Multi-Institutional Study of the Angiogenesis Inhibitor TNP-470 in Metastatic Renal Carcinoma

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Purpose: **Renal cell carcinoma is resistant to most chemotherapy, and only a minority of patients respond to immunotherapy. Its highly vascular nature suggests that antiangiogenesis therapy might be useful. We thus performed a phase II study of the fumigillin analog TNP-470 in previously treated patients with metastatic renal cell carcinoma.**

Patients and Methods: **Metastatic renal cell carcinoma patients with good organ function were entered onto the study through five separate institutions. There were no exclusion criteria for prior therapy. All patients were treated at a dose of 60 mg/m^2 of TNP-470 infused over 1 hour three times per week.**

Results: **Thirty-three patients were enrolled. Therapy was generally well tolerated, but asthenia, fatigue, vertigo, dizziness, sense of imbalance, and loss of concentration were common and severe enough to lead to therapy discontinuation in five patients. There was only one partial response of short duration (response rate, 3%, 95% confidence interval, 0% to 16%), but six patients (18%) remained on study for 6 or more months without toxicity or disease progression.**

Conclusion: **Long-term therapy with TNP-470 has manageable toxicities and is feasible in patients with metastatic renal cell carcinoma but does not lead to any significant objective responses. Further studies in this population using TNP-470 schedules that produce more prolonged drug levels and clinical trial end points other than objective tumor regression may be indicated.**

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TREATMENT OF METASTATIC renal cell carcinoma is woefully inadequate, and approximately 11,900 Americans will die of disease in 1999.[1] Although immunotherapy with interleukin-2 (IL-2), interferon alfa (IFNα), or both is considered standard, only 15% of individuals experience an objective response and only 5% experience a complete response.[2-5] Renal cell cancer is also resistant to most chemotherapeutic agents, in part due to overexpression of various multidrug resistance proteins.[6,7] Novel approaches to this disease are thus needed. Because of the vascular nature of renal tumors, an antiangiogenesis approach is attractive. Furthermore, experimental studies have suggested that such an approach may circumvent the development of drug resistance.[8]

One of the first antiangiogenesis compounds to undergo clinical testing is the fumigillin analog TNP-470. Fumigillin was originally isolated from *Aspergillus fumigatus* contaminating endothelial cell cultures.[9] Subsequent studies revealed that fumigillin was a potent inhibitor of endothelial growth in vitro as well as in vivo, but that administration to animals led to profound weight loss.[9] A number of analogs were then synthesized, and TNP-470 was selected as the least toxic compound with the greatest antiangiogenic activity.[9,10] TNP-470 inhibits in vitro endothelial cell proliferation, including growth stimulated by basic fibroblast growth factor (bFGF).[9-11] Additionally, it inhibits angiogenesis in the chorioallantoic membrane and rat corneal assays.[9-11] It can also inhibit tumor growth and metastases in a number of rodent tumor model systems, including a rodent renal cell carcinoma.[9,10,12-14]

Animal studies have revealed that the highest doses lead to myelosuppression, microhemorrhages in various organs, and neurotoxicity consisting of seizures, tremors, and ataxia.[15] Phase I studies in humans have revealed reversible neurotoxicity as the major dose-limiting toxicity. These consisted of fatigue/asthenia, weakness, nystagmus, diplopia, vertigo, dysmetria, and truncal ataxia.[16-18] Hemorrhage into a CNS lymphoma and into a cytomegalovirus retinitis lesion was also observed in AIDS patients with Kaposi's sarcoma.[16] One patient with biopsy-proven metastatic cervical carcinoma on a phase I study experienced a complete and long-lasting response in her lung lesions.[17]

We initiated a phase II study of TNP-470 in patients with refractory metastatic renal cell carcinoma using a three times weekly bolus infusion. We confirm previous observations of asthenia and cerebellar toxicities as the most significant adverse events. We also report one partial response and several patients with prolonged freedom from progression and suggest that this compound may be worthy of further study in this population.

