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Tetrathiomolybdate Copper Reduction Therapy as an Antiangiogenic Treatment for Lymphoma and Other Cancers

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Chapter I

Disclaimer

The following is written by lay individuals for information purposes only and is not to be used to diagnose or treat any disease. The document outlines therapeutic strategies which have not been clinically proven and may not be effective for lymphoma and other malignancies anc may present significant foreseen and unforeseen risks. Any and all treatment decisions should be made with the full cooperation of a qualified physician. The authors or any individual contributing to or distributing this document cannot be held responsible for any consequences resulting from the use of any of the medical strategies outlined in this review.

Introduction

The concept of treating cancer by restricting the blood supply to malignant tumors has recently gathered intense interest in the medical community and the press. In January 2000, University of Michigan researchers published a preliminary report of a human trial showing that reducing body copper levels can be a minimally toxic means to inhibit the growth of smal blood vessels feeding solid tumors. This information has not appeared to have much relevance to patients suffering from non-Hodgkin's lymphoma which is considered a blood-borne cancer and not a solid tumor. However, an overwhelming amount of scientific evidence shows that copper plays a role in the progression of cancers such as lymphoma, and this opens up the possibility of new therapeutic strategies outlined here.

The authors have been personally touched by non-Hodgkin's lymphoma, a disease where oncologists frequently advise postponing conventional treatment due to the toxicity and limited effectiveness of standard chemotherapy and radiation. This places patients into a category called Watch & Wait (W&W) where oncologists monitor cancer's progression withou beginning anticancer treatment. Although the author's interest leads much of the information in this report to be specific to lymphoma, other information may be of value to those facing different cancers as well.

What is Lymphoma?

Lymphoma, leukemia, and multiple myeloma are all blood-proliferative cancers. Lymphoma is a general term for a group of cancers originating in the lymphatic system - a vital part of the body's defense against infection. Lymphoma can be further categorized as non-Hodgkin's lymphoma (NHL) and Hodgkin's disease. NHL is a diverse group of diseases affecting many different types of lymphocytes at different times in their maturation. When a lymphocyte undergoes a malignant change and begins to multiply, cancerous cells start to outnumber healthy ones leading to tumors. Lymphoma results as the tumors enlarge, usually in the lymph nodes but sometimes other sites in the body.

Approximately 70,610 cases of lymphoma will be diagnosed in North American in 2001, and non-Hodgkin's lymphoma is the fifth most common cancer. The incidence of non-Hodgkin's lymphoma is increasing 3% to 4% every year. One out of every forty-seven men and one out of every fifty-seven woman will develop lymphoma in their lifetime. Over the past 15 years, th incidence of NHL has increased 50%, and its cause is unknown.

Antiangiogenesis - A New Approach to Cancer Treatment

The growth of new blood vessels in a process called "angiogenesis." "Angio" means blood vessel and "genesis" means new formation. Antiangiogenesis involves the hypothesis that cancer may be stopped by depriving tumors of the blood supply that nourishes them. As tumors grow larger than two millimeters in size, they require new blood vessel growth to supply oxygen and nutrients to the cells and carry waste away. Vascular endothelial cells are the cells involved in the genesis of new blood vessels. By specifically suppressing the growth of vascular endothelial cells, it is hoped that the tumor will no longer grow and will remain essentially harmless. Simply stated, the theory holds that tumors deprived of vascularization can no longer grow, and the cancer remains dormant indefinitely.

Angiogenesis Inhibitors - Dr. Judah Folkman

Dr. Judah Folkman has been a leading pioneer on the subject of angiogenesis and cancer growth since the early 1970s. He has developed this concept for many years in relative obscurity, but a New York Times article in 1998 suggested that his research could have profound effects on the treatment of cancer. Two of the drugs he developed from natural animal proteins are named endostatin and angiostatin. When combined, they showed stunning effects on cancer growth in test animals. In 1999, the first human trial to investigate antiangiogenesis using endostatin was opened at Dana-Farber Cancer Institute in Boston, and in 2000 an additional endostatin trial was initiated at MD Anderson Cancer Center in Houston. Preliminary results released in November 2000 have showed no toxicity and sufficient anticancer effects to support further trials. The investigational drug used, endostatin cannot be made widely available before full pharmacology and toxicology profiles have been established. Investigational drugs are generally not available through a compassionate use protocol. Endostatin trials have been for solid tumors only. None have included lymphoma patients.

Antiangiogenic Activity in Hematologic Malignancies

James M. Pluda, M.D., is a senior investigator at the Investigational Drug Branch of the National Cancer Institute (NCI). He was contacted and asked if antiangiogenesis therapy

would be useful in so-called "liquid malignancies." Dr. Pluda replied, "My expertise is in the field of drug development for antiangiogenesis inhibitors. I can tell you that the NCI is indeed interested in evaluating the activity of antiangiogenesis compounds against [non-Hodgkin's lymphoma] NHL and have placed trials administering thalidomide and SU5416 to patients wit NHL and consider the evaluation of the activity of antiangiogenesis inhibitors against NHL a priority."(1) He stated later in September 2000, "There currently are NCI-sponsored lymphoma trials administering angiogenesis inhibitors that are in review and should open shortly." (2)

Dr. Thomas A. Davis is a senior investigator for the Cancer Therapy Evaluation Program at the NCI. He has stated, "Given the recent interest in antiangiogenesis agents and the knowledge that angiogenesis is present in lymphoid malignancies via production of vascular endothelial growth factor (VEGF) and other growth regulatory factors, justification exists for the development of phase I and II trials using such agents in hematologic malignancies."(3)

Dr. George Brewer is a professor of human genetics at the University of Michigan Medical School who has worked for decades in devising a copper depletion treatment for Wilson's disease, a rare genetic disorder that causes excessive copper accumulation. In this disease, the body is unable to rid itself of copper, which leads to dangerously high concentrations of this mineral. The disease is fatal unless detected and treated before serious complications develop from copper toxicity. Since copper has been found to be a major growth nutrient required for angiogenesis, the consideration arose that limiting copper levels in the body could help control the growth of malignant tumors.

Angiogenesis Is a Factor in Non-Hodgkin's Lymphoma

1. In July 2000, the combination of the antiangiogenic drug endostatin with either Rituximab or chemotherapy was reported to produce better results than either Rituximab or chemotherapy alone. The anti-CD20 monoclonal antibody Rituximab is a effective agent for B-cell non-Hodgkin's lymphoma, but the risk of relapse is high. Mice with limited disease were given a low toxicity combination therapy of Rituximab followed by endostatin. They experienced no tumor growth as long as the endostatin was administered. Mice with bulky NHL were given chemotherapy and endostatin sequentially, and these mice exhibited significant tumor regression.(4)

2. Angiogenesis was shown to increase with the pathological progression of B-cell non-Hodgkin's lymphomas in a January 2000 study of lymph node biopsies where the microvessels were counted. The results suggest that angiogenesis in B-NHLs increases with their progression.(5)

3. In 1999, thalidomide was found to be a successful antiangiogenic treatment for Multipl Myeloma, a disease of mature proliferating B-cells, related to lymphoma.(6)

4. In March of 1999, the Lymphoma Lab at the University of Arizona at Tucson studied the expression of VEGF and its receptors in non-Hodgkin's lymphoma. Their findings were that the process of angiogenesis appeared to be active in NHL. Their data also raised the possibility that VEGF may play a role in the growth of NHL.(7)

5. In January 1996 lymph node samples were analyzed from 88 B-cell NHL patients and compared to 15 patients with benign lymphadenopathies. The microvessel number increased significantly from the benign to the low-grade lymphadenopathies. Intermediate-grade tumors displayed even further significant increase, and high-grade B-cell NHL showed the highest count of microvessels.(8)

6. A December 1995 study showed that angiogenesis is a necessary step in solid tumor progression (growth, invasion, and metastasis) beyond its primary site which can be indicative of an unfavorable prognosis. This study identified angiogenesis in lymph nodes of B-cell NHL intermediate and high grade categories, and concluded that

antiangiogenic therapy could be envisioned as a possible future development.(9)

7. Angiogenesis induced by B-cell non-Hodgkin's lymphomas was confirmed in March 1990. The angiogenic nature of lymphoma tumors was demonstrated using a standard lab test which uses the chorioallantoic membranes of chicken embryos.(10)

Microvessels

Leukemia and lymphoma are two similar blood-proliferative diseases. Both are characterized as "liquid tumors," unlike solid tumors that start at a primary site. Microvessels were not found in lymphoma or leukemia tissues when first examined under microscopic power. Several years ago, Dr. Folkman added a stain to leukemia cells. With stain under microscopic power, dense microvessels could be seen in leukemia cell tumors. In experimentation with mice that were allowed to grow excessively large leukemia tumors, normally fatal within two days, they were able to completely regress the tumors using antiangiogenic drugs. The intensity of angiogenesis varies according to the type of malignant tumor. Liver tumors are highly vascularized while lymphoma tumors are poorly vascularized. Dr. Folkman has shown that antiangiogenesis can effectively treat even poorly vascularized tumors in animal trials. These studies demonstrate new blood vessel growth supports soft leukemia tumors.

Growth Factors

Tumors need to grow, and to grow over two millimeters in diameter they need neovascularization. One way tumors do this is to provoke an inflammatory process by expressing arachidonic acid. This allows the enzyme cyclooxygenase-2 (COX-2) to cascade a sequence of reactions that results in the development of new blood vessels to support the tumor growth.

Additional factors supporting tumor growth in NHL (and many other cancers) are the metalloproteinases such as MMP-9. Growth factors such as thromboxane A2 (attracts endothelial migration), VEGF, basic fibroblast growth factor (bFGF) that scientists first found in solid tumors have now been identified in "liquid tumors." Control of this growth process can be attempted at various points such as COX-2 inhibition, thromboxane A2 inhibition, or at the growth factor level. The effectiveness of any given therapy will depend on the sensitivity of the particular cell line.

A study in 1999 evaluated angiogenic growth factors and endostatin in non-Hodgkin's lymphoma. The data suggested that bFGF and, particularly, VEGF might be considered prognostic factors in NHL staging and management.(11) This means that NHL is very typical compared to other cancers in terms of tumor stimulation and growth through angiogenesis.

A recent PubMed search for angiogenesis and NHL lists 47 abstracts most of which support the contention that angiogenesis is active in NHL (see Appendix III.)

Chapter II

Copper-Reduction Therapy

Knowing that reducing copper can effect vascularization of tumor masses, Dr. George Brewe applied his copper-reduction techniques to cancer. New blood vessels appear to have a very strong dependence on copper for growth, but low copper levels are unlikely to affect existing

vessels. By depriving cancer tumors of the copper they need to form new blood vessels, Dr. Brewer's research team at the University of Michigan stopped the growth and spread of the disease for over two years (as of December 2000) in a small group of patients with advanced cancer. The abstract of Brewer's Phase I trail results is shown in Appendix I, but the full ten page report released January 2000 is viewable on line, Treatment of Metastatic Cancer with Tetrathiomolybdate, an Anticopper, Antiangiogenic Agent: Phase I Study.(12) The (PDF version is available for printing if you have Adobe Acrobat Reader, and don't mind the few minutes it takes to load.)

