A Phase I and Pharmacokinetic Study of TNP-470 Administered Weekly to Patients with Advanced Cancer

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ABSTRACT

A Phase I study of angiogenesis inhibitor TNP-470 was conducted in patients with advanced cancer. TNP-470 (25–235 mg/m^2) was administered i.v. over 4 h once a week to patients who had solid tumors refractory to the best available treatment or with a high risk of recurrence and who had normal renal, hepatic, and hematological function and no evidence of coagulopathy. The aims of the study were to determine the maximum tolerated dose, dose-limiting toxicities (DLTs), and the pharmacokinetics of TNP-470 given on a once-weekly schedule. Thirty-six patients, ages 23–75 (median, 54 years), with an Eastern Cooperative Oncology Group performance status of 0–2 were treated. The number of patients at each dose level (mg/m^2) were 6 (25), 3 (50), 3 (75), 3 (100), 3 (133), 12 (177), and 6 (235). The principal toxicities of TNP-470 were dizziness, lightheadedness, vertigo, ataxia, decrease in concentration and short-term memory, confusion, anxiety, and depression, which occurred at doses of 133, 177, and 235 mg/m^2. Two patients treated at 235 mg/m^2 experienced DLT in the form of grade III cerebellar neurotoxicity after 6 weeks of treatment. Overall, these neurological symptoms were dose-related, had an insidious onset, progressively worsened with treatment, and resolved completely within 2 weeks of stopping the drug. One patient with malignant melanoma had stabilization of the previously growing disease for 27 weeks while on the treatment. Two patients, one with adenocarcinoma of the colon and the other with a soft tissue sarcoma, had no clinically detectable disease but were at high risk for recurrence at the initiation of treatment and received 13 months and >3 years of treatment, respectively, with no evidence of disease recurrence. The remaining patients had progression of their disease after 1–6 months of treatment. The mean plasma half-life ($t_{1/2}$) of TNP-470 and its principal metabolite, AGM-1883, were extremely short (harmonic mean, $t_{1/2}$ of 2 and 6 min, respectively) with practically no drug detectable in the plasma by 60 min after the end of the infusion. MII, an inactive metabolite, had a considerably longer $t_{1/2}$ of approximately 2.6 h. Mean peak TNP-470 concentrations were ≥400 ng/ml at doses ≥177 mg/m^2. On the basis of this study, the maximum tolerated dose of TNP-470 administered on a weekly schedule was 177 mg/m^2 given i.v over 4 h. The principal DLT was neurotoxicity, which appeared to be dose-related and was completely reversible. On the basis of the short plasma $t_{1/2}$ of TNP-470, exploration of a prolonged i.v. infusion schedule is warranted.

INTRODUCTION

Tumor-induced angiogenesis has been pursued as a therapeutic target for anticancer treatment since studies showed that angiogenesis is a critical process in the growth of primary and metastatic tumors (1). A property that makes angiogenesis an attractive target is that endothelial cell proliferation in an adult is limited to sites of wound healing, reproductive organs, and tumors (2–4). Therefore, drugs targeting angiogenesis can be expected to selectively effect growing tumors without significant effects on other organs. Because endothelial cells in tumors are genetically stable, drug resistance may be less likely to develop with antiangiogenic therapy (5). Fumagillin, an antibiotic secreted by *Aspergillus fumigatus fresenius* (6, 7), was identified as a potent inhibitor of endothelial cell proliferation *in vitro* and tumor-induced neovascularization *in vivo* (8). However, the clinical utility of fumagillin was limited by severe weight loss seen in mice, which prompted the development of several synthetic analogues. Alkaline hydrolysis of fumagillin yielded several compounds, of which TNP-470 [*O*-(chloroacetyl-carbamoyl) fumagillol; AGM-1470] was found to be the most potent and selected for clinical development (8). TNP-470 was demonstrated to be 50-times more potent than fumagillin in inhibiting endothelial cell proliferation, migration, and capillary tube formation. TNP-470 inhibited endothelial cell proliferation at concentrations in the picogram range, and cytotoxicity to tumor cells was not observed until concentrations exceeding 10 μg/ml (9). TNP-470 inhibited angiogenesis in several *in vivo* model systems, including chick embryo chorioallantoic membrane (CAM), rat dorsal air sac, rat and rabbit corneal micropocket assays, and surgically implanted sponges (10). Administered systemically, TNP-470 was shown to decrease growth and vascularity of primary tumor and metastases in several human tumors xenografts [breast (11), ovarian (12), and prostate cancer (11); glioblastoma (13); and neurofibrosarcoma (14)]. The molecular target of TNP-470 has been recently identified as methionine aminopeptidase (MetAP-2), an intracellular enzyme that is highly conserved between humans and the yeast *Saccha-*