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PATIENTS AND METHODS

Patients

Patients were required to have recurrent or inoperable renal cell carcinoma, a World Health Organization performance status of 0 to 2, and no history of other invasive cancers in the previous 5 years. There were no exclusions for prior therapy, but at least 3 weeks had to have elapsed since the last dose of the prior drug (6 weeks for nitrosoureas and 4 weeks for any investigational agent). At least 4 weeks had to have elapsed since any prior surgery or radiotherapy, and any previously irradiated lesions could not be used for response evaluation. Patients were required to have adequate organ function defined by serum creatinine $\leq$ 2.0 mg/dL; total bilirubin, ALT, and alkaline phosphatase $\leq$ 2 times the upper limit of normal; hemoglobin $\geq$ 9.0 g/dL; platelet count greater than 100,000/μL; WBC count greater than 3,000/μL; and absolute neutrophil count greater than 1,500/μL. In addition, patients were required to have a normal prothrombin time and partial thromboplastin time, no history of bleeding diathesis, and could not receive concomitant anticoagulation except for maintenance of central-line patency. Finally, all patients provided written informed consent before participating. Thirty-three patients were enrolled between January and June 1997 at five separate institutions: University of Chicago, Chicago, IL (eight patients); Northwestern University, Chicago, IL (nine patients); Dana Farber Cancer Institute, Boston, MA (nine patients); University of Illinois, Chicago, IL (six patients), and Loyola University, Chicago, IL (one patient).

Therapy and Dose Modifications

TNP-470 (60 mg/m^2) was administered by intravenous infusion over 1 hour three times per week. The first week of therapy was administered in the outpatient area of the parent institution. If no significant toxicities were encountered, subsequent infusions could be administered at the patient's home with the use of a home nursing service. Any grade 3 or 4 toxicity led to therapy discontinuation until toxicity resolved to patient's baseline or $\leq$ grade 1. Therapy could then be reinitiated at a dose of 45 mg/m^2 three times per week. Recurrent grade 3 or 4 toxicity required therapy discontinuation.

Patient Follow-up and Study End Points

The primary end points for this study were objective tumor response rate and toxicity assessment. Response evaluations were performed after an initial 12 weeks of therapy and every 8 weeks thereafter. Toxicity evaluations were performed every 2 weeks for the first 8 weeks and then every 4 weeks thereafter. Interim evaluations were performed in patients who experienced clinical signs or symptoms of toxicity or disease. Partial response was defined as a 50% or greater decrease in the sum of the products of diameters of all measurable lesions persisting for at least 4 weeks without the development of any new lesions. Complete response was defined as disappearance of evidence of tumor persisting for at least 4 weeks. Progressive disease was defined as a 25% or greater increase in the sum of the products of diameters of all measurable lesions or the appearance of any new lesion. All other situations were defined as stable disease. Toxicity grading was by the National Cancer Institute common toxicity criteria.

Statistical Considerations

Patient accrual was performed with a two-step mechanism. An initial 19 patients were accrued, with at least one response needing to be observed to proceed to the second accrual stage. The null hypothesis to be tested in the first stage was that the response rate was 15% or greater. If no patients responded then the null hypothesis could be rejected with a confidence of greater than 95%. The second stage was designed to accrue a cumulative total of 30 patients such that the SE on the response rate would be $\leq$ 0.09. To maintain simultaneous commitments made to patients at the various participating institutions, the total accrual was 33 patients. All patients were deemed assessable for response, survival, and toxicity. Event-free survival was defined as the time from the start of therapy until discontinuation as a result of either progressive disease or a toxic event. Overall survival was defined as the time from the start of therapy until death. All survival estimates were censured as of August 1, 1998, and calculated by the Kaplan-Meier method.

RESULTS

Table 1 depicts baseline characteristics of all patients. Because there was no exclusion criteria for prior therapy, a heavily pretreated group was enrolled. In fact, 30 (91%) of 33 patients had received at least one prior therapy, and in all cases this included IL-2 and/or IFNα. Twenty-seven percent of patients also underwent prior chemotherapy, usually in the

Table 1. Baseline Characteristics of All Enrolled Patients

Characteristic	No. of Patients
Total enrolled	33
Sex (M/F)	25/8
Age, years	
Median	58
Range	40-81
Performance status (World Health Organization)	
0	11
1	17
2	5
Number of metastatic sites	
1	7
2	16
$\geq$ 3	10
Sites of metastatic disease	
Lung only	4
Lymph node only	1
Liver (with or without other sites)	6
Bone (with or without other sites)	10
Prior nephrectomy	24
Interval, nephrectomy to therapy, months	
Median	41
Range	7-277
Interval, metastatic disease diagnosis to therapy, months	
Median	14
Range	2-84
Prior therapy for metastatic disease	
Immunotherapy	30
Chemotherapy	8
Radiation	10
Surgery	3
Other	4
Number of prior regimens	
Median	2
Range	0-7

NOTE. Metastatic sites are defined as the number of organs involved. Multiple enlarged lymph nodes were still considered one site of disease.

context of a clinical trial. This selection bias favored the accrual of patients with indolent disease, and the median interval from diagnosis of metastatic disease to study initiation was 14 months. The median interval between nephrectomy, in those patients who underwent this procedure, and study initiation was even longer at 41 months. Nevertheless, patients had a significant disease burden and 30% had three or more metastatic sites. In addition, only 33% of patients had no disease symptoms and a normal performance status.