The underlying hypothesis of antiangiogenesis using copper-reduction therapy is that the level of copper required for angiogenesis is higher than that required for essential copper-dependent cellular functions. The assumption is that there is "...a window of copper deficiency in which angiogenesis is impaired, but other copper-dependent cellular processes are not affected enough to cause clinical toxicity."

Dr. Brewer's clinical trial indicates that physicians might fight cancer by targeting copper as a "common denominator" of angiogenesis. Unlike other anti-cancer agents now being studied around the world, copper-reduction therapy is not limited to a single type of cancer. The relationship of copper to cancer is not causative but associative. Cancer cells in a high copper environment find it easy to proliferate into tumors. In an environment low in copper, cancer cells would remain dormant or very slow-growing, increasing survival time.(13)

For the cancer trials, Dr. Brewer teamed up with Sofia Merajver, Ph.D., M.D., a molecular genetics researcher and oncologist also at the University of Michigan Comprehensive Cancer Center. The promising results from the phase I trial prompted Merajver to state, "These initial results suggest that the tactic of preventing angiogenesis through copper deficiency holds significant promise. Through this and other therapies, we may one day be able to turn cancer into a chronic or controllable disease or to contribute to its eradication... *We also believe that the earlier TM [copper-reduction therapy] is given in the progression of a patient's cancer, the better it should work* [emphasis added]."(14)

Penicillamine and Trientine

There are a number of techniques that can be used to reduce copper stores. Most Wilson's disease patients use either penicillamine or trientine,(15) prescription drugs that act as chelating agents by binding to copper. Penicillamine (sold as Cuprimine or Depen) works by binding with copper and increasing renal excretion. It is effective in reducing copper levels, but up to 20 percent of penicillamine patients can suffer neurological symptoms. About half develop other adverse effects such as fever, rash, or joint pains. These people are usually changed to trientine (Syprine), another chelating compound which removes excess copper from the body. Unfortunately, trientine, like penicillamine, can also have significant side effects.(16)

In lieu of using either of the harsh drugs, penicillamine or trientine, Dr. George Brewer investigated zinc acetate to treat the presymptomatic or pregnant Wilson's disease patients. He also used a complex of molybdenum for the initial treatment of Wilson's disease patients with neurological symptoms who needed fast copper-reduction therapy, but this therapy required the patient to come to the University of Michigan.(17)

Molybdenum & Ammonium Tetrathiomolybdate

What is molybdenum? Molybdenum is an essential trace mineral that is needed for the proper function of certain enzyme-dependent processes, including the metabolism of iron. Some increased urinary excretion of copper will occur with daily molybdenum consumption of up to 10 - 15 mg (10,000 - 15,000 mcg) per day,(18) but intake of higher doses has produced clinical evidence of gout-like symptoms, such as joint pain and swelling.(19)(20) A combination of sulfur and molybdenum is required to form a metallothionein complex to effectively bind copper for excretion. This reaction does not occur naturally in the human

body. Thus, molybdenum (as ammonium tetramolybdate) is not an effective copper chelator by itself.

What is TM? Ammonium tetrathiomolybdate (TM) is a complex of sulfur and molybdenum designed as a fast-acting compound to quickly lower copper levels by oral chelation.

"Ammonia" is a salt which increases body absorption and known as "ammonium" when an extra hyrodgen atom is added to the molecule.

"Tetrathiomolybdate" is a complex word -- tetra-thio-molybdate.

"Molybdate" is the chemical compound of oxygen with the copper binding mineral molybdenum. (The "-ate" suffix indicates oxygen atoms are part of the molecule.)

"thio-" is the sulfur which aids the body's elimination of molybdate after it has bonded with copper.

"Tetra-" is four, as there are four sulfur atoms in one tetrathiomlybdate molecule.

Ammonium tetrathiomolybdate (TM) is a complex of sulfur and molybdenum designed as a fast-acting compound to quickly lower copper levels by oral chelation. This compound may be the world's safest and most potent anti-copper agent. It is extremely well tolerated, with few side effects, and TM is particularly useful to patients who wish to avoid the potential adverse reactions to the standard chelating agents, penicillamine and trientine.(21)

How does TM work? In his trials, Dr. Brewer wants to determine if copper deficiency is a feasible modality to stop cancer's growth by inhibiting tumor neovascularization. Dr. Brewer found that TM could be safely administered to his copper-reduction therapy patients in daily doses of 120 mg (six capsules of 20 mg each), at least ten times the maximum safe dosage of molybdenum alone. TM lowers the body's copper level by chelating (binding to) the copper and protein, making a stable compound that cannot be used by the tumor cells or any part of the body. Taken at mealtime, TM prevents the body from processing and absorbing the copper in food as well as the copper normally found in saliva and gastric secretions. When taken between meals separated from any food by at least two hours, TM is even more effective. (Capsules taken in the middle of the night if one awakens to go to the bathroom seems to be the most effective in lowering copper levels.) On a relatively empty stomach, TM is absorbed into the blood and binds copper to serum albumin, a protein in blood. The TM-protein-copper complex does not interact with other biological molecules and is excreted.(22)

How can TM be obtained? TM has been registered under the FDA's Orphan Drug Act (23) since 1994. The University of Michigan claims to be filing a new-use patent for TM in the treatment of cancer and other diseases that rely on angiogenesis.(24) The University of Michigan states that full-scale toxicity studies are required before TM can be made available to large numbers of people, and this would be a necessary step for the FDA to approve TM a a drug suitable for any therapy. According to the University of Michigan, the National Cancer Institute has accepted them into a program designed to speed access to new medications. Verification is impossible as the FDA considers all pending applications as confidential.

At the University of Michigan, TM is currently available only through their clinical research programs.(25) Dr. Brewer's source for TM is Sigma-Aldrich, Inc., in Milwaukee where they specifically refine the product for human consumption. Dr. Brewer further tests the product for freshness by spectrum analyses and biological assays. Apparently, any pharmaceutical TM purchased from Sigma-Aldrich outside of the University of Michigan's control with Dr. Brewer must be initiated by the patient's doctor, approved as patient specific through FDA, purchased in bulk, and encapsulated at a compounding pharmacy.(26) After a lot of customer prescreening, Sigma-Aldrich has sold their technical grade of TM in powder form which is guaranteed pure only up to 99.97%. Technical grades are not always pure enough for human ingestion as they can possibly contain toxic contaminants. However, there are other sources

available for obtaining TM.

The FDA Modernization Act of 1997 (27) changed availability of many drugs which have not been approved. TM is not a common substance, and Sigma-Aldrich is not the only source. Several compounding pharmacists (28) have found other companies to provide it. Only a doctor's prescription is required, and there is no requirement for patient specific FDA approval.

Every day more compounding pharmacists are becoming familiar with TM as they start supplying it to their customers, usually by mail order prescription. We have listed known suppliers in Appendix VI

In most locations, compounding pharmacists require a doctor's order for TM, but the prescription does not have to be from an oncologist. The usual price is $2.25 per capsule. Since the material is very sensitive needing an oxygen-free environment prior to the arduous compounding process, any offering of "cut rate" TM would be very suspicious. Dr. Brewer found that TM retained 90% of its potency in capsules for only 8 weeks before the exposure t oxygen causes it to slowly degrade (oxygen replaces the sulfur in the molecule rendering it inactive).(29)

To maximize the potency and shelf life of an unstable chemical such as TM, it needs a good and reliable compounding pharmacist to professionally prepared it to each patient's order ensuring freshness. The best way is to place the drug in capsules along with an inert, nontoxic gas such as argon. This method is very expensive, but an alternate method is to disperse the drug in a nonionic oil that has a poor solubility for oxygen or water. Oil based capsules should release the TM slowly minimizing any potential gastrointestinal discomfort and increase the body's absorption of the drug to chelate copper. Most people tolerate the oil base very well.(29.1)

Not all compounding pharmacists are using oil filled TM capsules. Some pharmacists are dry packing capsules. TM is a new and experimental drug, and actual clinical testing on the preferred encapsulating methods have simply not been done.

TM needs to be stored by the patient in a controlled environment low in oxygen, low in humidity, and away from light. Oxygen causes TM to decompose, and moisture can cause a chemical reaction in TM. Adding as many small desiccant packets as possible to the bottle (many can be found packed in other vitamin and drug bottles) can minimize any moisture. Do not store the TM in either the freeze or refrigerator because these environments are too wet. Put the TM bottle inside a second container to maximize darkness and store in a dry, cool location. Do not purchase more than a one month's supply.(29.2)

What is the physician's liability for prescribing TM or any other drug not approved for a specific medical indication? It is not legal to promote TM as a treatment for cancer since it lacks FDA approval for anything other than experimental use. A physician can order prescriptions for a drug if he thinks it is medically justified even if there is no specific approval for the specific condition he is treating. In general, there are two things a doctor might be worried about. One is having administrative sanctions imposed on him (the main one being losing his license), and the other is being sued by a patient for damages suffered because of inappropriate treatment that was not up to the generally recognized standard of care. This is a major reason doctors are reluctant to try drugs not officially approved. In relation to treating cancer patients, the only human data on the effect of TM in this group is from Brewer's limitec Phase 1 trial. If something goes wrong, the doctor could be judged according to whether his judgment in prescribing the medication was professionally reasonable. TM is an unproven experimental treatment for cancer which requires caution.

The Mineral Zinc

Zinc compounds

Zinc is a trace metal needed for more than 300 enzymes used by the body. It is not a copper chelator, zinc acts to block dietary copper in the intestines by preventing additional absorption of copper. It induces cells of the intestinal tract to produce a metallothionein protein (MT) which has a very high affinity for copper and is excreted in the stool. This means that any newly ingested copper does not reach the blood circulation system.(30)(31) Zinc is an alternative to older copper chelating agents, and Wilson's disease patients frequently use it in their maintenance programs.

In 1997 Dr. Brewer sought and received FDA approval for a compound of zinc known as zinc acetate or Galzin® (32) to treat Wilson's disease. According to Dr. Brewer, Galzin® is made by a reputable company and measured in precise doses untainted with lead or other contaminants. Galzin® is expensive and requires a prescription.

With a normal concentration of stomach acids, most forms of digestible zinc will break down and be absorbed into the bloodstream through the intestinal walls. Because zinc acetate undisputedly releases all of its zinc as zinc ions,(33) it is bioavailable. Before the advent of Galzin®, Dr. Brewer used zinc gluconate for his patients. Although easily absorbable, high doses of either zinc acetate or gluconate can cause stomach upset in sensitive patients. Zinc as amino acid chelates avoids nausea and naturally facilitates absorption as long as sufficien stomach acids are present.(34)(35) Another suggestion has been zinc citrate as a consideration of the best tolerated non-prescription form of zinc. Successful zinc therapy requires absorbing the proper amount of the mineral in the blood, but once in the bloodstream, it does not matter what form of zinc was ingested. Zinc serum level in the bloodstream may be easily measured by a common lab test.

