Original article

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A phase II trial of farnesyl protein transferase inhibitor SCH 66336, given by twice-daily oral administration, in patients with metastatic colorectal cancer refractory to 5-fluorouracil and irinotecan

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Background: *ras* genes encode Ras proteins that are important for signal transduction in cancer cells. Farnesyl protein transferase (FPTase) is an enzyme that is responsible for a critical post-translational modification of Ras.

Patients and methods: We report the results of a phase II trial of SCH 66336, an FPTase inhibitor, in patients with metastatic colorectal cancer. This is the first reported experience of an FPTase inhibitor in this disease. All patients were considered refractory to first- and second-line therapy. A total of 21 evaluable patients were treated with a starting dose of 200 mg b.i.d. given continuously.

Results: The major side-effects were fatigue (grade 1 in 42%, grade 2 in 42% and grade 3 in 14%), diarrhea (grade 1 in 23% and grade 3 in 42%) and nausea (grade 2 in 16%). Elevations in serum creatinine (grade 2 or 3) were observed in 19% of patients and appeared to be related to dehydration induced by diarrhea. Significant hematological toxicity was not observed (only grade 1 thrombocytopenia in 19% and grade 2 or 3 anemia in 28%). Pharmacological studies revealed adequate mean pre-dose plasma concentrations in this group of patients on day 15 of therapy. No objective responses were observed, although stable disease was seen in three patients for several months. Administration of SCH 66336 was accompanied by gastrointestinal toxicity.

Conclusions: Future development of this compound cannot be recommended as monotherapy in this disease.

Key words: colon cancer, farnesyl protein, *ras*

Introduction

ras genes encode 21 kDa proteins (Ras proteins). Ras proteins localize on the inner surface of plasma membrane, bind guanosine triphosphate (GTP) and guanosine diphosphate, and are important for signal transduction in cells [1]. Mutations in *ras* are present in >30% of human cancers and lead to constitutive activation of Ras protein [2]. Ras proteins stimulate cellular proliferation and lead to inhibition of GTPase activity, which is normally responsible for dissociation of Ras from its binding to GTP. The most commonly occurring mutations in *ras* are called Ha-, K- and N-*ras*, and gene products from these mutations are found in a variety of human tumors including colon, pancreas, lung, sarcomas, etc. [2].

Farnesyl protein transferase (FPTase) is an enzyme that catalyzes the addition of a farnesyl isoprene group to a variety of cellular proteins including Ras [3]. Ras is capable of signal transduction through multiple effector pathways (including raf, mitogen-activated kinases, phosphatidylinositol-3′-activated kinases, etc.). Farnesylation is recognized as an important target for cancer chemotherapy and several novel approaches are being designed for inhibition of the farnesylation reaction [4, 5].

SCH 66336 is a non-peptidic, small molecule inhibitor of FPTase [6]. It inhibits FPTase *in vitro* and blocks H-Ras processing in whole cells [7]. SCH 66336 blocks the transformed growth properties (e.g. anchorage-independent growth) of human tumor cell lines containing mutated *ras* and of normal cells that have been transformed with mutated *ras* [8].

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However, activity of SCH 66336 does not depend on the presence of a *ras* mutation. Although SCH 66336 specifically inhibits farnesylation of H-*ras*, *in vitro* activity has been observed against tumors containing mutated K-*ras* or wild-type *ras* [8].

In preclinical animal studies, oral administration of SCH 66336 resulted in antitumor activity in human xenograft models in mice [7]. The spectrum of activity includes colon, pancreatic, lung, prostate, bladder and breast carcinomas and melanomas.

In soft agar, several colon cancer cell lines containing activated K-*ras* including Lovo colon carcinoma and HCT 116 colon carcinoma were highly sensitive to inhibition by SCH 66336. The IC_{50} for Lovo and HCT lines in these experiments were 30 and 125 nmol/l, respectively [8]. When DLD-1 human colon cancer xenografts in nude mice were treated with SCH 66336, there appeared to be a dose-dependent inhibition of the tumors. The percentage inhibition varied from 32–49% at a dose of 10 mg/kg, to 76–77% at a dose of 50 mg/kg [8].

SCH 66336 has been evaluated in phase I clinical trials. In two phase I trials, SCH 66336 was given orally b.i.d. as continuous administration or on a 2 of 4 week schedule [9–11]. The recommended phase II dose in both trials was 200 mg b.i.d. The primary toxicities were diarrhea, anorexia, fatigue and nausea. These toxicities were described as being mild and reversible on discontinuation of therapy. In the intermittent administration trial, two patients with colon cancer exhibited stable disease for 4 months. Based on these data, the continuous dosing schedule was tested in the present trial in patients with metastatic colorectal cancer.

