Phase I and Pharmacokinetic Study of Prinomastat, a Matrix Metalloprotease Inhibitor

**Kenneth R. Hande,[1] Mary Collier,[2]
Linda Paradiso,[2] Jill Stuart-Smith,[2] Mary Dixon,[2]
Neil Clendeninn,[2] Geoff Yeun,[2] Donna Alberti,[3]
Kim Binger,[3] and George Wilding[3]**

[1]Vanderbilt University School of Medicine, Nashville, Tennessee;
[2]Agouron Pharmaceuticals, A Pfizer Company, La Jolla, California;
and [3]University of Wisconsin Comprehensive Cancer Center,
Madison, Wisconsin

ABSTRACT

Purpose: Prinomastat is a matrix metalloprotease (MMP) inhibitor with selectivity for MMPs 2, 3, 9, 13, and 14. Inhibition of these MMPs has been postulated to block tumor invasion and metastasis. This Phase I, dose-escalation study was designed to evaluate the acute and chronic toxicities of various doses of prinomastat and to determine prinomastat pharmacokinetics.

Experimental Design: Seventy-five patients with advanced cancer were given 1, 2, 5, 10, 25, 50, or 100 mg prinomastat orally twice daily until tumor progression or development of significant toxicities. Prinomastat pharmacokinetics were measured on day 29 of therapy.

Results: The primary toxicities identified were joint and muscle-related pain, which were generally reversible with treatment rest and/or dose reduction. No dose-limiting toxicities were noted within the first 4 weeks of treatment, but grade 2–3 arthralgias and myalgias were noted 2–3 months after initiation of therapy in >25% of patients at doses >25 mg twice a day. The frequency and severity of symptoms were dose related. Plasma prinomastat concentrations greater than the K_i for MMPs 2 and 9 were achieved at all of the dose levels.

Conclusions: Doses of 5–10 mg bid were recommended for additional trials, because this dose range was well tolerated for a treatment duration of at least 3 months and achieves trough plasma concentrations 10–100-fold greater than the K_i (*in vitro* inhibition constant) for the targeted MMPs (2 and 9).

INTRODUCTION

Matrix metalloproteases (MMPs) are a family of zinc- and calcium-dependent endopeptidases that participate in the remodeling of extracellular matrices (1). The healthy remodeling process is well ordered and necessary for tissue growth, angiogenesis, collagen turnover, and cellular migration. Perturbation of the remodeling process occurs under pathological conditions of tumor growth, invasion, metastasis, and neovascularization.

More than 20 members of the family of human MMPs have been identified to date. Of these, several are expressed by tumors and surrounding stroma (1–4). MMP-2 (gelatinase A), MMP-9 (gelatinase B), and MMP-14 (MT-MMP-1, a membrane-bound enzyme) have been associated with invasive tumors and neovascularization (5–9). Other MMPs also associated include MMP-1 (collagenase-1), MMP-3 (stromelysin-1), MMP-7 (matrilysin), MMP-11 (stromelysin-3), and MMP-13 (collagenase-3; Ref. 10,11). MMP-1 and MMP-7 have also been linked to the carcinogenic processes (12,13).

Inhibitors of the MMPs have the potential to block both tumor invasion and metastasis by preventing MMP degradation of the extracellular matrix proteins and angiogenesis. Several synthetic MMP inhibitors have undergone clinical testing in recent years. MMP inhibitors under evaluation differ in chemical class, patterns of MMP inhibition, and pharmacological characteristics (14). Prinomastat (also known as AG3340; Agouron Pharmaceuticals, Inc., a Pfizer Company, La Jolla, CA) is a small molecule, nonpeptidic, hydroxymate MMP inhibitor designed using X-ray crystallography. It was chosen for development based on its relative selectivity for MMPs -2, -3, -9, -13, and -14 (K_i values ranging from 0.03 to 0.33 nM), as opposed to MMP-1 ($K_i = 8.3$ nM). It was theorized that selective inhibition of MMPs 2 and 9 would result in optimal inhibition of tumor growth, invasion, metastasis, and angiogenesis. With reduced inhibition of MMP-1, prinomastat could potentially avoid the arthritis and arthragias thought related to MMP-1 inhibition that has been observed using broad-spectrum MMP inhibitors (15).

In the preclinical setting, prinomastat has demonstrated antitumor activity in models of murine and human xenograft tumors. Prinomastat treatment inhibited the growth of s.c. implanted human tumors in nude mice (including PC-3 prostate, MV522 colon, and COLO-320DM colon cancer models), reduced the number and size of metastases in both induced and spontaneous metastasis models, and significantly inhibited tumor angiogenesis (16,17).

Short-duration (1 week) Phase I dose-escalation studies of prinomastat have been conducted in healthy volunteers. Prinomastat was found to be well tolerated and associated with linear pharmacokinetics when administered orally as a single dose or twice daily for 1 week at doses between 10 and 100 mg.

This report discusses a dose-escalation study of prinomastat given for protracted periods conducted in patients with advanced cancer. The study objectives were to evaluate the

safety, dose tolerance, and pharmacokinetics of prinomastat given over a prolonged treatment course.

