

Phase I and pharmacokinetic study of lonafarnib, SCH 66336, using a 2-week on, 2-week off schedule in patients with advanced solid tumors

Carlos Castaneda · Kellen L. Meadows · Roxanne Truax · Michael A. Morse ·
Scott H. Kaufmann · William P. Petros · Yali Zhu · Paul Statkevich ·
David L. Cutler · Herbert I. Hurwitz

Received: 12 July 2010 / Accepted: 5 October 2010 / Published online: 24 October 2010
© Springer-Verlag 2010

Abstract

Purpose This phase I study was performed to determine the safety profile, maximum tolerated dose (MTD) and biological activity of lonafarnib (SCH 66336). Single-dose and multi-dose pharmacokinetics were conducted.

Methods Twenty-one patients with advanced solid tumors were enrolled. Each patient received single-dose administration on day 1, cycle 1 then switched to a twice daily (BID) dosing regimen on days 2–14 of a 28-day cycle; subsequent cycles continued BID dosing on days 1–14. Dose-limiting toxicity (DLT) was assessed during the cycle one; toxicity evaluation was closely monitored throughout the treatment. Radiographic scans were completed to assess tumor response. Blood and urine pharmacokinetics were evaluated on days 1 and 14 in cycle 1. SCH 66336-induced farnesylation inhibition was assessed via conversion of prelamin A to lamin in buccal mucosa.

Results DLT and most common adverse events were diarrhea, fatigue, nausea and anorexia. No grade 3 or 4 hematological toxicities were observed. Nineteen of 21 patients were evaluable for response; short-term stable disease was observed in 5 patients. SCH 66336 systemic exposure increased with dose; however, drug accumulation was higher than projected. Renal excretion of parent drug was negligible. Farnesyl transferase inhibition was detected at the 200 and 300 mg BID doses.

Conclusion The MTD and recommended phase II dose is 200 mg BID on days 1–14 of a 28-day dosing regimen. The plasma concentration profile suggests the pharmacokinetics of SCH 66336 is dose and time dependent. Farnesyl transferase target inhibition was observed at doses of lonafarnib recommended for further study.

Keywords Lonafarnib · SCH 66336 · Phase I · Pharmacokinetics · Advanced solid tumors

The deceased author is: Roxanne Truax.

C. Castaneda
Instituto Nacional de Enfermedades Neoplasicas, Lima, Peru

K. L. Meadows · R. Truax · M. A. Morse · H. I. Hurwitz (✉)
Duke University Medical Center, Durham,
North Carolina 27710, USA
e-mail: hurwi004@mc.duke.edu

S. H. Kaufmann
Mayo Clinic, Rochester, Minnesota 55905, USA

W. P. Petros
Mary Babb Randolph Cancer Center,
West Virginia University, Morgantown, WV 26506, USA

Y. Zhu · P. Statkevich · D. L. Cutler
Schering-Plough Research Institute,
Kenilworth, New Jersey 07033, USA

Introduction

The Ras protein family is comprised of four highly conserved functional ras proteins, K-Ras4A, K-Ras4B, N-ras and H-ras. These proteins are small GTPases which function as molecular switches transmitting extracellular growth signals downstream to the nucleus in signal transduction pathways that regulate cellular proliferation, differentiation, migration and survival [1, 2]. Cells with ras-activating mutations (primarily in codons 12, 13 and 61) are susceptible to neoplastic transformation because cellular response to the loss of normal mitogenic growth signals is abrogated, leading to unregulated cellular proliferation and survival. The high frequency of ras-activated mutations makes this gene family an attractive target for anti-cancer therapy [1–3].

Farnesyl transferase inhibitors (FTIs) are a novel class of anti-tumor drugs that selectively inhibit farnesylation, a post-translational modification of an isoprenyl lipid to a number of mammalian proteins including the Ras gene family, multiple Rheb and Rho family GTPases and centromeric proteins CENP-E and CENP-F [4–8]. Farnesylation is one of the several essential enzymatic steps ras proteins undergo to facilitate proper protein localization to the inner surface of the plasma membrane and is essential for the neoplastic transforming activity of Ras [1, 9–11]. This modification is sequence dependent on a tetrapeptide CaaX motif at the carboxyl terminal (C, cysteine, a, aliphatic amino acid, and X, serine, glycine or methionine) [12–14]. Interestingly, studies with FTIs led to the discovery that geranylgeranyl transferase (GGTase-1) can alternatively prenylate K-ras and N-ras isoforms in FTI-treated cells [13–16]. However, preclinical tumor models indicate FTI-mediated growth suppression is still achieved in models which express activated K-ras, suggesting that other farnesylated proteins are important in tumor growth [17, 18]. Although the exact biological mechanisms are not fully understood, FTIs are thought to exert anti-tumor effects through the inhibition of abnormal ras-mediated growth and proliferation as well as interruption of mitotic spindle formation during M phase [19, 20].

Lonafarnib (SCH 66336, *Sarasar*; Schering-Plough, Kenilworth, NJ) is a tricyclic, orally active, CaaX-competitive inhibitor which specifically inhibits farnesyl transferase activity in vitro with an IC₅₀ value of 1–2 nM and blocks protein farnesylation in cell culture with an IC₅₀ value of 10 nM [1, 21]. Lonafarnib does not inhibit GGTase-1 [20] and does not inhibit processing of GGTase-II substrates in cell-based assays (unpublished data). Preclinical studies have demonstrated that SCH 66336 exhibits selective anti-cancer activity in a broad range of solid and hematologic tumor cell lines, including those with wild type Ras [22–25]. Moreover, mouse models show clinical activity against human lung, prostate, pancreas, colon, bladder and glioblastoma xenografts as well as hematologic malignancies [23, 26].