The primary study end point was response rate. There was one response among the first 19 patients, and, therefore, the study proceeded to the second stage, in which no additional responses were observed. The overall response rate was thus 3% (95% confidence interval, 0% to 16%). The lone responder had a 1.4 × 1.4 cm paratracheal lymph node and two approximately 1.0-cm lung nodules, which regressed at the initial 12-week evaluation. He was, however, removed from the study at that time due to the development of asthenia and neurocortical toxicity and subsequently progressed and died 8 months after study discontinuation as a result of progressive metastatic renal cancer. Figure 1 depicts event-free survival, with events being defined as progressive disease or toxicity requiring study discontinuation. The median event-free survival was 12 weeks (Fig 1). Six patients (18%) remained on study without toxicity or progressive disease for 6 months or more. Five of these six patients had progressive disease on prior therapy, and one had recurrent disease after adjuvant IFNα therapy. As of this report, three patients remain on study at 59+, 60+, and 72+ weeks. Reasons for therapy discontinuation were progressive disease in 20 patients, drug toxicity in five, and intercurrent event (for which relatedness to drug therapy could not be definitively determined) in five. These latter events included a gastrointestinal hemorrhage on day 3 of therapy, depression and suicidal ideation on day 3, hypercalcemia and increasing back pain during week 4, a line infection during week 19, and a pulmonary embolism during week 47. At a median follow-up of 14 months, the median survival is 56 weeks (Fig 1).

Table 2 depicts observed toxicities that were interpreted to have some possible relation to the study drug by each investigator. Therapy, was, in general, well tolerated, although neurocortical toxicities were common. These symptoms were not usually associated with objective neurologic findings. Because no formal neuropsychiatric tests were performed, they were conveniently categorized as cerebellar symptoms, confusion, and other psychiatric symptoms. In total, 67% of patients experienced at least one of these toxicities. Although these toxicities were usually mild, in five patients they were considered severe and led to drug discontinuation. Fatigue and asthenia were also common

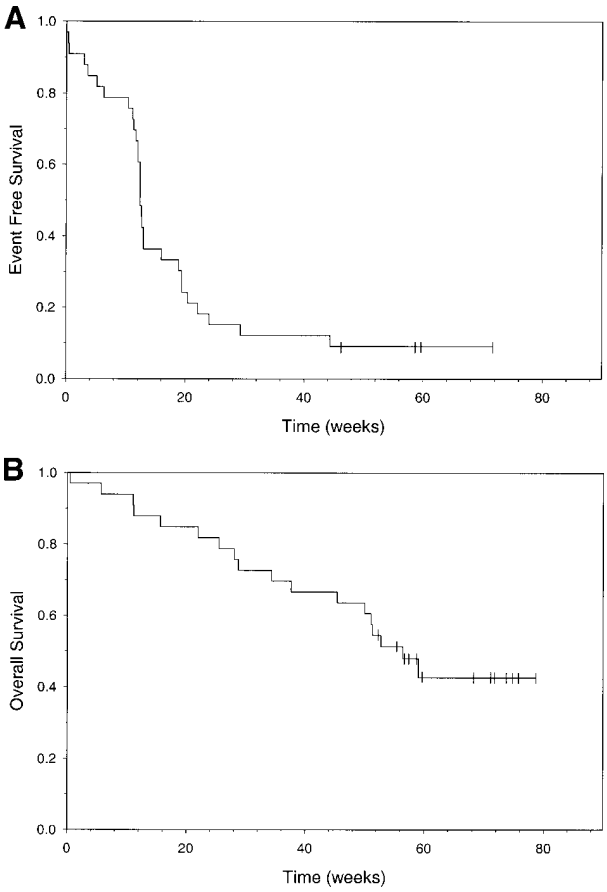

Fig 1. (A) Event-free and (B) overall survival for all enrolled patients. Tick marks represent censured patients. Events included progressive disease and toxicity necessitating discontinuation of therapy.

and occurred in 60% of patients. Because therapy was administered three times per week, and because there was poor documentation in the clinical notes, it was difficult to discern whether the fatigue was worse on the treatment days. However, patients did not seem to become tolerant to these effects, nor did there seem to be a cumulative effect, as is seen with prolonged IFN-α or IL-2 therapy. All fatigue and neurocortical symptoms resolved rapidly after TNP-470 was discontinued.