Patients and methods

Patient selection

Patients with histologically confirmed colorectal cancer with metastatic disease in whom treatment had failed with both 5-fluorouracil (5-FU) and irinotecan were included in the study. All patients were ≥18 years of age, signed a written informed consent and had an Eastern Cooperative Oncology Group (ECOG) performance status of 0–2. Other inclusion criteria included measurable or clinically evaluable disease, adequate hematological function (white blood cell count $\geq 3.0 \times 10^3/mm^3$, absolute neutrophil count $\geq 1.5 \times 10^3/mm^3$, platelet count $\geq 100 \times 10^3/mm^3$), renal function (serum creatinine <1.5 mg/dl) and hepatic function [serum bilirubin <1.5 mg/dl, aspartate aminotransferase <3.0 × upper limit of normal (ULN) or <5.0 × ULN if the patient had liver metastases]. The study was approved by the institutional review board of Memorial Sloan-Kettering Cancer Center and all patients signed written informed consent before entry on the study. Twenty-one patients were treated on this phase II study from November 1998 to May 1999.

Drug administration

Single agent SCH 66336 was administered for 28 consecutive days at a starting dose of 200 mg orally b.i.d., taken with food. SCH 66336 was supplied by Schering-Plough Research Institute (Kenilworth, NJ, USA) as solid gelatin capsules in 100 and 200 mg strengths. Patients were supplied with drug and a dosing diary when they left the study site on day 1 and were given enough drug for their 28 days of treatment. Patients were instructed to bring in their dosing diary at each clinic visit and their remaining pills were counted and reconciled against the doses that were to be administered. The number of missed doses (according to pill count) was recorded. Patients were instructed not to take additional pills to make up for a missed dose. If a patient vomited after dosing, additional drug was not given to make up for that dose.

Pretreatment evaluation and follow-up studies

Baseline evaluation was carried out within 14 days before treatment initiation and included informed consent, a detailed medical history, comprehensive physical examination, 12-lead ECG, complete blood count, differential, serum chemistries, electrolytes, carcinoembryonic antigen concentration, prothrombin time, pregnancy test and urinalysis. A chest X-ray and baseline tumor measurements were obtained up to 28 days before beginning therapy. Patients were seen by a physician weekly and before each treatment course. Tumor measurements/serum tumor markers were performed using appropriate tests at baseline and after each 28-day course of therapy. Standard response criteria were used [12].

An ophthalmological examination was obtained at screening, at the end of patients' participation in the study, and as needed for symptoms or complaints of visual abnormalities. In addition, patients underwent regular ophthalmological examinations repeated at the start of every third cycle while on study, even in the absence of visual disturbances. Evaluation included direct ophthalmoscopy, assessment of visual acuity and assessment of color vision. Patients also had baseline retinal photographs obtained before the start of study treatment and after each cycle of therapy.

Pharmacokinetic sampling and assay

Limited pharmacokinetic sampling was performed on all patients in order to determine pre-dose concentrations after establishment of steady-state levels. Blood samples for the determination of plasma SCH 66336 concentrations during the first cycle were obtained just before the morning dose on day 15.

Five milliliter blood samples were collected into a sodium heparin tube. Samples were then centrifuged at ~3000 *g* for 15 min at 4°C. The resulting plasma was divided into two equal aliquots of at least 1 ml each and was transferred to cryogenic tubes. The plastic tubes were labeled and all samples were immediately frozen to at least –70°C and maintained in the frozen state until assayed. Plasma SCH 66336 concentrations were determined using validated liquid chromatography with tandem mass spectrometric detection assays. The lower limits of quantification in plasma were 1.00 ng/ml when 1 ml of plasma was used and 5.00 ng/ml when 0.2 ml of plasma was used. The corresponding concentration ranges were 1.00–5.00 ng/ml (low range) and 5.00–2500 ng/ml (high range). The analyses were performed at Taylor Technology, Princeton, NJ, USA.

Statistical considerations

Accrual on the study was performed using a two-stage design. Fifteen eligible patients were first treated and if either (i) two or more patients continued therapy to begin the third cycle (i.e. stable or improved disease beyond 8 weeks), or (ii) if one or more patients demonstrated an objective tumor regression of >25%, an additional 15-patient accrual was planned. However, after re-evaluation of the safety and efficacy, the study was closed after 21 evaluable patients had been accrued due to lack of objective response and presence of toxicity to the study drug.

Results

Patient characteristics are listed in Table 1. Twenty-one patients were enrolled. Patients with metastatic colorectal cancer treated on this study had good performance status (83% had an ECOG performance status of 0 or 1). All patients had received prior irinotecan chemotherapy (20 parenterally, one orally). Twenty patients had received prior 5-FU therapy. One patient was not previously treated with 5-FU but was considered 5-FU-resistant on the basis of a high thymidylate synthase level in the tumor (done with a RT–PCR). Most patients had either metastatic disease to liver or lung. Two patients had received prior pelvic radiation therapy.