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romyces cerevisiae (15). However, methionine aminopeptidase is expressed in both endothelial and nonendothelial cells, and the mechanism(s) responsible for the specificity of TNP-470 for endothelial cell-cycle inhibition is under investigation.

In vitro studies by Kusaka *et al.* (9) had shown that cytostatic inhibition of human umbilical vein endothelial cells (HUVEC) continued for several days (up to 6 days) after a brief exposure to TNP-470 (2-h incubation at 100 ng/ml). Furthermore, antitumor activity was demonstrated in animal studies using a weekly s.c. dosing regimen of TNP-470 (11). Phase I studies of TNP-470 had used a dosing regimen of 1-h infusions i.v. either q.o.d. (16, 17) or on a Monday, Wednesday, and Friday (MWF) schedule (18). On the basis of preclinical evidence of prolonged antiangiogenic activity after a single dose (9) and antitumor efficacy of once-a-week administration of TNP-470 (11), this study was initiated using a once-weekly dosing regimen. The Phase I study was conducted in patients with solid tumors to determine DLTs,[2] MTDs, and pharmacokinetics of TNP-470 given as a 4-h i.v. infusion once a week.

PATIENTS AND METHODS

Patients. Adults (≥18 years of age) with a histologically confirmed malignancy refractory to the best available treatment or with a high risk of recurrence were eligible for this study. Patients had to have an Eastern Cooperative Oncology Group performance status of 2 or less and an anticipated life expectancy of at least 8 weeks. Laboratory criteria for eligibility included a WBC count ≥3000/mm^3, absolute granulocyte count ≥1500/mm^3, platelet count ≥100,000/mm^3, normal PT and PTT, bilirubin ≤1.2 mg/100 ml, transaminases (AST and ALT) ≤2 times the upper limit of normal, and serum creatinine ≤1.5 mg/100 ml (or creatinine clearance ≥60 ml/min). Patients with radiographically detectable brain metastases or brain tumors, recent (≤6 weeks) history of seizures, peripheral neuropathy (≥ grade II), or a history of bleeding diathesis were excluded from the study. All of the patients were informed about the investigational nature of the study drug and signed an informed consent. The study was approved by the institutional review board of Georgetown University Hospital.

Study Design. Before beginning treatment, patients had a complete medical history and physical examination including a stool hemoccult examination and electrocardiogram. Complete blood counts; serum chemistries; renal, hepatic, and coagulation parameters; and urinalyses were obtained. These studies were repeated every week for the first 4 weeks and every 2 weeks thereafter. Stool hemoccult examinations were repeated weekly. When a measurable tumor was present, it was defined by imaging studies (computerized tomography scans or magnetic resonance imaging). A complete eye examination was performed by an ophthalmologist at baseline, 4 weeks after starting treatment, and every 8 weeks thereafter. Formal tumor assessments were done every 8 weeks. For patients with measurable disease, standard response criteria were used (19).

Three patients were entered per dose level; if one of the three developed a DLT, the cohort was expanded to six patients. The MTD was defined as the dose at which less than two of six patients experienced DLT. Patients who tolerated therapy continued to receive TNP-470 as long as their tumor showed no evidence of progression. All of the patients were evaluated for toxicity.

Criteria for terminating treatment included disease progression, patient noncompliance, a request to withdraw, or the development of unacceptable toxicity. The National Cancer Institute common toxicity criteria were used to grade toxicities and define the MTD.

Treatment Regimen and Dose Escalation. TNP-470 was provided by TAP Holdings Inc. (Deerfield, IL). The drug was reconstituted with 10 ml of 5% dextrose in water, and the appropriate dose was further diluted in 200–250 ml of 5% dextrose in water to yield a final concentration of less than 10 mg/ml TNP-470 for i.v. infusion. TNP-470 was administered by a 4-h i.v. infusion once a week in the outpatient center.

