Copper deficiency as an anti-cancer strategy

V L Goodman1,3, G J Brewer1,2 and S D Merajver1,3

1Department of Internal Medicine, Division of Hematology and Oncology, University of Michigan Medical School, 1500 East Medical Center Drive, Ann Arbor, Michigan 48109, USA
2Department of Human Genetics, University of Michigan Medical School, 1500 East Medical Center Drive, Ann Arbor, Michigan 48109, USA
3Comprehensive Cancer Center, University of Michigan Medical School, 1500 East Medical Center Drive, Ann Arbor, Michigan 48109, USA

(Requests for offprints should be addressed to S Merajver, Department of Internal Medicine, Comprehensive Cancer Center, 1500 East Medical Center Drive, Ann Arbor, Michigan 48109-0948, USA; Email: semrajve@umich.edu)

Abstract

Copper is a tightly regulated trace element. Disruptions of copper homeostasis are rare and they cause serious disorders such as Wilson’s disease and Menkes disease. Copper also plays an important role in promoting physiological and malignant angiogenesis. Formation of new blood vessels by a tumor enables tumor growth, invasion and metastasis. The copper chelator tetrathiomolybdate (TM), which quickly and effectively depletes copper stores, is under investigation as an anti-angiogenic agent. Promising results in vitro, in pre-clinical animal models and in an early (phase I) clinical trial have led to ongoing phase II evaluation of TM in patients with advanced cancers.

Copper balance in biological systems

Copper is an essential trace element for most organisms. Critical proteins such as cytochrome oxidase, zinc–copper superoxide dismutase, lysyl oxidase and several transcription factors require copper for activity. Consequently, complex systems for acquiring and regulating copper influx and efflux have evolved. In the yeast Saccharomyces cerevisiae, for example, several essential enzymes are copper dependent. S. cerevisiae has therefore developed an elaborate system to ensure adequate levels of copper. In an environment where copper is scarce, genes are activated to enhance copper uptake (Dancis et al. 1994, Hassett & Kosman 1995, Lesuisse et al. 1996, Labbe et al. 1997, Yamaguchi-Iwai et al. 1997, Winge 1999). Two such genes encode the high affinity copper permeases Ctr1p (Dancis et al. 1994, Labbe et al. 1997) and Ctr3p (Labbe et al. 1997), and another codes for Fre1p, an enzyme responsible for iron and copper uptake from the environment (Lesuisse et al. 1996). In mammals, copper balance is established by maintaining equilibrium between copper absorption from dietary intake and copper excretion in the stool (Ravestyn 1944).

Based on the tightly controlled homeostasis of copper in primitive organisms, we have developed the hypothesis that copper availability was a primitive growth regulator, and that this regulatory aspect of copper has been maintained throughout evolution (Brewer 2001a). For example, the fungus Podospora anserina utilizes a copper-dependent transcription factor to maintain normal growth; organisms such as yeast grow more slowly when copper is unavailable, but survive by using fermentation for energy (Osiewacz & Nuber 1996, Borghouts et al. 1997, Borghouts & Osiewacz 1998). We hypothesize that primitive organisms required copper but, unlike other essential elements such as zinc and iron, copper may have been much more variable in the environment. Support for this concept comes from studies of metal content in soils of diverse geographical origins, which demonstrate that copper content is much more variable than the content of other trace metals (Bendell-Young et al. 1994, Kashin & Ivanoc 1999, Kudashkin 2000, Tebaldi et al. 2000). Consistent with this, copper deficiency is a much more frequent occurrence in grazing animals than is deficiency of other metals.

In the human and other mammals, the first observable evidence of copper deficiency is a drop in the level of blood ceruloplasmin (Cp) (Brewer et al. 2000). Cp is a copper-containing protein secreted by the liver into the blood. The copper in Cp accounts for about 90% of the
neuropathy in addition to pancytopenia. These are seen only very rarely in patients, involving a severe anemia, with or without leukopenia (Brewer et al. 2000). The next stages of copper deficiency may involve connective tissue defects, and possibly decreasing bone density if copper deficiency persists. These intermediate stages of copper deficiency are not well described in the human. A late stage of copper deficiency, which has been seen only very rarely in patients, involves a severe neuropathy in addition to pancytopenia.

