Advances in Brief

Treatment of Metastatic Cancer with Tetrathiomolybdate, an Anticopper, Antiangiogenic Agent: Phase I Study


Abstract
Preclinical and in vitro studies have determined that copper is an important cofactor for angiogenesis. Tetrathiomolybdate (TM) was developed as an effective anticopper therapy for the initial treatment of Wilson’s disease, an autosomal recessive disorder that leads to abnormal copper accumulation. Given the potency and uniqueness of the anticopper action of TM and its lack of toxicity, we hypothesized that TM would be a suitable agent to achieve and maintain mild copper deficiency to impair neovascularization in metastatic solid tumors. Following preclinical work that showed efficacy for this anticopper approach in mouse tumor models, we carried out a Phase I clinical trial in 18 patients with metastatic cancer who were enrolled at three dose levels of oral TM (90, 105, and 120 mg/day) administered in six divided doses with and in-between meals. Serum ceruloplasmin (Cp) was used as a surrogate marker for total body copper. Because anemia is the first clinical sign of copper deficiency, the goal of the study was to reduce Cp to 20% of baseline value without reducing hematocrit below 80% of baseline. Cp is a reliable and sensitive measure of copper status, and TM was nontoxic when Cp was reduced to 15–20% of baseline. The level III dose of TM (120 mg/day) was effective in reaching the target Cp without added toxicity. TM-induced mild copper deficiency achieved stable disease in five of six patients who were copper deficient at the target range for at least 90 days.

Introduction
The concept of antiangiogenic treatment for solid tumors, which was pioneered by Folkman (1–3), has a firm rationale and shows efficacy in animal tumor models (4–12). Compounds that interfere with critical steps in the angiogenesis cascade are reaching the clinic (13). The steps required for successful tumor angiogenesis at the primary and metastatic sites are diverse, and they depend on an imbalance between angiogenesis activators (14–15) such as vascular endothelial growth factor and basic fibroblast growth factor and inhibitors such as thrombospondin 1 (16–20), angiostatin (21–23), and endostatin (10). The relative importance of the different angiogenesis-modulating molecules in different tissues may determine the relative potency of antiangiogenic compounds to elicit a response at both the primary and metastatic sites. Therefore, it would be very desirable to develop an antiangiogenic strategy that would affect multiple activators of angiogenesis in order for it to be generally applicable to human tumors. Because copper is a required cofactor for the function of many key mediators of angiogenesis, such as basic fibroblast growth factor (24–27), vascular endothelial growth factor, and angiogenin (28), we have developed an antiangiogenic strategy for the treatment of cancer based on the modulation of total body copper status. The underlying hypothesis of this work is that a window of copper deficiency exists in which angiogenesis is impaired, but other copper-dependent cellular processes are not affected enough to cause clinical toxicity.

It has been amply demonstrated that copper is required for angiogenesis (29–31), and several years ago, some promising animal tumor model studies were carried out using an anticopper approach (32–33). The chelator penicillamine and a low-copper diet were used to lower copper levels in rats and rabbits with implanted intracerebral tumors. However, although they showed reduced tumor size, the animals treated with the low-copper regimen did not show improved survival over untreated controls.

For the past 20 years, we have developed new anticopper therapies for Wilson’s disease, an autosomal recessive disease of copper transport that results in abnormal copper accumulation and toxicity. One of the drugs currently being used, TM, shows unique and desirable properties of fast action, copper specificity, and low toxicity (34–36), as well as a unique mechanism of action.

1 Supported by NIH Grants RO3-CA-77122 (to S. D. M.), P30-CA46592 (to M. W.), and MO1-RR-00042 (to the General Clinical Research Center); the Tracy Starr Award from the Cleveland Foundation (to S. D. M.); a grant from the Tempting Tables Organization (to S. D. M.); and donors to the University of Michigan Comprehensive Cancer Center.

2 To whom requests for reprints should be addressed, at University of Michigan Comprehensive Cancer Center, 7217 CCGL, 1500 East Medical Center Drive, Ann Arbor, MI 48109-0948.

3 The abbreviations used are: TM, tetrathiomolybdate; Cp, ceruloplasmin; Hct, hematocrit; GI, gastrointestinal; CAT, computer-assisted tomography.
TM Treatment of Metastatic Cancer

Toxicity and Efficacy as an Anticopper, Antiangiogenic Therapy. Copper drugs, we evaluated it in animal tumor models for the toxicology and efficacy questions, we report preliminary observations on efficacy based on the use of TM in patients with metastatic cancer. This Phase I trial of TM yielded information on dose, dose response, evaluation of copper status in patients, and toxicity (37).

