A Phase I and Pharmacokinetic Study of SAM486A, a Novel Polyamine Biosynthesis Inhibitor, Administered on a Daily-times-five every-three-week Schedule in Patients with Advanced Solid Malignancies


ABSTRACT

Purpose: SAM486A is a novel inhibitor of the polyamine biosynthetic enzyme S-adenosylmethionine decarboxylase (SAMDC). This study was performed to characterize the toxicity profile and the pharmacological behavior and to determine the maximum tolerated dose (MTD) of SAM486A administered by a 1-h i.v. infusion daily for 5 days every 3 weeks in patients with advanced cancer.

Experimental Design: Twenty-three patients received 46 cycles of SAM486A at dose levels ranging from 3.6 to 202.8 mg/m²/day. SAM486A plasma concentrations were measured during the first cycle for pharmacokinetic and pharmacodynamic evaluations. Paired tumor biopsy specimens pre- and posttreatment were obtained in 1 patient to assess the impact of SAM486A on intratumoral enzymes and metabolites involved in the polyamine biosynthetic pathway.

Results: The dose-limiting toxicity of SAM486A on this schedule was myelosuppression. Nonhematological toxicities, including nausea, vomiting, anorexia, and fatigue, were mild to moderate in severity. The MTD of SAM486A was 102.4 mg/m²/day. Pharmacokinetic analyses demonstrated a rapid initial decrease in plasma drug concentrations at the end of infusion, followed by a long terminal elimination phase with a mean (± SD) terminal elimination half-life of 65.4 ± 55.6 h. Dose and area under the concentration-time curve correlated with the appearance of grade 4 neutropenia with correlation coefficients of 0.70 and 0.69, respectively. Analysis of paired tumor biopsy specimens taken before and after SAM486A treatment in 1 patient with metastatic melanoma revealed decreased SAMDC activity, increased ornithine decarboxylase activity, increased levels of putrescine, and depleted levels of decarboxylated S-adenosylmethionine and spermine, all of which are consistent with the proposed mode of action of SAM486A.

Conclusions: SAM486A was well tolerated on this schedule of administration with the MTD established at 102.4 mg/m²/day. Neutropenia was dose-limiting and correlated with dose and area under the concentration-time curve. Pharmacodynamic assessment of tumoral tissues in 1 study patient demonstrated changes in the levels of polyamines and their biosynthetic enzymes consistent with SAMDC inhibition.

INTRODUCTION

SAM486A (previously designated CGP 48664, Fig. 1) is the free base of 4-(aminoiminomethyl)-2,3-dihydro-1H-inden-1-one-diaminomethylenehydrazone, which exerts potent and specific inhibition of SAMDC3, the rate-limiting enzyme in polyamine biosynthesis. The polyamines spermidine and spermine are ubiquitously present in eukaryotic cells, where they play a key role in supporting cell proliferation via their interaction with diverse intracellular macromolecules (1, 2). Previous studies have demonstrated that tumor cells exhibit altered polyamine homeostasis, characterized by increased biosynthetic activity that results in elevated intracellular concentrations of polyamines (1, 3). This aberration in tumor tissue metabolism renders polyamine biosynthesis an attractive target for anticancer drug therapy (2).

The polyamine biosynthetic pathway is regulated at the

3 The abbreviations used are: SAMDC, S-adenosylmethionine decarboxylase; MTD, maximum tolerated dose; ODC, ornithine decarboxylase; dcSAM, decarboxylated S-adenosylmethionine; MGBG, methylglyoxal bis(guanylhydrazone); EKG, electrocardiogram; ANC, absolute neutrophil count; LVEF, left ventricular ejection fraction; CI, systemic clearance; mCRM, Modified Continual Reassessment Method; DLT, dose-limiting toxicity; HPLC, high-performance liquid chromatography; QC, quality control.
level of two rate-limiting enzymes (Fig. 1). The first of these, ODC, is responsible for the conversion of ornithine to the diamine, putrescine. This enzyme is the target of difluoromethyl-lornithine, a synthetic and irreversible inhibitor of ODC, which has been evaluated for its anticancer activity in advanced disease and is currently being investigated as a chemopreventive agent in premalignant disorders (4). The second rate-limiting enzyme is SAMDC, which provides the aminopropyl donor dcSAM, required for the sequential conversions of putrescine to spermidine, and of the latter to spermine.