The present study is a phase I, dose escalation trial investigating the safety, tolerability, pharmacokinetics, and biological activity of orally administered SCH 66336 using a twice daily 2 week on, 2 week off dosing schedule in patients with advanced solid tumors.

Materials and methods

Patient selection

Patients with histological evidence of advanced solid cancer (no clinical evidence of central nervous or bone marrow

involvement) for which there was no established curative or significant palliative therapies were eligible for this study. Additional eligibility criteria also included age ≥ 18 years; Eastern Cooperative Oncology Group (ECOG) performance status ≤ 2 ; life expectancy ≥ 12 weeks; measurable disease according to modified World Health Organization criteria; no chemotherapy, biologic therapy or radiotherapy within 4 weeks prior to the first administration of SCH 66336; no residual toxicity from prior chemotherapy, biologic or radiotherapy; no radiation therapy to $\geq 30\%$ of bone marrow; adequate bone marrow function defined as platelets $\geq 100 \times 10^9/\text{liter}$ (L), absolute neutrophil count (ANC) $>1.5 \times 10^9/\text{L}$, hemoglobin ≥ 10 gm/dL; adequate liver function defined as total bilirubin $\leq 1.5 \times$ upper limit of normal (UNL), aspartate transaminase $\leq 2.5 \times$ UNL; and serum creatinine $\leq 1.5 \times$ UNL. Patients were also required to be free of medical conditions which could complicate ingestion or absorption of oral medications; have received ≤ 2 prior chemotherapy regimens for metastatic cancer, not be pregnant or lactating (negative pregnancy test within 24 h prior to first administration of SCH 66336); no prior nitrosoureas or mitomycin C administration; no prior bone marrow or stem cell transplantation; no known HIV or AIDS-related illness. This study was a single-center study approved by the Duke Institutional Review Board (IRB) and followed the guidelines of the Helsinki Declaration. All patients provided informed written consent and were treated at Duke University Medical Center.

Patient evaluations

All patients completed an extensive medical history and physical examination prior to enrollment. Toxicity and safety clinical assessments were performed weekly during the first cycle and on the first day of each subsequent cycle. More frequent evaluations were performed as clinically indicated. Assessments included an interval history, performance status, complete blood count (CBC) with differential, electrolytes, liver function tests, serum chemistry panel, creatinine, urinary calcium and urinalysis with microscopy, electrocardiogram and ophthalmology examinations when clinically indicated. Radiographic imaging by computed tomography (or magnetic resonance imaging) was completed at baseline and every two cycles. Toxicities were graded using the National Cancer Institute (NCI) common terminology criteria version 1.0 for adverse events as the study was conducted from December 1997 to April 1999 [27].

Drug administration

SCH 66336 is a crystalline solid possessing one chiral center. The study drug was supplied by Schering-Plough

Research Institute (Kenilworth, NJ, USA) as oral gelatin capsules containing 25, 100 and 200 milligrams (mg). For each dosing cohort, patients received a single dose of SCH 66336 on day 1, cycle 1 followed by a 24-h observation period; dosing increased to twice daily on days 2 through 14 of a 28-day cycle. Cycles 2 and higher continued the twice daily dosing regimen on days 1 through 14 of a 28-day cycle. Study drug was administered in-house on days 1 and 14 of cycle 1 followed by pharmacokinetic sampling. On these pharmacokinetic sampling days, study drug was administered immediately after the patients consumed a standardized, continental breakfast. Protocol therapy was continued until the evidence of disease progression, unacceptable drug-related toxicity occurred or discontinuation at physician and/or patient discretion.

Dose escalation

The selection of the dosing range and the starting dose of SCH 66336 was based on safety results of the 15 mg/kg/day dose in preclinical 3-month rat and monkey toxicology studies [28]. The starting dose was 25 mg twice daily (BID). Dose escalation was not adjusted for BMI or weight.

For initial dose escalation, 1 patient was entered per cohort level until grade 2 toxicities were observed during cycle 1 (28 days). At that point, a minimum of 3 patients were enrolled to each cohort. If no DLT was observed in the first 3 patients, advancement to the next higher cohort was permitted. However, if DLT was observed in one of the first 3 patients, then 3 more patients were recruited to that same cohort level for a total of six patients. Advancement to the next higher dose level occurred only if ≤ 1 out of 6 patients experienced a DLT. If ≥ 2 patients in either a 3–6 patient cohort experienced a DLT, then the next 3 patients would be enrolled at the next lowest dose level. The MTD was defined as the dose level immediately below that where $\geq 30\%$ of patients experienced a DLT. Once the MTD was determined, 3 additional patients were recruited to this dose level to better define the safety, toxicities and clinical activity of the regimen (maximum total of 6 patients). There was no intra-patient dose escalation.

DLT was defined as $\geq$ Grade 3 neutropenia or thrombocytopenia, Grade 4 anemia, serum creatinine elevation ≥ 2 times the upper limit of normal and the occurrence of Grade ≥ 3 non-hematology toxicities except for nausea, vomiting, fever (in the absence of an infection) or alopecia. Grade ≥ 3 vomiting was only considered DLT when the toxicity persisted despite the use of adequate supportive care measures.

Dose interruption up to 3 weeks and 1 dose reduction due to toxicity was permitted for patients who were clinically benefiting.