Disorders of copper balance

Free copper is a potent oxidant. Cells therefore rigorously limit the amount of free copper by binding copper to other molecules. Chaperone proteins shuttle copper through the cell to copper-requiring enzymes in different cellular compartments. Copper toxicity can exist either because of the ingestion of excessive amounts of copper or because of genetic defects that interfere with copper homeostasis. Ingestion of toxic amounts of copper leads to acute gastrointestinal symptoms of nausea, vomiting and diarrhea (Commission on Life Sciences 2000). On the other hand, chronic copper toxicity is almost always due to a genetic defect in copper excretion, known in the human as Wilson’s disease (Brewer & Yuzbasiyan-Gurkan 1992, Brewer 2001b).

Wilson’s disease is a rare autosomal recessive disorder caused by mutations in ATP7B, a copper-binding ATPase and a key component of the biliary copper excretory pathway (Bull et al. 1993, Tanzì et al. 1993, Yamaguchi et al. 1993). Copper slowly accumulates in affected patients with subsequent liver damage and, in many cases, brain damage (Brewer & Yuzbasiyan-Gurkan 1992, Brewer 2001b). Most patients present in the second and third decades of life. Presentation of liver disease may vary from mild hepatitis or cirrhosis to acute fulminant hepatic failure requiring emergent liver transplantation.

About half of patients with Wilson’s disease develop central nervous system toxicity as the initial clinical manifestation of the disease. The basal ganglia and other parts of the brain co-ordinating movement are affected, producing a movement disorder. Speech, swallowing, coordination of fine movement and, later, more coarse movements are incrementally affected (Starosta-Rubinstein et al. 1987, Brewer & Yuzbasiyan-Gurkan 1992, Brewer 2001b). The neurological syndrome is often accompanied by tremor and patients may have a variety of behavioral abnormalities including depression, personality changes, emotional lability and difficulty in focusing on tasks. If undiagnosed and untreated, the disease is inexorably progressive and ultimately fatal.

Anti-copper drugs

The realization that Wilson’s disease was caused by a copper excess spurred the search for anti-copper drugs to treat this disease. The first successful oral drug was D-penicillamine, studied and developed by Walshe (1956). Penicillamine is a reductive chelator; the reduction step decreases the affinity of copper for protein and allows it to be more efficiently chelated. Initiation of penicillamine in patients with Wilson’s disease causes massive excretion of copper in the urine, and leads to a strong negative copper balance. Penicillamine is an effective therapy in Wilson’s disease except in the setting of neurological disease, where it worsens the patient’s neurological symptoms in about half the cases, perhaps by further elevating brain copper during the early copper mobilization process (Brewer et al. 1987). Half of the patients who worsen, or 25% of all patients, never recover from this treatment-related functional decline. Another drawback of penicillamine is the side-effect profile. This drug has multiple severe side-effects including hematologic and renal toxicities.

Other agents with efficacy in Wilson’s disease include trientine and zinc. Trientine was developed for patients who exhibited penicillamine intolerance (Walshe 1982). Trientine, like penicillamine, has never had a formal toxicity study, but experience indicates that it is effective and safer than penicillamine. It has not been studied with respect to the initial treatment of neurological patients. Zinc produces copper deficiency by inducing synthesis of intestinal metallothionein. This protein has a high affinity for copper and a copper–protein complex is formed (Hall et al. 1979, Menard et al. 1981, Yuzbasiyan-Gurkan et al. 1992). The complexed copper cannot be absorbed into the bloodstream and is excreted in the stool. As zinc is essentially non-toxic, it has become the drug of choice for maintenance therapy in Wilson’s disease.

