned to progressively higher doses of ABC294640 administered qd or bid in continuous 28-day treatment cycles. Extensive preclinical work with ABC294640 established a half-life of approximately 4 hours across several species, and so bid dosing of patients was expected to provide adequate exposure to the drug. The starting dose was defined by the IND-enabling animal toxicology studies with allometric scaling using the standard interspecies factors. ABC294640 was administered under fasting conditions (at least 1 hour before or 2 hours after eating). DLT was defined as any one of the following events, classified by the NCI Common Toxicity Criteria (NCI-CTCAE) Version 4.0, occurring during cycle 1 and attributable to ABC294640: grade 3 or 4 thrombocytopenia, anemia and/or neutropenia; grade 3 or 4 nausea, vomiting or diarrhea that persisted despite the use of adequate medical intervention and/or prophylaxis; any other grade 3 or greater nonhematologic toxicity; or failure to recover from toxicities in time to resume treatment within 4 weeks. The MTD was defined as the dose level below that which induced DLTs in at least 2 patients during the first cycle of treatment.

**Figure 1.**

Sphingolipid metabolism. This abbreviated summary of sphingolipid interconversions indicates proliferative and proinflammatory steps and lipids in red, whereas proapoptotic steps and lipids are indicated in green. ABC294640 targets SK2 and dihydroceramide desaturase, thereby reducing S1P levels and increasing ceramide, particularly dihydroceramides.



Patients  $\geq 18$  years of age with histologically confirmed solid organ cancers for which there was no standard treatment, or which were unresponsive to treatment, were eligible for study participation. In addition, patients were required to have an Eastern Cooperative Oncology Group performance status of 0 to 2 and adequate bone marrow, renal, and hepatic function. Patients were excluded if they had significant cardiovascular disease. In addition, the use of drugs known to be sensitive substrates of CYP1A2, 3A4, 2C9, 2C19, or 2D6 or strong inhibitors or inducers of all major CYP450 isozymes were contraindicated to provide the largest possible safety margin in this first-in-human study. After the first patient enrolled developed dose-limiting hyperglycemia, the protocol was amended to require fasting blood glucose  $\leq 160$  mg/dL. The clinical trial was approved by an independent ethics committee (Western Institutional Review Board) and was performed in accordance with the Declaration of Helsinki, Good Clinical Practice guidelines, and applicable local regulatory requirements and laws. All patients provided their written informed consent.

### Drug product

4-Pyridinylmethyl-3-(4-chlorophenyl)adamantinecarboxamide, hydrochloride salt (ABC294640) was synthesized by ChemPacific Corporation and manufactured into 250 mg gelatin capsules by UPM Pharmaceuticals.

### Study procedures

ABC294640 was administered continuously in 28-day cycles to sequential patient cohorts enrolled at the following dose levels: 250 mg qd (total daily dose = 250 mg), 250 mg bid (total daily dose = 500 mg), 500 mg bid (total daily dose = 1,000 mg), and 750 mg bid (total daily dose = 1,500 mg). Safety evaluations, including physical examination, vital signs, electrocardiograms, and clinical laboratory tests (hematology, blood chemistry, and urinalysis), were performed at baseline and weekly during cycle 1, biweekly during cycle 2 to cycle 4, and on day 1 of each cycle thereafter. All patients underwent a baseline echocardiogram, and electrocardiograms were obtained at multiple time points during cycle 1, day 1 concurrent with the collection of samples for plasma drug level analyses. Adverse events were graded according to the NCI-CTCAE, Version 4.0. Tumor response was determined by imaging performed every 2 cycles, using RECIST version 1.1 criteria. Blood samples for serial plasma drug level analyses were obtained at baseline, and at 1 to 24 hours after dosing on cycle 1 day 1 and on cycle 1 day 28. This period was selected to provide data over the course of several half-lives, predicted by animal data (~4.5 hours). ABC294640 concentrations were determined using HPLC/MS-MS by Ricerca Biosciences, using  $^{13}\text{C}$ -labeled ABC294640 as the internal standard. This assay demonstrated an analytic quantitation range of 0.0181 to 4.53  $\mu\text{g/mL}$ , with accuracy (% of theoretical)  $\pm$  precision (% coefficient of variation) of  $96.3 \pm 7.3$ ,  $94.1 \pm 6.0$ , and  $97.5 \pm 3.1$ , for low-, mid- and high-range standards, respectively. Noncompartmental pharmacokinetic analyses were performed using validated pharmacokinetic software (Phoenix WinNonlin, Certara).