Zinc Metabolism

Excessive supplemental zinc is not stored. The human body is extremely efficient in removing surplus zinc in fecal matter, urine, and sweat. Gram doses might be tolerated very well for a few days in some very ill people in need of zinc, but not likely for longer periods of time.

Some people do not absorb zinc well and live their entire lives in a state of poor health. Others absorb zinc very well and rarely are sick.(36) Zinc deficiency should be taken seriously as there is a direct correlation between cancer and zinc deficiency(37) Advanced cancer appears invariably associated with zinc deficiency.(38)

High serum copper, sometimes combined with low serum zinc, is associated with increased mortality from all cardiovascular disease (39) (40) and higher risk of subsequent cancer diagnosis.(41)

Zinc deficiency, low zinc serum levels below the normal range of 60 to 150 mcg/dL, is common in cancer and causes immune suppression since zinc is a key component of many enzyme systems necessary for T-cell function and regulation.

Studies such as Chrandra's (42) back in 1984 have claimed that zinc serum concentrations significantly above the optimal high-normal range of 140 to 160 mcg/dL can cause immune suppression. Other studies have concurred with Chrandra that the optimal zinc serum range of 140 to 160 mcg/dL is best for maximizing (doubling) T-cell function (43), (44), and (45). Back in 1997, Dr. Brewer did his own study on the effects of high dose zinc on Wilson's disease patients. He found no compromise in T-cell / lymphocyte function and stated, "We have seen no indications of immune suppression or increased susceptibility to infections in our patients, who have now been treated with zinc for up to 15 years. We conclude that any side effects from compromised lymphocyte function caused by administration of zinc are not of concern to patients of Wilson's disease."(46)

Absorption and excretion rates of zinc are subject to individual variability. The copper reduction protocol developed in Wilson's disease is 50 mg zinc three times a day without food. A different formula is to take a sufficient amount of zinc to maintain optimal blood serum

levels at the high normal range, between 140 and 160 mcg/dL. If 150 mg zinc per day does not increase zinc serum to this range, the dosage may be increased up to 1.5 mg per pound of body weight (or higher). For example, a 150 pound person could take as much as 150 multiplied by 1.5 mg or a total of 225 mg of zinc daily. The dosage may be adjusted to maintain zinc blood serum level between 140 to 160 mcg/dL, conservative enough to protect optimal immune function according to the Chrandra study while still producing copper-reduction effects. (47)

Zinc Toxicity

Zinc treatment is effective over long periods of time, even a lifetime, and poses no major health problems.(48) Harmless nausea can occur from individual doses exceeding 50 mg per dose. Extremely high doses of zinc have been consumed with minimal toxicity. Zinc taken in excess of two grams per day will usually cause gastrointestinal irritation and provoke vomiting.(49) However, this is thirteen times the dose prescribed by Dr. Brewer.

Zinc has been scientifically researched and is well documented as safe, and even the effects of gram doses of zinc given over many months was shown to be non-lethal. An interesting story of chronic zinc intoxication has been reported of a psychotic twenty-year-old woman who took an average of 2300 mg elemental zinc per day, in doses ranging from 400 mg to 5700 mg, for four months. She presented herself for a routine examination. The most striking laboratory findings were a serum zinc level of 1160 mcg/dL, a copper serum of 8 mcg/dL, anc an undetectable serum ceruloplasmin level. The patient was severely anemic and exhibited macrocytosis (excessively large red blood cells) and neutropenia. (Neutropenia is an adverse condition where white blood cells known as neutrophils have dropped so low that there is a much greater risk of developing infections, particularly in the mouth, throat, sinuses, lungs an skin.) Her only intolerance appeared to be some vomiting. Oral copper therapy and the cessation of excess zinc consumption produced a remarkable reversal of all parameters. Her rapid recovery from long-term zinc toxicity was essentially complete in one month, and an examination one and a half years later confirmed that there was no apparent permanent damage.(50)

Data indicates that as much as one-half of the world's population is at risk of zinc deficiency, but zinc toxicity is a rare phenomenon. High dose zinc supplementation has been used to treat patients with Wilson's disease to deplete copper for up to 15 years. Dr. Brewer saw no indications of immune suppression or increased susceptibility to infections in his patients.(51)

There are over 1800 MedLine citations about "zinc deficiency" and only about 15 on "zinc toxicity." Zinc can stimulate the immune system to increase the number of circulating effector T-lymphocytes and natural killer (NK) cells which kill cancer. Zinc deficiency is related to immunosuppression, which allows cancer cells to grow freely.(52)

Therapeutic Use of Zinc

History of Zinc Treatment in Cancer

In the early 1950s, discovery of abnormal zinc metabolism in chronic adult leukemia patients suggested use of zinc as a therapeutic drug in its treatment. Zinc may act by stimulating cell mediated immunity.

When in 1979 a child's acute T-cell lymphocytic leukemia (ALL) was put into permanent remission within two weeks of initiation of chemotherapy in conjunction with therapeutic doses of adult doses of vitamins and minerals, her oncologist questioned whether one of the supplements might have enhanced her therapy.(53) It was the child's father, George Eby, who researched to identify zinc as the element saving his daughter's life.(54) He found that raising zinc concentration in the serum to 140 mcg/dL resulted in numerous immunological and adjuvant to chemotherapy effects which had the effect of rapidly eliminating all detectable leukemic blasts in bone marrow and blood. The high normal zinc serum concentration was

maintained according to blood test each two weeks during the three year chemotherapy program. The young woman remains without relapse to this date.

About a dozen other families with leukemic children and one with lymphoma followed Eby's protocol of zinc plus chemotherapy between 1985 and 1987. They were all successful in putting their child's cancer into a very strong remission (zero blasts in bone marrow and blood) within about two weeks. When publicity ran out on the Eby case, no one followed up with human trials to supplement the diet with zinc in treating childhood leukemia.(55)

George Eby changed his career to biomedical research and published years of research in one of the largest zinc sites on the Internet starting in 1996.(56) He developed zinc cold lozenges which were proven effective in many trials, with the most recent clinical trial (57) in 2000 led by an expert in zinc studies, Ananda S. Prasad, M.D., Ph.D., a well-known research oncologist at Wayne State University in Detroit, Michigan.

Zinc's Role in Wilson's Disease

In 1987, doctors Brewer and Prasad, experts in copper and zinc metabolism, both joined together on a research project which developed the following standard zinc maintenance protocol used today for Wilson's disease, a genetic disorder in which the body is unable to excrete excess copper.(58) This therapy received approval by the Food and Drug Administration (FDA) early in 1997, and it is used as standard protocol today.(59)

1. Dose: 50 mg zinc three times a day (zinc acetate / Galzin®). Separate from food and beverages (other than water) by one hour.

2. Food: avoid liver, limit shellfish.

3. Check drinking water. Restrict intake if copper more than 0.1 ppm (0.1 mcg/L).

4. Monitor copper and zinc in 24-hour urine collection tests every six months. Good control in preventing copper loading is less than 125 mcg/24 h. Good compliance in zinc supplementation is more than 2 mg/24 h.

High urine copper levels indicate excessive amounts of toxic unbound copper in Wilson's disease patients. Ceruloplasmin (Cp) is a protein used to transport copper, and Wilson's disease patients typically present with low Cp levels. Despite excessive copper storage levels the liver of a Wilson's disease patient is not able to produce sufficient Cp to bind the free copper in the blood.

Dr. Brewer found that zinc is less effective when taken with meals. He advised separating each zinc dose from any food or beverage other than water by at least one hour before and two to three hours after eating. Since there is no known testing of combinations of vitamins or mineral supplements with zinc, it is best to take the zinc apart from any other substance.(60) Further, single daily doses of zinc are relatively ineffective in maintaining the blockade of intestinal copper absorption that is necessary to preclude significant copper reduction.(61) Some patients have found that 50 mg zinc is too upsetting on an empty stomach. One possible alternative might be to take a small piece of meat with the zinc which may inhibit any vomiting or nausea. Meat interferes with zinc absorption less than other foods.(62)(63)

Initial Copper Reduction and Maintenance in Wilson's Disease

The initial therapy for Wilson's disease patients was to use either penicillamine or trientine, prescription chelating agents that bind to copper. By the 1990s, Brewer had made a major advancement in copper reduction therapy when he started protocols with TM which the University of Michigan registered under the FDA's Orphan Drug Act in 1994. TM is very effective and quick-acting, and its method of excretion does not cause temporary increases ir

brain copper levels like penicillamine or trientine.

Zinc is not recommended as an initial treatment because it is slow-acting and produces little effect in the first few weeks. Its use as initial therapy for Wilson's disease patients is usually limited to those who are pregnant or without symptoms of copper toxicity.(64)

After initial therapy normalizes copper levels, the patient must be maintained to prevent further toxic copper accumulations. Zinc has been used by Dr. Brewer for Wilson's disease maintenance therapy for up to 15 years, and he has seen no indications of undesirable afflictions, immune suppression, increased susceptibility to infections, or other side effects in Wilson's disease patients treated with zinc.(65)

From Wilson's Disease to Cancer

Dr. Brewer noted several minor adverse effects from copper depletion in his Wilson's disease patients. These effects were easily controlled with treatment modifications, but the incidents drew Dr. Brewer's attention to other possible therapeutic uses for zinc.(66)

At the University of Michigan in 1999, Dr. Brewer applied his Wilson's disease protocol of copper reduction to cancer trial patients substituting TM during the actual copper-reduction therapy.(67) TM usually takes one and a half to two months to reach therapeutic levels of copper deficiency for cancer patients. Dr. Brewer stated that the main advantage of TM over zinc supplementation in reducing copper levels is speed of therapy. Although Dr. Brewer's original plan was to change the cancer trial patient's protocol from TM to zinc at the maintenance stage, his initial six successful trials patients were thriving so well on TM that they have continued to be maintained on it.

Copper Control in Cancer

Even with consistent, good compliance of 150 mg daily zinc and reaching the optimal zinc serum range of 140 to 160 mcg/dL, copper reduction with zinc therapy is extremely slow. Current estimates are that zinc therapy will lower Cu approximately 25 mcg/dL and Cp approximately 5-7 mg/dL over a 70-90 day period. High levels of zinc may be slow to reduce copper, but zinc is still a healthy supplement to enhance immune function and form an angiogenesis blockade to retard cancer's growth.

According to Dr. Brewer, even when the target Cp baseline is reached, it can take an additional four months to see tumor reduction because tumors sequester copper and will only reduce in size as the formation of new blood vessels is blocked. Given approximately two years, long-term oral zinc treatment can be a safe and effective alternative to copper chelatin agents. Assuming the more urgent necessity of control for a patient's cancer condition, more aggressive therapy with copper chelators is usually indicated.(68)

Dr. Brewer choose TM for the cancer trials because speed was needed to control the patient' disease, but once at baseline, zinc should be easy to substitute for TM in maintaining low copper levels. The dose would need to be individually tailored to maintain the patient's Cp within Brewer's copper deficiency window.