The underlying hypothesis of an anticopper, antiangiogenic approach to cancer therapy is that the level of copper required for angiogenesis is higher than that required for essential copper-dependent cellular functions, such as heme synthesis, cytochrome function, and incorporation of copper into enzymes and other proteins. Because of the unique and favorable characteristics of TM as an anticopper agent compared with other anticopper drugs, we evaluated it in animal tumor models for toxicity and efficacy as an anticopper, antiangiogenic therapy. These studies showed efficacy in impairing the development of de novo mammary tumors in Her2-neu transgenic mice (12), and TM showed no clinically overt toxicity as copper levels were decreased to 10% of baseline. Here we report the first human trial of an anticopper approach to antiangiogenesis therapy based on the use of TM in patients with metastatic cancer. This Phase I trial of TM yielded information on dose, dose response, evaluation of copper status in patients, and toxicity (37). Although the study was not designed to definitively answer efficacy questions, we report preliminary observations on efficacy and novel approaches to following disease status in trials of antiangiogenic compounds.

Patients and Methods

Patients. Eighteen adults with metastatic solid tumors exhibiting measurable disease, life expectancy of 3 or more months, and at least 60% Karnofsky performance status were enrolled. We excluded patients with effusions or bone marrow involvement as the only manifestations of disease and those who had severe intercurrent illness requiring intensive management or were transfusion dependent. Patients had to have recovered from previous toxicities and had to meet the following requirements for laboratory parameters: (a) WBC ≥ 3,000/mm³; (b) absolute neutrophilic count ≥ 1,200/mm³; (c) Hct ≥ 27%; (d) hemoglobin ≥ 8.0 g/dl; (e) platelet count ≥ 80,000/mm³; (f) bilirubin ≤ 2.0 mg/dl; (g) aspartate aminotransferase and alanine aminotransferase ≤ 4 times the upper limit of institutional norm; (h) serum creatinine < 1.8 mg/dl or calculated creatinine clearance ≥ 55 ml/min; (i) calcium < 11.0; (j) albumin ≥ 2.5 g/dl; (k) prothrombin time ≤ 13 s; and (l) partial thromboplastin time ≤ 35 s. Other requirements were demonstrable progression of disease in the previous 3 months after standard treatments such as surgery, chemotherapy, radiotherapy, and/or immunotherapy or progressive disease after declining conventional treatment modalities.

Treatment Schema: Doses and Escalation. Three dose regimens were evaluated. All dose levels consisted of 20 mg of TM given three times daily with meals plus an escalating (levels I, II, and III) in-between meals dose given three times daily for a total of six doses/day. Loading dose levels I, II, and III provided TM at 10, 15, and 20 mg, three times daily between meals, respectively, in addition to the three doses of 20 mg each given with meals at all dose levels.

Baseline Cp was taken as the nearest Cp measurement to day 1 of treatment (including day 1) because blood was drawn before TM treatment from all patients. The target Cp reduction was defined as 20% of baseline Cp. Due to Cp assay variability of approximately 2% at this institution, a change of Cp to 22% of baseline was considered as achieving the desired reduction of copper. In addition, if the absolute Cp was less than 5 mg/dl, then the patient was considered as having reached the target Cp. No patient reached the 5 mg/dl target without also being at least 78% reduced from baseline. After reaching the target copper-deficient state, TM doses were individually tailored to maintain Cp within a target window of 70–90% reduction from baseline.

Six patients were to be enrolled at each dose level. After four patients were enrolled at level I, if one patient experienced dose-limiting toxicity (defined as Hct < 80% of baseline), two more patients were enrolled at level I. If no dose-limiting toxicity was observed, patients were enrolled at the next dose level. Treatment was allowed to continue beyond induction of target copper deficiency if the patients experienced a partial or complete clinical response or achieved clinical stable disease by the following definitions. Complete response is the disappearance of all clinical and laboratory signs and symptoms of active disease; partial response is a 50% or greater reduction in the size of measurable lesions defined by the sum of the products of the longest perpendicular diameters of the lesions, with no new lesions or lesions increasing in size. Minor response is a 25–49% reduction in the sum of the products of the longest perpendicular diameters of one or more measurable lesions, no increase in size of any lesions, and no new lesions; stable disease is any change in tumor measurements not represented by the criteria for response or progressive disease; progressive disease is an increase of 25% or more in the sum of the products of the longest perpendicular diameters of any measurable indicator lesions compared with the smallest previous measurement or appearance of a new lesion. Because copper deficiency is not a cytotoxic treatment modality, the patients who provide information about the efficacy of TM for long-term therapy in this population of patients with advanced cancer are primarily those who remained within the target Cp window of 20 ± 10% of baseline for over 90 days without disease progression.