The initial starting dose was 25 mg/m^2 based on animal studies and two ongoing Phase I studies in which patients had been treated at doses of 34.2 and 31.5 mg/m^2 i.v. three times a week without toxicities (16, 20). Doses were escalated by 100% in level 1, 50% in level 2, and 33% in the subsequent dose levels.

Pharmacology Methods. Blood samples were obtained to study the plasma pharmacokinetics of TNP-470 and its metabolites, AGM-1883 and M-II, during the first 3 weeks of treatment. Samples were obtained on days 1 and 22 at the following time points: (*a*) immediately before the start of drug infusion; (*b*) at 20, 40, 60, 120, and 240 min during the infusion; and (*c*) at 5, 15, 30, 60, 120 min, and 20 h after the end of infusion. For the majority of samples, after harvesting the plasma, an aliquot of sulfuric acid was added to adjust the pH to 4–5, which minimized further *ex vivo* degradation of TNP-470. For the last 13 patients, blood samples were collected in vacutainer tubes containing citric acid, which immediately reduced the pH of the sample and minimized degradation of TNP-470.

Drug Analysis. Plasma was analyzed for TNP-470, AGM-1883, and M-II by a validated high-pressure liquid chromatography method with detection by mass spectrometry as described previously (21). Briefly, 1 ml of an acidified human plasma sample (either with a 10% by volume of a 2% sulfuric acid solution or with 5 mg of citric acid per ml of plasma) was supplemented with internal standard (d_3-TNP-470) and extracted with hexane:ethyl acetate (1:1). Analytes of interest were resolved by reverse-phase high-pressure liquid chromatography using a YMC ODS-AQ column (5 μm, 150 mm × 2.6 mm inside diameter; YMC Inc., Morris Plains, NJ). The mobile phase consisted of a mixture (v/v) of 60% acetonitrile and 40% of 2 mM ammonium acetate solution in deionized water. Detection was accomplished by selected reaction monitoring using a Sciex API III$^+$ tandem mass spectrometer with a heated nebulizer-atmospheric pressure chemical ionization source that was similar to a previously described method (22). The lower limit of quantitation was 0.25 ng/ml for TNP-470, and 0.39 to 0.41

[2] The abbreviations used are: DLT, dose-limiting toxicity; MTD, maximum tolerated dose; q.o.d., every other day; PT, prothrombin time; PTT, partial thromboplastin time (activated); AST, aspartate aminotransferase; ALT, alanine aminotransferase.

Table 1 Patient characteristics

No. of patients	36
Assessable	
Toxicity	36
Response	34
Median age (range)	54 (23–75)
Sex	
Male	18
Female	18
Performance status	
0	19
1	15
2	2
Prior therapy	
None	1
Chemotherapy	29
Hormones	8
Immuno- or biotherapy	20
Radiation	17
Tumor types	
Sarcoma	12
Colorectal cancer	7
Melanoma	4
Nonsmall cell lung carcinoma	3
Gastric adenocarcinoma	2
Head and neck squamous carcinoma	2
Breast carcinoma	3
Pancreatic adenocarcinoma	1
Meckel cell carcinoma	1
Spindle cell carcinoma	1

ng/ml (due to changes in concentrations of standards) for AGM-1883 and M-II, respectively.

Pharmacokinetic Analysis. The pharmacokinetic parameters were calculated with noncompartmental methods after the dose on study days 1 and 22. The maximum and minimum observed concentrations (C_{max} and C_{min}, respectively), time to C_{max} (T_{max}), and area under the concentration-time curve ($AUC_{0\text{-}t}$, in which t = time of the last sample collection, 20 h after the end of infusion) were estimated for TNP-470, AGM-1883, and M-II. Plasma half-life ($t_{1/2}$) values were estimated for TNP-470, AGM-1883, and MII.

RESULTS

Patient Characteristics

Thirty-six patients were enrolled in this study between January 1995 and October 1997. The characteristics of treated patients are listed in Table 1. All of the patients were evaluable for toxicity, and 34 patients were evaluable for response. Two patients entered the study without measurable or evaluable disease and were, therefore, inevaluable for antitumor response using standard criteria. All of the patients except one had received prior treatment (chemotherapy, radiation, or immuno- or biotherapy) for their cancer. There were equal numbers of males and females, and thirty-four patients had a performance status of 0 or 1.

