Phase II Trial of Tetrathiomolybdate in Patients with Advanced Kidney Cancer[1]

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ABSTRACT

Purpose: **Tetrathiomolybdate (TM), a copper-lowering agent, has been shown in preclinical murine tumor models to be antiangiogenic. We evaluated the antitumor activity of TM in patients with advanced kidney cancer in a Phase II trial.**

Experimental Design: **Fifteen patients with advanced kidney cancer were eligible to participate in this trial. TM was initiated p.o. at 40 mg three times a day with meals and 60 mg at bedtime to deplete copper. A target serum ceruloplasmin (CP) level of 5–15 mg/dl was defined as copper depletion. Doses of TM were reduced for grade 3–4 toxicity and to maintain a CP level in the target range. Once copper depletion was attained, patients underwent baseline tumor measurements and then again every 12 weeks for response assessment. Patients not exhibiting progressive disease at 12 weeks after copper depletion continued on treatment. Serum levels of Interleukin (IL)-6, IL-8, vascular endothelial growth factor (VEGF) and basic fibroblast growth factor (bFGF) were assayed pretreatment and at various time points on treatment. Dynamic contrast enhanced-magnetic resonance imaging (DCE-MRI) was performed on selected patients in an attempt to assess changes in tumor vascularity.**

Results: **All of the patients rapidly became copper depleted. Thirteen patients were evaluable for response. No patient had a complete response or PR. Four patients (31%) had stable disease for at least 6 months during copper depletion (median, 34.5 weeks). TM was well tolerated, with dose reductions most commonly occurring for grade 3–4 granulocytopenia of short duration not associated with febrile episodes. Serum levels of IL-6, IL-8, VEGF, and bFGF did not correlate with clinical activity. Serial DCE-MRI was performed only in four patients, and a decrease in vascularity seemed to correlate with necrosis of a tumor mass associated with tumor growth.**

Conclusions: **TM is well tolerated and consistently depletes copper as measured by the serum CP level. Clinical activity was limited to stable disease for a median of 34.5 weeks in this Phase II trial in patients with advanced kidney cancer. Serum levels of proangiogenic factors IL-6, IL-8, VEGF, and bFGF may correlate with copper depletion but not with disease stability in this small cohort. TM may have a role in the treatment of kidney cancer in combination with other antiangiogenic therapies.**

INTRODUCTION

Approximately 31,800 new cases of kidney cancer occur annually in the United States with an associated 11,600 deaths (1). The incidence and death rate from kidney cancer have increased over the last 2 decades, although the reason for this increase is unknown (2). One-third of the patients who receive a diagnosis of kidney cancer present with metastatic disease. Of the patients who present with local disease and are considered for surgery with curative intent, approximately one-third will go on to develop metastatic disease. Metastatic kidney cancer is resistant to all "standard" forms of radiation therapy, chemotherapy, and hormonal therapies used in the treatment of other kinds of carcinomas.

In the United States, IL-2[3] is the only Food and Drug Administration-approved systemic treatment for metastatic kidney cancer. High-dose bolus IL-2 has resulted in an overall response rate of 15% in patients with metastatic kidney cancer. Approximately 7% of patients achieve a CR, with 80% of these CRs maintained beyond 7 years.[4] Despite these encouraging results of durable CRs, the vast majority of patients with advanced kidney cancer are either not eligible to receive, or do not derive benefit from, IL-2. A critical goal, therefore, in the

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[3] The abbreviations used are: IL, interleukin; VEGF, vascular endothelial growth factor; bFGF, basic fibroblast growth factor; TGF-α, transforming growth factor α; TM, tetrathiomolybdate; CP; ceruloplasmin; MR, magnetic resonance; DCE, dynamic contrast enhanced; DCE-MRI, DCE-MR imaging; CR, complete response; PR, partial response; VHL, von Hippel-Lindau; AUC, area under the curve; IAUC, initial area under the enhancement curve.
[4] Proleukin: Update of Product License Application, 1996 (on file, Food and Drug Administration).

treatment of advanced kidney cancer is the development and evaluation of new therapies.