Monitoring of Copper Status. A method was required to monitor copper status easily and reliably, so that the TM dose could be adjusted appropriately during this trial. With TM administration, serum copper is not a useful measure of total body copper because the TM-copper-albumin complex is not rapidly cleared, and the total serum copper (including the fraction bound to the TM-protein complex) actually increases during TM therapy (34–36). The serum Cp level obtained weekly was used as a surrogate measure of total body copper status. Cp was measured by the oxidase method; the Cp measurements were made by nethelometry (differential light scattering from a
colored or turbid case solution with respect to a control solution) using an automated system and reagents available commercially (Beckman Instruments, Inc., Fullerton, CA). The serum Cp level is controlled by Cp synthesis by the liver, which, in turn, is determined by copper availability to the liver (38). Thus, as total body copper is reduced, the serum Cp level is proportionately reduced. The serum Cp level is in the range of 20–35 and 30–65 mg/dl for normal controls and cancer patients, respectively. Our objective was to reduce Cp to ≤20% of baseline and to maintain this level, within a window spanned by 20 ± 10% of baseline Cp, with typical Cp values in the range of 7–12 mg/dl. Because there appears to be no untoward clinical effects from this degree of copper reduction, we have termed this level of copper deficiency “chemical copper deficiency.” The first indication of true clinical copper deficiency is a reduction in blood cell counts, primarily anemia, because copper is required for heme synthesis as well as cellular proliferation (36). Thus, the copper deficiency objective of this trial was to reduce the Cp to ≤20% of baseline without decreasing the patient’s Hct or WBC to below 80% of baseline value at entry.

**Toxicity, Follow-Up, and Disease Evaluation.** Complete blood counts, liver and renal function tests, urinalyses, and Cp level were performed weekly for 16 weeks and then performed biweekly at the clinical laboratories of the University of Michigan Health System or at other affiliated certified laboratories. Physical examinations and evaluations of toxicity were carried out every 2 weeks for 8 weeks and then performed every 4 weeks for the duration of therapy. Toxicity was evaluated using the National Cancer Institute Common Toxicity Criteria. Extent of disease was evaluated at entry, at the point of achievement of copper deficiency (defined as Cp ≤20% of baseline), and every 10–12 weeks thereafter. CAT or magnetic resonance imaging was used as appropriate for conventional measurement of disease at all known sites and for evaluation of any potential new sites of disease. Angiogenesis-sensitive ultrasound with three-dimensional Doppler analyses was used in select cases as an adjunct to conventional imaging to evaluate blood flow to the tumors at different time points.

**TM Preparation and Storage.** TM was purchased in bulk lots suitable for human administration (Aldrich Chemical Company, Milwaukee, WI). Because TM is slowly degraded when exposed to air (oxygen replaces the sulfur in the molecule, rendering it inactive; Refs. 34–36), it was stored in 100-g lots under argon. At the time a prescription was written, the appropriate dose of TM was placed in gelatin capsules by research pharmacists at the University of Michigan Health System. Preparations were dispensed to each patient in 8-week installments throughout the trial.

**Measurement of Blood Flow.** Blood flow was measured by ultrasound in select patients with accessible lesions at the time they became copper deficient and at variable intervals of 8–16 weeks thereafter. Three-dimensional scanning was performed on a GE Logiq 700 ultrasound system, with the 739 L, 7.5 MHz linear array transducer. The scanning and vascularity quantification techniques were as described previously by the authors (39, 40).

**Results**

**Patient Characteristics**

Eighteen eligible patients (10 males and 8 females) with 11 different types of metastatic cancer who had progressed through or (in one case) declined other treatment options were enrolled in the trial in the order in which they were referred. Six, five, and seven patients were enrolled at the 90, 105, and 120 mg/day drug levels, respectively, following the protocol dose escalation schema. One patient originally assigned to the 105 mg/day level was removed early to pursue cytotoxic chemotherapy, due to rapid progression of disease. This same patient was later retreated at the 120 mg/day level for a longer duration; thus, he is counted only at the 120 mg/day level for the analyses. The average age was 59 years; the average baseline Cp was 47.8 mg/dl, which is elevated with respect to the normal level, reflecting the patients’ disease status. Table 1 summarizes the patient characteristics for each dose level.