The concept of SAMDC inhibition as an anticancer strategy was previously explored in studies of the first generation SAMDC inhibitor mitoguazone or MGBG. The therapeutic value of MGBG as an anticancer agent was most intensively evaluated in the early 1960s. Whereas antitumor activity was observed with MGBG against a wide variety of malignancies, including acute leukemia, chronic myelogenous leukemia, lymphoma, multiple myeloma, head and neck cancer, and esophageal cancer, the agent was associated with substantial schedule-dependent toxicity. Daily administration schedules of MGBG induced profound myelosuppression and mucositis, whereas severe muscular weakness, malaise, and gastrointestinal toxicity limited the utility of intermittent dosing schedules (5). Another obstacle in the clinical development of MGBG was that in addition to its inhibition of polyamine biosynthesis, the agent was found to interfere with mitochondrial function, resulting in swelling and vacuolization of the organelle (6). This effect on mitochondria was subsequently linked to cell proliferation and gastrointestinal toxicity (7). Nonetheless, the unique mechanism of action and antitumor activity of MGBG provided the impetus to search for new SAMDC inhibitors with improved therapeutic indices.

SAM486A inhibited SAMDC in vitro at a median concentration (IC\textsubscript{50}) of 4.7 nM, representing a ~200-fold greater potency and selectivity than MGBG and demonstrated growth inhibitory activity against a broad spectrum of human tumor cell lines, including multidrug resistant variants with IC\textsubscript{50}s ranging from 0.1 to 5 \mu M (8–11). Mechanistic indication of the specificity of SAM486A for SAMDC derives from the finding that Chinese hamster ovary cells made >1000-fold resistant to the drug exhibit stable, >10-fold amplification of the SAMDC gene (12). Additionally, at concentrations required for in vitro cytotoxicity, SAM486A did not induce structural or functional mitochondrial alterations. In contrast to MGBG, SAM486A did not appear to use the polyamine transport carrier system because it competes poorly with spermidine for uptake into L1210 murine leukemia cells, thereby even inhibiting the growth of polyamine transport-deficient cell lines (8, 11). In vivo, SAM486A demonstrated antitumor activity against a variety of malignancies, particularly malignant melanoma, including syngeneic (B16), human tumor xenograft (SK MEL-24 and MALME-3M), and metastatic (A375SM) models (8, 13–15). Additivity and synergism both in vitro and in vivo were noted when SAM486A was combined with 5-fluorouracil and paclitaxel (16). Results from the xenograft studies suggested that daily injections of SAM486A may be more effective than intermittent administration. Furthermore, the observation that SAMDC activity recovered very rapidly after withdrawal of SAM486A suggested that a continuous exposure of the drug may be desirable to achieve an optimal control of tumor growth.

In preclinical toxicology studies of rats and dogs, short-term single or repeat treatment with bolus injections of high doses of SAM486A induced acute cardiovascular and respiratory symptoms, described as hyperemia, tachycardia, cyanosis, and reduced body temperature and dyspnea, gasping, and deep respiration, respectively. After long-term treatment, heterogeneous electrocardiographic alterations in dogs were observed. Morphological changes were found in the liver and heart with the presence of reversible intracytoplasmic basophilic granules in hepatocytes and myocytes (17, 18). Preclinical studies with \textsuperscript{[\textsuperscript{14}C]}SAM486A revealed multiexponential elimination, characterized by a rapid initial decline followed by prolonged terminal elimination. The radiolabeled drug was distributed extensively from 0.1 to 5 \mu M (8–11). Mechanistic indication of the specificity of SAM486A for SAMDC derives from the finding that Chinese hamster ovary cells made >1000-fold resistant to the drug exhibit stable, >10-fold amplification of the SAMDC gene (12). Additionally, at concentrations required for in vitro cytotoxicity, SAM486A did not induce structural or functional mitochondrial alterations. In contrast to MGBG, SAM486A did not appear to use the polyamine transport carrier system because it competes poorly with spermidine for uptake into L1210 murine leukemia cells, thereby even inhibiting the growth of polyamine transport-deficient cell lines (8, 11). In vivo, SAM486A demonstrated antitumor activity against a variety of malignancies, particularly malignant melanoma, including syngeneic (B16), human tumor xenograft (SK MEL-24 and MALME-3M), and metastatic (A375SM) models (8, 13–15). Additivity and synergism both in vitro and in vivo were noted when SAM486A was combined with 5-fluorouracil and paclitaxel (16). Results from the xenograft studies suggested that daily injections of SAM486A may be more effective than intermittent administration. Furthermore, the observation that SAMDC activity recovered very rapidly after withdrawal of SAM486A suggested that a continuous exposure of the drug may be desirable to achieve an optimal control of tumor growth.

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throughout the tissues of rats with the greatest uptake in the salivary glands and liver.

The decision to pursue the clinical development of SAM486A was based upon the agent’s potent and specific inhibition of SAMDC, and its impressive antitumor activity against resistant malignancies such as melanoma. A daily-times-five schedule was selected in the present trial to approximate the dosing schedule associated with superior antitumor activity in preclinical studies. Two other concurrent Phase I trials exploring different schedules of drug administration were conducted (17, 18). One trial tested a continuous 120-h infusion schedule designed to maximize drug exposure (17). The second trial used a weekly intermittent schedule selected on the basis of an improved safety profile shown with MGBG in comparison with a daily schedule (18).