There has been speculation that since TM and zinc function differently in the body, a protocol could be used combining them both in copper reduction. Several anecdotal reports from patients who tried such combinations were negative. They found that their rapidly declining Cp was accompanied by bone marrow toxicity exhibited by platelets, neutrophils, WBC, and RCB counts dropping to unsafe levels. The problem could well have been dosage in that daily amounts of 120 mg TM combined with 150 mg zinc is too high. Alternate protocols such as 6(mg TM and 150 mg zinc are now being tested.

Although not yet standard protocol, new treatments of low-dose chemotherapy are being are

being tried where the patient is administered continuous low doses of standard anticancer drugs that target the tumor vasculature. Since there are no standard or well tested protocols, most oncologists are not participating in writing such therapies for their patients. A number of anecdotal reports have been heard of patients responding well to a combination of copper-reduction therapy with TM and low-dose chemotherapy. When these treatments are combined, extreme care must be taken by weekly monitoring of CBC counts because the combination of chemotherapy and TM can be more likely to produce bone marrow depression. Procrit or epogen may be needed to increase low RBC counts. In order to keep up your WBC, it may be necessary to take Leukine, also referred to as granulocyte macrophage colony stimulating factor (GM-CSF), a man-made form of a protein called a growth factor. Be sure to choose GM-CSF and not neupogen known as granulocyte colony stimulating factor (G-CSF). Neupogen is known as an angiogeneic agent where as leukine is mostly antiangiogenic.(68.1)

Captopril

The drug captopril is another copper chelator. It is an angiotensin I-converting enzyme (ACE) inhibitor that is approved and widely used for hypertension and congestive heart failure. Side effects may include anemia, rash, eosiniphila, low blood pressure and peripheral or facial edema (swelling). Although its positive effects on heart disease have been known for some time, only recently has its copper chelating and antiangiogenic properties been investigated. I appears that captopril inhibits angiogensis via three distinct pathways: conversion of plasminogen to angiostatin, copper chelation, and MMP inhibition.(69) Any use of captopril would require a doctor's supervision, probably a cardiologist since it is a potent heart medication. Since this drug chelates both copper and zinc, the advisability of supplementing with zinc during captopril therapy should be discussed with the administrating physician. Cancer patients who also have hypertension or a heart condition for which an ACE inhibitor is indicated may wish to contact their doctor to discuss the possibility of using captopril. Clinical trials of this drug for cancer are now underway at Northwestern University, but only as an agent to prevent lung injury in patients receiving a bone-marrow or stem cell transplant.

EDTA Chelation

Although chelation therapy has been used since the 1940's for a wide variety of ailments, it is still considered "alternative medicine" for anything other than heavy metal poisoning.(70) Chelation therapy usually consists of an intravenous solution containing a synthetic amino acid called ethylene diamine-tetraacetic acid (EDTA). When administered properly, it is a safe and effective way to deplete heavy metals and other toxins from the bloodstream. Although the authors could not locate any study results using EDTA chelation for copper-reduction therapy, one lymphoma patient known to them did make the attempt. He used standard EDTA chelation for five treatments, but he abandoned the therapy when test results indicated it was ineffective in lowering copper levels.

Chapter III

Medical Research -- Copper and Zinc in Cancer

The role of copper in cancer promotion through inflammation and angiogenesis is now well understood. Copper is incorporated in the extra-cellular matrix that forms the very structure of blood vessels. Copper acts as a co-factor to molecules known as bFGF, VEGF, and

angiogenin. Without it, they can not function, and growth of new blood vessels stops.(71) In other words, copper-reduction blocks angiogenesis by "switching" the endothelial cell into the apoptosis (programmed cell death) pathway, or quiescence, and the cancer remains dormant

For numerous malignancies (including lymphomas and leukemias) tumor incidence, progression, severity and relapse are all associated with high levels of copper serum (Cu). (72) Numerous studies since the 1970s established excess Cu levels and high copper/zinc ratios as prognostic indicators for lymphoma where higher levels correspond to more aggressive disease. (73), (74), (75), (76), and (77) Now that it is known that high copper levels promote angiogenesis, perhaps this can help explain the prognostic effect of high copper levels.

The objective of a 1995 copper/zinc ratio study in Mexico was to determine the diagnostic value of zinc serum (Zn), copper serum (Cu) and the copper/zinc ratios (CZR) in patients with hematological malignancies. Results showed that in healthy control subjects, Cu levels were significantly lower.(78)

	Healthy Controls	Lymphoma Remission	Lymphoma Untreated
Copper (Cu)	54 ± 8.9	--	93.7 ± 37.5
Cu/Zn Ratio	0.54 ± 0.13	--	1.21 ± 0.5

A 1988 study in Shanghai simultaneously determined the Cu, Zn, and CZR of 173 lymphoma patients by atomic absorption spectrophotometry.(79) The study concluded that Cu and CZR may be used as prognostic indicators for monitoring disease activity and response to therapy in malignant lymphoma.

	Healthy Controls	Lymphoma Remission	Lymphoma Untreated
Copper (Cu)	99.82	104.30	146.33
Cu/Zn Ratio	0.98	1.06	1.55

Ceruloplasmin (Cp) is a glucoprotein that transports Cu and was found to be significantly elevated in advanced stages of solid malignant tumors and increases up to four- to eight-fold during malignant progression. Data analysis in another study suggested Cp as a good diagnostic marker of cancer. Often before tumors become palpable, tumor regression returns Cp levels to normal.(80)

From this evidence it appears clear that tumors of all types have at their disposal the means to increase Cu and Cp levels for purposes of angiogenesis. Dr. Brewer's use of TM and zinc may be a method to thwart this tumor-driven conservation of copper and depletion of zinc.

Copper-Reduction Therapy in Trials for Cancer Patients

Dr. Henry Steven Brem

Dr. Steven Brem is a professor and Director of the Neuro-Oncology Research Laboratory at the Moffitt Cancer Center in Tampa, Florida. He has been a lead researcher in the field of angiogenesis and cancer (in collaboration with Dr. Folkman) for 30 years with a special interest in brain tumors, and he is the author of "Angiogenesis and Cancer Control: From Concept to Therapeutic Trial." (81)

Currently, Dr. Brem is the lead investigator in the Phase II Study of "Penicillamine and Reduction of Copper for Angiosuppressive Therapy of Adults with Newly Diagnosed Glioblastoma (brain cancer)". The new approaches to brain tumor therapy (NABTT) trial tests the combination therapy of radiation and copper depletion. It is closed to new patients, but trial participants are still continuing treatment.

The objectives are to determine in patients with glioblastoma whether penicillamine (PCT)(82

- yields a clinical improvement measured by an increase in the time from initial diagnosis to death (i.e., time of survival),
- improves the time from initial diagnosis to progression,
- reduces the level of serum copper, counteracting the normal response to brain tumor growth,
- controls the malignant phenotype by reduction on serial MRI scans of tumor volume, vascularity, invasion and edema.

A safety report for this trial has been published by ASCO 2000 which states, "penicillamine and a low copper diet are well tolerated and achieve clinically significant reduction of copper, an angiogenesis cofactor, in patients with newly diagnosed glioblastoma." (Appendix IV)

The rationale for the trial is that, "Tumor growth, invasiveness, and angiogenesis of experimental gliomas has been shown to be copper-dependent. Malignant brain tumors normally are associated with elevated tissue and serum levels of copper. The objective of the current clinical trial is to reduce the level of serum copper, countering the normal response to brain tumor growth. Patients with newly diagnosed glioblastoma, with or without macroscopic disease, are started post-operatively on a low copper diet (0.5 mg/day) and penicillamine, starting at 250 mg per day and escalated to 2 gm per day by the fifth week. The protocol begins simultaneously with the start of radiation therapy (6000 cGy in 30 fractions). Serum copper levels are obtained at baseline and each month thereafter." (Appendix IV)

Dr. George Brewer

The preliminary results(83) of a 1999 University of Michigan Comprehensive Cancer Center Phase I clinical trial appear to demonstrate that successful antiangiogenesis resulting in stable disease can be accomplished in some metastatic cancer patients by depleting systemi copper levels using oral TM. (84) Eighteen adult patients with metastatic solid tumors exhibiting measurable disease were selected for this clinical trial. The patient's minimum life expectancy was required to be three months or more with any condition of eleven different types of metastatic cancer. For a period of four to six weeks, was administered six times a day, for a maximum daily total of 120 milligrams. The protocol was to take TM three times a day with meals, and three times a day separated from food by a minimum of one hour before eating and one to three hours after ingesting any food.

For this trial, a new method to measure copper stores was needed for patient monitoring. While zinc acts to prevent copper absorption from food intake, TM therapy reduces body copper stores by oral chelation. In zinc therapy, Cu levels can be used as a gauge of total body copper level, but TM actually increases blood copper level making Cu an unreliable marker to actual copper status. Dr. Brewer needed a surrogate measure for copper status in his clinical trial patients.

Ceruloplasmin is a protein used to transport copper, and each mg of Cp contains 3 µg of

copper bound to it. Since Cp measurements appeared accurate with TM therapy, Dr. Brewer used Cp to gauge total body copper in his trials patients. Used in this way, Cp is actually a measurement of ceruloplasmin-bound copper in the blood, but about 95% of copper found in plasma is bound to ceruloplasmin.(85) The actual copper concentration of organ copper in the brain, heart, and liver would not be know during copper-reduction therapy although it might be substantially lowered as copper was reduced to Brewer's target level.(86)

Individual patient baselines were established by Dr. Brewer by taking their Cp and Cu levels the day copper-reduction therapy began. This provided the patient's baseline free copper level. The Cp baseline for control people without cancer was 20-35 mg/dL. For cancer patients, the average range was 30-65 mg/dL. The average trial patient's Cp was 47.8 mg/dL The goal was to reduce the patient's copper levels to about 10-20% of the patient's normal baseline for 90 days or more. Due to testing error, a drop to 22% of baseline was considered as achieving the desired reduction of copper. A typical Cp goal range was 7-15 mg/dL with a minimum level of 5 mg/dL in all cases.

"Free copper level" is defined as non-ceruloplasmin-bound plasma copper. Since each mg of ceruloplasmin contains 3 µg of copper, it is a mathematical computation of Cu - (3 x Cp). For example, if Cp = 29.3 and Cu = 106, free copper would be 106 - (3 x 29.3) = 18.

Dr. Brewer terms the 20% Cp baseline level "chemical copper deficiency." At this level, TM doses were individually tailored to maintain Cp within a target window of 70-90% reduction from baseline. This would result in mild copper deficiency without significant negative clinical signs. The body's important copper-based reactions, such as red blood cell functions, could continue normally.

Patient progress and health safeguards were monitored. Complete blood counts (CBC), liver and renal function tests, urinalyses, Cp levels, physical examinations, and CT scans were used to evaluate toxicity and disease progression. The first indication of true clinical copper deficiency is a reduction in blood counts. Because copper is required for the synthesis of heme, a protein critical for the production of red blood cells, a copper deficiency can result in anemia. Heme is also involved with leukocyte (white blood cell) proliferation. This is particularly true with neutrophils which combat acute infection. With this knowledge, the objective of the trial was to reduce Cp levels to less than or equal to 20% of baseline without decreasing the patients HCT (hematocrit), or white blood count, to below 80% of baseline value at entry. This was accomplished by reducing patient dosage of TM to slow therapeutic reduction of copper levels as critical levels were approached.