**Toxicity**

There were no cardiac, pulmonary, GI, renal, hepatic, hematological, infectious, skin, mucosal, or neurological toxicities observed for Cp levels at or above 20% of baseline. Mild (>80% of baseline Hct) reversible anemia was observed in four patients with Cp levels between 10–20% of baseline. Two of these patients had been treated with cytotoxic chemotherapy, and two patients had evidence of extensive bone marrow involvement with their disease at the time of entry into the trial. Although in the latter two cases, the anemia was most likely due to causes other than treatment, TM was temporarily discontinued until Hct was restored to acceptable levels with a transfusion of 2 units of packed RBCs. In one patient, it is very likely that the copper deficiency caused by TM produced the anemia. Stopping administration of the drug allowed the Hct to recover within 5–7 days without the need for transfusion; at the patient’s request, TM was restarted at a lower dose, without further complications of anemia. Several patients experienced transient, occasional sulfur-smelling burping, within 30 min of TM ingestion. No additional toxicities of any type were observed with long-term maintenance of mild clinical copper deficiency over 8–15 months. Of note, no evidence of GI or other mucosal bleeding or impaired healing of minor trauma were observed with long-term therapy. One premenopausal patient with extensive metastatic renal cancer experienced normal menstrual periods during TM therapy, including 2.5 months of observation while she was copper deficient with Cp < 20% of baseline.

**Cp as a Surrogate Measure of Copper Status**

Fig. 1 shows the response of Cp as a function of time on TM therapy, expressed as the ratio of Cp at time t to baseline Cp level for each patient enrolled at the 90, 105, and 120 mg/day dose levels. Increasing the in-between meals dose from 10 mg three times daily to 15 or 20 mg three times daily had no significant effect on the rate of decrease of the Cp level, reaching a level of 50% baseline at a mean of 30 days (median = 28 days). The response of Cp to TM therapy as a function of time exhibited only minor fluctuations; when TM was discontinued, a rapid rise in Cp was observed within 48 h.

Four patients were removed from study due to progression...
of disease before achieving the target Cp of 20% of baseline, whereas the remaining 14 patients achieved the target Cp level. Because all 14 patients who achieved the target Cp level wished to remain on study, they were allowed to do so, according to the protocol, as long as they did not exhibit disease progression or toxicity. The TM doses were adjusted in these patients to maintain the Cp level between 10–20% of baseline. These patients provide the preliminary evidence of the efficacy and long-term tolerance of this approach.

Dose Adjustments to Maintain Target Cp

TM doses were adjusted to maintain a Cp target level of 20% of baseline and to prevent absolute Cp values \(< 5 \text{ mg/dl}\). Due to the routine 7-day turn-around for the Cp test at our laboratory, these dose changes were made approximately 7–10 days after the blood for the Cp measurement was taken. After achieving the target Cp, the in-between meals dose was typically decreased by 20 mg. Further decreases of 15–30 mg were necessary during long-term therapy. A patient with metastatic chondrosarcoma secondary to radiation treatment for breast cancer on long-term therapy has stable disease after 12 months of copper deficiency, with stable quality of life. One biopsy-proven metastatic nodule on her third digit is easily measurable and has been stable. Other sites of suspected disease in the chest also remain stable. Interestingly, this patient has required only a minor adjustment to her TM dose from the initial loading dose level to maintain the target Cp throughout this relatively long period.

Table 1  Patient characteristics

<table>
<thead>
<tr>
<th>Assigned TM Dose (mg/day)</th>
<th>90</th>
<th>105</th>
<th>120</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of patients</td>
<td>6</td>
<td>5</td>
<td>7</td>
<td>18</td>
</tr>
<tr>
<td>Sex (M/F)</td>
<td>3/3</td>
<td>1/4</td>
<td>6/1</td>
<td>10/8</td>
</tr>
<tr>
<td>Mean age (SD) (yrs)</td>
<td>64 (12)</td>
<td>60 (12)</td>
<td>53 (17)</td>
<td>59 (14)</td>
</tr>
<tr>
<td>Primary Tumor</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Breast</td>
<td>2</td>
<td>2</td>
<td>0</td>
<td>4</td>
</tr>
<tr>
<td>Colon</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Lung</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Melanoma</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Pancreas</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Prostate</td>
<td>2</td>
<td>0</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>Angiosarcoma</td>
<td>0</td>
<td>0</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>Chondrosarcoma</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Nasopharyngeal tumor</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Hemangiendothelioma</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Renal tumor</td>
<td>1</td>
<td>0</td>
<td>2</td>
<td>3</td>
</tr>
</tbody>
</table>

Baseline

| Baseline Cp mean            | 52.6  | 49.3  | 42.7  | 47.8  |
| Baseline Cp range           | 36.6–74.1| 38.1–65.0| 31.9–52.7| 31.9–74.1 |
| Baseline Hct mean           | 31.9  | 37.2  | 41.9  | 37.3  |
| Baseline Hct range          | 26.6–35.6| 33.8–42.5| 35.4–45.7| 26.6–45.7 |

of disease before achieving the target Cp of 20% of baseline, whereas the remaining 14 patients achieved the target Cp level. Because all 14 patients who achieved the target Cp level wished to remain on study, they were allowed to do so, according to the protocol, as long as they did not exhibit disease progression or toxicity. The TM doses were adjusted in these patients to maintain the Cp level between 10–20% of baseline. These patients provide the preliminary evidence of the efficacy and long-term tolerance of this approach.

