A Phase I and Pharmacokinetic Study of Col-3 (Metastat), an Oral Tetracycline Derivative with Potent Matrix Metalloproteinase and Antitumor Properties

Samira Syed,[1] Chris Takimoto,[1] Manuel Hidalgo,[1] Jinee Rizzo,[1] John G. Kuhn,[1] Lisa A. Hammond,[1] Garry Schwartz,[2] Anthony Tolcher,[1] Amita Patnaik,[1] S. Gail Eckhardt,[1] and Eric K. Rowinsky[1]

[1]Institute for Drug Development, Cancer Therapy and Research Center and University Health Science Center at San Antonio, San Antonio, Texas; and [2]Brooke Army Medical Center, Fort Sam Houston, Texas

ABSTRACT

Purpose: **The purpose of this research was to assess the feasibility of administering Col-3, an oral chemically modified tetracycline derivative with potent inhibitory effects on matrix metalloproteinase activity and production, and recommend a dose on an uninterrupted once-daily schedule. The study also sought to characterize the pharmacokinetic behavior of Col-3 and seek evidence of anticancer activity.**

Experimental Design: **Patients with advanced solid malignancies were treated with escalating doses of Col-3 with dose level assignment according to an accelerated titration scheme. Because photosensitivity skin reactions were being reported in concurrent trials of Col-3, patients were instructed to apply sunscreen rigorously throughout the trial. The maximum tolerated dose was defined as the highest dose at which <2 of the first 6 new patients experienced dose-limiting toxicity. The pharmacokinetic behavior of Col-3 was characterized, and pharmacodynamic relationships were sought.**

Results: **Thirty-three patients were treated with 73 courses of Col-3 at four dose levels ranging from 36 to 98 mg/m^2/day. Unacceptably high incidences of photosensitivity skin reactions and malaise were noted in the first 28-day courses of patients treated with Col-3 at doses exceeding 50 mg/m^2/day. At 50 mg/m^2/day, severe toxicity occurred in 2 of 12 new patients in first courses, and no additional dose-limiting toxicities were observed in subsequent courses. Other mild to modest adverse effects included nausea, vomiting, liver function tests abnormalities, diarrhea, mucositis, leukopenia, and thrombocytopenia. The pharmacokinetics of Col-3 were dose proportional, and mean trough concentrations at steady state were similar to biologically relevant concentrations in preclinical studies. Major responses did not occur, but durable disease stability was noted in 3 patients, one each with carcinosarcoma of the uterus, pancreas, and ovary, all of whom had experienced disease progression before Col-3 treatment.**

Conclusions: **The recommended dose for Phase II studies of Col-3 administered once daily on an uninterrupted schedule is 50 mg/m^2/day accompanied by efforts that promote adherence to the use of sunscreen and other photoprotective measures. Pharmacokinetic results indicate that plasma concentrations above biologically relevant concentrations are readily maintained at this dose, and additional disease-directed studies, particularly in patients with soft tissue sarcoma, should be considered.**

INTRODUCTION

Over the last decade, there has been an accumulation of compelling experimental evidence indicating that matrix metalloproteinases (MMPs), which are produced by neoplastic, stromal, and endothelial components of tumors, play important roles in cancer invasion and malignant angiogenesis (1, 2). For these reasons, a wide range of small-molecule MMP inhibitors that delay tumor growth and formation of metastases in experimental models have been sought by concerted compound screening and structural biochemistry, and several have been broadly evaluated in the clinic. To date, however, these MMP inhibitors have not demonstrated convincing benefit in many types of clinical settings, albeit principally in the context of advanced disease (1, 3). Tetracycline derivatives are among the most potent inhibitors of MMP activity, and, therefore, these agents are being investigated in the treatment of nonmalignant diseases, such as degenerative arthritis and periodontitis, and malignant neoplasms, in which the pathogenesis, progression, and clinical manifestations relate to enhanced activity and production of MMPs (4–7). Several mechanisms to account for the actions of the tetracycline derivatives have been proposed and supported by experimental data, including MMP inhibition by divalent cation chelation of zinc at the active site of the MMPs, down-regulation of the production of the MMP proenzyme forms, inhibition of the oxidative activation of the proenzymes, and/or increased degradation of MMP proenzymes (8–12). Therefore, the tetracycline derivatives are attractive candidates for clinical development, because they reduce the production of MMPs and inhibit MMP activity.

The chemically modified tetracyclines are composed of a

Received 4/26/04; accepted 6/21/04.

Grant support: CA69853 and CA54174 and the Frederic C. Bartter Clinical Research Unit of the Audie Murphy Veterans Administration Hospital through NIH Grant MO1 RR01346.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked *advertisement* in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

Requests for reprints: Institute for Drug Development, Cancer Therapy and Research Center, 7979 Wurzbach Road, 4th Floor, Zeller Building, San Antonio, TX 78229. Phone: 210-616-5945; Fax: 210-616-5865; E-mail: erowinsk@idd.org.

group of at least 10 analogues that differ in their MMP specificity and potency from doxycycline and other antimicrobial tetracyclines (1). These agents were identified by efforts directed at developing potent MMP inhibitors that are devoid of antimicrobial activity. The chemically modified tetracyclines have several advantages over conventional tetracyclines in preclinical models, including a reduced incidence of gastrointestinal toxicity, which, in part, may explain the achievement of higher and more biologically relevant plasma concentrations. Nevertheless, gastrointestinal toxicity has been the principal dose-related toxicity of these agents in preclinical evaluations. In addition, the clearance rates of several chemically modified tetracyclines are relatively low, necessitating less frequent drug administration (1). Among the most potent chemically modified tetracycline is Col-3, 6-demethyl-6-deoxy-4-dedimethylamino tetracycline (Metastat; Collagenex Pharmaceuticals, Newton, PA; Fig. 1), which is devoid of antimicrobial properties and is a competitive and selective inhibitor of MMP-2 and -9 isoenzymes (13, 14). Col-3 also profoundly inhibits the activity of activated neutrophil gelatinase; expression of MMPs in breast, colon, and other cancers; and blocks the invasion of several types of tumors into Matrigel and basement membrane matrices at concentrations of 3 to 5 μg/mL (15). In addition, impressive growth-inhibitory activity has been noted after Col-3 treatment in a broad range of human tumor cell lines and xenografts (15–18). For example, treatment of DU145 and TSU-PR1 human prostate cancers with 12 μg/mL of Col-3 for 48 hours resulted in 50% inhibition of tumor growth (15, 16). Furthermore, Col-3 induced prominent inhibition of tumor growth and reduced lung and bone metastases in the rat Dunning MAT LyLu prostate cancer model (15, 17). At Col-3 concentrations of 10 μg/mL, profound apoptotic effects were also noted. Antiproliferative activity in most preclinical evaluations has been directly related to both drug concentration and the duration of treatment.