PATIENTS AND METHODS

Patient Selection. Patient enrollment took place at two institutions, the University of Wisconsin Comprehensive Cancer Center (Madison, WI), and the Vanderbilt/Ingram Cancer Center (Nashville, TN). Eligibility criteria included histologically confirmed advanced cancer for which no satisfactory treatment existed at the time of enrollment, age of at least 18 years, WHO performance status of 0 or 1, and full recovery from acute effects of previous chemotherapy or radiotherapy. Patients were required to be off any chemotherapeutic treatment for at least 4 weeks before study entry. Patients were required to have adequate laboratory parameters including hemoglobin level >9.5 g/dl, granulocyte count >1,500/µl, platelet count >100,000/µl, serum creatinine <1.5 mg/dl or calculated creatinine clearance >60 ml/min, serum bilirubin <1.5 mg/dl, and alanine transaminase and aspartate transaminase levels less than twice the upper normal limit unless clearly due to the presence of tumor (in which case <5-fold the upper normal limit was allowed). Patients with brain metastases were excluded from the study. Patients unable to swallow tablets, or with significant diarrhea or gastrointestinal disease were excluded from study. Women of childbearing age were required to have a negative pregnancy test and practice contraception. All of the patients provided written, informed consent.

Treatment Plan. Prinomastat was provided as 1-mg, 5-mg, and 50-mg tablets. Patients were instructed to take prinomastat twice daily at ~12-h intervals before meals on an empty stomach. At least 6 patients were enrolled into each dose cohort. Up to 6 additional patients could be enrolled at dose levels of selected interest to more carefully evaluate drug toxicity. The first dose evaluated was 10 mg of prinomastat twice daily (based on preclinical toxicology findings and pharmacokinetic information from short duration drug administration studies in volunteers). The next planned dose levels were 25, 50, 100, 150, and 200 mg twice daily. Dose escalation was permitted after at least 3 patients in a cohort had completed 4 weeks of treatment and 6 patients had been accrued to that cohort. An accrual of 6 patients per cohort was used to assure adequate pharmacokinetic data and toxicity assessment. Doses <10 mg twice daily (*i.e.,* 1, 2, and 5 mg twice daily) were subsequently studied when delayed joint related toxicities were noted after 2–6 months of therapy at higher drug doses. It was felt that continuous, long-term dosing would be required for this cytostatic agent. Dose escalation was not permitted in individual patients.

Definition of Maximum Tolerated Dose. Toxicity was graded according to version 2.0 of the National Cancer Institute Common Toxicity Criteria. Dose-limiting toxicity (DLT) was defined as any drug-induced effect > grade 3 in severity or any toxicity judged by the investigator to be intolerable for the patient that resulted in permanent discontinuation from treatment at the assigned dose level. Patients developing a DLT were managed by stopping drug until symptoms resolved. Prinomastat was then restarted at a lower dose determined during discussion between the sponsor and principal investigators (generally

50% of the previous drug dose). The maximum tolerated dose was originally defined as the dose below which a DLT occurred during the first 4 weeks of treatment in at least 2 of 6 patients. However, because prolonged treatment with this potentially cytostatic drug was anticipated, the final recommended doses for Phase II and III trials were those that could be tolerated for at least 3 months of continuous administration. All of the safety information was collected for a minimum of 28 days after the last administered dose of prinomastat.

Pretreatment and On-Study Evaluations. Before beginning treatment, patients had a complete medical history and physical examination, including WHO performance status, vital signs, and electrocardiogram. Complete blood cell counts, coagulation studies, serum chemistries, and urinalyses were performed at baseline, weekly for 8 weeks, and then every 2 weeks until 28 days after the last prinomastat dose. Extent of disease was assessed by radiological and/or physical evaluations within 4 weeks before the initial prinomastat treatment and every 8 weeks during treatment.

Pharmacokinetic Sampling and Analysis. A detailed 12-h pharmacokinetic profile after drug dosing on day 29 of the study was performed (plasma samples taken at 0, 0.5, 1, 1.5, 2, 3, 4, 6, 8, and 12 h). Limited plasma profiles over a 4-h interval were collected on days 1, 8, and 43. Trough samples were collected before dosing on days 15 and 22. Pharmacokinetic parameter estimates were obtained by noncompartmental methods using the WinNonlin software (Professional Version, v 1.5; Scientific Consulting Inc.). Pharmacokinetic parameters measured included maximum plasma drug concentration, time of observed maximum plasma concentration, plasma terminal elimination half-life, and area under the plasma concentration *versus* time curve.