Blood samples for pharmacodynamic analyses were obtained at baseline, in parallel with samples for pharmacokinetic analyses on cycle 1 days 1 and 28, and at each clinic visit. Plasma concentrations of S1P, dihydro-S1P, dihydro- $\text{C}_{16}$  ceramide, and other sphingolipids were determined by HPLC/MS-MS at the Lipidomics Core Shared Resource at the Medical University of South

**Table 1.** Patient characteristics

Characteristics	Study population (n = 21)
Mean age, years (range)	60 (22–73)
Gender, n (%)	
Male	15 (71)
Female	6 (29)
Race, n (%)	
White	14 (67)
Black	4 (19)
Other	3 (14)
ECOG performance status, n (%)	
0	10 (48)
1	11 (52)
Primary tumor type, n (%)	
Colon/rectum	6 (29)
Pancreas	4 (19)
Cholangiocarcinoma	3 (14)
Urothelial	3 (14)
Other <sup>a</sup>	5 (24)
Prior systemic therapy, n (%)	
1 Regimen	10 (48)
2 Regimens	3 (14)
$\geq 3$ Regimens	8 (38)

Abbreviation: ECOG, Eastern Cooperative Oncology Group.

<sup>a</sup>Adrenocortical cancer, hepatocellular cancer, malignant mixed mullerian tumor, non-small cell lung cancer, unknown primary (one each).

Carolina as described previously (38–40). Quantitation was conducted using an 8-point calibration curve for each lipid analyte (2.5–400 pmol/sample), and linear regression of the sample peak area, followed by normalization to the volume of plasma utilized (0.1 mL). The lower limits of detection for S1P, dihydro-S1P, and dihydro- $\text{C}_{16}$  ceramide were 4.2, 2.0, and 1.8 nmol/L, respectively.

Descriptive statistics were used to summarize results, with ranges and error included. Pharmacokinetic measures (AUC and  $C_{\text{max}}$ ) were compared between patients who had drug-related neuropsychiatric events and those who did not using Wilcoxon rank sum tests with comparisons made for day 1, day 28, and changes between day 1 and day 28.

## Results

### Patient characteristics

Twenty-two patients were enrolled at the Hollings Cancer Center (Charleston, SC) between September 2011 and July 2014, with characteristics of the 21 patients who received study drug summarized in Table 1. One patient (who was to be treated at 500 mg bid) was withdrawn prior to dosing due to onset of neurologic symptoms, and 2 (one at 250 mg bid and one at 500 mg bid) progressed prior to cycle 1 day 22 without DLT: all three were replaced. Altogether, patients received a total of 80 cycles of ABC294640, with a median of 3 (range, 0–19) cycles per patient.

### Safety and tolerability

The dose escalation summary is provided in Table 2. The first patient enrolled in the study (250 mg qd) developed dose-limiting grade 4 hyperglycemia in the setting of rapidly progressing pancreatic cancer and pancreatic failure, necessitating expansion of that cohort. Throughout the study, no other patient experienced drug-related hyperglycemia. Among the 4 patients enrolled at 750 mg bid, one ovarian cancer patient had dose-limiting grade 3 nausea and vomiting, and 2 patients were unable

**Table 2.** Dose escalation summary

Cohort	Dose level	Number of patients (evaluable for DLT <sup>a</sup> )	DLT
1	250 mg qd	6 (6)	Grade 4 hyperglycemia (1 patient)
2	250 mg bid	4 (3 <sup>b</sup> )	None
3	500 mg bid	4 (3 <sup>c</sup> )	None
4	750 mg bid	4 (2 <sup>d</sup> )	Grade 3 nausea/vomiting (1 patient)
5	500 mg bid	3 (3)	None

<sup>a</sup>Evaluable patients must have completed cycle 1 or experienced a DLT.

<sup>b</sup>One patient had clinical disease progression and was replaced.

<sup>c</sup>One patient had disease progression and was replaced.

<sup>d</sup>Two patients had dose interruption and/or dose reduction for intolerable grade 2 toxicity.

to complete cycle 1 due to drug-related toxicities (one with grade 2 dysarthria, which recurred with grade 2 noncardiac chest discomfort and grade 2 choking sensation when drug was restarted at 500 mg bid; the other with grade 2 acute kidney injury, grade 3 nausea, and grade 2 agitation). The 750 mg bid dose level was considered not to be tolerable, and the 500 mg bid dose level was expanded. Treatment of one patient in the 500 mg bid expansion cohort was interrupted, and dose reduced during cycle 3 due to grade 1 hallucinations, muscle spasms, and paresthesias, and this patient was ultimately

discontinued when spasticity recurred at 250 mg bid. The other 5 patients enrolled and treated at 500 mg bid tolerated the drug well, without dose modification or interruption for drug-related toxicity.