The ability of Col-3 to inhibit the activity, activation, and production of MMPs distinguishes this agent from other MMP inhibitors, which principally target the active enzyme. Furthermore, the results of preclinical studies suggests that Col-3 possesses greater antiproliferative activity and possibly conferred superior therapeutic indices compared with other MMP inhibitors, which served as the rationale for the clinical development of the agent. The principal objectives of this study were the following: (1) to determine the maximum tolerate dose (MTD) of Col-3 administered orally on a continuous daily schedule in patients with advanced solid malignancies, (2) to characterize the toxicities of Col-3 on this schedule of administration, (3) to describe the pharmacokinetic behavior of Col-3, and (4)to seek preliminary evidence for anticancer activity.

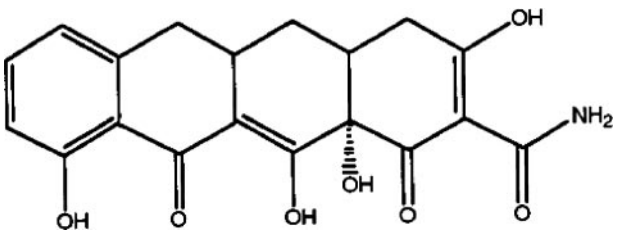

Fig. 1 Chemical structure of Col-3.

PATIENTS AND METHODS

Patient Selection. Patients with histologically confirmed advanced solid malignancies that were unresponsive to standard therapy or for whom adequate therapy was not available were eligible for this study. Eligibility criteria also included age ≥18 years; an Eastern Cooperative Oncology Group performance status ≤2; a life-expectancy ≥12 weeks; no prior chemotherapy or wide-field radiation therapy within 4 weeks of treatment (6 weeks for nitrosoureas and mitomycin C); adequate hematopoietic (absolute neutrophil count ≥1,500/μL, hemoglobin level ≥9 g/dL, platelet count ≥100,000/μL), hepatic [total bilirubin ≤ institutional upper normal limit, aspartate amino transaminase and alanine amino transaminase ≤2.5 times institutional normal upper limit (≤5 times institutional upper normal limit for patients with liver metastasis)], and renal (serum creatinine ≤2.0 mg/dL or calculated creatinine clearance ≥60 mL/minute according to the method of Cockcroft and Gault; ref. 19) functions; measurable or evaluable disease; no radiographic or clinical evidence of progressive brain metastases; no history of a gastrointestinal malabsorption that could interfere with bioavailability of the study drug; no history of hypersensitivity to tetracycline derivatives; and no coexisting medical problem of sufficient severity to limit compliance with the study. All of the concurrent medications were recorded in the case report form. Patients gave written informed consent according to federal and institutional guidelines before treatment.

Dosage and Drug Administration. The starting dose of Col-3, which was administered on an uninterrupted daily schedule, was 36 mg/m^2/day. This dose was equivalent to one-tenth of the MTD in rats (360 mg/m^2/day) and <50% of the lowest dose associated with toxicity of any type in monkeys (180 mg/m^2/day). Each course was defined arbitrarily as 28 consecutive days. The study used a modification of an accelerated titration design (permutation 4A), as initially described by Simon *et al.* (20), to guide dose escalation in cohorts of new patients. On the basis of safety data accumulated from a concurrent Phase I study of Col-3 (21), maximal dose escalation increments of ~40% for successive dose level assignments were allowed. This scheme resulted in an evaluation of the following dose levels: 36, 50, 70, and 98 mg/m^2/day. A single new patient was to be treated with Col-3 at each successive dose level that did not result in toxicity of at least grade 2 in the first course. In the event of grade 2 toxicity, the cohort size was to be increased to at least 3 new patients, and dose escalation was to proceed using more conservative increments. Furthermore, the size of subsequent dosing cohorts was to be increased to at least 3 new patients if at least two subjects in any dose level experienced grade 2 toxicity or any single patient experienced any grade 3 toxicity. In the event of dose-limiting toxicity (DLT) in any first course, at least six new patients were to be treated at the particular dose level. If patients experienced grade 2 drug-related toxicity lasting >7 days, treatment with Col-3 was temporarily discontinued until recovery of the toxicity to grade 1 or less, at which time treatment resumed at the same or the next lower dose depending on the effectiveness of the supportive measures. In the event of

DLT, treatment with Col-3 was interrupted and then resumed at the next lower dose level after resolution of the toxicity to ≤ grade 1. Patients who experienced DLT, which did not resolve to grade 1 or less within 3 weeks, were taken off study. Doses were not increased in individual subjects. The MTD was defined as the highest dose level at which <2 of the first 6 new patients experienced DLT in the first course. Ten to 12 additional patients were treated at the MTD to ascertain additional information about drug safety. DLT was defined as one of the following: (1) grade 3 nonhematological toxicity (excluding nausea or vomiting in the absence of optimal prophylactic and supportive measures); (2) any grade 4 nonhematological toxicity; or 3) grade 4 hematologic toxicity. Toxicities were graded according to the National Cancer Institute Common Toxicity Criteria (Version 2.0). Photosensitivity reactions that occurred in patients who were not compliant with specified photoprotective measures were graded and tabulated as toxicity in an intent-to-treat fashion.

Col-3 was supplied by the National Cancer Institute (Bethesda, MD) as 50-mg capsules. The calculated dose was rounded to the nearest 50 mg to accommodate capsule strength. Because the impact of food on the bioavailability of Col-3 in humans had not been investigated at the time of the study, patients were instructed to self-administer Col-3 with 8 ounces of water at 8 a.m. daily at least 1 hour before and 2 hours after eating. All of the patients were required to keep a study drug diary in which drug administration and potential toxicities were recorded. The diary had to be turned in before Col-3 could be dispensed for each additional course of treatment. All of the patients were counseled and given written instructions pertaining to the prevention and management of phototoxicity reactions induced by Col-3. Patients were instructed to minimize sun exposure and wear protective clothing and were provided with sunscreen (sun protection factor 30) for daily application beginning several days before Col-3 treatment.

Pretreatment and Follow-Up Studies. Histories that included recording of performance status and concurrent medications, physical examinations, and routine laboratory evaluations were performed pretreatment and weekly. Routine laboratory evaluations included complete blood counts, differential white blood cell count, electrolytes, blood urea nitrogen, creatinine, glucose, total protein, albumin, calcium, phosphate, uric acid, alkaline phosphatase, total and direct bilirubin, transaminases, clotting times, and urinalysis. After the first two courses, interval histories, physical examinations, and performance status recording were repeated every other week. Pretreatment studies also included an electrocardiogram, relevant radiologic studies for evaluation of all measurable or evaluable sites of malignancy, and an assessment of relevant tumor markers. Radiologic studies for disease status assessments were performed after every other course or as indicated to confirm response. Patients were able to continue treatment if they did not develop progressive disease. A complete response was scored if there was disappearance of all active disease on two measurements separated by a minimum period of 4 weeks, and a partial response required at least a 50% reduction in the sum of the product of the bidimensional measurements of all lesions documented to be separated by at least 4 weeks. Any concurrent increase in the size of any lesion by ≥25% or the appearance of any new lesion was considered disease progression.