Plasma concentrations of prinomastat and its *N*-oxide metabolite, AG3473, were determined by a validated high-performance liquid chromatography method with mass-spectrometric/mass-spectrometric detection. Plasma samples (0.05 ml) or standard samples containing blank plasma spiked with prinomastat and AG3473 were extracted with 2 ml of 10% 1-butanol in methyl-*t*-butyl ether. The extract was dried under nitrogen at 40°C, reconstituted with 0.1 ml of 1% formic acid: acetonitrile (85:15 v/v), and injected on a reverse-phase high-performance liquid chromatography system with separation using a Zorbax SB C18 column (2.1 mm i.d. × 50 mm, 5-µm particle size). The mobile phase flow rate was 0.2 ml/min with a gradient elution going from water:acetic acid (99.9:0.1, v/v) to acetonitrile:acetic acid (99.9:01 v/v). An API III Perkin-Elmer Sciex LC/MS using positive ion electrospray with Turbot Ion spray was used for detection and quantitation. The absolute recoveries of prinomastat and AG3473 from plasma were 85.3% and 63.4%, respectively. The within-day and between-day assay variabilities for prinomastat and AG3473 were <10.1% and 7.9%, respectively, and the assay bias was <7.1% for the two analytes. The lower limit of quantitation was 0.5 ng/ml for both compounds, using a 0.05-ml plasma sample.

Response Criteria. Complete response was defined as the disappearance of all of the clinical and radiological evidence of disease. Partial response was defined as a >50% reduction in the sum of products of perpendicular diameters of all of the measurable lesions with no new lesions appearing. Tumor size

Table 1 Patient characteristics

Patients treated	75
Median age, yr (range)	54 (25–87)
Gender (No. males/females)	44/31
Tumor types[a] (number)	
Colorectal	18
Non-small cell lung	15
Renal	13
Soft tissue sarcoma	9
Prostate	4
Ovarian	3
Carcinoid	2
Head and neck	2
Melanoma	2
Mesothelioma	2
Other	9
Previous treatment (no. pts)	
None	2
Chemotherapy	61
Surgery	53
Radiotherapy	39
Immunotherapy	15
Hormones	6
Other	6

[a] Patients may have more than one tumor type.

reduction for 30 days was required to define a response. Stable disease included all of the patients who did not meet the criteria for a complete or partial response or for progressive disease. Progression of disease was defined as a >50% increase or an increase of 10 cm^2 (whichever was smaller) in the sum of products of all of the measurable lesions over the smallest sum observed. Clear worsening of evaluable disease, reappearance of any lesion that had disappeared, appearance of a new lesion or disease site, or failure to return for evaluation due to death or deteriorating condition also qualified as disease progression.

RESULTS

Patient Characteristics. Seventy-seven patients were enrolled in the trial. Two patients never received study drug; 1 developed a second malignancy that required immediate surgery, and the other experienced disease progression between the time of screening but before receiving treatment.

Baseline characteristics of 75 treated patients are in Table 1. The majority of patients were male (59%). All but 2 of the patients had received therapy previously, including 61 patients who had received previous chemotherapy. The most common tumor types were colorectal, non-small cell lung, and renal cancer.

Results of Dose Escalation. Results of dose escalation are shown in Table 2. The starting prinomastat dose was 10 mg bid. Because no DLTs were demonstrated within 4 weeks, subsequent cohorts were escalated to 25, 50, and 100 mg bid. Again, no DLTs were reported within the first 4 weeks. However, the development of arthralgias 4–12 weeks after initiation therapy, which required a treatment rest, was noted at doses of 10–100 mg bid. When DLT was observed 1–4 months into therapy in each of the first four cohorts, doses of 1, 2, and 5 mg twice daily were subsequently evaluated.

No DLTs were seen in any patients at any time at doses of 1 or 2 mg bid. DLTs that occurred within 4 weeks of initial treatment were reported in 2 of 14 patients receiving 5 mg of prinomastat twice daily. One of these patients experienced grade 3 fatigue after 3 weeks of therapy that may have been either treatment or cancer related. This patient had permanent discontinuation of therapy. Another patient developed what was felt by the physician to be a grade 1 arthralgia in the shoulders after 3 weeks of treatment. Although only considered a grade 1 toxicity by the physician, this patient requested a treatment rest and was subsequently treated with a dose reduction.

Severe toxicity was uncommon at any dose studied. No grade 4 events were related to prinomastat treatment. Grade 2 or 3 adverse, drug-related events that occurred in at least 5% of patients included arthralgias (23% of patients), fatigue (11%), pain in limb (8%), and myalgia (5%).

DLTs reported after 4 weeks of treatment were noted in 14 patients (Table 2). These events occurred an average of 9 weeks (range, 4–19 weeks) after initial treatment, and consisted mainly of grade 2 to 3 arthralgia, limb pain, and other joint effects. The majority (10 of 14) of DLTs were in patients receiving prinomastat doses ≥25 mg twice daily (10 of 22 patients) as compared with patients receiving 1–10 mg bid (4 of 53 patients). The time of development and severity of arthralgias and arthritis appeared related to dose. Arthralgias appeared sooner (after 1–3 months of therapy) at high prinomastat doses (50 and 100 mg bid).