Pharmacokinetic studies

Pharmacokinetic sampling was performed in cycle 1 to measure SCH 66336 concentrations. Five mL blood samples were collected in sodium heparin tubes and centrifuged at approximately 3000 RPM for 15 min at 10°C. The plasma was divided into equal aliquots of at least 1 mL, transferred to cryogenic tubes and frozen at -70°C until assayed. On day 1, samples were collected immediately before the first dose of SCH 66336 (predose sample) and 0.5, 1, 1.5, 2, 3, 4, 6, 8, 12, 16, 20, and 24 h post-dose. On day 13, a blood sample was obtained immediately before the evening dose; on day 14, blood samples were collected at 0.5, 1, 1.5, 2, 3, 4, 6, 8, 12 and 24 h post-dose. The 12 h post-dose samples were collected before the evening dose. Plasma concentrations were determined using a validated liquid chromatography with tandem mass spectrometric detection assay. The lower limit of quantitation was 1.0 ng/mL plasma.

On day 14, urine samples were collected before the morning dose and then in block intervals of 0–6 and 6–12 h post-dose; samples were refrigerated during each collection period. The volume of each block interval was recorded. Two 25 mL samples were taken from each block interval and frozen at -70°C until assayed. Urine samples were analyzed using a validated liquid chromatography with tandem mass spectrometric detection assays. The lower limit of quantitation was 2.0 ng/mL urine.

Individual patients' SCH 66336 concentration, time profiles were analyzed using non-compartmental methods [29]. The maximum plasma concentration (C_{max}), predose plasma concentration and time of maximum plasma concentration (T_{max}) were reported as observed values. The terminal phase rate constant (K) was calculated as the negative slope of the log-linear terminal portion of the plasma concentration–time curve using linear regression. The terminal phase half-life, $t_{1/2}$, was calculated as $0.693/K$. The area under the plasma concentration–time curve from time 0 to 12 h post-dose [area under curve (AUC) (0–12 h)] and from time 0 to the time of final quantifiable sample [AUC (t_f)] was calculated using the linear trapezoidal method. The accumulation index (R) was described by the following equation: $R = \text{AUC}(0\text{--}12\text{ h}) \text{ day } 14 / \text{AUC}(0\text{--}12\text{ h}) \text{ day } 1$. The apparent volume of distribution was calculated: $V_{d_{\text{area}}} / F = [\text{Dose} / \text{AUC} (I)] / K$.

Pharmacodynamic studies

Buccal mucosa samples were collected for prelamina A analysis on day 1 prior to study drug administration and again on day 14 approximately 12 h after the previous dose was taken. Buccal sample processing and immunohistochemical methods have been previously described [30].

Table 1 Patient characteristics

Characteristics	No. of patients
Total Patients	21
Gender	
Male	11
Female	10
Age (Years)	
Median	59
Range	41–77
ECOG Performance Status	
Median	1
Range	0–1
Number of Prior Treatments	
0	0
1–2	14
3	3
≥4	3
Primary Tumor Type	
Colorectal	12
Pancreas	8
Renal Cell	1

Briefly, with each batch of buccal smears, A549 lung cancer cells treated with diluent or SCH66336 were included as negative and positive controls, respectively. Samples fixed in acetone and blocked as previously described were reacted with anti-rabbit prelamin A and mouse anti-lamin followed by fluorochrome-labeled secondary antibodies and examined on a Zeiss LSM310 confocal microscope. Sensitivity of the photomultiplier tubes was adjusted so that the signal for prelamin A in diluent-treated A549 cells was just below the limit of detection, a result consistent with the appearance of the specimens by conventional fluorescence microscopy. With the sensitivity of the confocal microscope fixed at this level, all other specimens were then examined in the conventional and laser scanning modes. When images were subsequently imported into Adobe Photoshop, any adjustments to brightness or contrast were applied identically to paired samples harvested before and after therapy. Multiple images from the same patient

stained and imaged at the same time were then imported into Canvas 8.0 to assemble the final figure.

Results

Patient characteristics are summarized in Table 1. Twenty-one patients (11 men and 10 women) with a median age of 58.7 years (range 40–77 years) were enrolled onto the study. The median number of treatment cycles administered per patient was 2 (range 1–5 cycles); 2 patients completed less than 14 days of study drug therapy. All primary tumor types were either colorectal or pancreatic cancer except for one patient. Nineteen of 21 were available for toxicity and tumor response.

The dose escalation schema and corresponding DLTs are listed in Table 2. Dose escalation was based on safety and tolerability of the investigational drug. Due to minimal study drug-related toxicity, one patient was enrolled in each of the lower dosing cohorts 25, 50 and 100 mg BID. One DLT (grade 3 diarrhea) was observed in one of the first three patients enrolled at 200 mg BID which triggered the enrollment of additional 3 patients to this cohort. At the 300 mg BID dose, 2 DLTs (grade 3 fatigue, nausea and anorexia) were observed during cycle 1, and as a result, the dose was de-escalated to 200 mg BID and MTD was confirmed.

Treatment-related toxicities are summarized in Table 3. There were no grade 3 or 4 hematological toxicities observed at any dose level; the highest treatment-related hematological toxicity was one grade 2 neutropenia in the 200 mg BID dosing cohort.

The non-hematological toxicities were primarily gastrointestinal (GI)-related and consisted of anorexia, diarrhea, and nausea and vomiting; these were consistently observed across the 200- and 300-mg dosing cohorts. Most patients experienced grade 2–3 fatigue concomitantly with the GI toxicities. Supportive measures such as anti-emetics and anti-diarrheal were provided to help manage these toxicities. Other treatment-related adverse events included pain, abdominal pain, cramping and swelling and constipation. Three patients reported grade 1 difficulty with balance.