Angiogenesis is an essential process necessary for the growth and progression of tumors and, therefore, a potential target for the treatment of cancer. Several proangiogenic molecules are known from experimental models of tumor angiogenesis such as VEGF, bFGF, IL-8, and TGF-α. These factors are increased in the serum of some patients with cancer and may even have prognostic significance (3).

Kidney cancer is a tumor that is characterized by a high degree of vascularity. Kidney cancer is associated with VHL hereditary syndrome, and the majority of sporadic kidney cancers have loss of heterozygosity at chromosome 3p (spanning the region of the *VHL* gene) or exhibit mutations of the *VHL* gene itself (4, 5). Inactivation of the *VHL* gene is associated with a loss of VEGF suppression and may be one potential explanation for the highly vascular nature of kidney cancer (6). Serum levels of proangiogenic molecules such as VEGF, bFGF, and IL-6 are elevated in patients with advanced kidney cancer, although no causal relationship between these factors and the malignant phenotype has been established (7, 8).

We have been investigating the role of copper as an essential trace element that is required for angiogenesis. It has been demonstrated that copper is required for angiogenesis and tumor growth in several murine tumor models (9–11). Recently, we have evaluated the role of TM in angiogenesis. TM is a copper-lowering agent that has been evaluated extensively in the treatment of Wilson's disease (12–14). TM forms a stable tripartite complex with copper and protein. When given with food, it complexes food copper with food protein and prevents absorption of copper. When TM is administered in the nonfed state, it is absorbed into the blood, in which it complexes either free or loosely bound copper with serum albumin. This TM-bound copper fraction is no longer available for cellular uptake, has no known biological activity, and is slowly cleared in bile and urine. TM has been shown to decrease serum copper levels as well as decrease angiogenesis and tumor growth in two different murine tumor models (15, 16).

In a Phase I trial of TM, we were able to consistently reduce copper levels to 20% of pretreatment baseline, as determined by the surrogate marker serum CP (17). We have subsequently found that higher initial doses than those reported in our Phase I trial result in a more rapid decline in serum CP levels.[5] On the basis of the results of our Phase I trial, we have evaluated TM in a Phase II trial for treatment of advanced kidney cancer.

A potential difficulty of evaluating antiangiogenic agents in the treatment of cancer is that the standard criterion for antitumor activity, tumor shrinkage, may not be applicable. Antiangiogenic agents may prevent tumor growth but not result in a measurable decrease in tumor size. This becomes especially difficult to judge in patients with advanced kidney cancer, a disease with a highly variable natural history. One-third of patients with advanced kidney cancer will meet standard criteria for stable disease while on observation (18). A minority of patients will exhibit stable disease out to 12 months (19).

To assist in the evaluation of TM therapy in patients with advanced kidney cancer, we also evaluated potential surrogate markers of antiangiogenic activity. DCE-MRI is emerging as a powerful noninvasive method of imaging the process relevant to the tumor microcirculation. Recent advances in MR technology provide the enhanced spatial and temporal resolution that allows the application of this methodology to the management of cancer patients. Several studies have shown that DCE-MRI measures correlate well with tumor angiogenesis and are sensitive to tumor physiology and to the pharmacokinetics of the contrast agent in individual tumors (20–24). The high diagnostic/prognostic value of DCE-MRI originates from its ability to provide high-resolution images that depict the perfusion and permeability of small vessels, especially the capillary network. Furthermore, MR studies are reproducible and can be used to monitor tumors longitudinally to detect changes in vascularity induced by treatment. As a result, DCE-MRI measurements offer the potential for a more accurate prediction of tumor response to therapy than do traditional tumor volume measurements.