All 21 patients treated with ABC294640 were included in the safety assessment. Treatment-related adverse events that were reported in >5% of patients, and all serious treatment-related adverse events, occurring in cycle 1 or in any cycle, are summarized in Table 3. Across all dose levels, the most common treatment-related toxicities were nausea, fatigue, and vomiting. In

**Table 3.** Most common (>5%) and all serious treatment-related adverse events by ABC294640 dose level, occurring in cycle 1 or any cycle

Adverse event	Cycle(s)	250 mg qd n (total = 6) Grades		250 mg bid n (total = 4) Grades		500 mg bid n (total = 7) Grades		750 mg bid n (total = 4) Grades		All patients n (%) Grades	
		1-2	3-4	1-2	3-4	1-2	3-4	1-2	3-4	1-2	3-4
Nausea	1	1	0	3	0	0	0	2	2	6 (29)	2 (10)
	All	4	0	3	0	0	0	2	2	9 (43)	2 (10)
Fatigue	1	0	0	0	0	3	0	2	0	5 (24)	0
	All	1	0	0	0	3 <sup>a</sup>	0	2	0	6 (29)	0
Vomiting	1	0	0	1	0	0	0	2	1	3 (14)	1 (5)
	All	1	0	1	0	0	0	2	1	4 (19)	1 (5)
Creatinine increase	1	0	0	0	0	0	0	2	0	2 (10)	0
	All	0	0	0	0	0	0	3	0	3 (14)	0
Diarrhea	1	0	0	0	0	1	0	2	0	3 (14)	0
	All	0	0	0	0	1	0	2	0	3 (14)	0
Dizziness	1	0	0	0	0	1	0	1	0	2 (10)	0
	All	0	0	0	0	1	0	1	0	2 (10)	0
Dysgeusia	1	1	0	0	0	1	0	0	0	2 (10)	0
	All	1	0	0	0	1	0	0	0	2 (10)	0
Gastroesophageal reflux	1	0	0	1	0	1	0	0	0	2 (10)	0
	All	0	0	1	0	1	0	0	0	2 (10)	0
Hot flashes	1	0	0	0	0	1	0	1	0	2 (10)	0
	All	0	0	0	0	1	0	1	0	2 (10)	0
Mood change	1	0	0	0	0	0	0	1	0	1 (5)	0
	All	0	0	0	0	1	0	1	0	2 (10)	0
Muscle spasm	1	0	0	0	0	1	0	1	0	2 (10)	0
	All	0	0	0	0	1	0	1	0	2 (10)	0
Muscle weakness	1	1	0	0	0	0	0	0	0	1 (5)	0
	All	1	0	0	0	1	0	0	0	2 (10)	0
Somnolence	1	0	0	0	0	2	0	0	0	2 (10)	0
	All	0	0	0	0	2	0	0	0	2 (10)	0
Vision change	1	0	0	0	0	0	0	0	0	0	0
	All	0	0	1 <sup>b</sup>	0	0	0	1	0	2 (10)	0
Hyperbilirubinemia	1	0	0	0	0	0	0	0	0	0	0
	All	0	0	0	1 <sup>c</sup>	0	0	0	0	0	1 (5)
Hyperglycemia	1	0	1	0	0	0	0	0	0	0	1 (5)
	All	0	1	0	0	0	0	0	0	0	1 (5)

<sup>a</sup>One patient was initially treated at 750 mg bid, but experienced this toxicity after dose reduction to 500 mg bid.