Table 2 Dose escalation scheme and dose-limiting toxicity events

Cohort level	SCH66336 mg BID	No. of subjects	No. of dose-limiting toxicity events	Dose-limiting toxicity
1	25	1	0	
2	50	1	0	
3	100	1	0	
4	200	11	1	Grade 3 diarrhea
5	300	7	2	Grade 3 nausea, fatigue and anorexia (2)

Table 3 Treatment-related Adverse Events (Highest grade per patient)

CTC grade	25 mg BID* (n = 1)		50 mg BID (n = 1)		100 mg BID (n = 1)		200 mg BID (n = 11)		300 mg BID (n = 7)	
	n (%)		n (%)		n (%)		n (%)		n (%)	
	1–2	3–4	1–2	3–4	1–2	3–4	1–2	3–4	1–2	3–4
Hematologic										
Hemoglobin	1 (100)	0	0	0	0	0	3 (27)	0	4 (57)	0
Thrombocytopenia	0	0	0	0	0	0	2 (18)	0	2 (28)	0
Neutropenia	0	0	0	0	0	0	2 (18)	0	1 (14)	0
Non-hematologic										
Diarrhea	0	0	0	0	0	0	6 (54)	2 (18)	5 (71)	0
Fatigue	0	1 (100)	1 (100)	0	1 (100)	0	8 (72)	2 (18)	4 (57)	2 (18)
Anorexia	1 (100)	0	1 (100)	0	1 (100)	0	6 (54)	1 (9)	2 (18)	1 (14)
Nausea	1 (100)	0	1 (100)	0	1 (100)	0	9 (82)	1 (9)	4 (57)	2 (18)
Vomiting	1 (100)	0	1 (100)	0	1 (100)	0	3 (27)	0	1 (14)	0
Constipation	1 (100)	0	0	0	0	0	3 (27)	0	1 (14)	1 (14)
Abdominal pain/cramping	0	1 (100)	0	0	0	0	2 (18)	1 (9)	1 (14)	0
Dehydration	0	0	0	0	0	0	1 (9)	0	0	1 (14)

* Subject died 37 days after receiving last study drug dose

Two patients one at 200 mg BID and another at the 300 mg BID experienced transient moderate elevation of liver enzymes. One patient at the 200 mg BID reported blurred vision that was not present during screening, but no other ophthalmologic changes were noted.

No partial or complete responses were observed. Five patients had stable disease for 4–5 months, including patients in the 50, 200, and 300 mg BID doses. Two deaths, both attributed to disease progression, occurred while patients were on the study and were not considered related to protocol therapy; these included a patient at 25 mg BID and another at 200 mg BID.

Pharmacokinetic studies were performed on all patients, although plasma concentration values for day 14 were missing for 3 patients (2 at 200 mg BID and 1 at 300 mg BID). A summary of the single and twice daily pharmacokinetic parameters is listed in Table 4. The mean plasma SCH 66336 concentrations for day 1 are illustrated in Fig. 1a. SCH 66336 was slowly absorbed following oral administration of SCH 66336 capsules with food. Peak concentration values on the first dose typically occurred at 6–8 h; the elimination half-life following single-dose administration ranged from 3 to 7 h. Apparent oral clearance values reflected a high degree of inter-patient variability. Mean systemic exposure (C_{max} and AUC) increased with dose (Fig. 1b); however, there was a trend toward reduced CL/F at the higher dose levels. SCH 66336 accumulated in plasma following twice daily oral administration under the fed state; the accumulation indices ranged from 2

to 4. These values were substantially higher than the predicted accumulation rate (1.1–1.4) based on the 3–7 h half-life observed on the first dose. Only a small amount of SCH 66336 was renally excreted, accounting for <0.04% of the dose in the 12-h dosing interval.

Pharmacodynamic studies were conducted on buccal mucosa samples to assess the farnesylation-dependent conversion of prelamin A to lamin. Accumulation of prelamin A after treatment with SCH 66336 is illustrated in Fig. 2. At the 200 mg BID dose level, 2 of the 3 samples analyzed (66.7%) showed prelamin A accumulation. At the 300 mg BID level, 5 of the 6 samples analyzed (83%) showed prelamin A accumulation; the only subject in this cohort who was negative had been dose reduced to 100 mg BID.

Discussion

Preclinical studies suggest that SCH 66336 induces potent anti-tumor activity through selective inhibition of farnesylation, which is an important mechanistic step for ras-dependent oncogenic transformation [1, 12]. We have conducted a Phase I open label, dose escalation study evaluating the safety profile, tolerability, MTD and pharmacokinetics and pharmacodynamics of this investigational drug using an oral BID 2 weeks on/2 weeks off dosing regimen. The MTD for this present study is 200 mg BID and is consistent with previously reported studies [31–33]. Overall, SCH66336 seemed to have an adverse event profile in this patient