Toxicity

Seven dose escalations were required to define the MTD. Neurotoxicity was the principal DLT of TNP-470 given as a 4-h infusion once a week. A listing of toxicities at all of the dose levels is shown in Table 2.

Neurotoxicity. Predominant symptoms reported were those of cerebellar dysfunction, characterized be dizziness, lightheadedness, vertigo, and ataxia. These were generally accompanied with the physical findings of abnormal finger-nose or heel-shin test and the inability to perform tandem walking. Other neurological side effects consisted of decreased concentration and short-term memory, confusion, forgetfulness, anxiety, depression, and insomnia. These symptoms were seen at dose levels 133, 177, and 235 mg/m^2 (Table 3). The median time to onset was 6 weeks (range, 4–11 weeks), and the symptoms resolved in all of the patients within 1–2 weeks of stopping treatment.

One patient who was treated at a dose of 133 mg/m^2 of TNP-470 developed lightheadedness and progressive loss of short-term memory after 6 weeks of treatment. The symptoms resolved within 2 weeks of stopping the drug. The patient was restarted on the same dose of TNP-470 and developed increasing emotional instability and depression after 4 weeks of treatment. These symptoms resolved within 1 week of stopping treatment. Tumor assessment at this time revealed stable disease, and the patient was not retreated with the drug.

Five patients treated at a dose of 177 mg/m^2 reported one or more episodes of dizziness and lightheadedness during their treatment course; three of the five patients had mild abnormalities in gait on examination during these episodes. Three patients reported episodes of decrease in short-term memory during treatment. There was no consistent temporal relationship between drug administration and the time of onset of these symptoms, which ranged from immediately after drug infusion to 2 days after completing infusion. These episodes lasted from a few minutes to 5 days and resolved completely before the next treatment. Symptoms did not consistently recur on successive treatments.

Two patients treated at a dose of 235 mg/m^2 developed grade III ataxia after 6 weeks of treatment. On examination, both patients had objective signs of cerebellar dysfunction with abnormal finger-nose and heel-shin test and the inability to perform tandem walking. These symptoms resolved on stopping treatment—within 1 week in one patient and within 16 days in the other. Another patient treated at 235 mg/m^2 developed incoordination and vertigo (grade I) after 11 weeks of treatment, which resolved completely within 12 days of stopping treatment. One patient presented with progressive decrease in short-term memory and confusion after 12 weeks of treatment at 235 mg/m^2, which resolved within 1 week after stopping treatment.

Cerebellar dysfunction was the DLT at the 235 mg/m^2 dose of TNP-470. It typically had a slow and insidious onset and progressively worsened with treatment. Recovery from this toxicity was complete and occurred within 2 weeks of stopping treatment. Because neurological symptoms resolved spontaneously, radiological studies of the brain were not performed on these patients. The symptoms were not temporally related to the timing of drug infusion, and none of the patients had an acute exacerbation of symptoms immediately after receiving the drug infusion. Symptoms were related to the dose and duration of treatment, which suggests a cumulative drug effect.

Table 2 A summary of all toxicities associated with TNP-470 administration[a]

	Dose level (mg/m^2)													
	25 (n = 6)		50 (n = 3)		75 (n = 3)		100 (n = 3)		133 (n = 3)		177 (n = 12)		235 (n = 6)	
	I–II[b]	III–IV	I–II	III–IV	I–II	III–IV	I–II	III–IV	I–II	III–IV	I–II	III–IV	I–II	III–IV
Nausea/Vomiting	1	0	0	0	0	0	1	0	1	0	1	0	1	2
Fatigue	0	0	1	0	1	0	1	0	1	0	1	0	2	0
Weight loss	1	0	0	0	0	0	0	0	0	0	0	0	0	0
Anorexia	1	0	1	0	1	0	1	0	0	0	3	0	0	0
Bleeding	0	0	0	0	0	0	0	0	1	0	0	0	1	0
Hematologic toxicity	0	0	0	0	0	0	0	0	0	0	1	0	0	0
Hepatotoxicity	0	0	0	0	0	0	0	0	0	0	1	0	0	0
Neurologic toxicity	0	0	0	0	0	0	0	0	4	0	8	0	2	2

[a] Values represent numbers of patients.
[b] I–II, grade I–II toxicity; III–IV, grade III–IV toxicity.