However, a significant therapeutic problem remained in terms of how to treat the acutely ill Wilson’s disease patient with neurological manifestations. As discussed above, penicillamine is contraindicated (Brewer et al. 1987), and zinc does not act rapidly enough to treat acute neurological disease. Previous work demonstrating that thiomolybdates promote copper deficiency suggested that
tetrathiomolybdate (TM) might be a useful agent in the treatment of Wilson’s disease. TM has dual mechanisms of action (Brewer et al. 1991). Given with meals it forms a tripartite complex of TM, copper and food protein, thereby preventing copper absorption. Given between meals, it is absorbed into the blood, and forms a tripartite complex with TM, albumin and the freely available serum copper. The complexed copper is rendered unavailable for cellular uptake, thus the amount of free copper is rapidly reduced.

Although TM had not undergone formal toxicity evaluation, there was enough veterinary literature to allow the Federal Drug Administration (FDA) to approve an 8-week trial of TM as first-line therapy in acute neurological Wilson’s disease. TM worked rapidly, titrating the potentially toxic copper of the blood and eliminating further copper toxicity (Brewer et al. 1991, 1994, 1996). Over 60 patients were studied in an open trial, and neurologic function was assessed with scored neurologic and speech tests. Two patients experienced deterioration in neurological function. This compares favorably with penicillamine treatment, which, as mentioned above, results in functional decline in 50% of patients. Many of these patients later had functional improvements while receiving zinc maintenance therapy. Toxicity is uncommon with TM use but includes further elevation of transaminase enzymes, and anemia and/or leukopenia related to overtreatment. The bone marrow toxicity is quickly responsive to decreasing the dose of TM treatment.

A comparison of penicillamine, trientine and TM treatment is shown in Table 1.

In an ongoing trial, TM is undergoing a double-blind comparison to evaluate it for superiority relative to trientine in the treatment of the neurological presentation of Wilson’s disease. The primary endpoint of the study is the frequency of neurologic deterioration over the first 8 weeks of therapy.

Thus, the remaining needs and questions with respect to anti-copper drugs in Wilson’s disease are as follows: (1) to establish the most effective available therapy for initial treatment of the neurological presentation through the double-blind comparison of trientine and TM. If TM proves superior to trientine, to pursue approval of TM through the FDA new drug approval process; (2) to establish the most effective available therapy for initial treatment of the patient presenting with acute liver failure. Currently, if these patients do not require immediate transplant, they are treated in most centers with penicillamine. At our institution, patients with Wilson’s disease are treated with a combination of trientine and zinc, which appears to be somewhat superior to penicillamine. Theoretical considerations backed by evidence in one patient suggest that TM may be an even better treatment for these patients, and a study of this hypothesis is planned; and (3) to evaluate and report the final outcomes of ongoing toxicology studies of TM.

**Anti-copper therapy in the treatment of cancer**

The essential role that angiogenesis plays in tumor development was initially hypothesized by Dr Judah Folkman over 30 years ago. In the absence of new blood vessel formation, solid tumors must receive necessary oxygen and nutrients by diffusion, restricting growth to 1–2 mm (Folkman 1971). Dr Folkman further postulated that tumor cells elaborate a growth factor termed ‘tumor angiogenesis factor’ (TAF), which would behave as an angiogenic switch (Folkman 1974). Once activated, TAF would promote vessel formation, allowing tumor growth, invasion and metastasis. Thus, blockade of angiogenesis via TAF inhibition might serve as a novel anti-neoplastic strategy.

In the ensuing years, it has been discovered that TAF activity is controlled by not one but several pro-angiogenic mediators including vascular endothelial growth factor (VEGF), fibroblast growth factor (FGF) and transforming growth factor-β (TGF-β) (Scappaticci 2002). These pro-angiogenic factors are counterbalanced by inhibitors of angiogenesis such as thrombospondin, angiostatin and endostatin. Tumors exploit an imbalance between the pro-angiogenic and anti-angiogenic factors to allow growth and metastasis (Hanahan & Folkman 1996, Iruela-Arispe & Dvorak 1997).