Measurement of Response of Metastatic Cancer to TM

Clinical Evaluation. Although the patients received different initial loading doses of TM, the Cp maintenance window of 20 ± 10% of baseline was used in all groups, regardless of the loading dose. Patients who maintained this degree of copper deficiency through tailored adjustments of the TM dose for over 90 days are likely to reflect the antiangiogenic activity of TM against their tumors. The period of 90 days is selected for two main reasons. First, TM is not cytotoxic to either cancer or endothelial cells and mainly impairs endothelial cell function and proangiogenic factor production. This mechanism of action is expected to have a very slow effect on the size of tumor masses. Second, as tumors sequester copper, the microenvironment of the tumor is expected to take a longer time to be rendered copper deficient. Table 2 summarizes the clinical course of the 18 patients.

Fourteen patients achieved the target copper deficiency before disease progression or other disease complications. Of these, eight patients either progressed within 30 days of achieving copper deficiency or have had stable disease for <90 days; it is unlikely that most of these tumors experienced an antiangiogenic environment long enough to evaluate clinical response to this type of therapy. In all patients removed from the protocol due to disease progression or choice and in one patient removed from the protocol due to the need for abdominal surgery to relieve a small bowel obstruction, much more rapid rates of progression of disease were noted clinically after discontinuation of TM therapy.

The remaining six patients experienced stable disease (five of six patients) or progression of disease at one site, with stable disease elsewhere (one of six patients). Two patients who have
stable disease by standard criteria also experienced complete disappearance of some lung lesions and a decrease in the size of other lung lesions during observation periods at target Cp of 120 and 49 days. The five patients on long-term (>90 days) maintenance therapy with stable disease have been copper deficient for 120–413 days at the time of this analysis.

Radiological Evaluation. Serial evaluations of tumor masses by conventional imaging with CAT scan or magnetic resonance imaging revealed that the radiographic appearance of certain masses changed significantly over time. In particular, areas of presumed central necrosis (corresponding to lower attenuation of the X-ray signal) were observed in a variety of tumor types, most notably renal cell cancer, angiosarcoma, and breast cancer. Seeking to evaluate the blood flow to the tumors as a function of time during copper deficiency on long-term TM therapy, lesions accessible to ultrasound were imaged with color flow three-dimensional ultrasound at the onset of copper deficiency and at 2–4-month intervals thereafter.

A representative example of the comparison between conventional CAT scan images and blood flow-sensitive three-dimensional ultrasound is depicted in Fig. 3. Here, a rib metastasis from renal cell carcinoma is depicted when the patient reached target copper deficiency (Fig. 3, A and C) and 8 weeks later (Fig. 3, B and D) by these two complementary imaging modalities. Fig. 3, A and B, shows stable size of this lesion by CAT scan over time, although a more distinct region of probable central necrosis is observed in Fig. 3B. In comparison, the color pixel density shown in Fig. 3, C and D, is the fraction of image voxels within the margins of the mass filled with color flow signals. There has been a 4.4-fold decrease in blood flow to this mass over a period of approximately 8 weeks. In addition to the mass depicted in Fig. 3, this patient had extensive disease in the chest, pelvis, and femurs.

TM in Combination with Other Treatment Modalities

During the long-term maintenance of copper deficiency, additional treatment modalities were added to TM as deemed appropriate for the optimal management of the patients. A patient with previously untreated metastatic breast cancer is doing well with a good-to-excellent quality of life after 12 months of treatment. She had metastases in the paratracheal, posterior cervical, and retroperitoneal lymph node chains but had declined all cytotoxic therapy. The patient had stable disease for more than 6 months on TM treatment, when, due to a
slight increase (less than 25% of baseline) in the bidimensional size of the paratracheal and retroperitoneal nodes, she began concurrent trastuzumab (Herceptin; Genentech) therapy after this drug became commercially available. This patient showed a rapid response to trastuzumab at all sites of disease: after one cycle, there was a clinical complete response in the neck; and after three cycles of trastuzumab, there was radiological confirmation of complete response at all previous sites of disease. The patient remains on TM, but the trastuzumab was discontinued after six doses. She continues to maintain her status as a complete responder on TM alone for more than 6 months after discontinuation of trastuzumab therapy. Because the complete response was achieved after the addition of trastuzumab therapy, this patient is classified as having only stable disease on TM on Table 2.