Plasma Sampling and Assay. Blood samples in heparinized tubes were collected pretreatment and at 15, 30, 60, and 90 minutes, and 2, 4, 6, 8, and 24 hours after treatment on the first day of the first (day 1) and second courses (day 29). Blood samples were also collected before treatment on days 3, 8, 15, and 22 of course 1 and before day 1 of all successive courses. The samples were centrifuged at 3,000 rpm at 4°C for 15 minutes immediately after collection, and the plasma was transferred to a cryostorage tube and stored at −80°C until assayed for Col-3.

Plasma concentrations of Col-3 were measured by high-performance liquid chromatography. Standard curves and quality control samples were prepared by spiking blank plasma (Biochemed Pharmacologicals, Winchester, VA) with Col-3 stock solutions prepared in DMSO:EtOH (1:9, v/v). Both Col-3 and the internal standard, Col-8 (CollaGenex Pharmaceuticals, Newtown, PA), were stored in amber glass tubes that were protected from light. Patient plasma samples were thawed at room temperature, and a 500-μL aliquot was transferred to a 5-mL glass extraction tube containing 20 μL of the internal standard (100 μg/mL of Col-8 in DMSO:EtOH, 1:9, v/v). A 4-mL aliquot of ethyl acetate/EtOH (9:1, v/v) extraction solvent was added, and the tubes were rotated at medium speed for 15 minutes and centrifuged at 2,500 rpm for 10 minutes. The organic solvent layer was transferred to a clean tube and evaporated under a gentle stream of nitrogen at room temperature. Next, the residue was reconstituted with 250 μL of mobile phase, vortexed, allowed to sit for 5 minutes, revortexed, and then transferred to a 0.45-μm nylon microfuge filter (Alltech, Deerfield, IL). Samples were centrifuged for 5 minutes at 3,000 rpm. The filtrate was transferred to a glass amber autosampler vial for high-performance liquid chromatography analysis.

Chromatographic separation was achieved with a Luna C8(2) (Phenomenex, Torrance, CA) column (5 μm, 4.6 mm × 250 mm) fitted with a Luna C8 guard column (10 μm, 4.6 mm × 30 mm) using a Waters Alliance 2690 separations module and a Waters 996 photodiode detector (Waters Corp., Milford, MA). After injecting 75 μL of analytical extract, Col-3 and the internal standard were eluted using a mobile phase of 0.10 mol/L sodium acetate buffer (pH 4.5)/acetonitrile/methanol (50:30:20, v/v/v) at a flow rate of 1 mL/minute. The column effluent was monitored by UV detection at 350 nm, and the autosampler temperature was set to 10°C. Under these conditions, Col-3 and the internal standard eluted with a retention time of 16 and 9 minutes, respectively. The total run time was 28 minutes to avoid late eluting peaks. The lower limit of quantitation for the assay was 0.250 μg/mL, and the upper limit of quantitation was 50.0 μg/mL. The slope of the calibration curve over the course of patient analyses averaged 0.3031 (SD, 0.0391; coefficient of variation, 13%) with correlation coefficients of at least 0.99. Quality control samples, prepared at 1, 2, and 10 μg/mL, were analyzed with each set of patient samples. For the analytical data to be considered acceptable, the quality control samples had to be within 15% of the theoretical value. In stability tests, Col-3 concentrations were stable when plasma was stored at −80°C for 19 months, which greatly exceeded the storage limit of patient plasma samples in this study.

Pharmacokinetic Analyses. Individual Col-3 plasma concentration data were analyzed by noncompartmental methods. Area under the plasma concentration-time curve measured until 24 hours post-treatment (AUC_{0-24}) values were calculated using the linear trapezoidal method as implemented in WinNonLin Standard, Version 3.1 (Pharsight Corporation, Mountain View, CA). Values of peak plasma concentration and time at which peak plasma concentration is achieved were determined by inspection of the data. Because patients were treated with Col-3 on an uninterrupted, daily schedule, pharmacokinetic sampling could not be performed at time points beyond 24 hours after any single dose without accounting for the impact of successive treatment. Thus, the terminal elimination rate constant and terminal half-life of elimination could not be estimated accurately. Individual values for apparent clearance were derived from the noncompartmental estimates of AUC_{0-24} after steady-state dosing on day 29 by dividing the daily dose by AUC_{0-24}. The accumulation index was calculated by dividing the AUC_{0-24} on day 1 by the AUC_{0-24} on day 29. Mean Col-3 average trough concentration at steady-state values were calculated by deriving the mean of the pretreatment Col-3 plasma concentrations measured on days 8, 15, 22, and 29. Dose proportional changes in AUC_{0-24} and peak plasma concentration values were examined by application of the intercept test, the power model (22), and by one-way ANOVA of the log-transformed dose-normalized pharmacokinetic parameters (23).

Differences between the pharmacokinetic parameters of drug exposure (AUC_{0-24}, peak plasma concentration, and average trough concentration at steady-state) in patients experiencing DLTs and/or various grades of adverse toxicity and patients treated at the same dose levels who did not experience such events were analyzed using the nonparametric Mann-Whitney test.

RESULTS

General. Thirty-three patients, whose pertinent characteristics are displayed in Table 1, received 73 total courses of Col-3 spanning four dose levels that ranged from 36 to 98 mg/m^2/day. Most patients were considered heavily pretreated with regard to prior myelotoxic radiation and/or chemotherapy. The median number of prior chemotherapy regimens was 4.5 (range, 1 to 10); 2 patients had received treatment previously with high-dose chemotherapy and autologous peripheral progenitor cell rescue. The total numbers of new patients treated at each dose level, numbers of courses, and dose escalation scheme are depicted in Table 2. Five courses involving 5 patients [50 mg/m^2/day (2), 70 mg/m^2/day (2), and 98 mg/m^2/day (1)] were not fully evaluable for toxicity due to patient noncompliance (1), development of progressive disease (2), patient election to discontinue treatment (1), and a serious adverse event (gastrointestinal hemorrhage) related to malignant disease (1). Two patients required dosage reduction from 70 to 50 mg/m^2/day due to unacceptable toxicity (rash/cutaneous photosensitivity reaction) at the higher dose level.