Among patients experiencing arthralgia, the most common site affected was the shoulder; other affected sites were knees, elbows, neck, wrists, hips, hands, and fingers. Limited range of motion in the shoulders was observed in a few patients. Changes

Table 2 Dose levels, and patients with dose-limiting toxicities (DLTs) and treatment rests

Prinomastat dose (mg BID[a])	No. of patients	No. of patients with DLT ≤28 days of treatment	No. of patients with DLT >28 days of treatment	No. of patients requiring treatment rest within 3 months
10[b]	14	0	1	1
25	8	0	3	2
50	8	0	3	2
100	6	0	4	3
1	13	0	0	0
2	12	0	0	0
5	14	2	3	2

[a] BID, twice daily.
[b] First dose level evaluated in study.

Table 3 Summary of laboratory and hematologic abnormalities
(National Cancer Institute toxicity criteria) for all dose groups
($n = 75$)

	Grade 1/2	Grade 3	Grade 4
Laboratory abnormality (no. pts.)			
↑ Alkaline phosphatase	43	1	0
↑ Aspartate aminotransferase	28	0	0
↑ Total bilirubin	7	4	1
↑ Creatinine	11	0	0
Hypercalcemia	6	1	0
Hypocalcaemia	20	1	0
Hematologic abnormality (no. pts.)			
Leukopenia	16	1	0
Thrombocytopenia	2	0	0
Anemia	33	2	0
↑ Prothrombin time	26	1	1

in the hands included edema (8 patients), thickening of finger joints or skin (5), contracture of the tendons (2), or nontender, fluid-filled cysts on the palm (2). The most advanced of these effects (*i.e.,* contractures of fingers, limited range of motion of the shoulders, and palmar nodules), all occurred among patients receiving prinomastat doses >25 mg twice daily.

Joint effects were managed successfully with brief (2–4-week) treatment rests followed by reinstitution of therapy at reduced drug doses (generally 1 dose level reduction). A total of 13 (17%) patients had treatment rests, the majority of whom were receiving doses >25 mg bid ($n = 9$). Nearly all of the patients had complete resolution of joint effects in 3–5 weeks. However, joint effects that occurred in patients who continued treatment without rest or dose reduction required a longer time to resolve. One patient receiving prinomastat 25 mg twice daily requested continued prinomastat therapy for 6 additional weeks after first complaining of arthralgias. He developed contractures of his fingers, which did not resolve before his death from progressive cancer.

Other grade ≥2 adverse events occurring in 1 or 2 patients each included anorexia, localized or generalized pain, weakness, constipation, dermatitis, facial edema, skin hypertrophy, taste disturbance, and urticaria. No pattern emerged with regard to these events and the prinomastat dose.

No pattern emerged to suggest a relationship between laboratory abnormalities and prinomastat treatment. Laboratory abnormalities that were seen were generally mild or moderate in severity (Table 3). Twenty-one patients had hypocalcaemia, and 7 had hypercalcemia. Almost all of the changes in calcium were

grade <2 and asymptomatic, but may be indicative of skeletal events seen with the MMP inhibitors (MMPIs). Seven patients had grade 3 or 4 elevations in some clinical chemistry parameter. Four of these events (three cases of hyperbilirubinemia and one hypercalcemia) were clearly related to disease progression and/or occurred after prinomastat treatment was stopped. Two patients experienced events (1 hyperbilirubinemia at week 2 and 1 hypocalcaemia at week 15) that occurred once and did not recur with continued prinomastat treatment. One patient [whose alkaline phosphatase level was elevated (grade 3) at study entry and which remained constant throughout] experienced a doubling in bilirubin level (from 1.2 to 2.4 mg/dl) after 6 weeks of treatment with 100 mg of prinomastat twice daily. After a 3-week treatment rest the bilirubin level decreased to 1.3 mg/dl; no subsequent bilirubin elevations occurred during 9 additional weeks of treatment with prinomastat at a reduced dose (5 mg bid). Grade 3 or 4 hematological abnormalities included prolonged prothrombin time in 2 patients, anemia in 2 patients, and leukopenia in 1 patient. Of the 2 patients with prolonged prothrombin time, 1 had a prolonged prothrombin time value at baseline that continued throughout the study, and the other had the prothrombin time increase steadily over 8 weeks of treatment, which the investigator attributed to progressive liver metastases from pancreatic cancer.

Four patients withdrew from the study due to treatment-related adverse events. Reasons for discontinuation included 1 case each of fatigue, arm pain, arthralgia in the hands, and hyperbilirubinemia. The fatigue was reported in the patient receiving 5 mg prinomastat twice daily (labeled as a DLT) and may have been tumor related; the other discontinuations occurred among patients receiving 100 mg twice daily. No treatment-related deaths occurred.