**Table 1** A comparison of penicillamine, trientine and TM treatment in Wilson’s disease

<table>
<thead>
<tr>
<th>Drug</th>
<th>Mechanism of action</th>
<th>Major usage in Wilson’s disease</th>
<th>Major toxicities in Wilson’s disease</th>
</tr>
</thead>
<tbody>
<tr>
<td>Penicillamine</td>
<td>Chelator</td>
<td>Liver disease</td>
<td>Renal/nephrotic syndrome, autoimmune, cytopenias, worsening of neurologic disease</td>
</tr>
<tr>
<td>Trientine</td>
<td>Chelator</td>
<td>Penicillamine intolerance</td>
<td>Anemia</td>
</tr>
<tr>
<td>TM</td>
<td>Forms complex with copper and protein</td>
<td>Neurologic involvement</td>
<td>Transaminase elevation, cytopenias</td>
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The clinical relevance of angiogenesis in cancer is illustrated by research data that correlate tumor expression of angiogenic growth factors with prognosis. In patients with renal cell carcinoma, for instance, increased basic FGF expression has been correlated with worsened survival (Nanus et al. 1993). Additionally, expression of VEGF in breast cancer correlates with a decrease in relapse-free survival (Toi et al. 1994).

Following these discoveries, the concept of anti-angiogenic therapy as an approach to treat malignancies has gained popularity, and many anti-angiogenic agents have been developed and are in clinical trials. This review will focus on the role of copper in neovascularization and copper depletion as an anti-angiogenic strategy.

The role of copper in angiogenesis was first appreciated in rabbit cornea models. Copper has been shown to be concentrated in the rabbit cornea during neovascularization induced by prostaglandin E1 (Ziche et al. 1982). Addition of copper in the rabbit cornea is sufficient to induce new vessel formation (Raju et al. 1982, Parke et al. 1988). Furthermore, copper-deficient rabbits were unable to mount an angiogenic response (Raju et al. 1982, Ziche et al. 1982).

Endothelial cell migration is an essential early step in angiogenesis. Copper has been shown to induce migration of bovine aorta endothelial cells (McAuslan et al. 1983). Furthermore, a heparin–copper complex has been shown to stimulate capillary migration in vitro as well as angiogenesis in vivo (Alessandri et al. 1984).

Although the angiogenic-promoting function of copper has been recognized for nearly two decades, the mechanism whereby copper exerts these effects is unknown. Recent work has been aimed at understanding the role of copper in promoting angiogenesis. It has become clear that copper interacts with several angiogenic factors; however, the functional significance of these interactions is in some cases unclear.

Among the angiogenic growth factors known to bind copper in vitro is angiogenin. Angiogenin is a protein with ribonucleolytic activity initially isolated from the conditioned media of the human colonic adenocarcinoma cell line, HT-29 (Fett et al. 1985). Angiogenin is secreted by vascular endothelial cells and aortic smooth muscle cells in addition to fibroblasts and tumor cells. It has been shown to induce new vessel formation in the chick chorioallantoic membrane (Rybak et al. 1987). An inhibitory antibody to angiogenin inhibits tumor growth of xenografted HT-29 cells (Olson et al. 1994) and breast cancer (Piccoli et al. 1998) in nude mice. Furthermore, angiogenin antisense oligonucleotides prevent establishment of primary human prostate tumors and metastases in athymic mice (Olson et al. 2001).

A functional interaction between angiogenin and copper has been hypothesized on the basis of in vitro data. Binding of radioiodine-labeled angiogenin to calf pulmonary artery endothelial cells is increased fourfold by the addition of copper (Badet et al. 1989). Using metal affinity chromatography, angiogenin was subsequently shown to bind copper (Soncin et al. 1997). A similar effect was seen with zinc but not nickel, cobalt or lithium. As copper is known to participate in angiogenesis, the authors postulate that copper binding to angiogenin results in an increased affinity between angiogenin and endothelial cells, thus promoting new vessel formation.