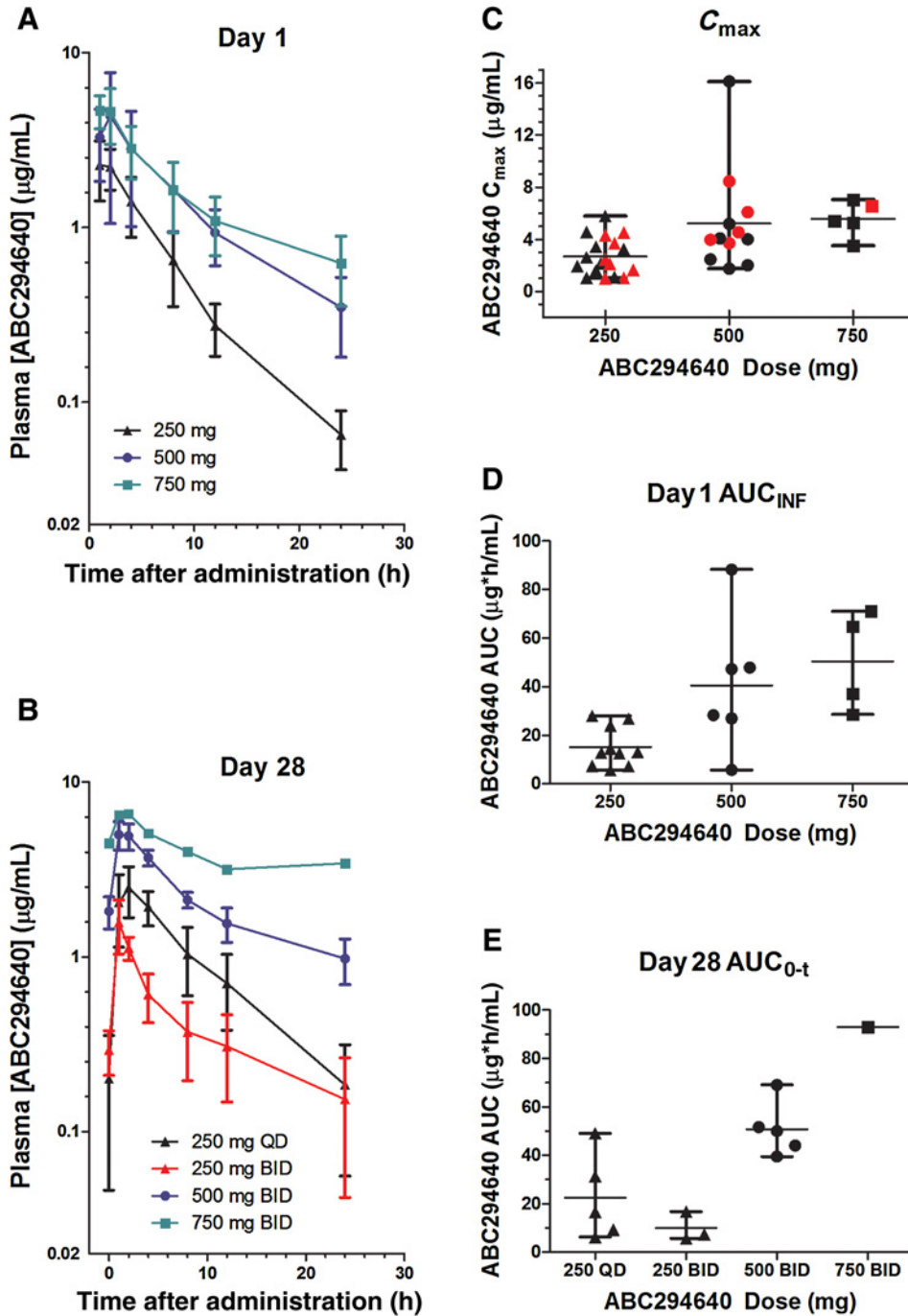
<sup>b</sup>Patient initially treated at 500 mg bid, but experienced this toxicity after dose reduction to 250 mg bid.

<sup>c</sup>A sigmoid colon cancer patient with liver metastases developed hyperbilirubinemia during course 3. Imaging revealed progressive liver metastases and biliary dilatation without an identifiable obstructive mass. Treatment was discontinued.

addition, 6 patients (1 at 250 mg qd, 1 at 250 mg bid, 2 at 500 mg bid, and 2 at 750 mg bid) experienced treatment-related nervous system disorders, including dizziness, dysarthria, dysgeusia, dysesthesia, headache, memory loss, muscle spasms, paresthesias, somnolence, and/or spasticity, and 2 of these patients (1 at 500 mg bid and 1 at 750 mg bid) also experienced psychiatric disorders, including agitation/anxiety, mood changes, and/or hallucinations. The neuropsychiatric effects were all grade 1 to 2 in severity and resolved upon interruption and/or discontinuation of ABC294640.

**Plasma drug exposure**

On cycle 1 day 1 and cycle 1 day 28, only one dose of ABC294640 was administered and blood was collected periodically over the next 24 hours. Plasma levels of ABC294640 typically peaked at 1 to 2 hours and declined with a mean half-life of 5 to 15 hours (Fig. 2A and B). There was a tendency toward increased  $t_{1/2}$  with higher doses of drug and at day 28 compared with day 1, suggesting that elimination may be saturated by high drug exposure. Data for the 250 mg qd and 250 mg bid cohorts is pooled for the analyses of day 1 samples (Fig. 2A),