Pharmacokinetics. Blood sampling to evaluate plasma pharmacokinetics of prinomastat and its metabolite, AG3473, was performed in 40 patients. Steady state (day 29) pharmacokinetic parameters for prinomastat and AG3473, the *N*-oxide metabolite, are provided in Tables 4 and 5. Plasma drug profiles are shown in Fig. 1. Prinomastat plasma pharmacokinetics was linear in the 2–100 mg twice-daily dose range. The drug was rapidly absorbed, with peak concentration occurring within the first hour after dosing. No time-related changes in the daily prinomastat area under the plasma concentration *versus* time curve (Table 6) or peak concentrations (data not shown) were noted over 43 days of plasma profiling. Steady-state plasma exposures and peak concentrations from the 1-mg dosing cohort were calculated as less than proportional compared with those

Table 4 Prinomastat steady-state plasma pharmacokinetics (mean ± SD)

Dose (mg BID[a])	n	C_{max} (ng/ml)	T_{max} (h)	AUC_{0-12} (ng.h/ml)	$C_{12h, trough}$ (ng/ml)	$t_{1/2}$ (h)
1	5	9.5 ± 3.8	0.70 ± 0.27	14 ± 5	BLOQ, n = 5	0.94 ± 0.26
2	6	58 ± 24	0.58 ± 0.20	86 ± 37	BLOQ, n = 4	4.76 ± 2.00
5	6	199 ± 100	0.75 ± 0.42	314 ± 173	2.3 ± 2.0	5.01 ± 1.97
10	6	291 ± 157	0.88 ± 1.53	413 ± 50	5.4 ± 5.1	2.70 ± 0.55
25	6	777 ± 239	0.42 ± 0.30	1108 ± 519	9.0 ± 6.0	2.22 ± 0.37
50	5	1861 ± 1296	0.45 ± 0.33	3107 ± 1603	20.4 ± 11.6	2.08 ± 0.34
100	6	2083 ± 1433	0.79 ± 1.09	5156 ± 3298	104.1 ± 80.5	2.46 ± 0.68

[a] BLOQ, below limit of quantitation; BID, twice daily; AUC, area under the curve; C_{max}, maximum plasma drug concentration; T_{max}, time of observed maximum plasma concentration.

Table 5 AG3473 steady-state plasma pharmacokinetics (mean ± SD)

Dose (mg BID[a])	n	C_{max} (ng/ml)	T_{max} (h)	AUC_{0-12} (ng.h/ml)	$t_{1/2}$ (h)
1	5	155 ± 180	2.60 ± 1.08	290 ± 153	6.36 ± 5.97
2	6	122 ± 55	1.83 ± 0.61	610 ± 352	6.33 ± 3.16
5	6	376 ± 195	2.17 ± 0.68	2172 ± 1082	5.63 ± 1.40
10	6	198 ± 144	2.17 ± 2.88	1688 ± 1462	7.03 ± 2.90
25	6	224 ± 189	1.33 ± 0.75	1883 ± 1679	6.15 ± 1.94
50	5	458 ± 521	2.05 ± 1.33	4078 ± 5085	5.13 ± 0.93
100	6	601 ± 323	2.50 ± 1.41	5659 ± 3294	10.52 ± 5.23

[a] BID, twice daily; AUC, area under the curve; C_{max} maximum plasma drug concentration; T_{max}, time of observed maximum plasma concentration.

from higher dose cohorts. However, prinomastat plasma exposures from the 1-mg dose were probably underestimated, because the β phase of the typically biexponential prinomastat plasma concentration profile could not be characterized due to plasma concentrations falling below the limit of quantitation after 4 h. Similarly, more variability in the calculated plasma half-life was seen at low drug doses (1–5 mg bid) due to low plasma drug concentrations at these doses.

AG3473 concentrations rapidly appeared in plasma, with peak concentrations usually occurring within 2 h of prinomastat dosing. AG3473 elimination half-life was approximately 6–10 h, slightly longer than that of prinomastat (*i.e.,* 2–5 h). AG3473 pharmacokinetics was linear between the 1 and 5 mg twice-daily dosing cohorts. However, at dosing cohorts >5 mg bid, a proportional increase in AG3473 exposure was not observed. At the 100-mg bid dosing, the AG 3473 area under the plasma concentration *versus* time curve decreased with more prolonged dosing perhaps suggesting inhibition of metabolism at this dose level.

Antitumor Effects. No confirmed tumor responses were observed. Three patients (1 mesothelioma, 1 rectal cancer, and 1 lung cancer patient receiving doses of 1–10 mg bid) were reported to have a minor tumor regression. These responses were not confirmed in any patient at the subsequent 30-day follow-up. Thirteen patients experienced stabilization of their

disease for at least 16 weeks, including 1 patient who remained stable for 1 year. The prinomastat dose assignment at study entry did not appear to influence which patient experienced stable disease.

DISCUSSION

The appropriate dose for Phase II or III studies of continuous long-term prinomastat therapy is 5–10 mg bid based on results of this Phase I study. Higher doses were tolerated for short periods. However, continuous therapy produced dose-limiting arthralgias in over one-third of patients within 6 months at doses of 25–100 mg bid. The results of the current study indicated that doses of 5–10 mg prinomastat bid can safely be given to patients on a continuous basis for periods of 3–6 months.

Prinomastat was designed to target members of the MMP family most associated with invasive tumors, MMPs 2 and 9. Inhibition of MMP-1 was avoided by design based on the hypothesis that this might limit joint effects at therapeutic exposures. The K_i value for prinomastat against MMP-2 (a target enzyme) is 166-fold more potent than the K_i for MMP-1. This therapeutic window was hypothesized to provide the opportunity to treat patients without inducing side effects.