Multiple potential proangiogenic molecules have been described. One of the mechanisms of the antiangiogenic effect of TM may be by decreasing levels of proangiogenic molecules such as IL-6, IL-8, bFGF, and VEGF secreted by the tumor and inflammatory cells (16). Serum levels of these molecules were monitored serially as part of this trial.

PATIENTS AND METHODS

Eligibility Criteria. Patients must have had documented metastatic kidney cancer who either had not responded to IL-2 or were not eligible to receive IL-2. Patients may also have received forms of therapy other than IL-2, and all prior therapy must have been completed at least 4 weeks before trial entry. Patients had to have a life expectancy of at least 5 months and a performance status 0 or 1. All of the patients had to have adequate organ function defined by: creatinine ≤20 mg/dl, total bilirubin ≤1.5 mg/dl, aspartate aminotransferase ≤2.5 times the institutional upper limit of normal, platelet count ≥100,000/mm^3, absolute granulocyte count >1000/mm^3, and hematocrit ≥29% (patients may be transfused to this level). Patients were excluded if they had an active infection, were pregnant or had active brain metastases requiring corticosteroid therapy for symptom control. Patients with a treated brain metastasis that had been stable for at least 2 months on imaging were allowed on trial. This study was approved by the University of Michigan Institutional Review Board (Medicine), and all of the patients signed an approved informed consent. This trial was also approved by the University of Michigan General Clinical Research Center.

Study Design. Before treatment, all of the patients had a complete history and physical and baseline laboratory evaluation including: complete blood count with differential and platelet count, serum chemistry profile to include creatinine, aspartate aminotransferase, and total bilirubin, β HCG in women of childbearing potential, and baseline CP level. Pretreatment serum levels of VEGF, bFGF, IL-6, and IL-8 were obtained. Patients underwent studies as indicated to determine the extent and sites of metastatic kidney cancer within 6 weeks of the start

[5] Unpublished observations.

of treatment (to include imaging of the brain). Those patients who were thought to have an appropriate lesion for DCE-MRI also underwent this procedure. Appropriate lesions for DCE-MRI were nonpulmonary, nonosseous lesions.

Patients initially received TM at 40 mg three times a day with meals and 60 mg at bedtime. Patients kept a medication log to document compliance. A CP level was obtained weekly for 8 weeks, then every 2 weeks. The target CP level was 5–15 mg/dl. Complete blood count was checked every 2 weeks and chemistry profile every month. Patients were evaluated with a history and physical at least monthly. Standard Common Toxicity Criteria version 2.0 was used for toxicity monitoring. If a patient had a decline in hematocrit to <80% of baseline or any other grade 3 or 4 toxicity, TM was held for 5 days. TM was then restarted at 40 mg p.o. with meals twice a day (omitting one with-meal dose) with the once daily 60 mg dose at bedtime maintained. After a TM dose adjustment, CP levels were again monitored weekly until a CP level of 5–15 mg/dl was achieved. If an additional dose reduction was required, then the bedtime dose was reduced to 40 mg once daily. Subsequent dose adjustments were made for toxicity in an attempt to maintain CP levels at 5–15 mg/dl. Dose adjustments were not to occur more frequently than every 2 weeks. Patients were not to be taking multivitamins or nutritional supplements that contain minerals and were asked to avoid organ meats in their diet.

When the CP level reached 5–15 mg/dl (defined as the onset of copper deficiency) patients underwent evaluation for baseline tumor measurement with appropriate radiological studies. Response Evaluation Criteria in Solid Tumors (RECIST) were used for response assessment. Patient were then evaluated for response at 12 weeks from this baseline evaluation, at which time they also underwent DCE-MRI of the same lesion that was evaluated pretreatment. Patients who did not exhibit progressive disease could remain on trial. In addition to CR and PR, stable disease for 6 months was also considered a beneficial response. For patients who remained on study, response assessments continued at 12-week intervals. Serum levels of VEGF, bFGF, IL-6, and IL-8 were obtained at the same time points as imaging studies.