Table 3 Characteristics of neurologic toxicity associated with TNP-470[a]

	Dose level (mg/m^2)		
Toxicity	133 (n = 3)	177 (n = 12)	235 (n = 6)
Dizziness, lightheadedness, vertigo			
Grade[b] I–II	1	5	3
Grade III–IV	0	0	0
Ataxia, incoordination			
Grade I–II	0	3	0
Grade III–IV	0	0	2[c]
Memory loss (short-term)			
Grade I–II	1	3	1
Grade III–IV	0	0	0
Confusion			
Grade I–II	1	1	1
Grade III–IV	0	0	0
Anxiety, depression			
Grade I–II	1	1	1
Grade III–IV	0	0	0
Insomnia			
Grade I–II	0	1	0
Grade III–IV	0	0	0

[a] Values represent numbers of patients.
[b] Using National Cancer Institute Expanded Common Toxicity Criteria V2.0.
[c] DLT.

Nausea. One patient each at dose levels 100, 133, and 177 mg/m^2 developed grade I-II nausea with one or more drug infusions. The symptoms resolved within 1–3 h after treatment and did not recur with subsequent infusions. Two patients at 235 mg/m^2 developed grade I nausea, and one patient developed grade III nausea and vomiting after 8 weeks of treatment. Subsequent treatments were administered with prophylactic antiemetics without recurrent nausea. In all of the patients, the nausea occurred during or up to 6-h after drug infusion, and responded well to oral antiemetic medications. No patient experienced protracted nausea or delayed emesis.

Bleeding. No significant bleeding was observed in any patient among all of the dose levels. Ophthalmological examinations did not reveal any evidence of retinal bleeding as was seen in animal toxicology studies. One patient each at 133 and 235 mg/m^2 dose levels were found to have hemoccult positive stools after 4 and 6 weeks of treatment, respectively. Neither of these patients had any gastrointestinal symptoms or a decrease in their hemoglobin or hematocrit associated with the episode. Subsequent weekly examinations for occult blood in the stools were negative. No elevations of PT or aPTT were seen during TNP-470 treatments.

Hematological Toxicity. One patient treated at a dose of 177 mg/m^2 developed grade II thrombocytopenia after the first treatment of TNP-470. A peripheral blood smear revealed thrombocytopenia, and a bone marrow examination revealed normal trilinear hematopoiesis with adequate megakaryocytes. The patient was treated with a prednisone (1 mg/kg/day) and had a complete recovery of platelet counts after 6 weeks of treatment. The pattern of thrombocytopenia was felt to be consistent with immune thrombocytopenic purpura. Upon discontinuation of prednisone, the patient was retreated with TNP-470 without recurrence of thrombocytopenia. No leukopenia or anemia was seen in patients treated with TNP-470.

Hepatotoxicity. One patient treated at 177 mg/m^2 was found to have abnormally high ALT (grade II), AST (grade I) and alkaline phosphatase (grade I) with a normal bilirubin after the third cycle of treatment. This patient had undergone a surgical procedure for placement of a central venous catheter 1 day before her third treatment, during which sedative and anxiolytic drugs were administered. A subsequent blood test obtained 6 days later revealed normal liver function tests. The patient had no clinical symptoms during this period, and a work-up for other causes was unrevealing. Treatment was resumed at 133 mg/m^2 after 2 weeks, and the patient received a total of 13 treatments of TNP-470 without a recurrence of these abnormalities.

Other. Grade I-II fatigue was seen in 13 patients, and anorexia was seen in 7 patients at dose levels 25, 50, 75, 100, and 177 mg/m^2. Weight loss ($<$10%) was seen in one patient treated at 25 mg/m^2. A patient with primary mucin-producing adenocarcinoma of the colon developed a unilateral deep venous thrombosis of the lower extremity after 13 weeks of treatment at 177 mg/m^2 of TNP-470. The thrombosis resolved with anticoagulant therapy and the patient did not receive further treatments. One patient treated at 235 mg/m^2 was found to have mild cataract changes on ophthalmological examination after 7 weeks

of treatment. This patient continued to receive a total of 24 treatments of TNP-470 without any change in the cataract.