What was most remarkable was that Dr. Brewer observed no patients with cardiac, pulmonary, gastrointestinal (GI), renal, hepatic, hematologic, infectious, skin, mucosal, or neurological toxicity for Cp levels at or above 20% of baseline (minimum 5-15 mg/dL).(87) The only drug-related toxicity observed was mild reversible anemia, usually when HCT dropped below 80% of baseline. TM doses were individually tailored to maintain Cp within a target window of 70-90% reduction from baseline levels, while maintaining HCT and white blood count at or above 80% of baseline. Cp was monitored weekly during copper-reduction therapy.

Only six of the original eighteen patients completed the trial, twelve dropping out because of severe cancer illness. All six achieved the 20% level and maintained it using TM for more tha 90 days. These six all achieved stabilizing tumors. "They were metastatic cancers that would have been expected to have progressed and caused mortality," Dr. Brewer said, "but instead the tumors stopped growing."(88) Success was measured in terms of "stable disease" where tumor growth was slowed or arrested.

Now, almost two years later, all six original trial patients are doing very well with a surprisingly high quality of life. Since Brewer's phase I trail results were published in January 2000, the four additional patients enrolled are also doing well. Although the original plan was to reduce the trial patient's copper levels with TM and switch to zinc for maintenance therapy, there was

no wish to discontinue TM since the therapy was so successful. Instead, after reaching sufficient copper reduction, individual reduced TM dosages were formulated to maintain Cp within the target copper levels. No therapy-induced detriment to patients' quality of life was observed. They continued with a low copper diet and daily TM to maintain copper levels.

Dr. Brewer concluded that it took a minimum of three months after reaching the 20% copper baseline in order for reduced levels to stop angiogenesis. This is because tumors sequester copper. As a result, it is expected to take a longer time for copper deficiency to occur in the tumor micro-environment. "It takes time to reduce copper levels," Dr. Brewer explained. "Tumors have extra copper; so, first you reduce the body's copper level, and then the tumor starts losing copper."(89) This trial validated Dr. Brewer's theory of a window of copper deficiency where antiangiogenesis occurs while other copper-dependent cellular processes can continue. The level of copper required for angiogenesis was found to be higher than that required for essential copper-dependent cellular functions. Dr. Brewer not only demonstrated that TM is remarkably nontoxic and safe at up to 120 mg/day, but he was also able to maintain stable disease after the patients had been made copper-deficient.

Dr. Brewer found antiangiogenesis to hold significant promise to help render cancer a chronic or controllable disease or to contribute to its eradication, most likely in combination with other therapeutic modalities. He envisions future trials formally incorporating the use of adjunct non toxic modalities while the patients are allowed to remain in a copper-deficient state.

The Phase I clinical trial of TM as a copper-reduction therapy and antiangiogenesis agent (Protocol Number: 9708)(90) at the University of Michigan was not closed with the publication of Dr. Brewer's report. He continued to accepting a few more patients with metastatic breast, gynecological, kidney, or skin cancers until his phase II trials were opened.(91) The University of Michigan Comprehensive Cancer Center planned phase II trials in copper-reduction therapy to enroll patients with various types of less-advanced malignant disease including breast, prostate, kidney, liver, sarcoma, and multiple myeloma. As of this writing, the phase II trials have already opened for kidney, liver, and multiple myeloma cancers.(92) (Multiple myeloma is a blood proliferative disease of b-cells with many similarities to lymphoma.) Dr. Brewer has considered expanding his clinical trials beyond the University of Michigan. He recently accepted that angiogenesis is a relevant process in NHL, and he indicated a willingness to supply TM and FDA approvals for a lymphoma trial outside of his own institution. As such an open protocol does not yet exist, no lymphoma patients will be accepted into the TM clinical trials at this time.

In formulating his TM trials for cancer patients, Dr. Brewer essentially copied his protocol for Wilson's disease. Both protocols use diets that prohibit liver and limit shellfish consumption. The diets also need to be supplemented with zinc to maintain low copper levels. Dr. Brewer has encouraged doctors wishing to use zinc to treat Wilson's disease patients to contact him for information concerning the appropriate dosage and regime to follow,(93) so perhaps he would be as amenable about sharing his Phase II treatment protocol. Dr. Brewer may be contacted by phone, US mail, or e-mail. He may be reached by other physicians via M-Line a the University of Michigan Comprehensive Cancer Center. M-Line is a physician to physician referral and communication line.(94)

Potential Health Risks of Copper Depletion

Copper is an essential mineral required for growth, the production of red and white blood cells, and many other cellular functions. According to Dr. Brewer, "Most of us take in about one milligram of copper per day in our diet, and that's about 25 percent more than we need."(95) Moreover, if not used or eliminated, excess copper is stored in the body, mainly in the liver.

The limited copper deficiency goal of Dr. Brewer would still allow the body's important copper based functions to continue normally with no significant clinical manifestations (see Appendix II). The fact that Dr. Brewer's original six cancer patients in the TM trials are still alive and

doing well after two years is tribute to the short-term safety of copper-reduction therapy.

It would seem logical to look to Wilson's disease patients and learn if copper deficiency has any long-term health problems. However, Wilson's disease patients only need to lower coppe levels to normal, while cancer patients need to lower copper levels further. Many articles have suggested that low copper status might play a role in some common degenerative diseases and conditions, but the extent of copper deficiency required for these conditions is not clearly defined.(96)

Personal interviews were obtained with both Dr. Prasad and Dr. Brewer where they were each asked what could be the health risks of copper-reduction therapy. Dr. Prasad was concerned only about neutrophil counts which are easily monitored. Dr. Brewer stated that copper deficiency first affected the bone marrow's ability to produce red blood cells and expressed itself immediately as anemia which could be easily corrected. He based his statement on over 20 years of both animal and human clinical studies.

Copper is sequestered by neoplasms and is a required factor for key mediators of angiogenesis. Copper acts as a co-factor to molecules known as bFGF, VEGF, and angiogenin, and without it, they can't function to growth new blood vessels. In simpler words, copper-reduction blocks angiogenesis by "switching" the endothelial cell into the apoptosis (programmed cell death) pathway, or quiescence, and the cancer remains dormant.(97)

Some patients may choose to deplete copper levels, but not to the goals set by Brewer either due to preexisting medical conditions or the fear of unknown long-term side-effects. When asked if some limited copper reduction could have an effect on cancer growth, he replied that he does not know. He was certain only that reducing Cp to the range between 5 and 15 mg/dL does work.

Chapter IV

Beginning Copper-Reduction Therapy

Cancer patients should understand the therapy they are receiving or requesting. There is a glossary of terms in Appendix V which might be useful to comprehend this review. Giving a copy to your oncologist would help you discuss copper-reduction therapy with him. Some doctors may prefer to contact Dr. Brewer directly for protocol advice.(98) It is important to have a doctor monitor the progress of any cancer therapy.

This next step should be to minimize copper intake.

1. WATER: Cooking and drinking water, including water used for ice cubes, should be purified by reverse osmosis or distilled. Any other water source needs to be tested to ensure that copper content it is not above 0.1 ppm (0.1 mcg/L). Simple and inexpensive copper testing equipment is available in pet stores for use with salt water aquarium fish.

2. SUPPLEMENTS: Supplements containing copper should be eliminated. Unless iron deficiency has been medically established, supplements containing iron should also be eliminated.

3. DIET: Dietary restriction should at least include the highest copper content foods which are liver and shellfish such as clams, crab, shrimp, oysters, mussels, and lobster.

4. COOKING APPLIANCES: No copper cook ware should be used.

Copper depletion therapy may aggravate preexisting metal imbalances. A heavy metals blood or urine test for arsenic, cadmium, lead, or mercury levels is recommended to test for metal toxins. Tests for iron serum (Fe), ferritin (sFt), transferrin saturation (%TS or %SAT), and Total Iron Binding Capacity (TIBC) should be performed. Serum iron and TIBC levels are used to diagnose anemia and iron metabolism problems. Iron deficiency anemia can be caused by internal bleeding in tumors. Serum ferritin detects conditions of iron overload and inflammatory conditions.(99) Elevated ferritin is an adverse indicator for malignant disease as ferritin levels closely parallel tumor progression.(102)

Serum ferritin is an iron storage protein which is used as a very reliable measure for the level of body iron stores. One ng/mL of serum ferritin equates to about 8-10 mg of storage iron. High levels of iron can be caused by:

1. Repeated blood transfusions.

2. Chemotherapy treatment. Ferritin is released from damaged cells during treatment, and it takes up to six months of high ferritin levels to normalize after chemo.(99.1)

3. Conditions involving liver cell damage as the liver is a substantial iron storage organ. (99.2)

4. Hereditary hemochromatosis, an iron metabolism condition prevalent in one out of every two or three hundred people.(100)

5. Either idiopathic or dietary iron overload affecting as much as 10% of adults.(101)

Hyperferritinemia (high blood ferritin levels), can also indicate inflammation independent of th level of body iron stores. There are other simple inflammation markers that can help us interpret a high ferritin reading. They are the Erythrocyte Sedimentation Rate (ESR) also called the Erythrocyte Sedimentation Rate (ESR, Sed Rate, Westergren Sed Rate) or C-Reactive Protein (CRP). If these tests are normal or low, then the high ferritin is closer to a true indicator of high iron status.

Lymphoma is an autoimmune cancer, and inflammation is part of the disease process. Various markers from inflammation are prognostic indicators and will increase as the disease progresses. One of those markers is serum ferritin. However, even cancer inflammatory episodes can produce only moderate increases in ferritin level, typically an increase of 50 or a total reading of serum ferritin (sFt) not exceeding 300 ng/mL.(102.1) Serum ferritin values greater than 300 ng/mL in persons with or without inflammation or anemia indicate that the tissues contain excessive amounts of iron deposits.(102.2)

Iron is a nutrient to cancer and promotes inflammation and increase of cancer cell growth. (103) (104) (105) (106) High iron levels encourages free-radical activity, and recently it was found that too much iron may actually stimulate angiogenesis.(112) (113) Iron overload conditions are associated with increased incidence of cancer, heart disease, and defective immune regulatory control, (107) and "Iron Loading and Disease Surveillance" (108) written by Eugene D. Weinberg, (109) Ph.D. microbiologist at Indiana University with 30 years of research into the effects of iron in humans.

Laboratory ranges for normal blood test levels are only the particular lab's idea of what levels are found in normal healthy people. Although usually similar, ranges can differ between separate labs. Many of the ranges for normal ferritin were determined when large proportion

of Americans used cigarettes, and nicotine smoking is iron loading. LabCorp's ranges range from 10 - 291 for females and 22 - 322 for males. These ranges claimed to be "normal" are not necessarily the best for optimal health.