Criteria for Discontinuing Protocol Treatment. Protocol treatment was discontinued for patient preference, unacceptable toxicity, or progressive disease. In this protocol, the first time point for progressive disease was 12 weeks after reaching copper deficiency. Evidence of tumor growth on radiological evaluation at the time of attaining copper deficiency compared with pretreatment was to be expected and was not accepted as treatment failure.

Assays for VEGF, bFGF, IL-6, and IL-8. Blood from patients and normal volunteers was collected in a serum separator tube and was allowed to clot for 30 min before centrifugation at 1000 × *g* for 10 min. Serum was immediately frozen (−70 C) in aliquots of 0.75 ml in microcentrifuge tubes. Human VEGF and human bFGF ELISAs were performed as directed by the manufacturer (R&D Systems, Minneapolis, MN). Briefly, serum (100 μl) was pipetted in triplicate onto the wells precoated for a monoclonal antibody specific for each factor and incubated for 2 h. After 3 washes to remove unbound substances, an enzyme-linked monoclonal antibody specific for each factor was added to the wells and incubated for 2 h. After a wash to remove unbound antibody-enzyme reagent, a substrate solution was added onto the wells and allowed to incubate for 30 min. Optical intensity of each well was measured using a microplate reader. ELISAs for IL-6 and IL-8 were performed by the University of Maryland Cytokine Core Laboratory (Baltimore, MD). For the analysis of cytokine levels, *t* tests were used to compare normal controls with the baseline levels for cases, after performing a log transformation on the levels to approach normality. For comparisons of posttreatment values with baseline levels, paired *t* tests were used.

MR Imaging and Analysis. The MR studies were performed on a 1.5T magnet (Signa; GE Medical System, Milwaukee, WI) with a torso phased-array coil. After a routine (noncontrast) MR exam, DCE-MRI was performed using a three-dimensional spoiled gradient-echo sequence: (TR, 5.2–7.5 ms; TE, 1–2 ms; flip angle, 30°; section thickness, 10–22 mm with zero interpolation yielding an effective slice thickness of 5–11 mm; sections, 12, spectral fat suppression, FOV, 38–42 cm; phase FOV, 0.75; matrix, 224 × 128, NEX 0.5; acquisition time, 6–8 s per temporal phase). Imaging plane was the sagittal or sagittal oblique to encompass the entire tumor and aorta, and to reduce the in- and out-of-plane effect of respiratory motion on the tumor. After a pre-contrast acquisition, DCE imaging was performed after a bolus injection of 0.1 mmol/kg gadolinium chelate (Gadopentetate Dimeglumine-Magnevist; Berlex Laboratories, Wayne, NJ) at a rate of 2 ml/sec via a power injector (Spectris, Medrad, Pittsburgh PA). Automated contrast-bolus detection (SmartPrep) was used to time the dynamic studies. The three-dimensional acquisitions were obtained in groups of three phases, each group requiring an 18–24-s breath-hold. Twenty-five acquisitions were obtained over 4 min. Two additional delayed phases were obtained at 6 min.

Quantitative analyses of the DCE-MRI data were performed using two analysis methods: the AUC, described by Evelhoch (25) with slight modification (26) using the aorta instead of muscle (as was initially described by Evelhoch) as the reference tissue; and two-compartment analysis (27, 28). The dynamic acquisitions were coregistered manually to minimize the effect of motion on the tumor volume. Tumor volume was manually defined by drawing volume of interest (VOI) around the tumor on all 12 slices. This VOI was also applied to calculate tumor statistics for each DCE-MRI parameter. For the AUC, regions of interest were defined over the aorta to provide reference tissue. Gadolinium concentration curves were generated using assumed T_1 values. The reference tissue concentration curves were then used to normalize the tumor AUC. The normalized AUC for each tumor; a hypothesized measure of tumor vascularity, was assessed at 30, 60, and 90 s after contrast injection, also called the IAUC (25).