The principal objectives of this Phase I and pharmacokinetic study were to: (a) characterize the toxicities of SAM486A administered daily for 5 days every 3 weeks in patients with advanced solid malignancies; (b) determine the MTD and recommended dose for subsequent Phase II trials; (c) characterize the pharmacokinetic behavior of SAM486A; and (d) seek preliminary evidence of antitumor activity and validation of drug action in patients with advanced cancers.

PATIENTS AND METHODS

Patient Selection. Patients with histologically documented solid malignancies (including lymphoma) refractory to standard therapy or for whom no effective therapy existed were eligible for this study. Other relevant eligibility criteria included: (a) age ≥18 years; (b) WHO performance status of ≤2; (c) life expectancy of at least 3 months; (d) adequate hematopoietic (white blood count ≥2500/μl, ANC ≥1500/μl, and platelets ≥100,000/μl), hepatic (total bilirubin <1.5 mg/dl and other liver function tests within twice the normal upper limit), renal (serum creatinine < 1.5 mg/dl), and cardiac (baseline LVEF within normal limits and New York Heart Association classification of I or II) functions; (e) no chemotherapy, immunotherapy, radiotherapy, or investigational therapy within 4 weeks (6 weeks for nitrosoureas, mitomycin C, and extensive radiotherapy) and full recovery from the toxicities of prior therapies; (f) no clinical signs of brain metastases or leptomeningeal disease; (g) no history of congestive heart failure; and (h) no previous history of congestive heart failure. All patients gave written informed consent according to institutional and federal guidelines before treatment.

Drug Administration. The starting dose of SAM486A was 3.6 mg/m²/day, which was equivalent to one-tenth of the lowest toxic dose in rats, the species most sensitive to the toxic effects of SAM486A. The mCRM was used in this trial for dose escalation in an attempt to minimize the number of patients treated at potentially subtherapeutic doses of SAM486A without inducing an excessive risk of drug-related toxicity (19–21). The mCRM is a sequential Bayesian estimation scheme in which a prior distribution function of the MTD and a dose-toxic response model are selected before the trial, generally based on preclinical toxicity data. This dose escalation method provides an updated estimate of the MTD after each patient has been evaluated for the occurrence of either acceptable or unacceptable (DLT, see below) toxicity after treatment. The updated MTD estimate is derived from a posterior distribution based on the experience of DLT in all previously treated patients during their first cycle of therapy. This estimate then enables a safe dose level to be recommended for the next patient. To ensure adequate safety in the dose escalation procedure, additional guidelines were applied as follows: (a) dose levels to be studied were predetermined based upon preclinical information with the selection of intermediate dose levels if ongoing trial data suggested that such a step was appropriate; (b) 3 patients were treated at the starting dose level and a single patient was treated at each dose level until moderate toxicity (see below) was encountered, at which point 2 additional patients were accrued; (c) the selected dose level could not be greater than one dose level above that assigned to the previous patient; (d) if DLT was observed in the previous patient, the dose level assigned to the subsequent patient could not be greater; and (e) no intrapatient dose escalation was permitted. Of note is the fact that a modified Fibonacci scheme was used for the selection of dose levels, however, the absence of toxicity up to the seventh dose level (51.2 mg/m²/day) led to an amendment of the protocol to allow 100% dose escalation steps above a prior dose in the absence of any drug-related toxicity.

Moderate toxicity was defined as grade 2 nonhematological toxicity (excluding alopecia, nausea, and vomiting without adequate antiemetic medications) or grade 3 hematological toxicity. Unacceptable toxicity, considered as dose-limiting in this trial, was defined as any one of the following occurring during cycle 1: (a) grade 3 nonhematological toxicity excluding alopecia but including grade 3 nausea and vomiting despite adequate antiemetic medications; (b) grade 4 thrombocytopenia; (c) grade 4 neutropenia of a duration of ≥4 days without fever; or (d) grade 4 neutropenia with fever.

Toxicities were graded according to the National Cancer Institute Common Toxicity Criteria, version 1.0 (22). The MTD was defined as the highest dose level at which no >30% of patients experienced DLT. The safety profile of SAM486A during cycle 1 only was taken into account in this assessment.

SAM486A (formulated as a salt with D,L lactic acid) was supplied by Novartis Pharmaceuticals Corp. (East Hanover, NJ) in 2-ml glass vials containing a sterile, light-yellow lyophilized product. The drug was reconstituted by the addition of 1 ml (for the 10-mg dosage strength) or 5 ml (for the 50-mg dosage strength) of 5% dextrose solution. The reconstituted solution was then mixed with 100 ml of 5% dextrose solution and infused i.v. ≥1 h. In the event of acute reactions (e.g., local reaction at infusion site, flushing, and so on), the infusion could be prolonged up to a maximum duration of 3 h.