Recent work in an animal model of post-angioplasty restenosis may shed some light on the effects of copper on two pro-angiogenic factors, FGF1 and interleukin (IL)-1α. Both these factors have been implicated in the vascular response to injury (Mandinov 2003). Human acidic FGF1 has been demonstrated to bind copper using a copper-affinity HPLC column (Watanebe et al. 1990). FGF1 is an angiogenic factor which requires secretion into the extracellular compartment for activity. As this protein contains no signal sequence for endoplasmic reticulum (ER)–Golgi-mediated secretion, the mechanism of FGF secretion has been investigated. These studies have demonstrated that copper plays a role in the formation of a multi-protein complex implicated in the release of FGF1 in response to heat shock (Landriscina et al. 2001). Similarly, IL-1α undergoes copper-dependent secretion into the extracellular compartment. In a rat model of balloon-mediated vascular injury, animals treated with the copper-lowering drug TM developed significantly less intimal thickening than did control rats. TM-treated rats also demonstrate a significant reduction in FGF1 and IL-1α as measured by immunohistochemistry (Mandinov 2003). Taken together, these data suggest that copper chelation may attenuate the restenotic response via inhibition of FGF1 and/or IL-1α release into the extracellular compartment.

In an effort to elucidate the mechanism of copper-mediated angiogenesis, Hu (1988) studied the effect of copper on proliferation of human endothelial cells. Addition of CuSO4 to human umbilical vein endothelial cell culture in a serum- and growth factor-free environment induced cell proliferation and [3H]thymidine incorporation. This effect was specific to endothelial cells, as human fibroblasts and smooth muscle cells did not proliferate under the same conditions. Zinc and iron did not induce endothelial proliferation to a significant extent.

In the same publication, Hu (1998) also studied the effect of antibody-mediated growth factor inhibition on copper-induced cell proliferation. Interestingly, the growth stimulation induced by copper was not blocked by the presence of antibodies to angiogenin, acid
fibroblast growth factor (aFGF), basic FGF, epidermal growth factor, tumor-necrosis factor-α, TGF-β, platelet-derived growth factor, macrophage monocyte chemotactic and activating factor (MCAF) or macrophage inflammatory protein 1α (MIP-1α). Hu (1998) therefore concluded that the proliferative activity of copper on endothelial cells was not mediated through any of these growth factors. Further, he demonstrated that the effects of copper and angiogenin on endothelial cell growth were additive, apparently functioning through discrete mechanisms. Notably, copper-induced proliferation was not studied in the presence of antibodies to VEGF.

VEGFs are a family of angiogenic proteins which are essential in vasculogenesis and hypoxia-induced angiogenesis (Bikfalvi & Bicknell 2002). Recent data suggest that copper may be a required cofactor of VEGF-mediated angiogenesis. Copper sulfate has been shown to induce VEGF expression in primary as well as transformed human keratinocytes at physiologically relevant concentrations (Sen et al. 2002). This effect was abrogated by addition of a copper-chelating agent. Furthermore, topical copper sulfate treatment resulted in accelerated wound contraction and closure in BalbC mice with full thickness excisional wounds. Immunohistochemistry of the wound site demonstrated increased VEGF in the copper-treated mice compared with saline-treated controls.

Clearly, copper plays an important role in angiogenesis. The data suggest several mechanisms through which copper may exert this effect: (1) copper may act through binding of angiogenic growth factors and increasing their affinity for endothelial cells, as seen with angiogenin; (2) copper may control the secretion of angiogenic cytokines, as demonstrated with FGF1 and IL-1β; and (3) copper may induce expression of angiogenic growth factors such as VEGF. Thus, therapy aimed at depleting copper may be a successful anti-neoplastic strategy which may target multiple angiogenic growth factors. In fact, as outlined below, inhibiting tumor growth via copper depletion has been a successful strategy in animal models.

In a rat xenograft model, copper depletion prevents invasive spread of gliosarcoma (Brem et al. 1990a). Fischer 344 rats were injected with 9L gliosarcoma cells. Half were then treated with a low copper diet and penicillamine. Control rats all developed a diffuse invasive pattern of tumor growth whereas 70% of the copper-depleted rats did not. Tumors from treated rats did not develop cytoplasmic extensions and pseudopodia, markers of invasiveness. A follow-up study had similar findings in a rabbit brain tumor model of VX2 carcinomas (Brem et al. 1990b). Copper-depleted animals developed small, relatively avascular tumors compared with controls. This was correlated with a decrease in serum copper in the treated animals.