For the two-compartment analysis, standard measures including the rate constant (κ_{ep}) and the transfer constant (K^{trans}) were assessed (28). These parameters are also hypothesized to be related to tissue vascularity. The aorta was used to provide the arterial input function (AIF).

When multiple tumor nodules were present, the most vascular tumor nodule was chosen for the analysis. The same nodule was analyzed at all time points.

Percentages of change in DCE-MRI parameters were calculated relative to pretreatment values using the following formula:

Table 1 Patient characteristics

Characteristic	*n*	%
Total	15	
Male	11	73
Female	4	27
Age (yr)		
Median	59	
Range	46–78	
Performance status		
0	13	86
1	2	14
Prior treatment		
IL-2	11	73
Chemotherapy	3	20
None	3	20
Nephrectomy	13	87
Dominant sites of disease		
Lung (lung only)	10 (5)	66
Liver	5	33
Bone	2	13
Primary	2	13
Adrenal	1	7
Time from diagnosis of stage 4 to study entry (mo)		
Median	21	
Range	5–34	

$$Posttreatment\ vascularity - \frac{Pretreatment\ vascularity}{Pretreatment\ vascularity} \times 100$$

Statistical Design. The original design for this study was a Minimax two-stage Phase II study design (29). This design suggested an initial accrual 13 eligible patients. If none of those 13 attained a positive response, then the trial would be stopped. Positive response was defined as CR, PR, or stable disease. If one or more patients had a positive response, then an additional 14 patients would be entered. According to this design, if the response rate was as low as 5%, there would be a 51% chance of stopping the trial after just 13 eligible patients and only a 4.2% chance of going on to a larger study (false positive error). However, if the true response rate was 20% for TM in renal cell cancer patients, there would be only a 5% chance of stopping the study early and a 19.9% chance of rejecting the therapy for further study (false negative). The average sample size, if the same design were used over and over, is 19.8 patients if the true response rate is 5%.

RESULTS

Fifteen patients were enrolled between October 2000 and February 2001. Patient characteristics are shown in Table 1. Most of the patients had received at least one prior therapy for metastatic kidney cancer (usually high-dose IL-2), and 13 had undergone nephrectomy. As expected for this population, the predominant site of metastases was the lung with six patients having only lung disease. Five patients had predominant liver metastases and two had bulky primaries in place (one with bilateral kidney cancer). The median time from initial diagnosis to metastasis was 2 years with a median time from metastasis to study enrollment of 21 months. The study population was healthy with only 2 patients exhibiting any symptoms of their disease and 13 patients asymptomatic (performance status, 0).

All of the patients reached copper deficiency as defined for this study. The median time to reach a CP level ≤15 mg/dl was 5 weeks, with a range of 1–15 weeks. The one patient who did not achieve copper deficiency until 15 weeks on study was taking multiple nutritional supplements, which were not reported to the investigators during this time. Once these supplements were discontinued, the patient rapidly reached the target CP level. When this patient was excluded from analysis, the median time to copper deficiency was 4.5 weeks.

Thirteen patients were evaluable for response. Two patients who were not evaluable discontinued therapy early for reasons other than progressive disease. Both patients discontinued protocol treatment at 9 weeks on study, 4 weeks after achieving copper deficiency. One discontinued therapy secondary to toxicity (fatigue) and the other for an inability to adhere to protocol therapy. As defined in this study, the time point for the first response evaluation was when a patient was copper deficient for 12 weeks. Eight of the 13 patients met this criterion. Five patients had evidence of progressive disease before 12 weeks (median, 7.5 weeks) while they were copper deficient.

Eight patients maintained copper deficiency for 12 weeks. Of these eight patients, four exhibited progressive disease on scheduled evaluations at 12 weeks and four had stable disease. The 4 patients with stable disease continued on therapy and had a total duration of copper deficiency of 28–45 weeks (median, 34.5 weeks). All of them ultimately had progressive disease, one in sites of known disease and three with new sites of disease. Overall, the 6-month progression-free survival was 31%, and the median time to progression was 13 weeks (range, 3–45 weeks).