Evaluation of Safety and Efficacy. Pretreatment evaluation included a history, physical examination, performance status assessment, and routine laboratory studies, which consisted of a complete blood cell count, differential white blood count, prothrombin time, activated partial thromboplastin time, serum chemistries, creatine phosphokinase, uric acid, triglycerides, and cholesterol. A urinalysis, calculated creatinine clearance, chest radiograph, EKG, and cardiac contractility assessment of LVEF by echocardiogram or multigated radionuclide scan were performed before the start of study treatment. Vital signs were measured before, during, and after SAM486A infu-
sion on treatment days and weekly thereafter. EKGs were done before and after SAM486A infusion on day 1 and after infusion on day 5 of every treatment cycle. LVEF assessment was repeated after every two cycles of treatment. Routine laboratory studies were obtained preinfusion on days 1, 4, 8, 11, and 15 of each cycle. Urinalysis and calculated creatinine clearance were measured preinfusion on days 1 and 8 of each treatment cycle. A chest radiograph was performed after every two cycles of treatment. Adverse experiences and concomitant therapies were recorded at every visit.

Efficacy was assessed by tumor measurements at baseline and after every two cycles of treatment. Response evaluation was performed according to the WHO tumor response criteria (23). Complete response was defined as the disappearance of all measurable or evaluable disease for at least two measurement periods separated by at least 4 weeks without worsening of cancer-related symptoms or performance status. Partial response required at least a 50% reduction in the sum of the bidimensional products of all measurable lesions documented by at least two measurements separated by at least 4 weeks. In addition, serum biochemical parameters of tumor response were to be recorded, where applicable, at baseline and if elevated, after completion of every treatment cycle. Reduction in any of these markers by ≥50% for at least 4 weeks was considered evidence of biochemical response. Stable disease was defined as a change not >50% decrease or 25% increase in the sum of the bidimensional products of all measurable lesions without the appearance of new lesions. Progressive disease was defined as an increase >25% in the sum of the products of diameters of all measurable lesions or appearance of new lesions. Patients were allowed to continue treatment in the absence of disease progression or intolerable toxicity.

Pharmacokinetic Sampling and Assay. To study the pharmacokinetics of SAM486A, whole blood samples were obtained from an indwelling venous catheter placed in the arm contralateral to the drug infusion. On days 1 and 3 of the first treatment course, samples were collected before the infusion and at the end of the infusion. On day 5 of the first treatment course, samples were collected before the infusion, and at 10, 30 min, and 1, 2, 4, 6, 8, 12, 24, 48, and 72 h after infusion. Samples were also collected on days 15 and 22 of course 1. The samples were collected in tubes containing sodium heparin, inverted several times, and immediately placed on ice. Within 15 min of blood collection, samples were centrifuged at room temperature to separate plasma, and then the plasma was frozen at −20°C until analysis. SAM486A was detected using a sensitive HPLC assay (24). After thawing, study and QC samples were homogenized by shaking on a vibration shaker for 30 seconds. Samples were diluted as necessary with blank human plasma. A 50-μl aliquot of the internal standard solution (CGP 51467) was added to study and QC samples. Aliquots of 1 ml were analyzed.

The drug and the internal standard were separated from other plasma matrix substituents across a cellulose membrane (molecular weight limit: Mw 15,000) using a Gilson Asted fitted with a flat-bed dialyzer. Chromatographic separation of the compounds was achieved using a 3.5-μm Zorbax SB-CN Rapid Resolution analytical column (4.6-mm inside diameter × 150 mm) with 0.01 M solution of octanesulfonate in 0.01 M potassium phosphate buffer (pH 2.5):acetonitrile (78:22) as the mobile phase at a flow rate of 1 ml/min. The analytes were detected with a UV detector monitoring at a wavelength of 230 nm.

Calibration curves (y = mx + b) were generated from the plots of the peak area ratios (y) of SAM486A to the internal standard versus the concentrations (x) of the calibration samples using weighted (1/y) linear least-squares regression as the mathematical model. Concentrations in QC and study samples were calculated from the resulting peak area ratios and interpolation from the regression equations of the respective calibration curves. Turbochrom II 2700 (Version 4.1) software from PE Nelson was used for these data analyses. Specificity of the method in blank human plasma was demonstrated by the lack of interfering peaks at the retention times of SAM486A and the internal standard.

The limit of quantitation was based on the accuracy and precision of sample determinations. It was set at the lowest concentration QC sample for which accuracy was in the range 80–120%, and precision was ≤20% coefficient of variation. During the course of the study, the dynamic range of the method was adjusted to the concentrations expected in the study samples. For different assay runs, therefore, the limit of quantitation varied from 5 to 50 ng/ml.