Twenty-one evaluable patients received a total of 142 weeks of therapy (median 7 weeks). There were no major objective responses observed in this trial. Three patients exhibited stable disease for >2 months of therapy (6, 4 and 4 months, respectively).

All patients were initially started at a dose of 200 mg b.i.d. Several patients required dose reductions. Ten patients (48%) could receive planned therapy for 4 weeks (one cycle) without delay or dose attenuations. Dose was reduced to 150 mg b.i.d. in eight patients (four in the first, three in the second and one in the third month of therapy) and to 100 mg b.i.d. in four patients (in the second month of therapy) because of fatigue or gastrointestinal toxicity.

Table 1. Patient characteristics

Characteristic	No. of patients
Total	21
Age (years)	
Median	64
Range	39–83
Sex	
Male	8
Female	13
ECOG performance status	
0	2
1	17
2	3
Prior chemotherapy	
5-Fluorouracil	20[a]
Irinotecan	21[a]
Prior radiation	4
Pelvic	2
Peritoneal	1
Abdominal wall	1
Thoracic	1
Sites of metastasis	
Liver	13
Lung	13
Other	3

[a]See text for explanation.
ECOG, Eastern Cooperative Oncology Group.

The major toxicity observed in this trial was fatigue. Twelve patients experienced moderate (grade 2) to severe (grade 3) fatigue (57%). Although fatigue is generally multifactorial, most fatigue appeared to be temporally drug related. No patient discontinued therapy due to fatigue only, but three patients required dosage adjustments to continue therapy.

The other major toxicity of SCH 6636 was gastrointestinal in nature. Overall, five patients (24%) experienced grade 1 and nine patients (43%) experienced grade 3 diarrhea. Mild diarrhea was controllable with loperamide and oral hydration. However, three patients (14%) needed intravenous fluids in addition to oral hydration and loperamide. Patients who experienced grade 3 diarrhea could tolerate therapy in the subsequent weeks on a reduced dose. Overall, diarrhea (grades 1, 2 and 3) was reported in 80 of 142 weeks (56%) of therapy administered. No patients reported grade 4 diarrhea. Interestingly, three of six patients who had grade 3 diarrhea had grade 3 or 4 diarrhea with irinotecan therapy, one patient had no diarrhea with irinotecan and information regarding diarrhea with irinotecan could not be verified in the two other patients. It is not clear whether the mechanism of diarrhea with SCH 66336 is similar to the mechanism of irinotecan-induced diarrhea. Nausea was generally mild [grade 1 or 2 in three patients (14%) and grade 3 in only one patient] and easily controllable with anti-emetics.

Four patients had elevations in serum creatinine from 1.6 to 4.2 mg/dl during therapy (grade 2 or 3, in 19% of patients). This was related to dehydration, poor oral intake and fatigue and was temporally related to drug administration. All of these toxicities resolved after intravenous hydration, encouraging oral hydration, stopping drug temporarily and resuming later at a lower dose.

Very little hematological toxicity was seen in the trial (Table 2). The most common hematological toxicity was mild thrombocytopenia, seen in four patients, but this had no clin-

Table 2. Toxicities observed in the trial

Toxicity	No. of patients (total = 21)		
	Grade 1	Grade 2	Grade 3
Diarrhea	5	0	9
Fatigue	9	9	3
Nausea	2	1	1
Vomiting	2	0	0
Thrombocytopenia	4	0	0
Anemia	2	4	2
Increased serum creatinine	0	3	1
Increased bilirubin	0	1	1

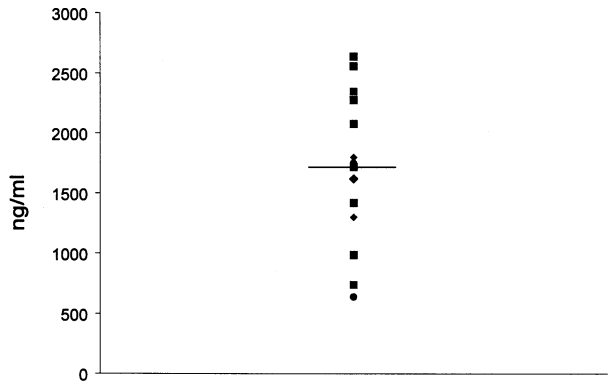

Figure 1. Graph showing pre-dose concentrations of farnesyl protein transferase inhibitor SCH 66336. The horizontal line is the median value.

ical relevance. Mild to moderate anemia was seen in a minority of patients.