Overall the accelerated titration design and use of single-patient cohorts at dose levels associated with negligible toxicity was not efficient at discerning the MTD in this study, because the starting dose closely approximated the biologically relevant dose range in humans. The first patient was treated uneventfully with Col-3 at the 36 mg/m^2/day dose level, and Col-3 doses were subsequently increased to 50 and 70 mg/m^2/day in cohorts of 1 to 3 evaluable patients each, resulting in an acceptable level of toxicity as defined *a priori*. At the 98 mg/m^2/day dose level, however, 3 of 5 patients encountered DLT, characterized by a wide gamut of unacceptable toxic events, including grade 3 anorexia (1 patient), grade 3 malaise and/or fatigue (2 patients), grade 3 photosensitivity rash (1 patient), grade 3 anemia (1 patient), and grade 4 hyperbilirubinemia (1 patient). Therefore, 11 additional patients were treated with Col-3 at the next lower dose level, 70 mg/m^2/day. Despite rigorous instructions as to the mandatory use and distribution of sunscreen to prevent photosensitivity phenomena, 4 of 9 evaluable patients developed grade 2 (3 patients) and grade 3 (1 patient) photosensitivity dermatitis in first courses, and 1 patient developed a grade 3 photosensitivity dermatitis in course 2. Furthermore, the symptoms of the grade 2 photosensitivity reactions were intolerable in 2 of 3 patients, both of whom required dose reduction to 50 mg/m^2/day. Therefore, despite the classification of these manifestations as grade 2 by strict interpretation of National Cancer Institute Common Toxicity Criteria, the skin rash/photosensitivity dermatitis was felt to represent DLT in both individuals. Overall, intolerable cutaneous toxicity was encountered in 3 of 15 new patients enrolled at the 70 mg/m^2/day dose level. Additionally, there were relatively high rates of mild to moderate (grade 1–2) fatigue, anorexia, nausea, anemia, and transaminitis in first and subsequent courses of Col-3 at this dose. On the basis of these results, 70 mg/m^2/day was not considered feasible for chronic administration, and additional patients were treated with Col-3 at the 50 mg/m^2/day dose level.

At 50 mg/m^2/day, 2 of 12 new patients experienced grade 3 rash/photosensitivity dermatitis in first courses. One of these events occurred in a patient who was not compliant with sunscreen use; however, the subject did not experience additional manifestations of photosensitivity in subsequent courses in

Table 1 Patient characteristics

Characteristic	Number of patients
Number of patients	33
Median number of courses/patient (range)	4.5 (1–10)
Median age (range), years	57 (24–80)
Gender (male:female)	17:16
Median performance status (ECOG)	1
0	7
1	20
2	6
Previous therapy	
Chemotherapy only	23
Radiation therapy only	1
Both	7
None	2
Tumor types	
Soft tissue sarcoma	11
Colorectal	10
Pancreas	4
Ovary	3
Breast, lung, head and neck, renal, mesothelioma	1 each

Abbreviation: ECOG, Eastern Cooperative Oncology Group.

Table 2 Dose escalation scheme

Col-3 dose (mg/m^2/day)	Number of patients			Number of courses	Number of patients with DLT	
	New	Dose reduction to	Total		First course	All courses
36	1		1	2	0/1	0/2
50	12	2	14	40	2/12	2/40
70	15		15	25	3*/15	5/25
98	5		5	6	3/5	3/6

Abbreviation: DLT, dose-limiting toxicity.

* Two episodes of grade 2 rash/photosensitivity dermatitis that were grade 2 by National Cancer Institute Common Toxicity Criteria; however, both events were considered intolerable by patients, required dose reductions, and, therefore, were considered dose-limiting.

which sunscreen was applied according to protocol instructions. On the basis of the acceptable incidence of DLT at the 50 mg/m^2/day dose level, which included 3 individuals who received protracted treatment for at least seven courses each, it was determined to be the MTD for Col-3 on this schedule of administration.

Toxicity. Cutaneous toxicity and malaise were the most common toxicities of Col-3 administered daily on an uninterrupted schedule. The distributions of grades of the most common adverse events as a function of dose level are displayed in Table 3.

Cutaneous. Cutaneous toxicity in a sun exposure distribution (*i.e.*, photosensitivity dermatitis) was the principal DLT. Both the incidence and the severity of the cutaneous manifestations appeared to be dose related, but the narrow dosing range and small increments between dose levels preclude definitive conclusions about the relationship between rash and dose. The onset of the cutaneous manifestations, which were qualitatively similar in most affected individuals, was ~2 weeks after starting treatment. The rash typically involved sun-exposed areas, especially the face and the dorsal aspects the hand, and was generally macular and erythematous. In more severe cases, desquamation and blistering developed on a bright red erythematous base, and patients often experienced discomfort. Inflammation and excoriation of adjacent mucous membranes, particularly of the outer lips and nares adjacent to the rash, were observed in severe cases. The severity of the cutaneous manifestations was clearly related to time spent outdoors, as well as the degree of noncompliance with instructions regarding the use of sunscreen. Nevertheless, all of the events irrespective of compliance issues were considered evaluable in an intent-to-treat fashion.

Although the initial protocol criteria defined grade 3 cutaneous toxicity as dose-limiting, 2 patients experienced grade 2 photosensitivity rashes, characterized by erythema, desquamation, and pain, which were clearly intolerable and, therefore, considered DLT. Both events occurred during first courses of Col-3 at the 70 mg/m^2/day dose level. At doses levels >50 mg/m^2/day, unacceptably high incidences of intolerable toxicities, principally rash and malaise, occurred. At the 50 mg/m^2/day dose level, 2 of 12 patients (16%) experienced DLT in the first course. However, no additional dose-limiting events occurred; 3 patients received 7 to 10 courses each without DLT; and intolerable toxicity occurred in only 2 of 40 (5%) total courses. One of these subjects was totally noncompliant with regard to sunscreen use in course 1 but adhered to the instructions during subsequent treatment without recurrence of intolerable photosensitivity dermatitis. The second individual developed a grade 3 photosensitivity reaction as well as progressive disease during course 1 and was not retreated.