Pharmacokinetic and Pharmacodynamic Analysis. The pharmacokinetic parameters maximum plasma concentration (Cmax), area under the concentration-time curve (AUC(0→∞)), and terminal elimination half-life (t1/2) after the day 5 dose were determined by noncompartmental analysis using WinNonlin Professional (version 1.5) software (Scientific Consulting, Inc.). Descriptive statistics of the pharmacokinetic parameters were calculated. For calculations of CI and volume of distribution at steady state (Vss), the data from all sampling time points and all five drug infusions during cycle 1 were fitted to a three-compartment mammillary model by nonlinear regression using WinNonlin Professional, version 1.5 (Scientific Consulting, Inc.). From the estimated model parameters, the program calculated CI and Vss. Scatterplots of AUC versus dose and Cmax versus dose were created to evaluate the effect of dose on the systemic exposure and peak plasma concentrations observed after SAM486A dosing. The dose proportional relationships of AUC and Cmax were analyzed by linear regression. To determine whether there was a relationship between the appearance of granulocytopenia and pharmacokinetic parameters, scatterplots of the nadir of absolute granulocyte counts versus dose, Cmax, and AUC were produced. The data were fitted to an inhibitory sigmoid Emax model by nonlinear regression (WinNonlin version 1.5, Scientific Consulting, Inc.). The model is expressed as the following equation:

$$E = E_{\max} \left(1 - \frac{C^E}{(C^E + C_{\text{IC}_{50}})^E}\right)$$

where E is the absolute granulocyte count; Emax is the maximum absolute granulocyte count from the regression; C is either dose, Cmax, or AUC; γ is a shaping factor; and EC50 is the value of the parameter in which 50% of Emax is elicited.

Tumor Tissue Analysis. For consenting patients with an accessible tumor for biopsy, paired biopsy specimens were taken for the purposes of determining polyamine and SAM486A concentrations. Tissue samples were procured before the infusion on day 1 and repeated on day 5 of treatment, sonicated, and...
Enzyme activities were expressed as nmol CO$_2$ released/minute using previously established methods (12). Polyamine pools, SAM pools, and polyamine pool analysis were confirmed by repeat analysis of the tumor tissue. SAM$_486$A concentrations were expressed as pmol/mg protein.

Prior systemic therapy was classified into three groups as follows: group 1, below MTD (102.4 mg/m$^2$/day); group 2, MTD (102.4 mg/m$^2$/day); and group 3, above MTD (>102.4 mg/m$^2$/day). The dose levels explored and respective assignment to groups are shown in Table 2. Seventeen of the 23 patients enrolled discontinued treatment because of disease progression. Three patients withdrew because of adverse events, all of whom were treated at dose levels above the MTD (group 3). Additionally, 3 patients had dose/infusion modifications because of toxicity during the trial: 1 patient (202.8 mg/m$^2$/day) had the infusion time prolonged to 2 h because of grade 1 urticaria at the injection site; 1 patient (202.8 mg/m$^2$/day) had study drug interrupted because of grade 3 back pain that was attributable to disease progression; and 1 patient (150 mg/m$^2$/day) had a dose reduction to 102.4 mg/m$^2$/day in cycle 2 because of grade 4 neutropenia and grade 2 fatigue.

### Hematologic Toxicity
Neutropenia was the primary dose-limiting toxicity of SAM$_486$A identified in this trial. Although transient grade 1–2 neutropenia was observed in groups 1 and 2 (group 1: grade 2, 1 patient; and group 2: grade 1, 2 patients, grade 2, 1 patient), grade 4 neutropenia only occurred in group 3. For example, 5 of the 6 patients in group 3 experienced grade 4 neutropenia with a median nadir ANC of 300/mm$^3$, which was ameliorated by i.v. antibiotics. Interestingly, no thrombocytopenia was recorded in any patient. Additionally, only 1 patient in group 3 experienced a grade 4 decrement in hemoglobin, which was transient and concomitant with grade 4 neutropenia.

### Nonhematologic Toxicity
The nonhematologic toxicities of SAM$_486$A were mild to moderate in severity and primarily consisted of nausea, vomiting, anorexia, and fatigue.
The nausea and vomiting appeared to be dose related, occurred in the peri-treatment period, and were ameliorated by the use of oral phenothiazines or serotonin antagonists. Prophylactic antiemetics were not routinely required. The fatigue and anorexia also appeared to be dose related and generally persisted for 7–10 days after completion of the 5-day treatment. Grade 1–2 phlebitis at the injection site was observed in 6 of 23 patients, was transient in nature, and did not result in local skin ulceration or necrosis.

Similar to MGBG, a constellation of symptoms such as facial flushing, circumoral numbness, and feeling of a warm sensation occurred during the infusion of SAM486A. Ten patients (group 1, 2 patients; group 2, 4 patients; and group 3, 4 patients) experienced these symptoms that abruptly ceased after the end of infusion. These symptoms were always mild in severity and prolongation of the infusion duration was only required in 1 patient.