Limited pharmacokinetic sampling in 18 evaluable patients revealed that the mean trough concentration of SCH 66336 in the plasma on day 15 (Figure 1) was 1757 ng/ml. This is comparable to those concentrations observed at this dose level in phase I trials [11]. The mean bioavailability on day 15 was 46%, which is also comparable to previous studies of this agent [8].

Discussion

Colorectal cancer is one of the most common cancer diagnoses in the USA, with an expected 130200 new cases being diagnosed in 2000 [13]. Treatment of metastatic colorectal cancer with chemotherapy is essentially palliative, with a minority of patients achieving complete responses [14, 15]. Recently, several newer chemotherapeutic agents such as irinotecan and oxaliplatin have been introduced into clinical practice [16, 17]. These agents produce modestly improved outcomes. However, there is an urgent need to develop new agents and novel targets for treatment of this lethal disease.

This is the first published experience of treatment of metastatic colorectal cancers with any oral FPTase inhibitor. We observed that treatment with SCH 66336 was associated with moderate to severe toxicity in a majority of patients at the 200 mg b.i.d. dose level, and that this could only be partly ameliorated with dose reductions. No responses were observed with this drug, although three patients had stable disease for 4–6 months. This trial has confirmed prior experience that there is practically no significant hematological toxicity seen with this drug [9]. The primary toxicity was diarrhea and fatigue, which is consistent with the experience in phase I trials of this drug [9–11]. Several patients [12 of 21 (57%)] required dosage reductions in order to continue SCH 66336 therapy. Dehydration resulting from diarrhea resulted in treatment with intravenous hydration in several patients. Although fatigue can be multifactorial, there was a strong temporal correlation between drug administration and onset of fatigue.

Given the novel mechanism of action, it was presumed that prior treatment with front-line chemotherapies such as 5-FU and irinotecan would not affect resistance to this agent. Although response rate as an end point may not be able to adequately demonstrate lack of activity of a cytostatic agent, SCH 66336 did not appear to have significant activity in this disease. In a recently published abstract, another non-peptidic farnesyl transferase inhibitor, R115777, was evaluated in patients with metastatic breast carcinoma [18]. Twenty-seven patients were treated in this study and three partial responses (11% response rate) were seen. Interestingly, this compound exhibited significant hematological toxicity (neutropenia and thrombocytopenia) and little gastrointestinal toxicity, in contrast to our experience with SCH 66336.

The exact mechanism of action of farnesyl transferase inhibitors is not clear. Although compounds such as SCH 66336 inhibit the transformed growth properties (e.g. anchorage-independent growth) of human tumor-cell lines containing mutated *ras* [8], newer evidence indicates that their principal target may not be *ras*. Presence of *ras* mutations does not seem to predict cytotoxicity to FPTase inhibitors [19–21]. In light of this work, we did not analyze the status of *ras* mutations in our patients and did not require its presence as an entry criterion to our study. A structurally related protein prenyl transferase, geranylgeranyl transferase-1 that prenylates critical proteins may alternatively prenylate K-Ras bypassing the effect of FPTase inhibition [22]. There is also some evidence that RhoB isoform RhoB-GG may be required as a mediator of apoptotic effects of FPTase inhibitors [19, 23]. RhoB-GG can induce the cell cycle kinase inhibitor p21 WAF1 in a p53-dependent manner, but it can also inhibit the growth of p53-null cells that lacked p21 WAF1 activation [24]. It is not clear whether inactivity of SCH 66336 is related to mutations leading to inactivation of RhoB in this study.

SCH 66336 has been shown recently to inhibit the farnesylation of a centromeric protein, CENP-E, leading to alterations in centromere-microtubule formation [25].Other farnesylated proteins may include lamins A and B, and other targets for FPTase inhibitors may include mitochondria in mammalian cells [26, 27]. In addition, Sebti and colleagues [28, 29] have demonstrated recently that in some models, FPTase inhibitors may inhibit progression through G_2 to M transition by blocking bipolar spindle formation and chromosome alignment.

As mentioned in a previously published review [5], evidence of activity in phase II trials with FPTase inhibitors may be limited to tumor growth inhibition or 'cytostasis' rather than objective tumor responses. We are unable to rule out some activity of SCH 66336 given that a refractory population was tested and a limited number of patients were treated. In addition, newer target-specific agents may only demonstrate activity by other measures such as lengthening time to progression and causing stability of disease. Also, the greatest potential for agents such as SCH 66336 may be in combination with other traditional chemotherapies.

Although preclinical tumor regressions have been obtained with SCH 66336 in animal models, in order for tumor stabilization to be clinically useful, it must be accomplished with acceptable side-effects. Given these facts and our present experience, we cannot recommend further evaluation of SCH 66336 as monotherapy in patients with refractory metastatic colorectal cancers.

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