Table 3 Toxicities of Col-3 in the first course (total courses)

Col-3 dose level	36 mg/m^2/day				50 mg/m^2/day				70 mg/m^2/day				98 mg/m^2/day			
No. of first courses (total courses)	1 (2)				12 (40)				15 (25)				5 (6)			
Grade of toxicity	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4
Malaise/fatigue	1 (2)				1 (2)	2 (4)			3 (7)	4 (7)				1 (1)	2 (2)	
Anorexia		1 (1)			2 (3)				3 (6)	3 (4)			1 (1)	1 (1)	1 (1)	
Nausea and/or vomiting	1 (2)				1 (2)	1 (1)			6 (6)				1 (1)	3 (4)	1 (1)	
Transaminitis					1 (2)	1 (1)			1 (2)	1 (1)			2 (2)			
Hyperbilirubinemia					1 (1)				2 (3)							1 (1)
Diarrhea		1 (1)							1 (1)							
Rash/photosensitivity dermatitis*	1 (2)	1 (1)			4 (6)	1 (1)	2 (2)		4 (8)	3*(4)	1 (2)			1 (1)	1 (1)	
Stomatitis					1 (1)				2 (2)							
Dizziness					1 (1)									1 (1)		
Anemia		1 (2)			3 (5)	3 (4)			2 (3)	4 (7)	1 (1)			1 (2)	1 (1)	
Leukopenia									2 (4)					1 (1)		

* Rash graded as grade 2 by National Cancer Institute Common Toxicity Criteria; however, two of the events were considered intolerable by patients, required dose reductions, and, therefore, considered dose-limiting.

Miscellaneous. Several patients developed malaise within 2 to 4 weeks after beginning Col-3. In general, the subjects described these events as total body weakness. Most of these events occurred in the first course of treatment in the absence of obvious confounding factors such as disease progression. Both the incidence and severity of malaise appeared to relate to the dose of Col-3, with two grade 3 events occurring in 2 patients in the first course of treatment at the 98 mg/m^2/day dose level. These events resolved soon after drug discontinuation, which supports the assertion that malaise was drug-related. Other mild to moderate (grade 1 or 2) complaints and nonhematologic effects that were possibly related to Col-3 included headache, anorexia, transaminitis, hyperbilirubinemia, anorexia, diarrhea, nausea, vomiting, and dizziness. However, these events were noted across the entire Col-3 dosing range, and definite temporal relationships could not be discerned for any of these potential toxicities, indicating that the underlying malignant process may have been contributory. Hepatic function test abnormalities always occurred in patients with liver metastases. One such individual with profound liver metastases developed grade 4 hyperbilirubinemia during the first course of Col-3 at the 98 mg/m^2/day dose level. The course of toxicity resolution after drug discontinuation could not be characterized, because the patient expired due to progressive malignancy soon after discontinuation of treatment. Several patients also developed leukopenia and anemia, which were mild to moderate in severity and uncomplicated. Most events occurred in heavily pretreated individuals, and it is likely that the cytopenias represent reduced hematopoietic reserves from prior treatment.

Pharmacokinetics. Plasma sampling for pharmacokinetic studies was performed in 30 of 33 patients treated with Col-3 at doses ranging from 36 to 98 mg/m^2/day. Pertinent pharmacokinetic parameters derived using noncompartmental methods are listed in Table 4. Col-3 was absorbed slowly. The values for median time at which peak plasma concentration is achieved and ranges on both days 1 and 29 were identical, but interindividual variability in these values was large (median, 6.0 hours; range, 4.0 to 24.0). Plasma concentration-time curves, reflecting mean dose-normalized concentration values after treatment on days 1 and 29 at the 50 mg/m^2/day dose level, are shown in Fig. 2, *A* and *B*. Because the mean percentage AUC extrapolated beyond the last time point to infinity was unacceptably large due to the low clearance rate of Col-3, a terminal elimination phase could not be accurately estimated from plasma sampled in this uninterrupted dosing study. To examine the dose-proportionality of Col-3 pharmacokinetics, both peak plasma concentration and AUC_{0-24} parameter values were calculated for all of the individual patients. An inspection of the scatter plots of dose *versus* peak plasma concentration and $AUC_{0-24,}$ shown in Fig. 3, *A* and *B*, revealed substantial overlap in peak plasma concentration and AUC_{0-24} values within the Col-3 dosing range evaluated in the study ($R^2 = 0.24$ and 0.25, respectively). However, the data met the criteria for dose proportionality over the entire dose range using the intercept test (23), power model (22), and ANOVA testing of the dose-normalized, log-transformed AUC_{0-24} as a function of dose level (data not shown; ref. 22).

A high degree of interindividual variability in average trough concentration at steady-state values was observed as shown in the scatter plot in Fig. 4. Average trough concentration at steady-state values averaged 9.37, 8.24 ± 3.78, 9.97 ± 5.06, and 12.84 ± 1.36 μg/mL in patients treated with Col-3 doses of 36, 50, 70, and 98 mg/m^2/day, respectively, and the relationship between Col-3 dose and average trough concentration at steady-state was weak ($R^2 = 0.11$). Apparent clearance values averaged 602.80 ± 334.25 mL/hour. Interpatient variability in individual apparent clearance values was moderate, with coefficients of variation of 49% and 55% at the 50 and 70 mg/m^2/day dose levels, respectively. No individual patient covariates were strongly predictive of apparent clearance on day 29. Similarly, the relationship between body surface area and apparent clearance was weak ($R^2 = 0.11$), as shown in Fig. 5.

Table 4 Pharmacokinetic parameters of Col-3*

Col-3 dose level (mg/m^2/day) (n)†	$C_{ss,\ min}$ (μg/ml)	C_{max} (μg/ml)		T_{max} (h)		AUC_{0-24} (μg-h/ml)		AUC_{0-24} ratio (Day 29/1)	CL/F (ml/h)
		Day 1	Day 29	Day 1	Day 29	Day 1	Day 29		
36 (n = 1/1)	9.37	1.71	11.24	1.25	1.25	28.4	224.4	7.91	–
50 (n = 7/10)	8.24 (3.78)	2.06 (0.51)	11.65 (4.79)	8.00 (6.00–24.00)	6.00 (1.50–24.00)	37.13 (15.79)	241.4 (91.8)	5.81 (2.06)	413.27 (111.61)
70 (n = 14/10)	9.97 (5.06)	2.76 (0.76)	13.47 (4.80)	6.00 (4.00–24.00)	6.00 (1.50–24.00)	52.97 (13.21)	243.4 (115.0)	4.70 (1.97)	703.85 (385.97)
98 (n = 5/1)	10.95 (1.34)	3.14 (1.18)	23.290	8.00 (6.00–24.00)	8.00	58.48 (19.18)	430.9	13.59	464.1
Total	NA	NA	NA	6.00 (1.25–24.00)	6.00 (1.25–24.00)	NA	NA	5.74 (2.80)	602.80 (334.25)

Abbreviations: AUC_{0-24}, area under the concentration-time curve until 24 hours after treatment; CL/F, clearance at steady-state; C_{max}, peak plasma concentration; $C_{ss,\ min}$, trough concentration at steady-state; NA, not applicable; $T_{max,}$ time to C_{max}.

* Values represent mean (SD) values. Median (range) values are listed for T_{max}.

† Numbers of patients with pharmacokinetic sampling on days 1/29.