<table>
<thead>
<tr>
<th>Group</th>
<th>Total no. of patients</th>
<th>Nausea</th>
<th>Vomiting</th>
<th>Anorexia</th>
<th>Fatigue</th>
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<td>9</td>
<td>44</td>
<td>22</td>
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<td>6</td>
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Antitumor Activity. Although no complete or partial responses were observed, 4 patients (adrenal, renal, sarcoma, and head and neck cancer) maintained stable disease during 3–5 cycles of therapy.

Pharmacokinetic Analysis. Pharmacokinetic studies were performed in all 23 patients, whereas 4 patients were excluded from the analysis because of insufficient plasma sampling on day 5. Mean plasma concentration-time profiles on day 5 after administration of 3.6–202.8 mg/m²/day of SAM486A and the mean plasma concentration-time profile of all 6 patients treated at the 102.4 mg/m²/day dose level are depicted in Figs. 2 and 3. The disposition of SAM486A was characterized by a rapid increase in plasma concentration after the start of infusion, followed by a multiphasic elimination and prolonged terminal half-life. On day 5 the mean $t_{1/2}$, $V_{ss}$, and CI values for SAM486A were 65.4 ($\pm 55.6$) h, 811 ($\pm 515$) l/m², and 7.79 ($\pm 6.43$) l/h/m², respectively (Table 4). Although there was substantial variability, both $C_{max}$ and AUC appeared to be linearly related to dose (Figs. 4 and 5) with correlation coefficients ($r$) of 0.85 and 0.92 for $C_{max}$ and AUC, respectively. There was accumulation of SAM486A with daily treatment as
reflected in a mean ratio of preinfusion drug concentrations between day 5 and day 3 of 1.5.

**Pharmacodynamic Analysis.** The relationships between SAM486A systemic exposure and the ANC nadirs are depicted in Fig. 6. The EC50 for dose was 130 mg/m²/day, and doses of 150 and 202.8 mg/m²/day resulted in grade 4 neutropenia, whereas the EC50 for AUC was 51,200 ng/h/ml, and grade 4 neutropenia occurred at AUC values 55,000 ng/h/ml. Only 1 patient with an AUC that exceeded 55,000 ng/h/ml did not experience grade 4 neutropenia. The correlation coefficients of observed versus predicted values were 0.70 and 0.69 for dose and AUC, respectively. Thus, dose and AUC appeared to be equivalent in predicting grade 4 neutropenia.

**Tumor Tissue Analysis.** Pre- and posttreatment biopsies of tumors were obtained from a 51-year-old man with melanoma who received SAM486A at a dose of 202.8 mg/m²/day. The results of the polyamine and SAM486A analyses are depicted in Table 5. Consistent with the proposed mechanism of action of SAM486A, SAMDC activity was reduced by 50% and the enzyme product dcSAM was lowered by 95%. An unusual finding was that dcSAM was present in this particular tumor in high quantities before treatment. As per the role of dcSAM in the forward conversion of putrescine to spermidine (Fig. 1), tumor putrescine pools were increased 16-fold after treatment, and spermine pools were decreased by 71%. As a typical compensatory response to apparent SAMDC inhibition, ODC activity was increased by 6-fold in treated tumors (12). Finally, SAM486A concentrations within the tumor were similar to those observed in preclinical studies (25).

**DISCUSSION**

The mCRM was used in this study to minimize the number of patients treated at potentially ineffective dose levels while maintaining patient safety. In support of this objective, six initial dose level escalations (from 3.6 to 51.2 mg/m²/day) were accomplished with a total of 9 patients, representing a 57% reduction in the number of patients required. However, the reduction in the number of patients was principally attributable to the novel reduced cohort size and not the mCRM. In this study, the first occurrence of DLT was at a dose 56-fold of the starting dose, thereby limiting the applicability of the preselected dose-toxic response model because lower doses were originally predicted to have a 95% probability of exceeding the MTD. None-