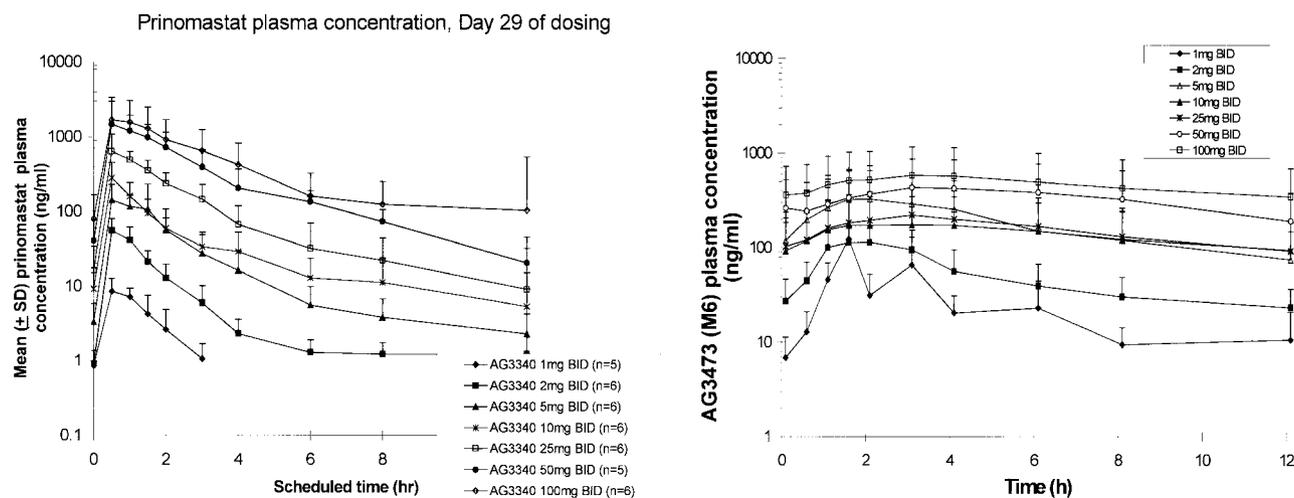It is apparent from this Phase I study that dose escalation of

Fig. 1 Steady-state plasma concentrations of prinomastat (*AG3340; left*) and AG3473 (*right*) on day 29.

Table 6 Plasma AUC for prinomastat and AG3473 over days 1–43[a]

Prinomastat dose	AUC[b] (ng.hr/ml)							
	Prinomastat				AG3473			
	Day 1	Day 8	Day 29	Day 43	Day 1	Day 8	Day 29	Day 43
1 mg BID	19 ± 12	18 ± 10	17 ± 10	22 ± 17	79 ± 37	97 ± 40	169 ± 120	113 ± 30
2 mg BID	59 ± 36	72 ± 45	76 ± 32	69 ± 31	203 ± 156	256 ± 34	344 ± 185	262 ± 127
5 mg BID	193 ± 93	216 ± 101	262 ± 132	246 ± 147	491 ± 229	798 ± 364	1086 ± 618	1025 ± 685
10 mg BID	429 ± 260	468 ± 328	365 ± 159	439 ± 109	357 ± 280	632 ± 733	635 ± 452	816 ± 592
25 mg BID	1168 ± 273	1170 ± 295	1104 ± 379	1029 ± 379	474 ± 329	799 ± 609	725 ± 605	939 ± 785
50 mg BID	2723 ± 1059	2836 ± 1173	2876 ± 1525	3346 ± 1875	1096 ± 862	1862 ± 1435	1421 ± 1611	1308 ± 1302
100 mg BID	4329 ± 273	3593 ± 1892	3849 ± 3281	2740 ± 862	1001 ± 646	1975 ± 1063	2028 ± 1079	1726 ± 510

[a] Data include evaluations of 4 to 8 patients on each day at each dose.
[b] AUC, area under the curve; BID, twice daily.

prinomastat sufficient to cause joint complaints is possible. Reversible joint-related effects, consisting of arthralgias, stiffness, and swelling, were the most common adverse events observed in this study. Arthralgias were seen at doses as low as 5 mg bid. Prinomastat plasma concentrations at 5 mg bid were above the K_i for MMP-1 for roughly 18 of every 24 h. Minimal joint complaints were noted at 2 mg bid where the K_i of MMP-1 was exceeded for only 8 h/day. The joint effects may be attributable to the intermittent inhibition of MMP-1 or to the pharmacological action of prinomastat on other enzymes involved in collagen remodeling in joints and tendons.

Prinomastat inhibits MMP-2 and MMP-9 in the picomolar range (K_i = 0.05 and 0.26 nM, respectively). Adjusting for binding of prinomastat to plasma proteins (69% bound in human plasma), plasma drug concentrations in excess of 1.73 ng/ml and 9.0 ng/ml, respectively, would be required to inhibit these MMPs in plasma. Results from preclinical studies with COLO-320DM tumor models indicated that regimens that maintained plasma concentrations of 1–2 ng/ml had efficacy superior to regimens associated with lower trough concentration (16, 17). Hence, minimum target trough plasma concentrations for clinical trials were set at 1–2 ng/ml.