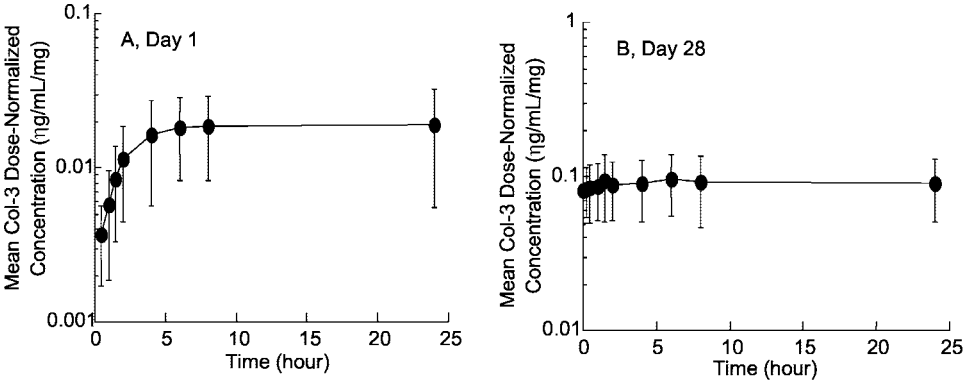

Fig. 2 Mean dose-normalized plasma concentration *versus* time curves of Col-3 after treatment of patients at the 50 mg/m^2/day dose level on: *A*, day 1 ($n = 7$) and *B*, day 29 ($n = 10$). *Bars*, ±SD.

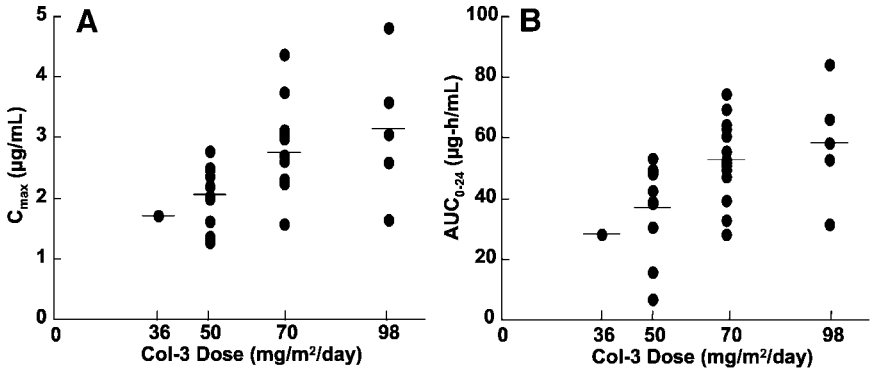

Fig. 3 Scatterplot of peak plasma concentration and AUC_{0-24} values as functions of Col-3 dose level. *A*, peak plasma concentration values; and *B*, AUC_{0-24} values ($R^2 = 0.24$ and 0.25, respectively). *Bars*, ±SD.

No relationships were evident between pertinent pharmacokinetic parameters reflecting drug exposure (AUC_{0-24}, peak plasma concentration, and average trough concentration at steady-state) in patients experiencing each of the principal toxicities (cutaneous, malaise) or all of the DLTs, together. Average trough concentration at steady-state values averaged 7.47 ± 3.56 µg/mL and 9.68 ± 3.69 µg/mL in patients who did and did not experience DLT in course 1, respectively. Nevertheless, the small numbers of patients experiencing these events limit the statistical power of such analyses.

Antitumor Activity. No major responses were observed. Three patients who had disease progression documented before treatment with Col-3 had prolonged stable disease (>6 months). All 3 of the individuals were treated with Col-3 at the 50 mg/m^2/day dose level. One patient with a progressive carcinosarcoma of the uterus that had been treated previously with four

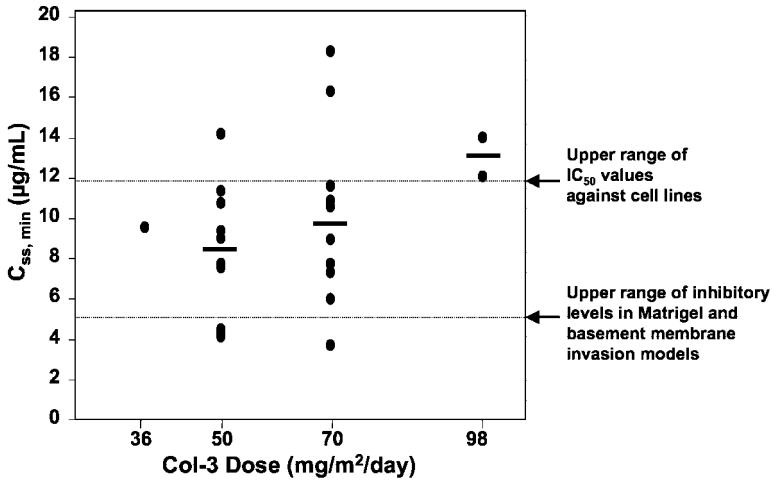

Fig. 4 Scatterplot of average trough concentration at steady-state ($C_{ss, min}$) values as functions of Col-3 dose level ($R^2 = 0.11$). *Upper bar*, upper range of IC_{50} values in tumor growth assays *in vitro*. *Lower bar*, upper range of inhibitory concentrations in Matrigel and basement membrane invasion assays.

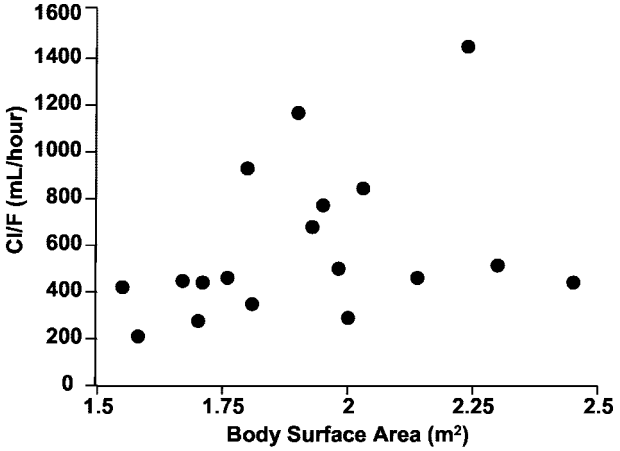

Fig. 5 Scatterplot of apparent clearance values for individual patients as a function of body surface area ($R^2 = 0.11$).

prior chemotherapy regimens experienced stable disease lasting 10 months during treatment with Col-3. Another patient with metastatic pancreatic cancer that progressed during treatment with gemcitabine experienced stable disease lasting 7 months. Lastly, a patient with metastatic ovarian cancer that had progressed during three prior chemotherapy regimens had stable disease for 7 months during treatment.