The copper-chelating agent trientine has demonstrated anti-tumor activity in a murine model of hepatocellular carcinoma (HCC) (Yoshii et al. 2001). In mice treated with trientine as well as a copper-deficient diet, HCC development was nearly abolished. In addition, copper depletion inhibited neovascularization and led to an increase in apoptosis of malignant cells.

Our laboratory has been investigating TM as a novel anti-angiogenic agent. In a murine model of head and neck cancer, TM has shown efficacy in suppressing tumor growth (Cox et al. 2001). Mice were injected in the floor of the mouth with 1.5 × 10⁵ SCC VII/SF cells, a squamous cell carcinoma cell line. After achieving measurable tumor growth, mice were treated with fresh water with or without TM added. Copper stores were measured by serum Cp levels, which reflect the pool of bioavailable copper. Cp levels were reduced by 28% in the treated animals. After 7 days of treatment, tumor volume in the control group was 4.7 times that of the TM-treated mice. This was accompanied by a reduction in microvessel density in TM-treated mice versus control.

We have demonstrated that TM-induced copper deficiency suppresses tumor growth in two animal models of breast cancer (Pan et al. 2002). First, TM was studied in a mouse xenograft model of inflammatory breast carcinoma. Inflammatory breast cancer is a highly aggressive subgroup of locally advanced breast cancer. These tumors are termed ‘inflammatory’ because they cause skin thickening, warmth and erythema of the breast, which is mediated by dermal lymphatic invasion and obstruction; interestingly, inflammatory cells are absent. The highly angiogenic and invasive nature of these tumors makes them an ideal model for studying anti-angiogenic strategies. TM inhibited tumor growth of injected SUM 149 cells, a cell line derived from a patient with inflammatory breast cancer. Ten-week-old female athymic mice received intra-mammary injections of 1 × 10⁶ cells, and were subsequently gavaged with water or TM beginning on the day of xenograft transplantation. Cp levels were monitored weekly, and maintained at approximately 20% of baseline. TM treatment resulted in a 69% reduction in the size of primary breast tumors, as well as decreased tumor vascularity as measured by immunohistochemical staining for CD31 an endothelial cell marker.

TM also prevents tumor growth in a transgenic murine model (Pan et al. 2002). HER2/neu transgenic mice overexpress a protein associated with poor prognosis in patients with breast cancer. Female mice expressing this gene develop intra-mammary adenocarcinomas which metastasize to the lungs. Mice were treated with TM
beginning at 100 days of age so that they would be copper deficient during the key period of tumor growth. Cp levels were maintained at 10–30% in treated mice, who appeared to tolerate this level of copper deficiency well. Although 50% of control mice developed clinically overt tumors by 218 days, none of the TM-treated mice did, as long as their Cp levels were maintained at ≤30% of baseline. Upon release from therapy, TM-treated mice developed measurable tumors in 13 ± 5 days.

To investigate the mechanism of the anti-angiogenic effects of TM, SUM 149 cells were grown in culture and treated with TM (Pan et al. 2002). Decreased secretion of pro-angiogenic mediators including VEGF, FGF2, IL-1α, IL-6 and IL-8 was seen in treated cells. Furthermore, conditioned media derived from treated cell cultures had impaired ability to promote new vessel formation in the rat aortic ring assay. TM-treated SUM 149 cells also showed reduced activity of the transcription factor nerve factor-kB (NF-kB), a protein known to be involved in tumor invasion, angiogenesis and metastasis. This effect was reversed by the addition of copper, suggesting that induction of copper deficiency is responsible for reduced NF-kB activity. Because VEGF, IL-6 and IL-8 are NF-kB-regulated genes, we postulate that reduced secretion of these proteins is a direct consequence of the inhibition of NF-kB by TM.

TM may also potentiate the effect of cytotoxic therapies. Cultured SUM 149 cells were treated with TM in combination with doxorubicin. The combination therapy was shown to induce apoptosis in a synergistic fashion (Pan et al. 2003). Following these promising results, the combination was utilized in a mouse xenograft model of breast cancer. Female nude mice were transplanted with SUM 149 cells, and tumors were allowed to grow to 0.5 cm³. Mice were then assigned to receive no treatment, single agent doxorubicin, single agent TM or the combination. Mice receiving the combination regimen had stabilization of tumor volume to a greater extent than mice treated with either single agent with no additional toxicity.