**Figure 2.** Plasma drug levels of ABC294640. **A**, Cycle 1 day 1: Levels of ABC294640 were measured in plasma samples prepared from patients treated with 250 mg (▲), 500 mg (●), or 750 mg (■) of ABC294640. Data for patients in the 250 mg qd and 250 mg bid cohorts are combined because only one dose of drug was administered on day 1. Values indicate the mean ± SEM. The axis terminates at 0.02 µg/mL, which is the lower limit of detection for the ABC294640 assay. **B**, Cycle 1 day 28: Levels of ABC294640 were measured in plasma samples prepared from patients treated with 250 mg qd (▲), 250 mg bid (▲), 500 mg bid (●), or 750 mg bid (■) of ABC294640. Values indicate the mean ± SEM. **C**, The  $C_{max}$  concentration for each patient is shown with values from day 1 indicated by black symbols and values from day 28 indicated by red symbols. **C-E**, Vertical bars indicate the range of the values, with the average indicated by the horizontal hash mark. **D**, Day 1  $AUC_{INF}$  values for each patient are indicated. Data for patients in the 250 mg qd and 250 mg bid cohorts are combined because only one dose of drug was administered on Day 1. **E**, Day 28  $AUC_{0-t}$  values for each patient are indicated. Data for patients in the 250 mg qd and 250 mg bid cohorts are separated because patients experienced different exposures to ABC294640 over the previous 27 days.

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whereas separate curves are indicated for day 28 (Fig. 2B) because of the difference in total drug received during the cycle. The plasma  $C_{\max}$  levels of ABC294640 were comparable at day 1 and day 28 at all dose levels, and as shown in Fig. 2C, the plasma  $C_{\max}$  (combining data for day 1 with day 28, as well as 250 mg qd with 250 mg bid) was approximately dose proportional between 250 and 500 mg [ $2.68 \pm 1.44$  ( $n = 18$ ) and  $5.16 \pm 4.29$  ( $n = 12$ )  $\mu\text{g/mL}$ , respectively,  $P < 0.05$ ]. However, there was no further increase at 750 mg [ $5.56 \pm 1.38$  ( $n = 5$ )  $\mu\text{g/mL}$ ]. Trough drug levels (sampled on day 28 prior to drug administration) were observed for the 500 mg bid and 750 mg bid treatment cohorts [ $1.82 \pm 0.85$  ( $n = 5$ ) and  $4.48$  ( $n = 1$ )  $\mu\text{g/mL}$ , respectively], but were low in the 250 mg qd and 250 mg bid treatment groups [ $0.20 \pm 0.34$  ( $n = 5$ ) and  $0.29 \pm 0.15$  ( $n = 3$ )  $\mu\text{g/mL}$ , respectively].  $\text{AUC}_{\text{INF}}$  values on day 1 (Fig. 2D) indicate an approximately dose-proportional increase between the 250 and 500 mg doses [ $15.23 \pm 8.23$  ( $n = 10$ ) and  $40.70 \pm 28.00$  ( $n = 6$ )  $\mu\text{g}^*\text{h/mL}$ , respectively,  $P < 0.05$ ]; however, further increases in drug exposure were not obtained at the 750 mg dose level [ $50.40 \pm 20.62$  ( $n = 4$ )  $\mu\text{g}^*\text{h/mL}$ ]. As shown in Fig. 2E, the  $\text{AUC}_{0-t}$  on day 28 showed an approximately dose-proportional increase between doses of 250 (pooling 250 mg qd and 250 mg bid) and 500 mg bid [ $17.82 \pm 15.24$  ( $n = 8$ ) and  $50.84 \pm 11.29$  ( $n = 5$ )  $\mu\text{g}^*\text{h/mL}$ , respectively,  $P < 0.05$ ], with the possibility of further increase suggested by the single data point obtained for the 750 mg bid dose (93.00  $\mu\text{g}^*\text{h/mL}$ ).  $\text{AUC}_{0-t}$  values at day 28 were greater than  $\text{AUC}_{\text{INF}}$  at day 1 for the 500 mg bid and 750 mg bid cohorts (ratio = 1.25 and 1.85, respectively), suggesting the possibility of drug accumulation at higher doses. The relatively small sample size may underlie the moderate interpatient variability observed; however, differences in mechanisms for the accumulation, elimination, and metabolism of ABC294640 may account for the variability. In rats, ABC294640 is metabolized by CYP3A4 and CYP2D6 to two products (M1 = N-oxide and M2 = hydroxypyridyl), which reach  $C_{\max}$ s of approximately 14% and 7%, respectively, of that of the parent drug (unpublished data). For the patient samples, only the parent drug was quantified, and so ABC294640 metabolism in humans remains to be studied. There was no significant correlation between drug-related neuropsychiatric effects and systemic exposure as measured by AUC or  $C_{\max}$  values on days 1 and 28. However, patients who experienced these effects exhibited a larger increase in  $C_{\max}$  from day 1 to day 28 compared with those who did not ( $P = 0.02$ ). This analysis is limited by small sample size and absence of a specific neuropsychiatric screening tool.