DISCUSSION

Although the MMP inhibitors have been evaluated extensively in the clinic and have shown little clinical benefit when used as monotherapy in a variety of clinical settings, profound antimetastatic, antiangiogenic, and tumor growth inhibition have been demonstrated consistently in preclinical models (1). The disparity between expectations based on preclinical observations and actual clinical results can be attributed to many factors including the inadequacy of preclinical models to predict for success of the MMP inhibitors in patients with advanced disease and the innate inadequacy of specific classes of MMP inhibitors in the clinic. The widely disparate characteristics of the MMP inhibitors that have been evaluated to date, including their potencies, selectivity, and pharmacological and pharmaceutical properties, suggest that additional optimization of the overall therapeutic indices of this class of antiproliferative agents is possible (1–3).

The tetracyclines are among the most important classes of MMP inhibitors. Not only are some tetracycline analogues innately potent at inhibiting MMPs, but they possess pleiotropic antiangiogenic effects (1, 24). In addition to inhibiting MMP activity, the tetracycline analogues inhibit the production of MMPs and have demonstrated impressive activity in models of both malignant and nonmalignant diseases (1). Although MMP expression is low or undetectable in most normal tissues, both MMP production and activity are substantially increased in most malignant neoplasms, including carcinomas of the lung, breast, colon, and pancreas (1). Increased MMP production and activity confer local invasive properties and facilitate the production of a new blood supply by malignant tumors (1–3, 24, 25). The distinct mechanistic, pharmacological, and pharmaceutical properties of the prototypic chemically modified tetracycline Col-3 compared with predecessor MMP inhibitors, as well as its impressive antiproliferative activity in preclinical models, served as the rationale for its clinical development.

Photosensitivity skin reactions and malaise were the principal DLTs of Col-3 in the present study. At doses >50 mg/m^2/day, patients experienced unacceptably high incidences of intolerable photosensitivity reactions and malaise in their first course, whereas the overall incidence of severe toxicities at the 50 mg/m^2/day dose level in first and subsequent courses was acceptable. At 50 mg/m^2/day, 2 of 12 (16%) patients experienced DLT in the first course. One individual who was totally noncompliant with regard to the use of sunscreen developed a grade 3 photosensitivity skin reaction in the first course, but no additional toxicity was noted after subsequent treatment and strict adherence to prophylactic measures. Furthermore, no additional dose-limiting events occurred with continued treatment beyond course 1 in the other patients including 3 subjects who received 7 to 10 courses each. Overall, intolerable toxicity occurred in 2 of 40 (5%) total courses at the 50 mg/m^2/day dose level. These results suggested that 50 mg/m^2/day is an appropriate dose for disease-directed trials of Col-3. Nevertheless, treatment with Col-3 at this dose must be accompanied by efforts that promote adherence to the use of sunscreen and other photoprotective measures.

The tetracyclines are well-known photosensitizers. Until the development of Col-3, the most prominent photosensitizing tetracycline derivative was doxycycline (26). Several mechanisms have been proposed to explain this phenomenon including photo-oxidation or an oxygen-dependent pathway that involves a tetracycline photoproduct and a singlet oxygen after absorption of UV radiation by the tetracycline molecule (27). There are many structural elements of the molecular structure of tetracycline, including its phenolic ring, tertiary amino groups, and various conjugated double bonds, which may serve as reactive sites for singlet molecular oxygen-mediated photo-oxidation (28). However, Col-3 contains only a phenolic ring in its tetracycline backbone and lacks the other aforementioned sites that could potentially react with a singlet oxygen. Nevertheless, the agent is the most potent photosensitizer among the tetracyclines. Photosensitivity skin reactions were determined to be the principal dose-limiting phenomena in two other Phase I studies of Col-3 administered on a similar schedule (21, 29). In a Phase I study performed at the NCI in patients with advanced solid malignancies, cutaneous photosensitivity reactions precluded treatment at Col-3 doses exceeding 70 and 36 mg/m^2/day in patients who were and were not using prophylactic sunscreen, respectively, and the lower dose was recommended for subsequent disease-directed trials (21). An unacceptably high incidence of severe cutaneous photosensitivity reactions also precluded dose escalation of Col-3 >25 mg/m^2/day in a Phase I study of Col-3 in patients with AIDS-related Kaposi's sarcoma performed by the AIDS Malignancy Consortium (29). For the most part, the toxicities in all three of the trials were nearly identical except for a constellation of manifestations that resembled drug-induced systemic lupus erythematosis in the National Cancer Institute study (21). In this study, 3 patients developed arthralgia, fevers, and elevations of antinuclear and antihistone

antibodies, all of which responded to discontinuation of Col-3 and treatment with corticosteroids (21). The pathogenesis of these drug-induced manifestations is not known; however, a similar autoimmune process that typically occurs ~1 to 2 years after initiation of treatment has been reported with minocycline (21). Although the most prominent clinical manifestations of the lupus-like syndrome, such as arthralgia and fever, were not noted in the present study, the profound drug-induced malaise observed in some patients might represent an element of this possible drug-induced syndrome.

The pharmacokinetic results of the present study suggest that Col-3 behaves in a dose-proportional manner, and pharmacokinetic parameters reflecting drug exposure were similar to those observed in other clinical trials to date (21, 29). In addition, intraindividual variability was moderate; coefficients of variation in AUC_{0-24} values were 25% and 55% on days 1 and 29 at the MTD, respectively. Because the systemic clearance of Col-3 is very low, a terminal elimination phase could not be accurately estimated from plasma sampled in this uninterrupted daily dosing study. Therefore, it was elected not to report estimated terminal half-life values due to the unacceptably large errors inherent in such estimates. However, a mean terminal half-life value of 56.7 hours (range, 23.7 to 144.4) was reported by Rudek *et al.* (21) who evaluated an identical Col-3 schedule using a similar plasma-sampling scheme. The mean accumulation ratio of 5.74 ± 2.80 is consistent with this pharmacokinetic behavior. Most importantly, values of average trough concentration at steady-state at all dose levels were in the range of Col-3 concentrations capable of substantially inhibiting invasiveness in Matrigel and basement membrane assays and IC_{50} values required to inhibit the growth of a wide range of tumors in preclinical studies, and peak plasma concentration values generally exceeded biological relevant concentrations (1, 25). The fact that a continuous dosing schedule maintains average trough concentration at steady-state values for much longer periods than the duration of treatment in preclinical studies must also be considered in evaluating the biologically relevance of the magnitude of pharmacokinetic parameters achieved in the clinic. Lastly, the weakness of the relationship between apparent clearance and body surface area suggests that the administration of fixed doses of Col-3 is reasonable in subsequent clinical evaluations.