Two patients with extensive angiosarcoma of the face and scalp achieved stable disease on TM. In one patient with severe chronic bleeding from an ocular lesion that threatened the orbit, IFN-α2 was added to TM to attempt to enhance tumor response. Given the suggestion that, based on studies of progressing glandular behavior, treatment with TM alone may be adjusted to achieve a target Cp of 20% of baseline.

**Fig. 2** Management of long-term therapy with TM to maintain a Cp target of 20% of baseline in two patients. A, patient required a decrease in dose at two time points that were 60 days apart. To prevent his Cp from falling below 5 mg/dl, this patient will likely require a decrease in TM dose in the future. B, TM dose was increased after day 100 to prevent a drifting of the Cp above the target range. Heterogeneity of diet and tumor behavior (such as tumor cell lysis) may account for the individual variability in dose adjustment needs.
hemangiomas, the use of low-dose IFN may be efficacious for the treatment of hemangioma (41), IFN-α was administered to both of these patients at a dose of 500,000 units s.c. twice a day. Radiotherapy was also given to these two patients while on TM to attempt to control actively bleeding (but not progressing) lesions. Both patients had disease stabilization for >60 days, with one of these patients remaining with stable disease for over 5 months before discontinuation of therapy due to patient choice. No exacerbation of toxicity was observed by the addition of any of these treatment modalities to TM.

Discussion

This is the first human trial of induction and maintenance of copper deficiency with TM as an antiangiogenic therapy for cancer. In a group of patients with advanced cancer, we have demonstrated that TM is remarkably nontoxic when Cp is lowered to 10–20% of baseline levels for up to 17 months of treatment. The only drug-related toxicity observed was mild anemia, which was easily reversible with adjustment of the TM dose to bring the Cp level to the desired target. Despite the diverse roles that copper plays in essential biological processes including heme synthesis and superoxide dismutase and cytochrome function, no lasting significant adverse effects were observed on reduction of Cp to approximately 20% of baseline or to a range between 5 and 15 mg/dl. From our data, we surmise that this level of copper reduction constitutes the lower limit of chemical copper deficiency and the beginning of mild clinical copper deficiency, the first manifestation of which is mild anemia. Table 3 summarizes the stages of copper deficiency in humans and their clinical characteristics. This information was derived from studies of patients with Wilson’s disease, from occasional patients with chemical and clinical copper deficiency, and from copper-deficient small rodents. Note that as Cp is reduced below 5 mg/dl, it becomes an insensitive marker of the degree of copper deficiency. However, based on observations in humans with normal copper metabolism from this trial, we find that Cp is a sensitive and valid marker of copper status for levels above 5 mg/dl. This key finding allows the targeting of the antiangiogenic window of copper deficiency that appears to be required to slow or arrest tumor growth.

The Cp response to TM-induced copper deficiency is monotonic and exhibits little intersubject variability; therefore, there is essentially no risk of sudden changes or unpredictable fluctuations that might make dose management difficult. Following Cp levels once every 1–2 weeks is adequate to monitor copper status early in therapy. As a corollary, overtreatment is easily detectable and correctable. Using the six times/day dose regimen borrowed from our Wilson’s disease work and initial TM doses ranging from 90–120 mg/day, the serum Cp was reliably lowered to 50% of baseline in 17 of 18 patients and to 20% of baseline in 14 of 18 patients. Reduction to 50% of baseline was achieved, on average, in 30 days, with further reduction to Cp levels of 5–10 mg/dl taking 20–30 days. Although this rate of decrease in Cp is reasonable for the initial treatment of early malignant lesions or in the adjuvant setting, in widely metastatic advanced cancer, this rate of decrease will not be sufficiently rapid to prevent some disease progression during induction of copper deficiency in a significant number of patients. Because loading dose variations of 90–120 mg/day do not appear to affect the rate of Cp reduction, and given the typical daily intake of copper with food, we conclude that higher doses in-between meals will likely be required to accelerate the rate of induction of copper deficiency. A follow-up trial is under way to test this hypothesis.