In the current study, all of the dosing cohorts >2 mg twice daily had steady state plasma prinomastat concentrations above the target concentration of 1–2 ng/ml (Fig. 1). The pharmacokinetic studies associated with this Phase I trial demonstrate adequate plasma drug concentrations to inhibit MMPs 2 and 9. However, there may be differences in drug concentrations found in plasma and drug concentrations in tumor or stromal tissues.

Prinomastat is minimally eliminated through the kidneys, with <2% of an administered dose recovered unchanged in urine (data on file, Agouron Pharmaceuticals). There was no plasma accumulation of prinomastat with repeat twice daily dosing over 43 days of evaluation, although accumulation of the metabolite AG 3473 was noted. There was greater intersubject variability in prinomastat pharmacokinetics in cancer patients compared with healthy volunteers (area under the plasma concentration *versus* time curve coefficient of variation 47% *versus* 21%, respectively; data not shown).

The major circulating prinomastat metabolite in human plasma, AG3473, is an *N*-oxide product and was assayed in all of the plasma samples in this study. Oxidation of AG3473 primarily occurs via the isoenzyme CYP2D6. AG3473 12-h

plasma exposures were nonlinear at doses >5 mg bid, indicating saturation in the pathway responsible for AG3473 formation. Saturation of the AG3473 metabolite in the presence of linearity in the pharmacokinetics of the parent drug suggest that prinomastat metabolism is probably diverted to another pathway(s) at prinomastat doses >5 mg bid. AG3473 concentrations are not expected to impact on the efficacy of prinomastat due to the significantly lower potency of the metabolite toward key MMPs (*in vitro* K_i values are 10–100-fold lower than those of prinomastat). However, it is possible that this metabolite could play a role in drug toxicity through mechanisms other than MMPI inhibition.

Clinic trials of MMPIs as cancer therapy have yielded disappointing results (18–21). No documented tumor responses were noted in our trial. In other reported studies, addition of marimastat (British Biotech) to standard therapy did not improve survival over standard therapy for small cell lung cancer (19). Treatment with Bayer's MMPI (Bay 12–95656) worsened survival in a similarly designed study in small cell lung cancer (18).

Two Phase III trials of prinomastat in therapy of metastatic non-small cell lung cancer have been completed. No survival benefit has been noted when 5 or 15 mg bid prinomastat has been added to standard chemotherapy such as paclitaxel plus carboplatin (20) or gemcitabine plus cisplatin in these studies (21). One explanation for lack of tumor response in these studies could be that the doses of prinomastat were inadequate. However, our study demonstrates that plasma concentrations of prinomastat were adequate to continuously inhibit MMPs 2 and 9. This would suggest that inhibition of additional MMPs might be necessary for antitumor effect, that inhibition of MMPs does not affect tumor growth, or that inhibition of MMPs may be useful only in very early stages of cancer. A final possibility is that inhibition of all of the MMPs may be required for antineoplastic activity. In this case, unacceptable joint and muscle toxicities would limit the usefulness of MMPIs.

To date, clinical trials of MMPIs have been directed at patients with well-established metastases. MMPs do more than degrade extracellular matrix proteins. MMPs also promote cancer growth through activation of insulin growth factor, cleaving FAS ligand, and increasing the bioavailability of vascular endothelial growth factor and fibroblast growth factor 2. Additional trials of MMPIs such as prinomastat may be warranted in

earlier-stage cancer, but only after there is a better understanding of the role of individual MMPs in carcinogenesis and in tumor control.

REFERENCES

1. Nelson, A. R., Fingleton, B., Rothenberg, M., and Matrisian, L. M. Matrix metalloproteinases: biologic activity and clinical implications. J. Clin. Oncol., *18:* 1135–1149, 2000.

2. Chambers, A. F., and Matrisian, L. M. Changing views of the role of matrix metalloproteases in metastasis. J. Natl. Cancer Inst., *89:* 1260–1270, 1997.

3. Wojtowicz-Praga, S. M., Dickson, R. B., and Hawkins, M. Matrix metalloproteinase inhibitors. Investigational New Drugs, *15:* 61–75, 1997.

4. Levy D. E., and Ezrin A. M. Matrix metalloproteinase inhibitor drugs. *In:* Bowman, W. C., Fitzgerald, J. D., Taylor, J. B. (eds.) Emerging Drugs: The Prospective for Improved Medicines. Ashley Publications Ltd., 1997.

5. Stetler-Stevenson, W. G., Aznavoorian, S., and Liotta, L. A. Tumor cell interactions with the extracellular matrix during invasion and metastasis. Annu. Rev. Cell Biol., *9:* 541–573, 1993.

6. Sato, H., Takino, T., Okada, Y., Shagawa, A., Yamamoto, E., and Seiki, M. A. matrix metalloprotease expressed on the surface of invasive tumor cells. Nature (Lond.), *370:* 61–65, 1994.