Although no objective antitumor responses were observed, apparent clinical benefit in several patients with soft tissue sarcoma in the National Cancer Institute and present studies, as well as a 44% overall response rate in patients with AIDS-related Kaposi's sarcoma, suggest that Col-3 should undergo additional evaluation for the treatment of various types of sarcoma and other malignancies in which MMPs may play an important role in invasion, proliferation, and metastases (1, 21, 29). These preliminary observations, as well as the reasonably well-tolerated toxicity profile of Col-3, have served as the rationale for an ongoing two-stage Phase II study of Col-3 in patients with soft-tissue sarcoma, in which both objective responses and the proportion of patients who do not experience disease progression in the first stage guide additional patient accrual.

REFERENCES

1. Hidalgo M, Eckhardt SG. Development of matrix metalloproteinase inhibitors in cancer therapy. J Natl Cancer Inst 2001;93:178–93.

2. Chambers AF, Matrisian LM. Changing views of the role of matrix metallaproteinases in metastasis. J Natl Cancer Inst 1997;89:1260–70.

3. Brown PD, Giavazzi R. Matrix metalloproteinase inhibition: a review of anti-tumor activity. Ann Oncol 1995;6:967–74.

4. Golub LM, Lee HM, Lehrer G, et al. Minocycline reduces gingival collagenolytic activity during diabetes: Preliminary observations and a proposed new mechanism of action. J Periodontal Res 1983;18:516–26.

5. Golub LM, Ramamurthy N, McNamara TF, et al. Tetracyclines inhibit tissue collagenase activity: A new mechanism in the treatment of periodontal disease. J Periodontal Res 1984;19:651–5.

6. Golub LM, Goodson JM, Lee HM, Vidal AM, McNamara TF, Ramamurthy NS. Tetracyclines inhibit tissue collagenases: Effects of ingested low-dose and local delivery systems. J Periodontal Res 1985; 56:93–7.

7. Golub LM, Wolff M, Lee HM, et al. Further evidence that tetracyclines inhibit collagenase activity in human crevicular fluid and from other mammalian sources. J Periodontal Res 1985;20:12–23.

8. Golub LM, Ramamurthy NS, McNamara TF, Greenwald RA, Rifkin BR. Tetracyclines inhibit connective tissue breakdown: New therapeutic implications for an old family of drugs. Crit Rev Oral Biol Med 1991;2:297–321.

9. Hanemaaijer R, Visser H, Koolwijk P, et al. Inhibition of MMP synthesis by doxycycline and chemically modified tetracyclines (CMTs) in human endothelial cells. Adv Dent Res 1998;12:114–8.

10. Ramamurthy NS, Vernillo AT, Greenwald RA, et al. Reactive oxygen species activate and tetracyclines inhibit rat osteoblast collagenase. J Bone Miner Res 1993;8:1247–53.

11. Smith GNJ, Brandt KD, Hasty KA. Procollagenase is reduced to inactive fragments upon activation in the presence of doxycycline. Ann NY Acad Sci 1994;732:436–8.

12. Smith GNJ, KD, Hasty KA. Activation of recombinant human neutrophil procollagenase in the presence of doxycycline results in fragmentation of the enzyme and loss of enzyme activity. Arthritis Rheum 1996;39:235–44.

13. Seftor REB, Seftor EA, De Larco JE, et al. Chemically modified tetracyclines inhibit human melanoma cell invasion and metastasis. Clin Exp Metastasis 16:217–25.

14. Myers SA, Wolowacz RG. Tetracycline-based MMP: Inhibitors can prevent fibroblast-mediated collagen gel contraction in vitro. Adv Dent Res 1998;12:86–93.

15. Lokeshwar BL. MMP inhibition in prostate cancer. Ann NY Acad Sci 1999;878:271–89.

16. Lokeshwar BL, Selzer MG, Zhu BQ, Block NL, Golub LM. Inhibition of cell proliferation, invasion, tumor growth and metastasis by an oral non-antimicrobial tetracycline analog (Col-3) in a metastatic prostate cancer model. Int J Cancer 2002;98:297–309.

17. Selzer MG, Zhu B, Block NL, Zhu B, Lokeshwar BL. CMT-3, a chemically modified tetracycline, inhibits bony metastases and delays the development of paraplegia in a rat model of prostate cancer. Ann N Y Acad Sci 1999;878:678–82.

18. Seftor REB, Seftor EA, De Larco JE, et al. Chemically modified tetracyclines inhibit human melanoma cell invasion and metastasis. Clin Exp Metastasis 1998;16:217–25.

19. Cockcroft DW, Gault MH. Prediction of creatinine clearance from serum creatinine. Nephron 1976;16:31–41.

20. Simon R, Freidlin B, Rubinstein L, Arbuck SG, Collins J, Christian MC. Accelerated titration designs for phase I clinical trials in oncology. J Natl Cancer Inst 1997;89:1138–47.

21. Rudek MA, Figg WD, Dyer V, et al. Phase I clinical trial of oral Col-3, a matrix metalloproteinase inhibitor, in patients with refractory metastatic cancer. J Clin Oncol 2001;19:584–92.

22. Gough K. Assessment of dose proportionality: Report from the statisticians in the pharmaceutical industry/pharmacokinetics UK joint working party. Drug Inform J 1995;29:1039–48.

23. Noe DA. Noncompartmental pharmacokinetic analysis, in a clinician's guide to chemotherapy: pharmacokinetics and pharmacodynamics. LB Grochow and MM Ames, editors. Williams & Wilkins: Baltimore, 1998. pp. 515–30.

24. Stetler-Stevenson WG. Matrix metalloproteinases in angiogenesis: a moving target for therapeutic intervention. J Clin Investig 1999;103: 1237–41.

25. Khokha R, Denhardt DT. Matrix metalloproteinases and tissue inhibitor of metalloproteinases: A review of their role in tumorigenesis and tissue invasion. Invasion Metastasis 1989;9:391–405.

26. Layton AM, Cunliffe WJ. Phototoxic eruptions due to doxycycline—a dose-related phenomenon. Clin Exp Dermatol 1993;18:425–7.

27. Hasan T, Kochevar IE, McAuliffe DJ, Cooperman BS, Abdulah D. Mechanism of tetracycline phototoxicity. J Investig Dermatol 1984;83: 179–83.

28. Miskoski S, Sanchez E, Garavano M, Lopez M, Soltermann AT, Garcia NA. Singlet molecular oxygen-mediated photo-oxidation of tetracyclines: Kinetics, mechanism and microbiological implications. J Photochem Photobiol B 1998;43:164–71.

29. Cianfrocca M, Cooley TP, Lee JY, et al. Matrix metalloproteinase inhibitor Col-3 in the treatment of aids-related kaposi's sarcoma: A phase I aids malignancy consortium study J Clin Oncol 2002;20: 153–9.