### Pharmacodynamics

Changes in plasma sphingolipid levels were evaluated as exploratory biomarkers for ABC294640 action. Blood was collected in parallel with the sampling for pharmacokinetics on cycle 1 days 1 and 28, as well as at each clinic visit, providing plasma samples at 0, 1, 2, 3, and 4 weeks of drug treatment for most patients, along with longer term treatment samples from patients that completed multiple cycles of treatment with ABC294640. The predosing plasma S1P level measured in 15 patients across all dose levels averaged  $1.08 \pm 0.11$   $\mu\text{mol/L}$  (range = 0.42–1.72  $\mu\text{mol/L}$ ). As shown in Fig. 3A–D, plasma S1P levels in samples harvested from individual patients for up to 72 weeks demonstrated variability, even between screening (typically day –7) compared with day 1 (prior to ABC294640 administration). Consequently, there were no consistent long-term changes in

plasma S1P levels that appear to be drug-related, although patients in the 250 qd cohort who were followed for several months seemed to have a progressive increase in plasma S1P levels that may be related to disease progression (Fig. 3A). However, ABC294640 treatment caused reproducible acute decreases in plasma S1P levels that reached a minimum at 12 hours after ABC294640 treatment and recovered to baseline by 24 hours (Fig. 3E). Combining data from days 1 and 28, as well as combining 250 mg qd with 250 mg bid, the maximum S1P decreases for patients receiving 250, 500, and 750 mg of ABC294640 were  $51\% \pm 6\%$  ( $n = 10$ ),  $46\% \pm 9\%$  ( $n = 5$ ), and  $54\% \pm 14\%$  ( $n = 2$ ), respectively. The kinetics and magnitude of reduction of dihydro-S1P in the plasma virtually paralleled the data for S1P, consistent with the known ability of SK2 to phosphorylate either sphingosine or dihydrosphingosine (data not shown). In contrast, plasma samples from the 250 and 500 mg treatment groups contained elevated levels of dihydro $C_{16}$ -ceramide, which reached a maximum at 8 to 12 hours and then returned to baseline at 24 hours (Fig. 3F). It is noteworthy that the changes in sphingolipid levels were not dose related, suggesting that the pharmacodynamic maximal effect is achieved by the 250 mg dose of ABC294640.