Two patients refused further treatment after 4 and 5 weeks, respectively, because of reasons not related to drug side effects. Treatment in the remaining patients was stopped only for disease progression. The median duration of treatment was 7.5 weeks (range, 2 weeks to >3 years). Three patients continued treatment for > 6 months.

Responses

No objective disease regression was documented. There was stabilization of previously growing disease in a patient with malignant melanoma who had undergone surgery, radiation, and chemo- and immunotherapy with progression of metastatic disease in the lymph nodes and bones. Treatment was started at 25 mg/m^2, and the patient had stabilization of measurable disease for 27 weeks. Two patients who had undergone surgical resection of all of the macroscopic disease before starting treatment had prolonged disease-free intervals while on the treatment. One patient had metastatic adenocarcinoma of the colon and had undergone resection of lung metastases with no remaining measurable disease. This patient was treated at 235 mg/m^2 and showed no evidence of disease progression for 13 months while on treatment. Liver metastases were noted 4 months after the patient elected to stop treatment. The other patient had undergone resection of a high-grade malignant fibrous histiocytoma and was found to have microscopic involvement of the margins of excision, for which he underwent radiation treatment for a total dose of 6480 cGy to the tumor bed. The patient had no measurable disease at the start of treatment and received TNP-470 at 50 mg/m^2 for 22 months, during whichtime, there was no evidence of disease recurrence. This patient elected to resume treatment after a break of 4 months. He continues to receive TNP-470 at 50 mg/m^2 i.v. once a week with no evidence of recurrent disease more than 3 years after first starting treatment.

Pharmacokinetics

Day 1 pharmacokinetic samples were available for 32 patients, and day 22 samples were available for 25 patients. The plasma concentration, $t_{1/2}$ and *AUC* of TNP-470, its principal metabolite, AGM-1883, and MII were evaluated. The concentration-time profiles of TNP-470 and its metabolites were found to be highly variable in the first 23 patients because of the rapid degradation of TNP-470 after collection. This was corrected in the remaining patients by immediate acidification of the blood samples with citric acid at the time of collection, thus yielding consistent serum levels. Therefore, only the data from these patients were evaluated for pharmacokinetic determinations.

The plasma concentrations of TNP-470 and AGM-1883 generally plateaued by 20–40 min into the infusion. The $t_{1/2}$ of TNP-470 and AGM-1883 were extremely short (overall harmonic mean $t_{1/2}$ values of 2 min and 6 min, respectively; Table 4). TNP-470 plasma concentrations were relatively high during the 4-h infusion but declined rapidly after the infusion was stopped (Fig. 1*A*). For patients who received a dose of 235 mg/m^2, the mean TNP-470 concentration decreased from about 405 ng/ml at the end of infusion to about 51 ng/ml 5 min postinfusion. TNP-470 and AGM-1883 were generally undetectable in plasma after 60 min from the end of infusion. AGM-1883 concentrations were typically much lower that those for TNP-470 (usually less than 10 ng/ml during the infusion). The mean $AUC_{0\text{-}t}$ of AGM-1883 was only about 2% that of TNP-470 (29 *versus* 1459 ng·hr/ml, respectively). MII, which is a metabolite formed from AGM-1883 by the action of epoxide hydroxylase, reached maximum plasma concentration (C_{max}) within 4 h after the start of infusion, and showed a considerably longer $t_{1/2}$ of approximately 2.6 h. Pharmacokinetics on day 22 were essentially identical to those on day 1, and no plasma accumulation of TNP-470 or the measured metabolites was detected on weekly dosing (Fig. 1*B*).