The patients with stable disease differed from the rest of the study population in time from first metastasis to study entry. These four patients had a median time from metastasis to study enrollment of 27 weeks compared with the nine patients who progressed more rapidly and who had a median of 13.5 weeks ($P = 0.0018$).

All of the patients were evaluable for toxicity. TM was relatively well tolerated. Only one patient discontinued therapy secondary to toxicity (fatigue). Grade 1–2 fatigue was reported by almost all of the patients, as was the occurrence of sulfurous eructation after taking TM. Other toxicities reported that did not require a dose reduction were as follows: four patients had grade 1 nausea; three patients had grade 1–2 diarrhea; four patients complained of occasional episodes of feeling dizzy without documented blood pressure changes; and two patients developed a self-limiting macular rash on their trunk.

Eleven patients had dose reductions in TM. The most frequent cause for a dose reduction of TM was grade 3 or 4 granulocytopenia. Four patients had one reduction; two patients had two dose level reductions; and one patient each, had three and four dose level reductions. After stopping TM, the granulocytopenia decreased to grade 1 or 2 by 5 days. There were no episodes of febrile neutropenia. Granulocytopenia was not seen before 12 weeks on study. Despite dose reductions in TM, the CP level remained within the study-specified target range. One patient had a single dose level reduction for anemia. Two

patients had one dose reduction of TM for a CP level that was below the range defined by the study.

Serum levels of VEGF, bFGF, IL-6, and IL-8. Serum levels of VEGF, bFGF, IL-6, and IL-8 were determined pretreatment, at the time of reaching copper deficiency, and after 3 and 6 months of copper deficiency (Table 2). For control, we used the serum levels from 29 healthy adults obtained at two different time points. The pretreatment levels of VEGF, IL-6, and IL-8 were significantly higher then control levels ($P \leq 0.0003$). Pretreatment levels of bFGF were not significantly different from unaffected controls ($P = 0.10$).

At the time of reaching copper deficiency, the levels of all four of the factors were significantly reduced from pretreatment levels ($P \leq 0.035$). At this time point, only the serum level of IL-6 remained significantly greater then control levels. At 3 months of copper deficiency, the levels of VEGF, bFGF, and IL-8 were not significantly different from pretreatment levels. Only IL-6 at 3 months remained significantly reduced from pretreatment levels.

DCE-MRI Imaging. Four patients had tumor nodules accessible for DCE-MRI and were on treatment long enough for posttreatment evaluations. The results of the two methods of analysis are shown in Table 3. There was a significant increase (>25%, WHO criteria) in the bi-product measurements of the tumor mass in all four of the patients (mean, 77%; range, 26–147%). Tumor vascularity, as assessed by the IAUC/aorta, had increased (5 and 73%) in two patients, and reduced (−5 and −22%) in two patients. Likewise, K^{trans} was increased in one patient (63%) and reduced in three patients (−11, −13, and −21%). In the three patients with increase in tumor size and reduction (or minimal increase) in tumor vascularity, there was extensive tumor necrosis. There was no necrosis in the tumor that increased in both size and vascularity.

DISCUSSION

We have evaluated TM, a novel antiangiogenic molecule in patients with advanced kidney cancer. Although the process of angiogenesis is a multifactorial process that has not been fully elucidated, copper appears to be an essential requirement of this process. TM has been shown in a Phase I cancer clinical trial and in Wilson's disease to consistently deplete copper. In this Phase II trial, we have once again confirmed the ability of p.o. administered TM to rapidly deplete copper as evaluated by serum CP levels.