According to a prominent iron and cancer scientist, the most ideal ferritin value are between 10 and 25. The reason ferritin ever goes above 25 is to detoxify excessive iron. If ferritin remains low that indicates that toxic "free" iron is not building up in the body. When part of the ferritin level is due to inflammation, the increased ferritin is used to help scavenge the "free" iron that is causing damage in the inflamed site.(109.5) Cancer patients should maintain ferritin at no higher than 80-100 ng/mL to restrain malignancy growth,(110) but the optimal goal is to restrict ferritin levels to between 10-25 ng/mL).(109.6)

Iron overload patients should consider completing any aggressive phlebotomizes or other therapy required to reduce iron levels before attempting reduce copper levels. Deironing requires taking the patient close to iron deficiency anemia, and this will cause the liver to synthesize significantly more ceruloplasmin(111) conflicting with the Cp reduction goal of copper-reduction therapy.

High iron levels encourage free-radical activity.(112) (113) Some antioxidant dietary supplements including vitamins E, C, alpha lipoic acid, grape seed extract, and CoQ10 may also help in reducing free-radicals. Additionally, Cp can be either an oxidant or an antioxidant depending on mineral levels. Increasing zinc levels while reducing copper and iron levels will decrease Cp and encourage its antioxidant behavior.

There is an important link between ceruloplasmin, iron, and copper. The Cp protein cannot function without copper, and Cp is needed for iron metabolism. (114) People who cannot produce enough Cp become iron loaded because Cp functions to convert ferrous iron absorbed from the intestine into ferric iron that can be transported by transferrin to all the tissues in the body. When Cp is too low, the body is forced to absorb much excess iron to compensate for the low amount of ferric iron.(115) Most likely, the copper reduction therapy will not be extensive enough to interfere with normal iron metabolism. This can be easily monitored by checking ferritin levels 2-4 times a year.(116)

High ferritin can indicate inflammation independent of the level of body iron stores. Ferritin levels over 100 ng/mL in blood proliferative malignancies can be due to tumor cell activities (117) (118) causing inflammation without regard to actual iron storage amounts. Early minima disease indolent NHL has been found to have surprisingly low ferritin levels, even stages III and IV.(118.1) Even with inflammation, iron deficiency cannot exist until ferritin drops below 100 ng/dL. Therefore, it is safe to lower ferritin to the 80-100 ng/dL level even in conditions of inflammation common in cancer patients.

Monitoring red blood cells (RBC) and iron levels is recommended because a possible adverse reaction to any form of copper depletion can be anemia. Copper reduction lowers Cp which is essential to red blood cell formation with iron. A warning sign that copper levels have been over-depleted is if RBC counts indicate anemia. If Cp is very low at the same time iron levels are low, such could increase the risk of iron deficiency anemia. Conversely, if RBC counts do not indicate anemia, such is indirect evidence that copper and iron levels are not at an extreme deficient level.

White blood counts including differentials are needed to monitor neutrophils. It is recommended that neutrophil counts not drop below 2500 µL.(119) Repeated testing of complete blood counts (CBC), liver and renal function tests, urinalyses, and Cp level were all required in Dr. Brewer's trial using TM, first weekly, then decreasing to monthly. Dr. Brewer may have included periodic heart function and liver enzyme levels as well.

Medical monitoring of copper at low levels by blood tests is extremely important. If copper levels fall below Brewer's "window" and become deficient, then the production of hemoglobin from iron (which requires sufficient copper) might be decreased and cause the "back up" of

excess iron in the liver, particularly in people with iron overload. Conversely, people with iron deficiency anemia, common in cancer patients, risk worsening their anemia if copper levels g down to the deficient state and further decrease the production of hemoglobin. If copper-reduction therapy is induced too rapidly, bone marrow toxicity can occur involving both lowered RBC and WBC counts, bone marrow depression, anemia, neutropenia, and leukopenia. Such conditions are confirmation of deficient copper levels below target requiring immediate corrective action by decreasing or stopping TM and possibly by taking some copper supplementation to raise copper to safer levels.

Blood tests that establish an accurate level for Cp and Cu prior to beginning therapy are important. The same testing laboratory should be used if possible to maintain consistency. If there is any significant time delay in beginning treatment, new tests should be taken on the day therapy is begun. Copper reduction therapy needs to be continuously applied because any suspension will result in the body quickly replenishing copper supplies. In other words, if copper-reduction therapy was started before pretesting, it is not too difficult to regress in orde to establish baseline copper level.

It may be best to refrain from using any vitamins or supplements for a week before taking pretests. This will help establish a baseline free of interactions with other minerals or vitamins One should fast for a minimum of 12 hours before the blood draw. Proper care requires regular monitoring of blood and urine levels during the copper-reduction regime.

Your physician will probably order a number of blood pretests before beginning therapy. He may include the ones described below and possibly more.

1. Copper

a. **Ceruloplasmin (Cp):** This is a glucoprotein that transports serum copper. Monitoring Cp is particularly important as some copper-reduction therapies can invalidate copper serum test readings.(120) Cp level is also a good lymphoma marker.
b. **Copper serum level (Cu):** Doctors may choose to monitor either Cp, Cu, or both, during copper-reduction therapy.

2. Zinc serum level (Zn): Studies have shown the diagnostic value of serum zinc, copper an their ratios in patients with hematologic malignancies.(121) This is discussed in this report under "Medical Research -- Copper and Zinc in Cancer."

3. Copper/zinc ratio (CZR): This ratio is obtained by dividing the two numbers, copper serun and zinc serum.

4. Complete Blood Count (CBC):

a. **Hemoglobin (HGB):** This is the chemical compound that combines with oxygen from the lungs and carries the oxygen and iron to cells throughout the body. People with a low hemoglobin level have iron deficiency anemia.
b. **Hematocrit (HCT):** This is the percentage of red blood cells (RBCs) in total blood, one check for iron deficiency anemia (the first indication that too much copper has been depleted). The goal of Dr. Brewer's phase I was to reduce Cp to 20% of baseline value without reducing hematocrit below 80% of baseline.
c. **ABS Neutrophils:** Absolute count of neutrophils, white blood cells that fight infection, should be maintained above 2500 µL and monitored closely as copper serum < 80. (122)

5. Iron:

a. **Ferritin level (sFt):** This is a protein transporter for iron. High ferritin levels can indicate tumor activity or iron overload disease which needs to be treated while

avoiding iron supplements.

b. **Serum iron (Fe):** This measures the amount of iron in the bloodstream to help diagnose anemia and other problems.

6. Lymphoma Marker(s):

a. **Lactate dehydrogenase (LDH):** This is the main lymphoma marker and a protein that is elevated in response to high levels of lactic acid. Elevated LDH levels can be caused by the progression of cancer, as well as many other diseases.
b. **Beta-2 Microglobin (B2MG):** A protein secreted by white blood cells that has recently been used as a tumor marker for lymphoma.

7. Total Cholesterol: Extreme copper depletion can result in increased cholesterol in blood plasma (123) and increased risk of cardiovascular disease.(124) High cholesterol levels are associated with heart disease and may depress the immune system.

a. **High-density lipoprotein (HDL) cholesterol, or "good" cholesterol:** High HDL level indicates low risk of a heart attack.
b. **Low-density lipoprotein (LDL) cholesterol, or "bad" cholesterol:** A high LDL level indicates high risk of heart disease.

8. Prostate Specific Antigen (PSA): Prostate cancer baseline and monitoring. The PSA test can help determine if the prostate is normal, enlarged, or if cancer is present. The normal PSA value is less than one. High PSA readings correspond with high prostate tumor burden. Zinc supplementation may increase PSA levels regardless of disease progression.(125) (126) Prostate cancer patients should consider alternative methods to deplete copper to retain the validity of their PSA marker.

9. Electrocardiogram (EKG / ECG): In patients with a prior history of heart disease, an echocardiogram is also indicated. Patients should discuss with their doctor which tests to perform periodically to avoid any potential complications.

A chart detailing levels as therapy progresses might be useful. Monitored levels might include Cp, percent Cp reduction from baseline (calculation), Cu, percent copper reduction from baseline (calculation), free copper (Cu minus three times Cp), Zn, CZR (calculation), white blood cell count, absolute count of neutrophils, platelets, RBC, HCT, and ferritin.

Upon starting TM therapy, it is possible for Cu and Cp to elevate. Cp can go up temporarily because tumor lysis releases copper which the liver then converts to additional Cp. This could happen with no obvious symptoms. Cu goes up because some of the copper chelated from the liver by TM is released into the bloodstream instead of directly into the bile ducts. This is harmless copper because it is bound to albumin and is eventually excreted. Cancerous tumors do not use copper bound to albumin (about 19% total Cu); they use or store copper bound to Cp (about 70% total Cu is bound to Cp).(127) That is why Cp is such an appropriate marker for copper status in copper-reduction therapy for cancer control.

Target levels are copper reduction by 70-90% as measured by Cp (minimum 5-15 mg/dL). If zinc therapy is used, serum Zn should be maintained at 140 to 160 mcg/dL which medical reports show as optimizing immune function. Studies have shown that a healthy person has a CZR of one or less. Maximum reduction in HCT limited to 20% of baseline. Neutrophil counts should not drop below 2500 µL. Ferritin levels kept at a range of no more than 80 to 100 ng/mL should help minimize cancer's growth.

The point at which Cp level will decrease depends on the total amount of copper stores, compliance and type of copper-reduction therapy, and individual absorption factors. When Cp levels reach 20% of an individual's baseline or an absolute minimum of 5 mg/dL, zinc therapy may be used to maintain low copper status. Zinc supplementation level should be started at the protocol 150 mg per day and adjusted to individual requirements to permit copper level

maintenance. A low copper diet and zinc supplementation may be necessary for a prolonged period of time, as long as the threat of cancer growth remains, or until more effective therapies become available. Strict adherence to the dosing schedule is probably the most significant part of the zinc therapy.

It is crucial to involve a physician from the beginning of copper-reduction therapy. Any copper reduction effort must be stringently monitored to avoid over-depleting copper levels and avoid reducing hematocrit or white blood count below 80% of baseline values. Only accurate monitoring will allow a physician to safeguard a patient's health, particularly for those with progressive disease. Proper supervision may also provide valuable information to other patients and the medical community.

Special Medical Conditions

Patients should always consult with their doctor before attempting this therapy, especially those with any special medical condition.

If copper depletion therapy is to be attempted in anyone with a pre-existing heart condition, (128) a cardiac specialist should be involved in both approving the therapy and in continual monitoring. Such conditions would include prior heart attack, cardiomyopathy, congestive heart failure, uncontrolled high blood pressure, cardiac arrhythmia requiring medication, and uncontrolled angina.

Since copper depletion therapy can in theory also aggravate any pre-existing liver disease, as well as anemia and leukopenia (low blood counts), patients with these problems will need careful consultation with specialist physicians before attempting this therapy.

Women who are pregnant as well as those attempting to become pregnant may wish to forgo copper-reduction therapy. Angiogenesis plays an important role in ovulation as well as healthy development of a fetus.(129)

Zinc should be avoided as a copper depleting method in patients with prostate cancer because of the potential risk of losing their PSA readings as a reliable marker of cancer progression. There also is some evidence that zinc fuels prostate cancer cell proliferation. (130) (131)

Since elevated iron levels can confuse results of copper-reduction therapy, it should be discussed with your doctor whether or not iron levels should be normalized before beginning copper-reduction therapy.