Table 4 Pharmacokinetic data

	TNP-470	AGM-1883	M-II
177 mg/m^2 TNP-470[a]			
T_{max}[b] (h)	2.1 ± 1.6	3.7 ± 0.8	4.0 ± 0.0
C_{max} (ng/ml)	498 ± 352	7.7 ± 2.9	560 ± 244
$AUC_{0\text{-}t}$ (ng · h/ml)	1239 ± 659	24 ± 7	2012 ± 827
$t_{1/2}$ (min)	3 ± 3	9 ± 4	163 ± 27
235 mg/m^2 TNP-470[a]			
T_{max} (h)	2.6 ± 1.8	2.3 ± 2.1	4.1 ± 0.1
C_{max} (ng/ml)	490 ± 148	13.0 ± 9.5	415 ± 215
$AUC_{0\text{-}t}$ (ng · h/ml)	1459 ± 820	29 ± 14	1445 ± 755
$t_{1/2}$ (min)	2 ± 1	9 ± 7	156 ± 13

[a] Day 1, $n = 6$; day 22, $n = 5$.
[b] T_{max}, time to maximum plasma concentration; C_{max}, maximum plasma concentration; $AUC_{0\text{-}t}$, area under the curve, time 0 to t; $t_{1/2}$, half-life of elimination.

DISCUSSION

Because of its broad antitumor activity demonstrated in preclinical studies, TNP-470 is being pursued for clinical development. TNP-470 inhibited endothelial cell proliferation at concentrations of 10 pg/ml, whereas cytotoxicity against malignant cells was achieved only at concentrations in the microgram range (9). Clinical trials have attempted to use this two-log difference in specificity of TNP-470 to achieve antiangiogenic (and hence antitumor) response at concentrations that would cause minimal systemic toxicity. Although plasma pharmacokinetics of TNP-470 suggest a very short half-life, preclinical studies suggest that the biological effects of TNP-470 on endothelial cells may last longer.

This study describes our clinical experience with TNP-470 given on a once-weekly schedule. The principal DLT was cerebellar neurotoxicity, which had been observed in animal toxicology studies and also reported in Phase I studies using a shorter duration of infusion with more frequent dosing (18). Interestingly, the pattern of neurotoxicity seems to correlate with the cumulative weekly dose of TNP-470 irrespective of the schedule. Kudelka *et al.* (17) reported similar neurotoxicity in patients treated at 71.2 mg/m^2 i.v. q.o.d. (cumulative weekly dose of 284.8 mg/m^2), whereas we encountered dose-limiting neurotoxicity at 235 mg/m^2 i.v. once a week. Dezube *et al.* used lower doses (up to 70 mg/m^2 i.v.) once a week (as 1-h infusion) without observing neurotoxicity, which also argues for a dose-related effect. The neurotoxicity developed after a few weeks of treatment, was dose-related, and completely reversible on stop-

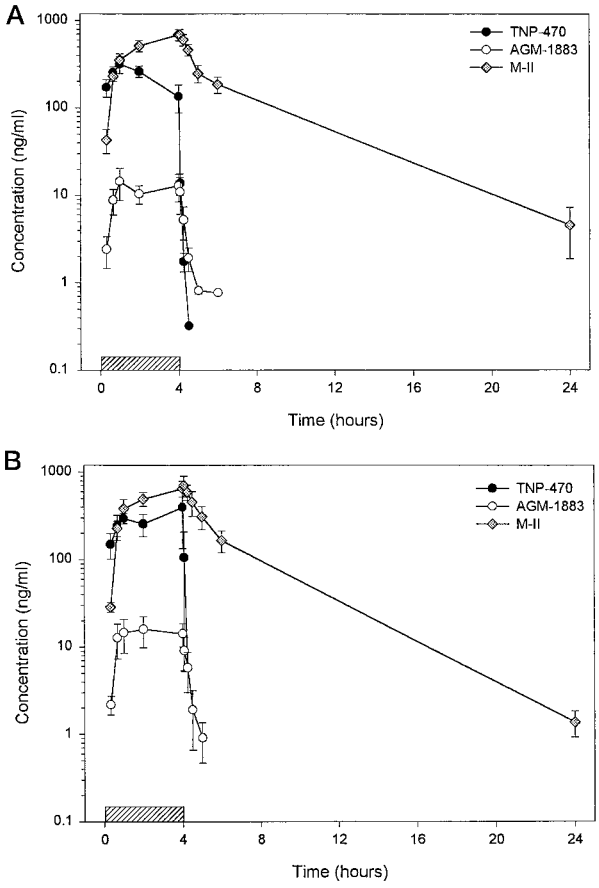

Fig. 1 Plasma concentrations of TNP-470, AGM-1883, and MII as a function of time on (*A*) day 1 ($n = 6$) and (*B*) day 22 ($n = 5$) of treatment in patients treated with TNP-470 at a dose of 177 mg/m^2 weekly as a 4-h infusion. *Error bars*, SD; *horizontal hatched bar*, duration of infusion.