TM has been relatively well tolerated. All of the patients experienced some degree of fatigue, which was generally mild, with only one patient discontinuing therapy because of this symptom. The other side effect seen in most patients was sulfurous eructation that occurred after taking TM. The toxicity that required a dose reduction of TM was granulocytopenia. However, this was never associated with a febrile episode and was rapidly reversible with holding TM for 5 days. It is difficult to state the exact etiology of the granulocytopenia, but we have made some potentially useful observations. It is not an acute toxicity, because it did not occur before 12 weeks on study. Also it seems to exhibit a complex relationship to copper deficiency as assessed by CP levels because, after dose reductions for granulocytopenia, the CP level remained in the deficient range

Table 2 Serum levels

Patient	VEGF (pg/ml)					bFGF (pg/ml)					IL-6 (pg/ml)					IL-8 (pg/ml)				
	Base	Cu def	3 mo	6 mo	9 mo	Base	Cu def	3 mo	6 mo	9 mo	Base	Cu def	3 mo	6 mo	9 mo	Base	Cu def	3 mo	6 mo	9 mo
1	261.61	16.16	294.54			12.07	6.76	6.25			42.17	28.71	36.24			45.87	21.54	51.38		
2	267.82	222.2	222.09	220.45		5.02	3.28	3.43	6.12		4.46	0	2.13	3.86		37.54	6.39	14.36	16.24	
3	424.21	294.82	273.8			6.56	2.26	2.35			29.8	9.51	15.69			30.76	3.99	4.1		
4	344.47	356.65	398.6			3.18	4.07	4.67			7.17	8.96	7.96			13.86	14.09	15.63		
5	401.25	138.32	274.43	463.23		8.11	4.71	4.5	7.43		42.85	2.84	14.63	30.57		50.03	40.53	36.57	46.2	
6	297.92	283.78	234.05			23.47	13.81	15.63			6.45	3.78	3.36			13.22	8.97	9.68		
7	925.35	766.01	252.89			2.97	1.49	6.32			12.15	9.54	11.63			15.02	7.95	9.48		
8	211.97	218.51	206.09	NA	648.87	5.33	2.49	2.83	NA	8.54	6.78	6.57	3.87	NA	7.63	63.88	72.37	63.45	NA	86.31
9	474.17	354.03				2.26	ND				6.12	4.13				6.46	1.08			
10	646.08	240.12				6.98	4.41				20.67	20.54				9.02	8.25			
11	448.42	432.04				1.05	8.58				45.5	41.63				10.84	11.67			
12	204.64	163.77	256.84			3.13	2.13	4.06			14.05	11.73	16.43			36.85	33.24	37.5		
13	128.5	130.15	132.86			4.29	4.78	4.63			26.48	21.65	19.67			9.31	11.78	11.28		
14	127.8	111.98	109.89	128.76		6	4.35	4.3	6.19		9.67	11.31	10.53	9.46		15.34	12.97	14.63	13.01	
15	345.62	304.69	368.24			5.46	6.36	6.59			33.86	24.63	30.62			42.62	35.64	40.12		

[a] Base, baseline; Cu def, copper deficient; ND, not determinable; NA, no sample available.

Table 3 DCE-MRI results

Patient no.	Pretreatment vascularity	Posttreatment-1[a] vascularity	% change	% change in biproduct diameter	Necrosis
Analysis 1, IAUC/aorta					
5	750.0582	787.4162	4.98	147	++
6	869.5683	827.1792	−4.87	26	+
8	426.5111	331.5037	−22.28	51	+++
13	231.9096	400.8148	72.83	84	No
Analysis 2, two-compartment model (K^{trans})					
5	295.4349	258.3588	−12.55	147	++
6	297.7969	236.1423	−20.70	26	+
8	114.4119	102.3782	−10.52	51	+++
13	69.0811	111.7936	61.83	84	No

[a] Posttreatment-1 vascularity, 1st on treatment evaluation at 12 weeks of copper deficiency: biproduct diameter, product of two bidimensional measurement; +, minimal; ++, moderate; +++, large.