Other severe toxicities included pain, pulmonary embolism and gastrointestinal hemorrhage (as noted previously), and hypotension. The treating physician interpreted the first three events to be most likely disease-related, but contribution from TNP-470 could not be ruled out. The hypotension event was correlated with drug infusion and persisted during treatment and was thus considered probably drug-related. Five patients underwent a protocol-defined dose reduction from 60 to 45 mg/m^2 due to neurologic toxicities.

Table 2. Total Number of Patients Who Experienced an Adverse Event Interpreted by Investigator to Have Some Relation to the Study Drug

Toxicity	Grade 1	Grade 2	Grade 3	Grade 4
Cerebellar symptoms*	21	8	4	0
Confusion	8	2	0	0
Other psychiatric†	15	5	1	0
Fatigue/asthenia	9	7	2	0
Pain‡	16	4	1	0
Fever/chills	7	0	0	0
Anorexia	8	4	0	0
Nausea/vomiting	11	1	0	0
Other§	0	0	2	1

NOTE. A large number of unusual toxicities were described in various ways by individual investigators. Several have been grouped as symptom complexes. Some patients had more than one toxic event recorded. See Results for percentage of patients experiencing selected toxicities.

*Abnormal gait, ataxia, dizziness, incoordination, tremor, and vertigo.

†Abnormal dreams, anxiety, depression, emotional lability, insomnia, nervousness, and somnolence.

‡Abdominal pain, back pain, chest pain, flank pain, headache, pelvic pain, and pain not otherwise specified.

§Gastrointestinal hemorrhage (grade 4), pulmonary embolism, hypotension.

DISCUSSION

We have demonstrated that TNP-470, when administered to a group of heavily pretreated patients with metastatic renal cell carcinoma, is well tolerated, even when administered for prolonged time periods. TNP-470 administration, however, does not lead to significant objective responses; thus this trial did not meet its primary objective, and TNP-470 must be considered ineffective per the original trial design. Based on animal data, it has been hypothesized that TNP-470 may only prevent further tumor growth and not necessarily lead to tumor shrinkage.[12-14] Whether the prolonged median survival and prolonged progression-free survival in several patients was due to TNP-470 or simply a reflection of these patients' natural disease history cannot be determined by this study.

TNP-470 has now been evaluated in several clinical trials.[16-18] Our study supports observations in previous studies of low response rate and good general tolerability, but asthenia and neurocortical toxicity are dose-limiting. Nevertheless, our observations suggest that further studies with TNP-470 may be indicated. Such studies should seek to specifically answer the question of whether TNP-470 truly delays progression and may be best performed in the minimal disease or adjuvant setting.[9,12] Future trials should also include more formal neuropsychiatric evaluations to better delineate the neurocortical toxicities and perhaps identify patients who are particularly vulnerable to such difficulties. Combination studies of TNP-470 and other known or putative angiogenesis inhibitors may also be indicated. A potentially attractive candidate for metastatic renal cell carcinoma is IFNα, which has both antiangiogenesis and direct antitumor properties.[19]

Before such studies commence, it is likely that a more convenient formulation for TNP-470 will need to be developed. Animal studies suggest that prolonged exposure to TNP-470 may be necessary to fully realize all of its antiangiogenic properties. Pharmacokinetic studies, however, have shown that the half-life of TNP-470 and its active metabolite are only 2 and 6 minutes, respectively (M. Dordal, personal communication, September 1998).[16,18,20,21] Ongoing studies should determine the feasibility and toxicity of more prolonged exposure to TNP-470.

In conclusion, TNP-470 is one of the first specific antiangiogenesis drugs to undergo wide clinical evaluation. Our study shows that prolonged therapy with TNP-470 is feasible and tolerable in patients with metastatic renal cell cancer but does not lead to an appreciable objective response rate. Additional studies with formulations or schedules that give more prolonged TNP-470 exposure to determine its effect on tumor progression in renal cell carcinoma may be indicated.

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