As a result of this study, it is apparent that with our present TM dose regimens, there is considerable lag between the initiation of TM therapy and the reduction of copper levels in tumors to a likely antiangiogenic level. Further retarding the ability to reach antiangiogenic levels of copper deficiency is the likelihood that most tumors sequester copper (42–45). Thus, it is reasonable to hypothesize that additional time may be required to deplete the tumor microenvironment to an effectively low level of copper, which is defined as a level low enough to inhibit angiogenesis. It is difficult to estimate this time accurately from our study. Thus, patients with very rapidly progressive large tumors may be relatively poor candidates for this approach to antiangiogenesis therapy as a single modality.

Another level of complexity is added by the fact that in bulky disease, initially effective antiangiogenesis may cause brisk tumor necrosis, as was documented in the mass shown in Fig. 3. Tumor lysis may result in the release of additional copper from the dying cells. In the case of the patient whose mass is shown in Fig. 3, a transient rise in Cp was observed at approx-

<table>
<thead>
<tr>
<th>Type of response</th>
<th>No. of patients (Total = 18)</th>
<th>Duration in days of copper deficiency (average)</th>
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<tbody>
<tr>
<td>Did not achieve target Cp</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td>Achieved target Cp</td>
<td>14</td>
<td></td>
</tr>
<tr>
<td>Target Cp &lt;90 days</td>
<td>8/14</td>
<td></td>
</tr>
<tr>
<td>Disease progression</td>
<td>7</td>
<td></td>
</tr>
<tr>
<td>Stable disease with partial regression of lung lesions</td>
<td>1</td>
<td>49&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Target Cp &gt;90 days</td>
<td>6/14</td>
<td></td>
</tr>
<tr>
<td>Stable disease with partial regression of lung lesions</td>
<td>1/6</td>
<td>120&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Stable disease</td>
<td>4/6</td>
<td>159&lt;sup&gt;a&lt;/sup&gt;, 329&lt;sup&gt;a&lt;/sup&gt;, 351&lt;sup&gt;a&lt;/sup&gt;, 413&lt;sup&gt;a&lt;/sup&gt; (313&lt;sup&gt;a&lt;/sup&gt;)</td>
</tr>
<tr>
<td>Disease progression at one site, stable elsewhere</td>
<td>1/6</td>
<td>120&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>a</sup> On therapy.
<sup>b</sup> Patient discontinued therapy.

另外，当使用TM诱导铜缺乏时，Cp的反应是单调的，并且在不同受试者之间表现出很小的差异；因此，基本上没有风险突然变化或不可预测的波动，这可能使剂量管理困难。使用6次/天的剂量常规，从我们的Wilson’s病工作和初始TM剂量范围从90–120 mg/day，血清Cp可靠地降低到50%的 baseline在17的18患者和20%的 baseline在14的18患者。降低到50%的 baseline被实现，平均，在30天，进一步降低到Cp水平为5–10 mg/dl取20–30天。尽管这种降低Cp的速率在 Cp是合理的初始化治疗的早期恶性病变或在辅助设置，在广泛转移的进展性癌症，这种速率的降低将不会足够快速，以防止一些疾病进展期间诱导铜缺乏在显著数量的患者。因为加载剂量的变化90–120 mg/day不出现影响Cp的降低速率，和给定的典型日常摄入铜与食物，我们得出结论，更高的剂量在-between餐将很可能需要加速Cp的速率降低。一个后续的实验在进行中用于测试这个假设。

作为一个结果的研究，这是显而易见的，与我们当前的TM剂量方案，有相当的延迟在TM治疗的开始和铜水平在肿瘤到一个可能的反血管内皮水平。进一步推迟反血管内皮水平的铜缺乏的可能性是，肿瘤可能储存铜（42–45）。因此，合理假定，额外的时间可能需要被用来耗尽肿瘤微环境到一个有效低水平的铜，这定义为一个低水平来抑制血管生成。这是很难估计这个时间准确地从我们的研究。因此，进展迅速的大肿瘤可能较差的患者候选人这种方法的反血管生成性治疗作为单一的模态。

另一个复杂层面是增加了事实，在 bulky疾病，初始有效的反血管生成可能引起迅速的肿瘤坏死，如在图3中显示的。细胞死亡的释放可能增加铜。在图3的患者，其肿瘤的铜含量在48小时后显著增加，在细胞死亡时观察到一个暂时的上升Cp。
imately the same time as the ultrasound suggested that the large tumor mass might be undergoing central necrosis due to a significant decrease in blood flow. For these reasons, we conclude that a period of 60–90 days of Cp at the target level of 20% of baseline is a reasonable starting point for evaluation of response to anticopper therapy in future trials in patients with measurable disease. In the two patients who exhibited partial regression of lung lesions, tumor control may have begun ear-