7. Strongin, A. Y., Collier, I., Bannikov, G., Marmer, B. L., Grant, G. A., and Goldberg, G. I. Mechanism of cell surface activation of 72-kDA type IV collagenase. J. Biol. Chem., *270:* 5331–5338, 1995.

8. Goodsell, D. A. The molecular perspective: matrix metalloproteinase 2. The Oncologist, *4:* 509–511, 1999.

9. Brooks, P. C., Stromblad, S., Sanders, L. C., von Schalscha, T. L., Aimes, R. T., Stetler-Stevenson, W. G., *et al.* Localization of matrix metalloproteinase MMP-2 to the surface of invasive cells by interaction with integrin α v β 3. Cell, *85:* 683–693, 1996.

10. Fini M. E., Cook J. R., Mohan R., and Brinckerhoff C. E. Regulation of matrix metalloproteases gene expression. *In:* W. C. Parks and R. P. Mecham (eds.), Matrix Metalloproteinases, pp. 300–339. San Diego: Academic Press, 1998.

11. Basset, P., Bellocq, J. P., Wolff, C., Stoll, I., Hutin, P., Limacher, J., *et al.* A novel metalloproteinase gene specifically expressed in stromal cells of breast carcinomas. Nature (Lond.), *348:* 699–704, 1990.

12. Crawford, H. C., and Matrisian, L. M. Mechanisms controlling the transcription of matrix metalloproteinase genes in normal and neoplastic cells. Enzyme Protein, *49:* 20–37, 1996.

13. Wilson, C. L., Heppner, K. J., Labosky, P. A., Hogan, B. L., and Matrisian, L. M. Intestinal tumorigenesis is suppressed in mice lacking the metalloproteinase matrilysin. Proc. Natl. Acad. Sci. USA, *94:* 1402–1407, 1997.

14. Hidalgo, M., and Eckhardt, S. G. Development of matrix metalloproteinase inhibitors in cancer therapy. J. Natl. Cancer Inst., *93:* 178–193, 2001.

15. Rothenberg, M. L., Nelson, A. R., and Hande, K. R. New drugs on the horizon: matrix metalloproteinase inhibitors. Oncologist, *3:* 271–274, 1998.

16. Shalinsky, D. R., Brekken, J., Zou, H., McDermott, C. D., Forsyth, P., Edwards, D., Margosiak, S., Bender, S., Truitt, G., Wood, A., Varki, N. M., and Appelt, K. Broad antitumor and antiangiogenic activities of AG3340, a potent MMP inhibitor undergoing advanced oncology clinical trials. Ann. N. Y. Acad. Sci., *878:* 236–270, 1999.

17. Egeblad, M., and Werb, Z. New functions for the matrix metalloproteinases in cancer progression. Nat. Rev., *2:* 161–174, 2002.

18. Bonomi, P. Matrix metalloproteinases and matrix metalloproteinase inhibitors in lung cancer. Sem. Oncol., 29 (Suppl. 4)*:* 78–86, 2002.

19. Shepherd, F. A., Giaccone, G., Debrayne, C., Hirsh, V., Smylie, M., Rubin, S., *et al.* Randomized double blind, placebo-controlled trial of marimatat in patients with small cell lung cancer (SCLC) following response to first line chemotherapy: an NCIC and EORTC study. Proc. Am. Soc. Clin. Oncology, *20:* 4a, 2001.

20. Smylie, M., Mercier, R., Aboulafia, D., Tucker, R., Bonomi, P., Collier, M., *et al.* Phase III study of the matrix metalloproteinases (MMP) inhibitor prinomastat in patients having advanced non-small cell lung cancer. Proc. Am. Soc. Clin. Oncol., *20:* 307a, 2001.

21. Bissett, D., O'Bryne, K., von Pawel, J., Mercler, R., Price, A., Nicolson, M., *et al.* Phase III study of the matrix metalloprotease inhibitor prinomostat in combination with gemcitibine and cisplatin in non-small cell lung cancer. Proc. Am. Soc. Clin. Oncol., *21:* 296a, 2002.

Phase I and Pharmacokinetic Study of Prinomastat, a Matrix Metalloprotease Inhibitor

Kenneth R. Hande, Mary Collier, Linda Paradiso, et al.

Updated version	Access the most recent version of this article at: http://clincancerres.aacrjournals.org/content/10/3/909

Cited articles	This article cites 18 articles, 5 of which you can access for free at: http://clincancerres.aacrjournals.org/content/10/3/909.full#ref-list-1
Citing articles	This article has been cited by 5 HighWire-hosted articles. Access the articles at: http://clincancerres.aacrjournals.org/content/10/3/909.full#related-urls

E-mail alerts	Sign up to receive free email-alerts related to this article or journal.
Reprints and Subscriptions	To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.
Permissions	To request permission to re-use all or part of this article, use this link http://clincancerres.aacrjournals.org/content/10/3/909. Click on "Request Permissions" which will take you to the Copyright Clearance Center's (CCC) Rightslink site.