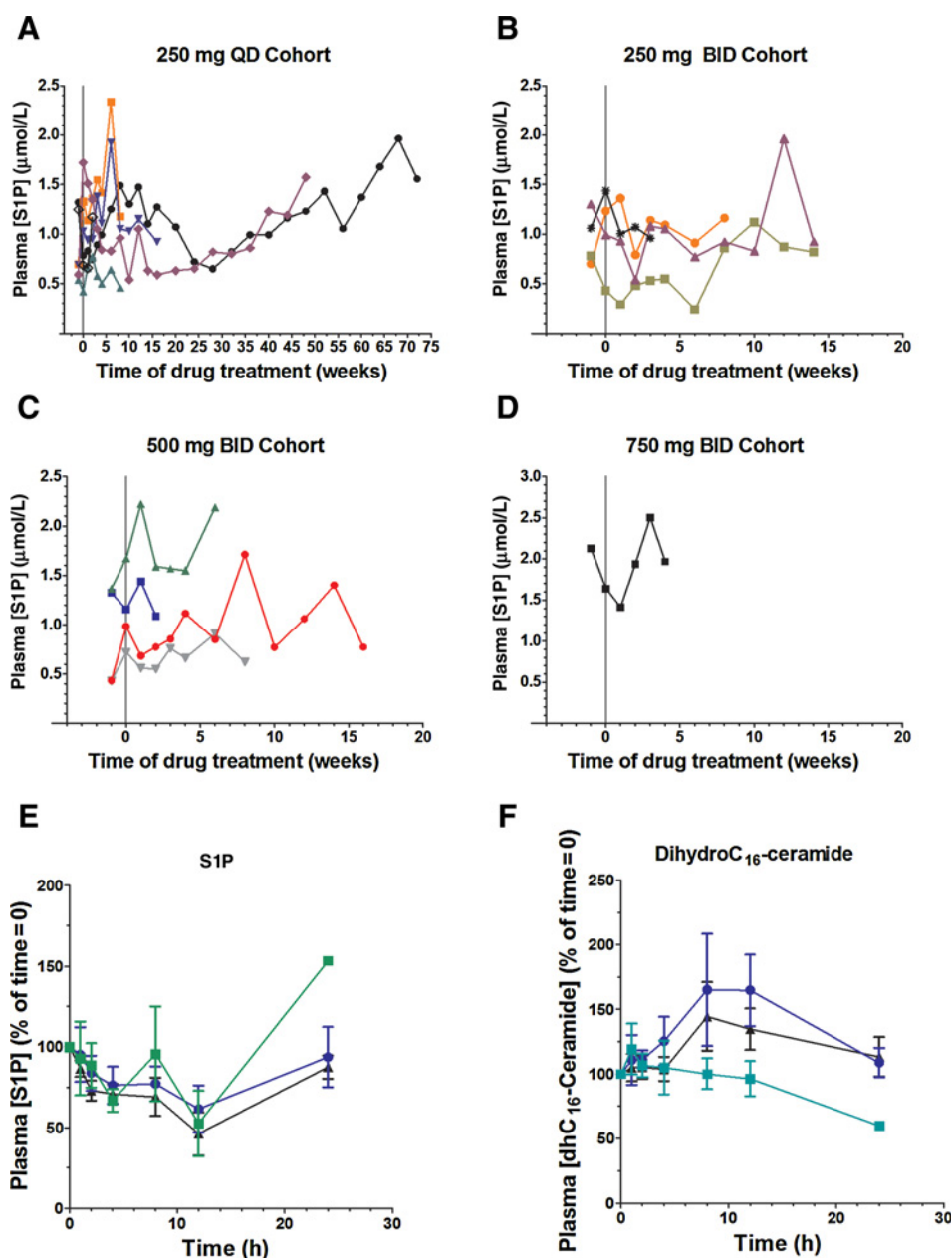
### Efficacy

Of the 21 patients who received ABC294640, 6 (29%) did not complete two cycles of treatment and thus were not evaluable for efficacy. Among the 15 patients evaluable for antitumor activity across all dose levels, one (7%) heavily pretreated cholangiocarcinoma patient developed a partial response after 8 cycles at 250 mg qd and continued on study for a total of 18 cycles before developing progressive disease. Six patients (40%) had a best response of stable disease: one metastatic urothelial carcinoma patient (at 250 mg qd) ultimately developed progressive disease after 12 cycles; one cholangiocarcinoma patient (at 500 mg bid) came off study in cycle 3 for toxicity; and 4 patients (1 at 250 mg qd, 2 at 250 mg bid, and 1 at 500 mg bid) came off study for progressive disease at the second disease evaluation (8 weeks). The remaining 8 patients (53%) had progressive disease as their best response.

### Discussion

Although sphingolipid metabolism, in general, and sphingosine kinases, in particular, have been widely discussed as targets for new anticancer drugs, very little knowledge on this pathway has been translated into clinical investigation. Safingol (L-threo-dihydrosphingosine) was tested as an anticancer agent in humans, but it is nonspecific, targeting SK1, SK2, and protein kinase C (41–43). Also, safingol must be administered intravenously in an emulsified formulation, and it causes dose-limiting liver toxicity (44). The only FDA-approved drug targeting the sphingolipid pathway is fingolimod, an S1P receptor modulator that prevents the recirculation of lymphocytes, thereby reducing relapse rates in patients with multiple sclerosis (reviewed in refs. 45, 46). Fingolimod also inhibits SK1 and has anticancer activity in preclinical models; however, its immunosuppressive effects are negative indicators for its use against cancers (47). Pharmaceutically tractable inhibitors of SK1 and/or SK2, such as ABC294640, provide a new approach for harnessing medical benefits potentially offered from modulating the ceramide/S1P rheostat.





**Figure 3.** Effects of ABC294640 treatment on plasma sphingolipid levels. Plasma was prepared from patient blood samples at the indicated times after the administration of ABC294640 and plasma S1P (**A–E**) or dihydroC<sub>16</sub>-ceramide (**F**) were quantified as indicated in the Materials and Methods section. **A–D**, Data are shown for individual patients in the following cohorts: 250 mg qd (**A**); 250 mg bid (**B**); 500 mg bid (**C**); and 750 mg bid (**D**). Values at time <0 represent samples drawn during patient screening, typically 7 days prior to initiation of drug therapy. **E** and **F**, S1P (**E**) and dihydroC<sub>16</sub>-ceramide (**F**) were measured in plasma samples prepared at the indicated times from patients treated with 250 mg (▲), 500 mg (●), or 750 mg (■) of ABC294640. Values represent the mean  $\pm$  SEM for the treatment groups after normalization to time = 0 for individual patients.