Fig. 3  Evaluation of antiangiogenic response to copper deficiency. CAT scans of the chest of a 59-year-old male with metastatic renal cell carcinoma shortly after achievement of target Cp (A) and 8 weeks later (B) are shown. Comparable frequency-shift color Doppler image volumes from the superficial renal cell carcinoma rib metastasis were evaluated at the same time points (C and D). Three-dimensional rendering of the vascularity is shown in green superimposed on three orthogonal image planes extracted from the reconstructed grayscale volume. The back plane shown exemplifies one of the frequency-shift color Doppler image planes acquired during the patient scan. Vascularity at initiation of TM therapy (C) is markedly greater than that seen 8 weeks later (D). Quantitatively, color pixel density is 4.4 times greater in the tumor volume scanned at the first time point; the mean flow velocities are equivalent at both times.
Table 3  Stages of copper deficiency and its clinical effects in humans

<table>
<thead>
<tr>
<th>Type of copper deficiency</th>
<th>Cp level</th>
<th>Clinical manifestations</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>% Baseline Cp</td>
<td>Absolute Cp level (mg/dl)</td>
</tr>
<tr>
<td>Chemical</td>
<td>10–30 (target range)</td>
<td>5–15 (target range)</td>
</tr>
<tr>
<td>Clinical Mild</td>
<td>&lt;10%</td>
<td>&lt;5</td>
</tr>
<tr>
<td>Moderate</td>
<td>&lt;10%</td>
<td>&lt;5</td>
</tr>
<tr>
<td>Severe</td>
<td>&lt;10%</td>
<td>&lt;5</td>
</tr>
</tbody>
</table>

<sup>a</sup> Normal serum Cp levels are 20–35 mg/dl. Cp levels in cancer patients are elevated (20–75 mg/dl).

<sup>b</sup> Bone marrow effects such as anemia and/or neutropenia may occur if the induction of copper deficiency is very rapid, as with high doses of TM, at higher levels of Cp than shown here.

<sup>c</sup> In general, signs and symptoms other than bone marrow depression require severe copper deficiency to have been present for weeks to months.

lier. It is also interesting to note that in both of these patients, the lung parenchymal metastases were the sites of tumor regression. It is possible that mild clinical copper deficiency impairs superoxide dismutase function (46) so that under conditions of high oxidant stress, such as those present in the lung, the metastatic foci are more susceptible to oxidative damage.

Despite individual differences, the use of three-dimensional ultrasound to determine the total blood flow to a given mass demonstrates that maintenance of mild copper reduction to 20% of baseline for at least 8 weeks appears sufficient to alter tumor blood flow. Due to the relative insensitivity of CAT to the blood flow or metabolic status of the lesions, parallel imaging modalities, as demonstrated here for three-dimensional ultrasound, will be required to assess functional response in addition to tumor size.

In light of the data presented above, we advance the preliminary conclusion that the size of solid tumors of a variety of types may be stabilized or decreased by TM, given sufficient time in a state of mild clinical copper deficiency represented by a decrease in Cp to or below 20% of baseline, as defined by this study. Among the patients maintained at the target Cp level for more than 90 days, a significant proportion of cases (five of six) were stabilized, with no detriment to their quality of life. However, in this population of patients with advanced disease, only 39% of those treated were able to be maintained at the target Cp for this duration.

The pattern and speed of progression observed in these patients have also provided useful preliminary information. One patient achieved stable disease at all sites but one and has chosen to remain on TM therapy due to disease stabilization at the more life-threatening sites of disease (bowel and paratracheal lymph nodes; the site of progression in this patient with melanoma is a large adrenal metastasis. This and other observations in this trial suggest that whereas copper deficiency may be generally inhibitory of angiogenesis, heterogeneity of tumor type and the specific location of metastases may modulate the response to this therapeutic modality. The small number of patients in this study and the design of this study preclude more detailed conclusions regarding efficacy at specific metastatic sites. Because it appears that lesions progress at a much faster rate on copper repletion than while on TM therapy, future trials may formally incorporate the use of adjunct modalities, either systemically or loco-regionally, to address the specific sites of progression while allowing the patients to remain in a copper-deficient state.

We report preliminary observations of combination therapies of TM with radiotherapy, trastuzumab, and IFN-α without apparent exacerbation of toxicity of the added modality. Taken as a whole, the safety and preliminary efficacy data derived from this trial support the conduct of additional studies designed to test the specific efficacy of TM alone or in combination for the treatment of early metastatic disease, minimal disease, and in adjuvant high-risk clinical settings, including chemoprevention.

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