These are only a few examples of special medical conditions which may preclude patients from copper-reduction therapy, need dose modification, or require more stringent medical monitoring.

Dietary Considerations

Brewer's dietary considerations of cancer patients undergoing copper-reduction at his trials are no greater than his recommendation for Wilson's disease patients(132) who are instructed to use distilled water for drinking and cooking,(133) avoid liver, and limit shellfish such as clams, crab, shrimp, oysters, mussels, and lobster.(134) Almost all foods contain some copper, but when Dr. Brewer used modern, sensitive instruments to measure the copper content of foods thought to contain very high levels of copper, he found they contained much less than had been previously realized.(135)

In another new study, Dr. Henry S. Brem used penicillamine as a copper chelator for brain tumors, as well as a stricter low-copper diet. Copper is limited to less than 0.5 mg per day, with restrictions including not only liver and shellfish, but also dark meats, pizza, white bread,

tea, French fries, pork, ham, white potatoes, whole wheat bread, carbonated soft drinks, fruit flavored drinks, whole milk, chicken, peanut butter, and bananas.(136)

A number of nutritional supplements have been suggested for patients in copper-reduction therapy. Garlic has been found to be very beneficial in reducing the toxicity of copper deficiency(137), providing anti-bacterial protection, and even inhibiting tumor cell growth.(138 If zinc is used to aid copper elimination, Vitamin B-6 supplementation should enhance zinc absorption, as any B-6 deficiency would result in impaired zinc absorption.(139) High doses c zinc can block selenium intake as well as copper, thus a selenium supplement can be taken at a meal either separately or in conjunction with vitamin E. FOS (fructooligosaccharides) supplementation is encouraged for its ability to assist the gastrointestinal tract. If a multivitamin is desired for maintaining general health, it should be free of iron and copper. Vitamin E and fish oil are other angiogenesis inhibitors that may be used in a nutritional program. For those who are not severely immune suppressed, supplementation with curcumi has the benefits of an antioxidant, an anti-inflammatory, and an angiogenesis inhibitor. Although other references suggest N-acetylcysteine (commonly called NAC) as an antioxidar and minor copper reducer, it is not recommended because NAC can actually inhibit apoptosis protecting cancer cells from being killed.

Other immune augmenting products that may be considered for lymphoma patients who wish to further stabilize and support immune function are ginseng (endurance and natural killer cel stimulation), Phytosterols/sterolins (i.e. beta sitosterol and its glycoside), and IP-6 (inositol hexaphosphate).

Animal toxicology studies suggest that items to avoid during copper-reduction therapy are alcoholic beverages,(140) fructose (fruit sugar),(141) (142) and iron supplements.(143) Interestingly enough, Dr. Brewer did not treat his two trial patients that developed anemia with supplemental iron. Instead, he treated one with a blood transfusion, and the other by decreasing the dosage of TM. Although Dr. Brewer does not feel that the toxicity studies of fructose in animals are applicable to humans, it is recommended to err on the side of caution and limit the consumption of sweets. Foods containing fructose are honey, fruits, and berries. Fructose is a component of table sugar and is used as a product sweetener and a preservative. Animal studies have shown that alcohol, iron, and fructose exacerbate any copper deficiency toxicity to the heart, liver, and pancreas.(144)

A low fat diet which is low in red meat and plentiful with fruit and vegetables appears to be appropriate for cancer patients. Such a diet can be naturally low enough in copper that it can by itself be sufficient for the maintenance required for Wilson's disease.(145) An example of a mostly vegetarian diet is the macrobiotic diet which is often touted as a cancer prevention lifestyle. Macro-biotic means long-life and the diets consist largely of grains, vegetables, beans, and soups. All protocols are low in copper and sweets which makes them very suitabl for use during copper-reduction therapy. Low copper diets are typically low in iron. There are also positive testimonials from lymphoma patients using this diet approach.(146) However, strict vegetarians (not lacto-ovo) in general have a higher risk of developing dietary zinc deficiency(147), and those not receiving zinc supplements should have zinc serum levels periodically monitored.

Conclusion

Copper-reduction therapy is a plausible new option to treat and control cancer since it is proven to inhibit angiogenesis. Scientific evidence shows that this therapy should work in

hematologic malignancies such as non-Hodgkin's lymphoma.

Comparing risk to benefit, cancer can immediately be treated by copper-reduction therapy and maintenance of a low copper diet to inhibit angiogenesis. Dr. Brewer's research is tantalizing, and very convincing from a scientific standpoint. Not all the potential long-term health risks of reduced copper levels are well known, but it appears to be an effective low-risk strategy. Continuing this therapy for many years might not have anywhere near the negative side-effects of a single course of chemotherapy. The problems of copper deficiency have been identified by Dr. George J. Brewer and Dr. Ananda S. Prasad as anemia and neutropenia. These conditions can be readily determined by standard blood tests and corrected by reducing the dose of zinc or TM. The risk seems small and manageable compared to the possible benefit of gaining some control over this disease. Copper deficiency is easily correctable -- cancer is not.

Considerable time is required to reduce body copper levels. The therapy does not directly attack malignant tumors. The goal is to create a sub-clinical copper deficiency to the point of blocking cancer tumor neovascularization. Therapeutic objectives are disease stabilization, increased survival time, and increased time to disease progression.

Copper-reduction therapy is simple, inexpensive, and easily combined with almost all other treatment modalities. It may be an effective low-risk strategy for cancer, especially during "Watch and Wait" (W&W), when the medical procedure is to monitor cancer progression but not begin anti-cancer therapy.

Will copper-reduction therapy have any effect on the progress of lymphoma? The answer will not be available from science until at least several years after Dr. Brewer's results are finally published and other researchers duplicate his trials on lymphoma patients. Many more years could pass before mainstream medicine would accept this therapy. After all, it took over 10 years for mainstream medicine to accept Dr. Brewer's therapy for Wilson's disease.

It is undeniable that promising drug therapies like angiostatin and endostatin will take many years of clinical trials to win FDA approval. The medical establishment is now busily testing both angiostatin and endostatin on solid tumor malignancies. Lymphoma patients with soft tumors have been left sitting on the trials' sidelines watching as their cancerous tumors grow. With 560,000 Americans lives lost to cancer each year, some of us cannot wait while medical science works to perfect the ultimate therapy. We are dying of cancer.

Remember that the work in this field, although very suggestive and on solid scientific grounds is preliminary. It may not work. It may work, then fail. It may work in some people, and not others. It might work and save lives.

PubMed

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APPENDIX I

PubMed Abstract Summary

Title: Treatment of metastatic cancer with tetrathiomolybdate, an anticopper, antiangiogenic agent: Phase I study.

Author: Brewer GJ; Dick RD; Grover DK; LeClaire V; Tseng M; Wicha M; Pienta K; Redman BG; Jahan T; Sondak VK; Strawderman M; LeCarpentier G; Merajver SD

Address: Department of Human Genetics, University of Michigan Health System, Ann Arbor 48109, USA.

Source: Clin Cancer Res, 2000 Jan, 6:1, 1-10

Abstract: Preclinical and in vitro studies have determined that copper is an important cofacto 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 o 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 marke for total body copper. Because anemia is the first clinical sign of copper deficiency, the goal o 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.

Language of Publication: English

Unique Identifier: 20120218

The full article of Brewer's preliminary Phase I trial results released January 2000 is viewable on-line entitled Treatment of Metastatic Cancer with Tetrathiomolybdate, an Anticopper, Antiangiogenic Agent: Phase I Study.

(The PDF version is available for printing if you have Adobe Acrobat Reader, and don't mind the few minutes it takes to load.)

APPENDIX II

Stages of Copper Deficiency and its Clinical Effects in Humans

"Treatment of Metastatic Cancer with Tetrathiomolybdate, an Anticopper, Antiangiogenic Agent: Phase Study" by George J. Brewer etal., Clinical Cancer Research, Vol. 6, pp. 1-10, January 2000, page 7.

Table 3 Stages of copper deficiency and its clinical effects in humans: Treatment of Metastatic Cancer with Tetrathiomolybdate

	Cp level[a]		
Type of copper deficiency	% Baseline Cp	Absolute Cp level (mg/dl)	Clinical manifestations
Chemical	10–30	5–15	None[b]
	(target range)	(target range)	Probable inhibition of tumor angiogenesis
Clinical			
Mild	<10%	<5	Mild anemia, Hct ~ 80% of baseline
			Mild neutropenia
Moderate	<10%	<5	Moderate anemia, leukopenia, possibly symptomatic
Severe	<10%	<5	Severe bone marrow depression, diarrhea, cardiac arrhythmias may occur rarely, peripheral neuropathy[c]
			In children, inhibition of epiphyseal bone growth

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

[b] Bone marrow effects such as anemia and/or leukopenia may occur if the induction of coppe deficiency is very rapid, as with high doses of TM, at higher levels of Cp than shown here.

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

Table 3 Stages of copper deficiency and its clinical effects in humans

"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 dimutase 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 limits of chemical copper deficiency and the beginning of mild clinical copper deficiency, the first manifestation of which is mild anemia. Table 3 summarized the states 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 chemica 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 metabolisms 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...

Following Cp levels once very 1-2 weeks is adequate to monitor copper status early in therapy. As a corollary, overtreatment is easily detectable and correctable..."

APPENDIX III

PubMed Search -- Angiogenesis and NHL

There is no longer any controversy over whether or not angiogenesis is involved in NHL tumc formation. The following list of 47 abstracts is the result of a PubMed search for angiogenesis and non-Hodgkin's lymphoma (NHL). To summarize, most of these studies support the contention that angiogenesis is active in NHL. The major growth factors found are basic Fibroblast Growth Factor (bFGF), Vascular Endothelial Growth Factor (VEGF-C) and the metalloproteinases (MMP-2 and MMP-9) which are all commonly found in many other cancers. In addition, the amount of these growth factors increases as the severity of the disease increases.

"Angiogenesis" is a broad term that applies to all cancers. A more accurate term for angiogenesis in lymphoma is 'lymphangiogenesis' which has specific unique characteristics. Many cancers, such as breast cancer also metastasize through the lymph system and so have some characteristics in common with lymphoma.

VEGF is one of the most important angiogenic growth factors that is expressed in lymphoma. An important characteristic of lymphangiogenesis is the active form of VEGF which is VEGF-

C and its receptor VEGFR-3. This occurs only in the lymph system or in lymphatic tissue elsewhere in the body. This is why a drug that blocks the VEGFR-1 receptor won't work in lymphoma. Any drug that blocks VEGF should be reviewed for its sensitivity to VEGF-C and its receptor to assess its efficacy in lymphoma. Reference 38 is a pertinent abstract. It shows that endothelial growth factors and their receptors may provide important therapeutic tools for the treatment of pathological conditions characterized by defective or aberrant angiogenesis. Vascular endothelial growth factor (VEGF) is pivotal for vasculogenesis and for angiogenesis in normal and pathological conditions. VEGF-B and VEGF-C provide this gene family with additional functions, for example, VEGF-C also regulates lymphangiogenesis.