TM has also been combined with external beam radiation therapy in a mouse model of lung cancer, the Lewis lung high metastatic carcinoma model. Combination therapy demonstrated an additive effect on reduction of tumor volume, again with no added toxicity over radiation alone (Khan et al. 2002). The addition of TM may also provide a modest survival benefit in these animals.

Given the genetic complexity of human solid tumors, it is not surprising that anti-angiogenic strategies that target a single angiogenic growth factor have failed to inhibit tumor growth in clinical trials. Blockade of one pathway of endothelial proliferation and migration may be overcome by exploitation of redundant pathways to promote development of new blood vessels. In fact, tumors have been shown to express multiple angiogenic factors (Relf et al. 1997, Bunn 2002). Effective targeting of malignant neovascularization may thus require development of a global inhibitor of angiogenesis rather than an inhibitor of one angiogenic cytokine.

As outlined above, copper is an essential angiogenic cofactor which appears to exert its effects through several pro-angiogenic mediators. In animal models, copper depletion via TM is a useful anti-neoplastic therapy which may be combined with traditional cytotoxic therapy with additive effects and little or no additional toxicity. Furthermore, TM has a demonstrated safety record in humans where it has been utilized for the treatment of Wilson’s disease. Induction of copper deficiency can be easily monitored via measurement of serum Cp. For these reasons, TM is currently being evaluated in clinical trials.

A phase I trial of TM in metastatic cancer has been completed (Brewer et al. 2000). Eighteen patients with a variety of tumor types (including breast, colon, lung and prostate) and measurable disease were enrolled. Patients were required to have progressive disease at the time of enrollment. The target Cp level was ≤20% of baseline; 14 of 18 patients reached the target Cp level. Eight of these patients were copper deficient for less than 90 days; the majority (seven of eight) of these patients had progressive disease. These patients likely did not achieve stable copper deficiency long enough to have a clinical effect. However, among the six patients achieving adequate Cp levels for > 90 days, four maintained stable disease, one had stable disease with partial regression of lung lesions, and one had disease progression at a single site, with stable disease elsewhere. Toxicity was minimal. Mild anemia was noted in four patients whose Cp reached 10–20% of baseline. Anemia was reversible upon discontinuation of the drug. Several patients reported sulfurous belching as the only other reported toxicity.

Results of a phase II trial of TM in advanced kidney cancer have recently been published (Redman et al. 2003). Fifteen patients with metastatic kidney cancer who had not responded to IL-2 or were not eligible for IL-2 were treated with TM. All patients were able to achieve the target Cp level of 5–15 mg/dl. Of the 13 patients evaluable for response, four (31%) had stable disease for at least 6 months; none had a partial or complete response. Grade 3 or 4 granulocytopenia developed in eight patients without episodes of febrile neutropenia. Neutropenia improved with temporary cessation of the drug in all cases. Serum levels of VEGF, basic FGF, IL-6 and IL-8 measured at the onset of copper deficiency were significantly reduced.
compared with pretreatment levels; by 3 months, however, only IL-6 remained significantly depressed.

These studies raise several interesting points. First, TM is likely to have a cytostatic, rather than cytotoxic, effect in bulky cancers and is therefore most likely to result in disease stabilization rather than reduction of disease burden. Furthermore, achieving adequate levels of copper deficiency may require several months. Thus, TM monotherapy would probably not be beneficial in patients with a large burden of disease and/or rapidly progressive disease. In the metastatic setting, TM may be most beneficial for patients with minimal disease burden (such as after induction of a complete or partial remission), or concurrently with cytotoxic agents or modalities such as chemotherapy or radiation. Combination therapy has been well tolerated in animals, as noted above. Additionally, TM may be useful in an adjuvant setting or as chemoprevention in high-risk patients. Ongoing phase II studies as well as future trials will attempt to exploit this knowledge to define the role of TM in cancer treatment.

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