Table 4 Summary of SCH 66336 pharmacokinetic data^a

	25 mg (<i>n</i> = 1)	50 mg (<i>n</i> = 1)	100 mg (<i>n</i> = 1)	200 mg (<i>n</i> = 11)	300 mg (<i>n</i> = 7)
Single-dose administration, cycle 1, day 1 only					
C_{max} (ng/mL)	26.5 (NA)	22.9 (NA)	461 (NA)	1,170 (57)	1,759 (52)
T_{max}^b (h)	8 (NA)	6 (NA)	6 (NA)	6 (2–24)	8 (4–12)
AUC (0–12h) ng–h/mL	176 (NA)	116 (NA)	4,069 (NA)	6,673 (58)	13,092 (54)
AUC (0–24 h) ng–h/mL	276 (NA)	138 (NA)	5,958 (NA)	11,575 (66)	21,572 (52)
$t_{1/2}$ (h)	3.81 (NA)	3.09 (NA)	7.18 (NA)	5.81 ^c (31)	7.14 (21)
tf (h)	24 (NA)	20 (NA)	24 (NA)	24 (0)	24 (0)
CL/F (mL/min)	1,444 (NA)	5,872 (NA)	243 (NA)	423 ^c (63)	301 (95)
Vd area/F(L)	476 (NA)	1,570 (NA)	152 (NA)	213 ^c (73)	171 (77)
	25 mg (<i>n</i> = 1)	50 mg (<i>n</i> = 1)	100 mg (<i>n</i> = 2)	200 mg (<i>n</i> = 9)	300 mg (<i>n</i> = 5)
Twice daily administration, Cycle 1, Day 14					
C_{max} (ng/mL)	59.1(NA)	38.2 (NA)	784 (NA)	2,780 (67)	3,868 (35)
T_{max}^b (h)	2 (NA)	4 (NA)	6 (NA)	4 (0–12)	0 (0–6)
AUC (0–12h) ng–h/mL	274 (NA)	298 (NA)	6,221 (NA)	25,589 (73)	35,821 (27)
tf (h)	12 (NA)	12(NA)	12 (NA)	12 (0)	12 (0)
CL/F (mL/min)	1,521 (NA)	2,796(NA)	273 (NA)	179 (53)	150 (32)
Accumulation or R	1.56 (NA)	2.57(NA)	1.73 ^d (NA)	3.89 (34)	2.51 (25)

C_{max} Peak plasma concentration, T_{max} Time to peak concentration, AUC Area under the plasma concentration–time curve, $t_{1/2}$ Terminal phase half-life, tf Time of quantifiable sample, CL/F Apparent total body clearance, R Accumulation index

NA Not applicable, sample size <3

^a Mean values $\pm$ coefficient of variation expressed as a percentage (%)

^b median value (range)

^c *n* = 9

^d *n* = 1

population that would be amenable to chronic administrations. Adverse events were primarily non-hematologic. The most frequent toxicity was fatigue which often presented with nausea, diarrhea, vomiting and anorexia. Hematological toxicities were very minimal with only one grade 2 neutropenia. Stable disease as best radiographic response was observed in 5 patients and lasted 4–5 months in duration. There were two patient deaths during the study due to disease progression that were not considered study related.

The pharmacokinetic profile suggests single and twice daily SCH 66336 administrations may be dose dependent, time dependent and variable, which has also been observed in other studies [32, 33]. SCH 66336 was slowly absorbed with food and displayed a corresponding lag time of 1–2 h, independent of single or twice daily drug administration. Accumulation of the drug on day 14 was higher than anticipated from day 1 pharmacokinetics. Additionally, the AUC (0–12 h) values after twice daily administration on Day 14 were higher than the corresponding AUC(I) values after single dose (Day 1) which also suggests non-linear pharmacokinetics. The etiology of this phenomenon is unclear, but could be related to either a time-dependent change in drug

metabolism and/or absorption. The negligible renal clearance compared to the total body clearance indicates that renal excretion is not a major elimination pathway for SCH 66336.

Processing of prelamins A to mature lamin A is known to require farnesylation of prelamins A. Therefore, accumulation of prelamins A in cells was evaluated in buccal mucosal cells as a potential marker for farnesyl transferase inhibition. Sample analysis was available for the second half of the study; therefore, only cohorts 200 and 300 mg BID were available for prelamins A detection. Although there was no correlation between prelamins A accumulation and minimum plasma concentrations for twice daily dosing, our results indicate clinically attainable levels of farnesyl transferase inhibition are feasible.

In conclusion, the MTD/RPTD for this phase I trial is 200 mg twice daily on days 1–14 of a 28-day dosing regimen. Our results suggest oral SCH 66336 monotherapy possesses a tolerable adverse event profile and may achieve concentrations which interfere with farnesyl transferase-dependent processes. Other Phase I SCH 66336 dose-finding studies have been conducted; twice daily dosing for SCH66336 has been found tolerable in