(≤15 mg/dl) and granulocytopenia did not always recur. Two case reports of pancytopenia (30), occurring in patients who had Wilson's disease and were receiving TM, show a clinical pattern of leukopenia similar to the one that we report. These two cases, after chronic TM exposure (2 and 5 months) at high doses, also exhibited leukopenia that was rapidly reversible with the discontinuation of TM (several days). Bone marrow biopsies performed on these patients were thought to be consistent with a myeloid maturation arrest. However, these two cases, as well as two other cases associated with TM use in Wilson's disease reported in the literature (31), exhibited pancytopenia, which was not seen in our patients. The mechanism of this toxicity warrants further investigation in patients with normal copper homeostasis.

Using standard criteria of CR and PR, TM alone has shown no efficacy in patients with advanced kidney cancer. However, the standard response criteria may have no value in evaluating a drug that is not known to be cytotoxic but rather may be cytostatic. Stable disease also has its pitfalls in evaluating efficacy in a Phase II trial. Using criteria of stable disease for at least 6 months after copper depletion as defined in this trial, we found that our overall response rate was 31%. Interestingly, the four responding patients differed markedly from the rest of the study population. The median time of having metastatic kidney cancer before entering this trial was significantly greater for these responders (median of 27 weeks *versus* 13.5 weeks). In retrospect, this group of responding patients might have been expected to have a longer progression-free survival, based on the natural history of their disease before study entry. Nonetheless, we cannot say for sure that they would have; therefore, by our entry criteria and the more inclusive definition of response that includes stable disease, 31% of patients in this cohort were responders. In the future, methods will need to be developed to facilitate the clinical development of cytostatic agents and, at the same time, appropriately use patient numbers. One method is to better define the population before study entry so that it is more uniform.

Prognostic subgroups of patients with advanced kidney cancer have been defined (32). However, this method has been seen as more suited as a means to stratify patients for larger randomized Phase III trials than for Phase II exploratory trials. Another method that may be more appropriate in the Phase II setting has been used before in a trial of IFN in advanced kidney cancer (19). In this type of trial, patients are entered into the first phase, which consists of monitoring time off therapy for evidence of disease progression. Once the patients have disease progression, they are then begun on the investigational therapy. The time to disease progression on observation *versus* treatment can then be compared in each individual patient with each patient being used as his or her own control. This method may be best suited for kidney cancer trials because of the variable natural history of this disease.

Another method of evaluating cytostatic agents in small Phase II trials would be to use surrogate end points of efficacy. In antiangiogenic trials, there is no consensus as to what these surrogate end points should be (33). In our trial, we evaluated serum levels of several factors that have been associated with angiogenesis, and we also used specialized imaging to assess the vascularity of tumors. The serum levels of VEGF, IL-6, and IL-8 were significantly reduced at the time of reaching copper deficiency as compared with pretreatment levels, indicating that the attainment of copper deficiency, at least initially, correlates with a decrease in these mediators, as predicted from the laboratory. However, this effect did not persist at a later study time point. For individual patients, there was no direct correlation between these levels and response, but the trial, with only 13 patients evaluable for response, was not powered to evaluate this relationship.

We also attempted to analyze tumor vascularity by using DCE-MRI. In the four patients in whom we were able to image, a decrease in vascularity as measured by DCE-MRI did not correlate with a decrease or stability in the size of the tumor. In the few examples from this study, it appears that DCE-MRI vascularity correlates better with the degree of necrosis than with actual tumor growth as measured by tumor mass size.

The factors responsible for the neovascularity associated with malignant growth are not well delineated in all tumor types. Copper seems to be one of many necessary substrates required for this process. TM is a relatively well-tolerated copper-depleting agent that, as a single agent, achieved stable disease in 31% of patients with advanced kidney cancer. Given the natural history of this disease, this percent of stable disease may not be significantly different from that observed without treatment. Because of the multiple pathways involved in angiogenesis, TM

may be of benefit in combination with other antiangiogenic therapies.

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