---

**Table 4** SAM486A pharmacokinetic parameters

<table>
<thead>
<tr>
<th>Dose (mg/m²/day)</th>
<th>No. of patients</th>
<th>C&lt;sub&gt;max&lt;/sub&gt; &lt;sup&gt;a&lt;/sup&gt; (ng/ml)</th>
<th>t&lt;sub&gt;1/2&lt;/sub&gt; &lt;sup&gt;a&lt;/sup&gt; (h)</th>
<th>AUC&lt;sub&gt;0-24&lt;/sub&gt; &lt;sup&gt;a&lt;/sup&gt; (ng·h/ml)</th>
<th>Cl (l/h/m²)</th>
<th>V&lt;sub&gt;SS&lt;/sub&gt; (l/m²)</th>
</tr>
</thead>
<tbody>
<tr>
<td>3.6</td>
<td>3</td>
<td>97.8&lt;sup&gt;b&lt;/sup&gt; (81.7)</td>
<td>18.5&lt;sup&gt;b&lt;/sup&gt; (20.1)</td>
<td>956&lt;sup&gt;b&lt;/sup&gt; (1,216)</td>
<td>4.17&lt;sup&gt;b&lt;/sup&gt; (5.90)</td>
<td>771&lt;sup&gt;b&lt;/sup&gt; (172)</td>
</tr>
<tr>
<td>7.2</td>
<td>1</td>
<td>100</td>
<td>51.3</td>
<td>1,460</td>
<td>9.14</td>
<td>998</td>
</tr>
<tr>
<td>14.4</td>
<td>1</td>
<td>341</td>
<td>50.7</td>
<td>4,350</td>
<td>7.96</td>
<td>376</td>
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<td>21.6</td>
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<td>28.8</td>
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<td>277</td>
<td>14.2</td>
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<td>38.4</td>
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<td>880</td>
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<td>6</td>
<td>2,470&lt;sup&gt;b&lt;/sup&gt; (590)</td>
<td>62.2&lt;sup&gt;b&lt;/sup&gt; (18.0)</td>
<td>39,200&lt;sup&gt;b&lt;/sup&gt; (16,700)</td>
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<tr>
<td>150</td>
<td>2</td>
<td>6,160&lt;sup&gt;b&lt;/sup&gt; (1,490)</td>
<td>45.5&lt;sup&gt;b&lt;/sup&gt; (3.3)</td>
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<tr>
<td>202.8</td>
<td>2</td>
<td>7,450</td>
<td>78.5&lt;sup&gt;b&lt;/sup&gt; (42.4)</td>
<td>73,000&lt;sup&gt;b&lt;/sup&gt; (19,600)</td>
<td>7.07&lt;sup&gt;b&lt;/sup&gt; (1.42)</td>
<td>556&lt;sup&gt;b&lt;/sup&gt; (24)</td>
</tr>
</tbody>
</table>

All doses: 19 patients

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<sup>a</sup>Parameter determined after dosing on day 5.
<sup>b</sup>Value is mean value (with SD in parentheses). Unless otherwise indicated, n = value in no. of patients column.
theless, the use of reduced cohort sizes that was introduced by the Continual Reassessment Method clearly demonstrated that safety can be maintained while minimizing patient resources.

The polyamine biosynthetic pathway appears to be aberrantly activated in tumor cells and thus is an attractive target for anticancer therapy. SAM486A is a potent and specific inhibitor of SAMDC, one of the rate-limiting enzymes in polyamine synthesis. Preclinical studies of SAM486A revealed that the agent was associated with substantial antitumor activity in vitro and in vivo (8–10, 13–16) and appeared to have superior attributes over the first generation SAMDC inhibitor MGBG. This Phase I and pharmacological study was designed to evaluate the feasibility of administering SAM486A as a 1-h i.v. infusion daily for 5 days every 3 weeks to approximate the schedule that was associated with optimal antitumor activity in preclinical models.

The main toxicities of SAM486A on this administration schedule were neutropenia, nausea, vomiting, fatigue, and anorexia. The principal dose-limiting toxicity observed at doses > 102.4 mg/m²/day was neutropenia. Five of 6 patients treated at SAM486A doses exceeding 102.4 mg/m²/day experienced grade 4 neutropenia, whereas no significant myelosuppression was observed in the 8 patients treated at 102.4 mg/m²/day. The lack of thrombocytopenia at all tested dose levels in this study suggests that the myelosuppressive effect of SAM486A may primarily influence mature neutrophil precursors rather than the earlier progenitor cells. Additionally, nonhematologic toxicities encountered at the 102.4 mg/m²/day dose level were mild to moderate in severity and nondose-limiting. Thus, the recommended Phase II dose of SAM486A for subsequent disease-directed studies is 102.4 mg/m²/day for 5 consecutive days every 3 weeks. In two other Phase I studies of SAM486A using different treatment schedules, neutropenia was also observed, whereas thrombocytopenia was mild and not dose dependent (17, 18).

Fig. 4 Relationship of Cmax to dose.

Fig. 5 Relationship of AUC to dose.

Fig. 6 A, relationship of absolute granulocyte count nadir to dose. B, relationship of absolute granulocyte count nadir to AUC.
Nonhematologic toxicities of SAM486A that were not dose limiting but were related to dose included nausea, vomiting, fatigue, and anorexia. A constellation of symptoms that included facial flushing, sensation of warmth, and circumoral numbness were observed after treatment with SAM486A and are characteristic of the first generation SAMDC inhibitor MGBG (5). However, a distinction between the safety profile of MGBG and SAM486A that emerged from this study was the absence of severe mucositis in patients treated with SAM486A, although this was one of the most clinically significant and problematic toxicities associated with MGBG. Only 3 patients experienced grade 2 mucositis with SAM486A treatment, 2 of these were in group 3 (i.e., at intolerable doses). Laboratory studies with MGBG strongly implicated mitochondrial toxicity as the etiology of gastrointestinal mucositis, and this particular drug action was intentionally minimized in the development of SAM486A (8, 26). The gastrointestinal side effects of SAM486A noted in two other parallel Phase I trials were similar to this study, suggesting that they were not schedule dependent (17, 18).