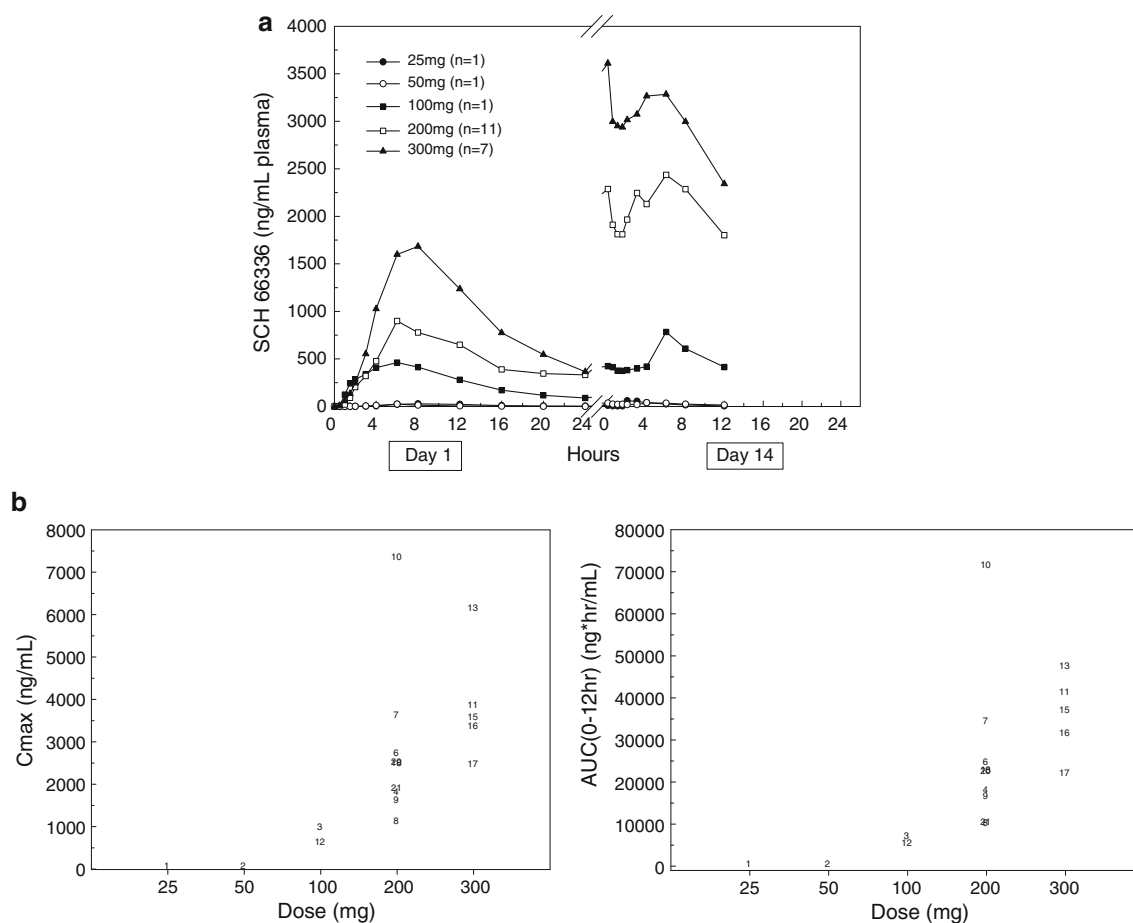
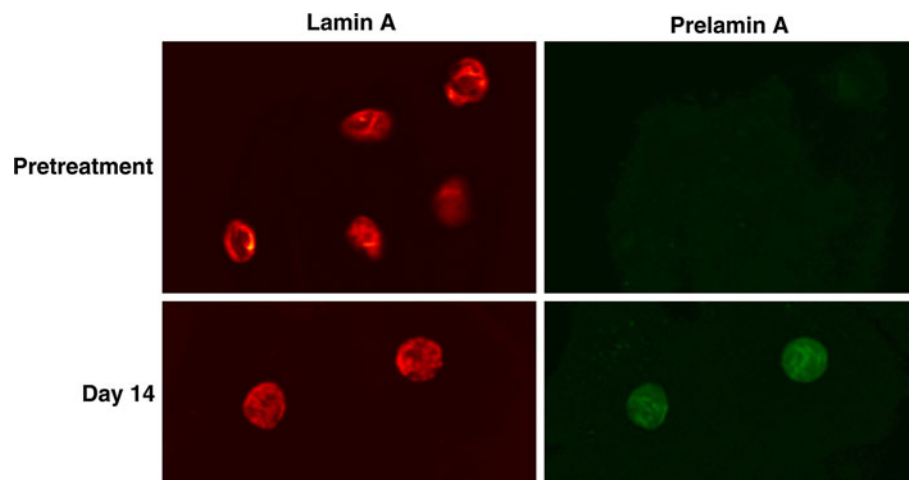


Fig. 1 Mean plasma SCH 66336 concentrations versus time. **a** Days 1 and 14, **b** Individual C_{max} and AUC Values of SCH 66336 on day 14

Fig. 2 Prelamin A accumulation after treatment with SCH 66336



monotherapy and in combination with other chemotherapy agents in multiple tumor types [34–37]. SCH 66336 in combination with chemotherapy agents is being evaluated in tumor types not typically associated with Ras mutations, such as breast, ovarian, head and neck, non-small cell lung cancer, hematologic malignancies and

brain [36, 38–41]. Thus, farnesyl transferase inhibition and the inhibition of other prenyltransferases such as geranylgeranyl transferase and Rab geranylgeranyl transferase in tumors with mutated and wild-type Ras and other relevant prenylated targets remain an area of ongoing interest [36, 38–41].

Acknowledgments This work was supported by Schering-Plough Research Institute. We gratefully acknowledge the invaluable contributions of the patients who participated in this study and their families. We also gratefully acknowledge the Duke University Phase I Oncology clinical team and health care providers who participated in this clinical study and Dr. O. Michael Colvin, former director of the Duke Comprehensive Cancer Center, who helped enable this study.