This first-in-human phase I trial of ABC294640 reflects the first attempt at targeting SK2 for cancer therapy. The most frequent ABC294640-related toxicities of nausea, vomiting, and fatigue were generally tolerable and/or manageable with supportive care, and the low-grade neuropsychiatric effects were fully reversible upon discontinuation of the drug. The mechanisms responsible for the reversible low-grade neuropsychiatric symptoms observed with ABC294640 are not known, but may be on-target effects. Beginning with the observation that some patients with hereditary diseases of sphingolipid metabolism (such as Tay–Sachs disease) present with mood disorders, ceramide pathway activation has emerged as a common theme in psychiatric conditions and pain perception (48). Furthermore, S1P receptors are expressed throughout the ner-

vous system, leading to the recent suggestion that fingolimod slows multiple sclerosis not only through a peripheral immunologic effect, but also through direct effects on the central nervous system (45). The association of ceramide/S1P signaling with neuropsychiatric conditions implies that SK2 inhibition may be the direct cause of ABC294640-related symptoms, such as hallucinations, anxiety, and spasticity. Regardless of the mechanism, in the future, if dose adjustments are required to mitigate neuropsychiatric effects, current data suggest that the drug may still provide benefit.

The plasma drug exposure and pharmacodynamic profiles of ABC294640 support future clinical investigation at or below a recommended phase II dose of 500 mg bid. Specifically, plasma ABC294640 concentrations peaked at 1 to 2 hours, and the

half-life of 5 to 15 hours is appropriate for bid dosing. There appears to be no advantage to doses greater than 500 mg bid, because the  $C_{max}$ , AUC, and trough drug levels are similar following administration at 500 and 750 mg. In addition, the declines in plasma S1P levels were equivalent at all doses of ABC294640, indicating that the 250 mg bid dose may be sufficient to achieve the maximum pharmacodynamic response.

The one objective response in this study occurred in a heavily pretreated patient with cholangiocarcinoma, treated at 250 mg qd. Cholangiocarcinoma is associated with bile stasis and conjugated bile acids, which activate signaling pathways mediated by the S1P receptor 2 (49). SK2 is overexpressed in human cholangiocarcinoma cell lines compared with normal cholangiocytes (26). In cholangiocarcinoma cells, ABC294640 inhibits proliferation and induces caspase-dependent apoptosis, an effect that is enhanced by pharmacologic inhibition of autophagy or concurrent use of sorafenib (26). These data suggest a potential novel strategy for the treatment of cholangiocarcinoma.

Despite the important roles of sphingolipid metabolism in cancer biology, there are very few agents targeting this pathway in clinical trials. This phase I trial of ABC294640 demonstrates pharmacologic inhibition of SK2 resulting in anticancer activity. In addition to cholangiocarcinoma, nonclinical studies demonstrate the potential for ABC294640 in multiple tumor types, including lung, prostate, colorectal, ovarian and liver cancers, multiple myeloma, lymphoma, and leukemia. Clinical trials of ABC294640 are ongoing in hepatocellular carcinoma (NCT02939807), relapsed/refractory diffuse large B-cell lymphoma (NCT02229981), and refractory/relapsed multiple myeloma (NCT02757326). In addition, because of its anti-inflammatory activity in animal models, clinical evaluation of the ability of ABC294640 to prevent mucositis in head and neck cancer patients receiving chemoradiation is expected to be initiated in mid-2017.

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## Disclosure of Potential Conflicts of Interest

S.L. Cusack is an employee of Cusack Pharmaceutical Consulting and holds ownership interest (including patents) in Apogee. C.D. Smith holds ownership interest (including patents) in Apogee. No potential conflicts of interest were disclosed by the other authors.

## Authors' Contributions

**Conception and design:** C.D. Britten, M.B. Thomas, E. Garrett-Mayer, S.L. Cusack, L.W. Maines, C.D. Smith

**Development of methodology:** M.B. Thomas, Y. Zhuang, C.D. Smith

**Acquisition of data (provided animals, acquired and managed patients, provided facilities, etc.):** C.D. Britten, M.B. Thomas, S.H. Chin, K. Shirai, B. Ogretmen

**Analysis and interpretation of data (e.g., statistical analysis, biostatistics, computational analysis):** C.D. Britten, E. Garrett-Mayer, S.H. Chin, B. Ogretmen, C.D. Smith

**Writing, review, and/or revision of the manuscript:** C.D. Britten, M.B. Thomas, E. Garrett-Mayer, S.H. Chin, B. Ogretmen, K. Anderton, S.L. Cusack, L.W. Maines, C.D. Smith

**Administrative, technical, or material support (i.e., reporting or organizing data, constructing databases):** C.D. Britten, M.B. Thomas, T.A. Bentz, A. Brisendine, K. Anderton, S.L. Cusack

**Study supervision:** C.D. Britten, M.B. Thomas, S.H. Chin, T.A. Bentz

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