The plasma concentration-time profiles of SAM486A were characterized by a rapid initial distribution, followed by a gradual prolonged elimination phase and are consistent with results from two parallel Phase I clinical studies exploring different schedules of SAM486A administration, namely a 120-h continuous infusion regimen repeated every 4 weeks and a weekly regimen given for 4 weeks every 6 weeks (17, 18). The linear relationship between AUC and dose implies that there are no saturable processes involved with the distribution and elimination of SAM486A throughout the dose range tested in this trial. The mean CI of 7.79 l/h/m² corresponds to 130 ml/min/m², which is comparable with the glomerular filtration rate. This is consistent with preclinical studies in rats and dogs, indicating that SAM486A is primarily eliminated by renal excretion. The large volume of distribution noted in this study (811 l/m²) is also compatible with the extensive distribution noted in preclinical studies. The data from this study (19 patients) were combined with those from the two other Phase I studies (60 patients) for use in a population pharmacokinetic analysis (27). The data were best fit to a three-compartment-linear model, and the estimated values of CI and Vss, 6.2 l/h/m² and 672 l/m², respectively, were similar to those obtained in this study. The plasma concentrations of SAM486A achieved at the 102 mg/m²/day dose level (~11 μM) clearly exceeded both the IC50 for in vitro SAMDC inhibition, as well as the IC50 for growth inhibition of human tumor cell lines in vitro (4.7 nM and 0.1–5 μM, respectively; Refs. 8–11).

From this study, the pharmacokinetic parameters AUC and, to a lesser extent, Cmax appeared to correlate with the degree of hematological toxicity observed. Modeling analysis using pharmacokinetic data from the three completed Phase I studies of SAM486A corroborate the correlation between AUC and neutropenia (27). Using all of the pharmacokinetic and pharmacodynamic data from the three Phase I studies, the cumulative AUC was the best predictor of the decrement in the ANC, the absolute ANC nadir, and the probability of grade 3 or 4 neutropenia. Interestingly, the duration of SAM486A exposure above the threshold concentrations of 0.05–0.1 μM was a less sensitive indicator of significant neutropenia. These data suggest that the dosing schedule is less important than the total drug exposure in predicting toxicity.

The clinical relevance and applicability of SAMDC inhibition are based upon established data that both ODC and SAMDC are rate-limiting enzymes in polyamine biosynthesis (Fig. 1). Because of its action early on in the polyamine biosynthetic pathway, ODC would seem to represent a more attractive drug target because inhibition of this enzyme should theoretically deplete all three major polyamine pools: putrescine, spermidine, and spermine. However, it has been demonstrated that despite being an effective and specific inhibitor of ODC, difluoromethylornithine may actually result in a paradoxical increase in spermine pools, primarily via the forward synthesis of putrescine and spermidine to spermine, leading to subsequent promotion of cell growth (25). In contrast, inhibitors of SAMDC tend to increase putrescine while reducing both spermidine and spermine pools. In the single biopsy specimen obtained in this study, the metabolite dcSAM was present in the pretreatment specimen with a subsequent marked decrease after therapy. The presence of dcSAM in the pretreatment tumor sample was unexpected based upon previous data from numerous clinical samples and human tumor cell lines. However, the unusually high pretreatment concentrations of dcSAM did allow the detection of its decline with SAM486A treatment, an observation that indicates direct inhibition of the target enzyme SAMDC. This conclusion was supported by the decrease in spermine pools and an expected compensatory increase in ODC activity which, together with inhibition of SAMDC, led to a 6-fold rise in putrescine pools. Obviously, additional studies are required to validate these effects and should be incorporated, where possible, into subsequent disease-directed studies of this agent.

Overall this trial has established a safe and recommended Phase II dose of SAM486A when given as a daily 1-h infusion for 5 consecutive days every 3 weeks. Compared with the other schedules tested, the daily-times-five schedule appears the most favorable based upon its feasibility, lack of EKG abnormalities (observed in both the infusional and weekly schedules), and mild spectrum of nonhematological toxicities (17, 18). In terms of potential tumor targets, the differential SAMDC enzymatic activity in colon tumors and normal colonic mucosa provides a sound, scientific rationale to assess this compound in the treatment of colorectal cancer (28, 29). Such findings, along with the
preclinical observation of additive antitumor effect when SAM486A is combined with 5-fluorouracil, support this combination for additional investigation in colorectal cancer (16). Prior experience with MGBG also raises the possible use for SAM486A in the treatment of lymphoma and other hematological malignancies (5, 30, 31).

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REFERENCES
A Phase I and Pharmacokinetic Study of SAM486A, a Novel Polyamine Biosynthesis Inhibitor, Administered on a Daily-times-five every-three-week Schedule in Patients with Advanced Solid Malignancies


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