ping the drug. The mechanism of neurotoxicity remains unexplained. However, because TNP-470 has a short plasma half-life, the neurotoxicity may be related to the accumulation of a metabolite(s) in the nervous system. This pattern of neurotoxicity is reminiscent of that seen with cytotoxic drugs like 1-β-D-arabinofuranosylcytosine (23), 5-fluorouracil (24) and ifosfamide (25), in which the accumulation of parent drug and/or metabolite in the nervous system has been implicated.

During toxicology studies of TNP-470, bleeding complications and neurotoxicity in animals were seen as potential hurdles precluding human use (18). Animals treated at high doses of TNP-470 developed brain hemorrhages, pulmonary and retinal hemorrhages, narrowing of retinal vessels, and bone marrow hypocellularity. Investigators studying TNP-470 in patients with HIV-associated Kaposi's sarcoma have reported retinal hemorrhages (26). However, no bleeding complications or changes in retinal vasculature were seen among patients treated in this study. Weight loss and cataract formation, also observed in animal studies, were seen in one patient each, and thought not to be related to TNP-470 administration. Three patients received treatment for >6 months without any cumulative toxicities.

Evaluation of metabolism in human hepatic microsomes had revealed rapid and extensive metabolism of TNP-470 into at least six metabolites within 30 min (27). The principal metabolite, AGM-1883 (M-IV) is formed by the cleavage of the chloroacetyl moiety, which is further metabolized by microsomal epoxide hydroxylase to M-II. TNP-470 was undetectable 60 min after being added to cells. Pharmacokinetics in the rhesus monkey showed a rapid clearance of TNP-470 with a plasma half-life of 30 min or less (28). Consistent with animal experiments, pharmacokinetic analysis from this study revealed a very short half life ($t_{1/2}$) for TNP-470 and its principal active metabolite, AGM-1883 (3 ± 3 and 9 ± 4 min, respectively, at the 177-mg/m^2 dose level). MII, which is thought to be an inactive metabolite, had a longer $t_{1/2}$ of approximately 2.6 h. Similar results have been reported in a study of patients with HIV-associated Kaposi's sarcoma (29). Significant variability was seen within C_{max} and *AUC* values of patients at the same dose level, which probably reflects the large interindividual differences in metabolism observed in studies with human hepatic microsomes (27). No plasma accumulation of the parent drug or metabolites was observed on weekly dosing. Concentrations achieved in plasma were significantly higher than those required for inhibiting endothelial cell proliferation *in vitro*, and comparable to concentrations required for inhibiting tumor-induced neovascularization *in vivo*. Although studies *in vitro* show sustained inhibition of endothelial cells after a single exposure to TNP-470 (9), plasma pharmacokinetics in humans would argue for a protracted infusion schedule.

Investigators have reported rare and often minor objective tumor responses in patients with HIV-associated Kaposi's sarcoma, cervical carcinoma, and renal cell carcinoma using a once-weekly (26) or q.o.d. (17, 30) schedule. Similarly, no significant antitumor activity was seen in this study using a weekly dosing schedule of TNP-470. However, some patients had prolonged periods of disease stabilization or extended recurrence-free intervals. Although the disease course of these patients may reflect the biological behavior of their cancers, these observations are also consistent with the hypothesis that antiangiogenic agents can prevent (or delay) the recurrence of cancer if instituted when the tumor burden is minimal. The observation that TNP-470 inhibited the development of lung/liver metastases in human tumor xenograft models in a dose-dependent manner also supports this hypothesis (31).

In conclusion, this study demonstrates that TNP-470, a potent antiangiogenic agent, can be given safely to patients as a weekly 4-h infusion up to a dose of 177 mg/m^2. The predominant toxicity from TNP-470 was neurotoxicity that occurred in a dose-dependent manner and was fully reversible on stopping treatment. The short plasma $t_{1/2}$ and the absence of objective antitumor activity seen with this regimen support exploration of protracted infusion schedules.

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