Reference 39 is absolute proof of angiogenesis in lymph nodes as seen in a CT scan study. Tumour angiogenesis is known to produce capillaries that exhibit increased permeability and CT measurements of permeability could therefore potentially provide a marker of tumour viability.

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APPENDIX IV

Dr. Steve Brem's clinical trial with copper reduction in brain cancer

Publication Year: 2000

Phase II Study of Penicillamine and Reduction of Copper, for Angiosuppressive Therapy of Glioblastoma: Preliminary Safety and Feasibility Study. Steve Brem, Stuart A A Grossman, Pamela New, Surasak Phuphanich, Regina Priet, Kathryn Carson, NABTT CNS Consortium, Baltimore, MD.

Tumor growth, invasiveness, and angiogenesis of experimental gliomas has been shown to be copper-dependent. Malignant brain tumors normally are associated with elevated tissue and serum levels of copper. The objective of the current clinical trial is to reduce the level of serum copper, countering the normal response to brain tumor growth. Patients with newly diagnosed glioblastoma, with or without macroscopic disease, are started post-operatively on a low copper diet (0.5 mg/day) and penicillamine, starting at 250 mg per day and escalated to 2 gm per day by the fifth week. The protocol begins simultaneously with the start of radiation therapy (6000 cGy in 30 fractions). Serum copper levels are obtained at baseline and each month thereafter. There have been 16 patients placed on study and 4 patients have been on the study for more than 5 months. A consistent trend has been observed that the baseline levels of copper (mean ± S.D.) are slightly elevated 138.6 (± 31.6 µg/dL)(normal @ 70 to 140 µg/dL). After one month (n=12), the levels are normalized to 119.3 (± 31.7). After 2 months (n=8), the levels are 57.5 (± 27.5), and clinically significant hypocupremia, 29.7 (± 31.7) is achieved by 3 months (n=7), and sustained (n=4) on the fourth month, 25.5 (± 18.4) and fifth month (n=4), 32.8 (±18.4), $p < 0.01$ by the Tukey-Kramer test and ANOVA. The diet and penicillamine are well tolerated and compliance is excellence. There has been no neurological toxicity noted (e.g., vasogenic edema). There has been one grade 3 toxicity (rash) related to penicillamine, and no laboratory or clinical toxicity linked to copper deficiency Future analysis will determine efficacy in terms of time of survival and time to tumor progression. In summary, penicillamine and a low copper diet are well tolerated and achieve clinically significant reduction of copper, an angiogenesis cofactor, in patients with newly diagnosed glioblastoma. A multi-institutional trial is currently occurring within NABTT CNS Consortium using the NABTT GBM database to evaluate the impact of copper reduction on survival outcome. abstract

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APPENDIX V

Glossary of Terms

Angiogenesis: The process by which new blood vessels are formed.

Antiangiogenesis: The hypothesis developed by Dr. Judah Folkman that cancer may be stopped by depriving malignant tumors of the blood supply that nourishes them. As tumors grow larger than two millimeters in size, they require new blood vessel growth to supply oxygen and nutrients to the cells and carry waste away. Simply stated, the theory holds that tumors deprived of vascularization can no longer grow, and the cancer remains dormant indefinitely.

Beta-2 Microglobin (B2MG): A protein secreted by white blood cells that has recently been used as a tumor marker for lymphoma.

Ceruloplasmin (Cp): This protein synthesized in the liver is an essential component of both iron and copper transport. Dr. Brewer monitored Cp as a surrogate measure of copper status in copper-reduction therapy.

Chemical Copper Deficiency: Dr. Brewer's term for 80% reduction of ceruloplasmin, the target level for copper-reduction therapy. Brewer's theory was that at this level, cellular processed could continue but tumor angiogenesis would be prevented.

Complete Blood Counts (CBC): An inexpensive group of tests used by physicians to determine a number of general health parameters.

Computer-Assisted Tomography (CAT)/(CT): An x-ray technique allowing a rapid examination of inaccessible areas of the body by taking pictures of the internal anatomy from different angles. In copper-reduction therapy, CATs were used to evaluate toxicity and disease progression.

Copper (Cu): Copper is an essential heavy trace mineral required for growth, the production of red and white blood cells, and many other cellular functions. The human body normally contains about 75-100 mg of copper, less than that in a copper penny. According to Dr. Brewer, out diets supply about one milligram of copper per day which is about 25% more than we need. Excessive copper is very toxic. The body is not able to eliminate excess copper, so it is stored, mainly the liver. Scientist's theorize that without high enough copper levels, blood vessels cannot grow to nourish cancerous tumors.

Copper/zinc ratio (CZR): This ratio is obtained by dividing the two numbers, copper serum and zinc serum.

Electrocardiogram (EKG/ECG): A noninvasive, electrical test that records the activity of the heart.

Ferritin (sFt): Ferritin is an important detoxification protein. By storing up to 4500 molecules of iron in each ferritin shell, free iron is prevented from harming the body. Synthesis of ferritin is induced when iron is available, and it is repressed in cases of iron deficiency. Ferritin is also elevated in cases of inflammation, so high ferritin can mean either high iron stores or inflammation.

Free Copper: Plasma copper that is not bound to ceruloplasmin. Since each mg of ceruloplasmin contains 3 µg of copper, free copper is calculated as copper serum minus three times ceruloplasmin [Cu - (3 x Cp)].

Galzin®: Zinc acetate available by prescription only.

Hematocrit (HCT): The percentage of red blood cells (RBCs) in total blood, one check for iron deficiency anemia (the first indication that too much copper has been depleted).

Hemoglobin (HGB): The chemical compound that combines with oxygen from the lungs and carries the oxygen and iron to cells throughout the body. People with a low hemoglobin level have iron deficiency anemia.

Hereditary Hemochromatosis (HH): One of several iron loading diseases prevalent in 1 out of 200 Americans. The concern must be to detect and remove any excess iron usually by bloodletting (phlebotomy) instead of establishing a definite diagnosis of hemochromatosis. Iron loading can both cause cancer and lead to a poor outcome unless the excess iron is removed. Iron Overload Fact Disease Association

Iron (Fe): An essential mineral for all living organisms, but in excess it can lead to serious health problems. The body's normal excretion can only handle about 1 mg of iron per day, so the excess is absorbed as iron stores in various vital organs, predominately the liver. Since iron is necessary for proliferating malignant cells, iron deprivation as a component in cancer therapy is being tested in clinical trials.

Lactate dehydrogenase (LDH): This is the main lymphoma marker and a protein that is elevated in response to high levels of lactic acid. Elevated LDH levels can be caused by the progression of cancer, as well as many other diseases.

Leukopenia: A reduction in the circulating WBC count to < 4000/µL usually characterized by a reduced number of blood neutrophils, although a reduced number of lymphocytes, monocytes, eosinophils, or basophils may also contribute to the decreased total cell count. (See "Neutropenia")

Molybdenum: An essential trace mineral needed for the proper function of certain enzyme-dependent processes. An essential ingredient in the compounding of tetrathiomolybdate.

Neutropenia: A reduction in the blood neutrophil count, often leading to increased susceptibility to bacterial and fungal infections. Neutropenia may be classified by the neutrophil count (total WBC x % neutrophils and bands) and the relative risk of infection: mild (1000 to 1500/µL), moderate (500 to 1000/µL), or severe (< 500/µL). (See "Leukopenia")

Neutrophils: A type of white blood cell that fight infection. They should be maintained above 2500 µL, and monitored closely as copper serum < 80 mcg/dL.

Non-Hodgkin's Lymphoma (NHL): A diverse group of cancers starting in and affecting the immune system.

Penicillamine: A prescription oral copper chelator sold as Cuprimine or Depen.

Prostate Specific Antigen (PSA): Prostate cancer baseline and monitoring marker. The PSA test can help determine if the prostate is normal, enlarged, or if cancer is present.

Red Blood Cell (RBC): A blood test used to evaluate anemia and loss of red cells.

Tetrathiomolybdate (TM): Ammonium tetrathiomolybdate (TM) is a complex of sulfur and molybdenum designed as a fast-acting compound to quickly lower copper levels. "Ammonia" is a salt which increases body absorption. "Molybdenum" is a mineral which binds with copper and is known as "molybdate" when chemically compounded. "Tetrathio-" is the sulfur which aids the body's elimination of molybdate after it has bonded with the copper.

Trientine: A prescription oral chelator of copper sold as Syprine.

Vascular Endothelial Growth Factor (VEGF): Vascular endothelial cells are the cells involved in the genesis of new blood vessels. By specifically suppressing the growth of vascular endothelial cells, it is hoped that the tumor will no longer grow and will remain essentially harmless.

Zinc (Zn): Zinc is a trace metal needed for more than 300 enzymes used by the body. It is not a copper chelator, zinc acts to block dietary copper in the intestines by preventing additional absorption of copper.

APPENDIX VI

Compounding Pharmacies Selling Ammonium Tetrathiomolybdate(TM)

Montana Compounding Pharmacy
Tim Calcagno, R.Ph.
111 N. Higgins, Suite 5
Missoula, MT 59802
Toll Free Voice: (800) 600-2009
Voice: (406) 542-2888
Fax: (406) 542-9380
e-mail: MCP@BigSky.net
Website: Montana Compounding Pharmacy

Green Hills Health & Wellness
Mark Binkley, Pharmacist
3900 Hillsboro Road
Nashville, TN 37215-2714
Toll Free Voice: (800) 338-7994
Voice: (615) 292-0066
Fax: (615) 292-2762
e-mail: MarkB@RXCompounder.com

The Prescription Center
Wayne Loveland, R.Ph.
1907 West Avenue South
LaCrosse, WI 54601
Toll Free Voice: (800) 203-9066
Voice: (608) 788-4500
Fax:(608) 788-4501
e-mail: RXCenter@Charter.net
Credit Cards Accepted: Mastercard, Visa, American Express, Diner's Club, Discover

Health Dimensions, Inc.
Scott Popyk, Pharmacist
32985 Hamilton Ct., Suite G200
Farmington Hills, MI 48334
Toll Free Voice: (800)836-2303
Voice: (248) 489-1573
Fax: (248) 489-1586
e-mail: Info@HealthDimensionsRX.com
Website: Health Dimensions Pharmacy

DeGarmo's Compounding Pharmacy
Richard DeGarmo, Pharmacist
1907 Harrison Avenue N.W.
Olympia, WA 98502
Toll Free Voice: (800) 892-5834
Voice: (360) 709-9999
Fax: (360) 705-2869
Credit Cards Accepted: Mastercard and Visa
e-mail: DeGarmoCompounding@home.com
Website: DeGarmo Compounding Pharmacy

Hopewell Pharmacy and Compounding Center
1 West Broad St.
Hopewell, NJ 08525
Mon/Fri 9AM - 8PM, Sat 9AM - 5PM
Toll Free Voice: (800) 792-6670
Toll Free Fax: (800) 417-3864
Hours: Mon-Fri. 9am to 8pm
Sat. 9am to 5pm

Payment Accepted: Visa, MasterCard, Amex, Discover, Checks and COD
e-mail: info@hopewellrx.com
Website: Hopewell Pharmacy and Compounding Center

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webmaster ElCajonSue@peoplepc.com

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