References

1. Basso AD, Mirza A, Liu G, Long BJ, Bishop WR, Kirschmeier P (2005) The farnesyl transferase inhibitor (FTI) SCH66336 (lonafarnib) inhibits Rheb farnesylation and mTOR signaling. Role in FTI enhancement of taxane and tamoxifen anti-tumor activity. *The Journal of biological chemistry* 280:31101–31108
2. Bos JL (1989) ras oncogenes in human cancer: a review. *Cancer Res* 49:4682–4689
3. Sebt SM, Adjei AA (2004) Farnesyltransferase inhibitors. *Semin Oncol* 31:28–39
4. Ashar HR, Armstrong L, James LJ, Carr DM, Gray K, Taveras A, Doll RJ, Bishop WR, Kirschmeier PT (2000) Biological effects and mechanism of action of farnesyl transferase inhibitors. *Chem Res Toxicol* 13:949–952
5. Ashar HR, James L, Gray K, Carr D, Black S, Armstrong L, Bishop WR, Kirschmeier P (2000) Farnesyl transferase inhibitors block the farnesylation of CENP-E and CENP-F and alter the association of CENP-E with the microtubules. *The Journal of biological chemistry* 275:30451–30457
6. Kho Y, Kim SC, Jiang C, Barma D, Kwon SW, Cheng J, Jaunbergs J, Weinbaum C, Tamanoi F, Falck J, Zhao Y (2004) A tagging-via-substrate technology for detection and proteomics of farnesylated proteins. *Proceedings of the National Academy of Sciences of the United States of America* 101: 12479–12484
7. Lebowitz PF, Casey PJ, Prendergast GC, Thissen JA (1997) Farnesyltransferase inhibitors alter the prenylation and growth-stimulating function of RhoB. *The Journal of biological chemistry* 272:15591–15594
8. Zhang FL, Kirschmeier P, Carr D, James L, Bond RW, Wang L, Patton R, Windsor WT, Syto R, Zhang R, Bishop WR (1997) Characterization of Ha-ras, N-ras, Ki-Ras4A, and Ki-Ras4B as in vitro substrates for farnesyl protein transferase and geranylgeranyl protein transferase type I. *The Journal of biological chemistry* 272:10232–10239
9. Hancock JF, Cadwallader K, Paterson H, Marshall CJ (1991) A CAAX or a CAAL motif and a second signal are sufficient for plasma membrane targeting of ras proteins. *The EMBO journal* 10:4033–4039
10. Hancock JF, Paterson H, Marshall CJ (1990) A polybasic domain or palmitoylation is required in addition to the CAAX motif to localize p21ras to the plasma membrane. *Cell* 63:133–139
11. Kato K, Cox AD, Hisaka MM, Graham SM, Buss JE, Der CJ (1992) Isoprenoid addition to Ras protein is the critical modification for its membrane association and transforming activity. *Proceedings of the National Academy of Sciences of the United States of America* 89: 6403–6407
12. Brunner TB, Hahn SM, Gupta AK, Muschel RJ, McKenna WG, Bernhard EJ (2003) Farnesyltransferase inhibitors: an overview of the results of preclinical and clinical investigations. *Cancer Res* 63:5656–5668
13. James GL, Goldstein JL, Brown MS (1995) Polylysine and CVIM sequences of K-RasB dictate specificity of prenylation and confer resistance to benzodiazepine peptidomimetic in vitro. *The Journal of biological chemistry* 270:6221–6226
14. Whyte DB, Kirschmeier P, Hockenberry TN, Nunez-Oliva I, James L, Catino JJ, Bishop WR, Pai JK (1997) K- and N-Ras are geranylgeranylated in cells treated with farnesyl protein transferase inhibitors. *The Journal of biological chemistry* 272:14459–14464
15. Rowell CA, Kowalczyk JJ, Lewis MD, Garcia AM (1997) Direct demonstration of geranylgeranylation and farnesylation of Ki-Ras in vivo. *The Journal of biological chemistry* 272:14093–14097
16. Sun J, Qian Y, Hamilton AD, Sebt SM (1998) Both farnesyltransferase and geranylgeranyltransferase I inhibitors are required for inhibition of oncogenic K-Ras prenylation but each alone is sufficient to suppress human tumor growth in nude mouse xenografts. *Oncogene* 16:1467–1473
17. Cox AD, Der CJ (1997) Farnesyltransferase inhibitors and cancer treatment: targeting simply Ras? *Biochim Biophys Acta* 1333: F51–F71
18. Lebowitz PF, Prendergast GC (1998) Non-Ras targets of farnesyltransferase inhibitors: focus on Rho. *Oncogene* 17:1439–1445
19. Lackner MR, Kindt RM, Carroll PM, Brown K, Cancilla MR, Chen C, de Silva H, Franke Y, Guan B, Heuer T, Hung T, Keegan K, Lee JM, Manne V, O'Brien C, Parry D, Perez-Villar JJ, Reddy RK, Xiao H, Zhan H, Cockett M, Plowman G, Fitzgerald K, Costa M, Ross-Macdonald P (2005) Chemical genetics identifies Rab geranylgeranyl transferase as an apoptotic target of farnesyl transferase inhibitors. *Cancer Cell* 7:325–336
20. Pan J, Yeung SC (2005) Recent advances in understanding the antineoplastic mechanisms of farnesyltransferase inhibitors. *Cancer Res* 65:9109–9112
21. Adjei AA, Davis JN, Bruzek LM, Erlichman C, Kaufmann SH (2001) Synergy of the protein farnesyltransferase inhibitor SCH66336 and cisplatin in human cancer cell lines. *Clin Cancer Res* 7:1438–1445
22. Feldkamp MM, Lau N, Roncari L, Guha A (2001) Isotype-specific Ras.GTP-levels predict the efficacy of farnesyl transferase inhibitors against human astrocytomas regardless of Ras mutational status. *Cancer Res* 61:4425–4431
23. Liu M, Bryant MS, Chen J, Lee S, Yaremko B, Lipari P, Malkowski M, Ferrari E, Nielsen L, Prioli N, Dell J, Sinha D, Syed J, Korfmacher WA, Nomeir AA, Lin CC, Wang L, Taveras AG, Doll RJ, Njoroge FG, Mallams AK, Remiszewski S, Catino JJ, Girijavallabhan VM, Bishop WR et al (1998) Antitumor activity of SCH 66336, an orally bioavailable tricyclic inhibitor of farnesyl protein transferase, in human tumor xenograft models and wap-ras transgenic mice. *Cancer Res* 58:4947–4956
24. Peters DG, Hoover RR, Gerlach MJ, Koh EY, Zhang H, Choe K, Kirschmeier P, Bishop WR, Daley GQ (2001) Activity of the farnesyl protein transferase inhibitor SCH66336 against BCR/ABL-induced murine leukemia and primary cells from patients with chronic myeloid leukemia. *Blood* 97:1404–1412
25. Petit T, Izbicka E, Lawrence RA, Bishop WR, Weitman S, Von Hoff DD (1999) Activity of SCH 66336, a tricyclic farnesyltransferase inhibitor, against human tumor colony-forming units. *Ann Oncol* 10:449–453
26. Reichert A, Heisterkamp N, Daley GQ, Groffen J (2001) Treatment of Bcr/Abl-positive acute lymphoblastic leukemia in P190 transgenic mice with the farnesyl transferase inhibitor SCH66336. *Blood* 97:1399–1403
27. National Institutes of Health (1982) Common terminology criteria for adverse events
28. Schering-Plough Corporation (1997) SCH 66336: Investigator's Brochure information for the investigational product. Schering-Plough Research Institute, Kenilworth
29. Gibaldi M, Perrier D (1982) Pharmacokinetics. Marcel Dekker, New York
30. Adjei AA, Davis JN, Erlichman C, Svingen PA, Kaufmann SH (2000) Comparison of potential markers of farnesyltransferase inhibition. *Clin Cancer Res* 6:2318–2325

31. Adjei AA, Erlichman C, Davis JN, Cutler DL, Sloan JA, Marks RS, Hanson LJ, Svingen PA, Atherton P, Bishop WR, Kirschmeier P, Kaufmann SH (2000) A Phase I trial of the farnesyl transferase inhibitor SCH66336: evidence for biological and clinical activity. *Cancer Res* 60:1871–1877
32. Awada A, Eskens FA, Piccart M, Cutler DL, van der Gaast A, Bleiberg H, Wanders J, Faber MN, Statkevich P, Fumoleau P, Verweij J (2002) Phase I and pharmacological study of the oral farnesyltransferase inhibitor SCH 66336 given once daily to patients with advanced solid tumours. *Eur J Cancer* 38:2272–2278
33. Eskens FA, Awada A, Cutler DL, de Jonge MJ, Luyten GP, Faber MN, Statkevich P, Sparreboom A, Verweij J, Hanauske AR, Piccart M (2001) Phase I and pharmacokinetic study of the oral farnesyl transferase inhibitor SCH 66336 given twice daily to patients with advanced solid tumors. *J Clin Oncol* 19:1167–1175
34. Cortes J, Jabbour E, Daley GQ, O'Brien S, Verstovsek S, Ferrajoli A, Koller C, Zhu Y, Statkevich P, Kantarjian H (2007) Phase I study of lonafarnib (SCH 66336) and imatinib mesylate in patients with chronic myeloid leukemia who have failed prior single-agent therapy with imatinib. *Cancer* 110:1295–1302
35. Khuri FR, Glisson BS, Kim ES, Statkevich P, Thall PF, Meyers ML, Herbst RS, Munden RF, Tendler C, Zhu Y, Bangert S, Thompson E, Lu C, Wang XM, Shin DM, Kies MS, Papadimitrakopoulou V, Fossella FV, Kirschmeier P, Bishop WR, Hong WK (2004) Phase I study of the farnesyltransferase inhibitor lonafarnib with paclitaxel in solid tumors. *Clin Cancer Res* 10:2968–2976
36. Kieran MW, Packer RJ, Onar A, Blaney SM, Phillips P, Pollack IF, Geyer JR, Gururangan S, Banerjee A, Goldman S, Turner CD, Belasco JB, Broniscer A, Zhu Y, Frank E, Kirschmeier P, Statkevich P, Yver A, Boyett JM, Kun LE (2007) Phase I and pharmacokinetic study of the oral farnesyltransferase inhibitor lonafarnib administered twice daily to pediatric patients with advanced central nervous system tumors using a modified continuous reassessment method: a Pediatric Brain Tumor Consortium Study. *J Clin Oncol* 25:3137–3143
37. Ready NE, Lipton A, Zhu Y, Statkevich P, Frank E, Curtis D, Bukowski RM (2007) Phase I study of the farnesyltransferase inhibitor lonafarnib with weekly paclitaxel in patients with solid tumors. *Clin Cancer Res* 13:576–583
38. ClinicalTrials.gov. National Institutes of Health
39. Hanrahan EO, Kies MS, Glisson BS, Khuri FR, Feng L, Tran HT, Ginsberg LE, Truong MT, Hong WK, Kim ES (2009) A phase II study of Lonafarnib (SCH66336) in patients with chemorefractory, advanced squamous cell carcinoma of the head and neck. *Am J Clin Oncol* 32:274–279
40. Kim ES, Kies MS, Fossella FV, Glisson BS, Zaknoen S, Statkevich P, Munden RF, Summey C, Pisters KM, Papadimitrakopoulou V, Tighiouart M, Rogatko A, Khuri FR (2005) Phase II study of the farnesyltransferase inhibitor lonafarnib with paclitaxel in patients with taxane-refractory/resistant nonsmall cell lung carcinoma. *Cancer* 104:561–569
41. Ravoet C, Mineur P, Robin V, Debusscher L, Bosly A, Andre M, El Housni H, Soree A, Bron D, Martiat P (2008) Farnesyl transferase inhibitor (lonafarnib) in patients with myelodysplastic syndrome or secondary acute myeloid leukaemia: a phase II study. *Ann Hematol* 87:881–885