

The background features a dark, almost black, field with several large, flowing, translucent waves. The top-left wave is a vibrant green, while the bottom-right wave is a bright orange. These waves overlap and curve across the frame, creating a sense of movement and depth. The text is centered in the upper-middle portion of the image.

AMMONIUM TETRATHIOMOLYBDATE AND CANCER

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AMMONIUM TETRATHIOMOLYBDATE

- Chelates copper
- Initially introduced as a treatment in Wilson's disease, a hereditary copper metabolism disorder, in humans; it acts both by competing with copper absorption in the bowel and by increasing excretion
- Copper is essential for angiogenesis

AMMONIUM TETRATHIOMOLYBDATE II

- Abnormally high serum copper levels are found in patients with many types of progressive tumors; in addition, cancer cells sequester copper.
- Copper-binding molecules [ceruloplasmin, heparin (Heparin is usually stored within the secretory granules of mast cells and released only into the vasculature at sites of tissue injury), and tripeptide glycly-histadyl-lysine] are non-angiogenic when free of copper, but they become angiogenic when bound to copper

AMMONIUM TETRATHIOMOLYBDATE III

- Angiogenic activity of BFGF, VEGF/VPF, TNF alpha, and IL-1 are copper dependent (require binding to copper to function properly)
- TM is known to decrease angiogenesis and cancer cell growth through the inhibition of cellular antioxidant copper zinc superoxide dismutase (SOD) and to elevate levels of cellular reactive oxygen species.
- TM in tumor animal studies activated pro-apoptotic MAPK signaling, down regulated survival proteins, such as XIAP, and reduced cancer cell motility and invasiveness.
- On January 21, 2000, the University of Michigan reported that researchers had "successfully stopped the growth and spread of cancer by depriving the tumors of the copper supply they need to form new blood vessels"



BIOAVAILABLE COPPER MODULATES OXIDATIVE PHOSPHORYLATION AND GROWTH OF TUMORS

Seiko Ishida; PNAS | November 26, 2013 | vol. 110 | no. 48 | 19507–19512

BIOAVAILABLE COPPER MODULATES OXIDATIVE PHOSPHORYLATION AND GROWTH OF TUMORS

- Using a genetically engineered mouse model of human cervical carcinoma, we previously observed and reported that cancer cells express higher levels of the copper transporter Ctr1 and that the tumors were differentially sensitive to reduction in systemic copper levels compared with normal tissues, leading us to hypothesize that cancer cells might have a greater demand for and dependence upon copper.
- Copper plays an essential role in OxPhos by forming a catalytic core of cytochrome c oxidase, the terminal enzyme complex of the electron transport chain that produces ATP in the mitochondria. The complex contains two hemes, a cytochrome a and cytochrome a₃, and two copper centers, the CuA and CuB centers.

BIOAVAILABLE COPPER MODULATES OXIDATIVE PHOSPHORYLATION AND GROWTH OF TUMORS

- Treatment of β TC3 (mouse insulinoma) cells with 10 μ M TM resulted in accumulation of cells in the G2 phase of the cell cycle: the percentage of cells in G2 was 9% in untreated controls vs. 22% in TM-treated cells after 24 h.
- The investigators hypothesized that the above effect might be due to decreased activity of cytochrome c oxidase and concomitant decrease in ATP levels. Indeed, TM treatment reduced the cytochrome c oxidase activity by twofold in β TC3 cells.
- We also observed a twofold decrease in the mitochondrial membrane potential in TM-treated β TC3 cells, indicating diminished activity of the electron transport chain.
- Together these results imply that copper limitation reduces oxidative phosphorylation in cancer cells.

COPPER-DEFICIENT CANCER CELLS HAVE INCREASED DEPENDENCY ON GLYCOLYSIS.

- We observed a transient decline in cellular ATP levels in TM-treated β TC3 cells further supporting the hypothesis that mitochondrial ATP production is impaired in cancer cells that are under copper limitation. However, the ATP level recovered subsequently, suggesting a compensatory mechanism.
- We found that TM treatment increased glucose uptake by 30% and cellular lactate levels by 47% in β TC3 cells, indicative of enhanced glycolysis for ATP production.
- Thus, when copper is limiting, glucose is primarily consumed for ATP production in cancer cells.

COPPER-DEFICIENT CANCER CELLS HAVE INCREASED DEPENDENCY ON GLYCOLYSIS

- These observations collectively imply that copper-starved cancer cells may rely on glycolysis for supplementary energy production and thus might be hypersensitive to glycolysis inhibition.
- To test this idea, we treated copper-deficient cancer cells with oxamate, an inhibitor of lactate dehydrogenase that converts the glycolytic end-product pyruvate into lactate and provides NAD⁺ to glycolysis. We found that oxamate, dosed at a concentration that does not suppress cell proliferation, enhanced the inhibitory effect of TM on β TC3 cells, as well as on human ovarian carcinoma cells SKOV3.
- We observed a similar combinatorial effect with another glycolysis inhibitor, 2-deoxyglucose, which inhibits the hexokinase that catalyzes the first step of glycolysis.
- Together these results support our hypothesis that copper-deficient cancer cells depend on glycolysis for viability and proliferation.

COPPER-CHELATOR TREATMENT DOES NOT PROMOTE TUMOR INVASION OR INCREASE REACTIVE OXYGEN SPECIES

- Enhanced glucose uptake, reflected by increased ^{18}F FDG-PET signals, is clinically associated with aggressiveness of tumors. Although we observed increased ^{18}F FDG accumulation in TM-treated tumors, they were not more invasive: in fact, the percentage of invasive tumors was slightly decreased, consistent with previous reports showing inhibition of invasion and metastasis by TM.



AMMONIUM TETRATHIOMOLYBDATE TREATMENT
TARGETS THE COPPER TRANSPORTER ATP7A
AND ENHANCES SENSITIVITY OF BREAST
CANCER TO CISPLATIN

Cristine L. Chisholm; *Oncotarget*. 2016 Dec 20; 7(51): 84439–84452.

TM AND ATP7A

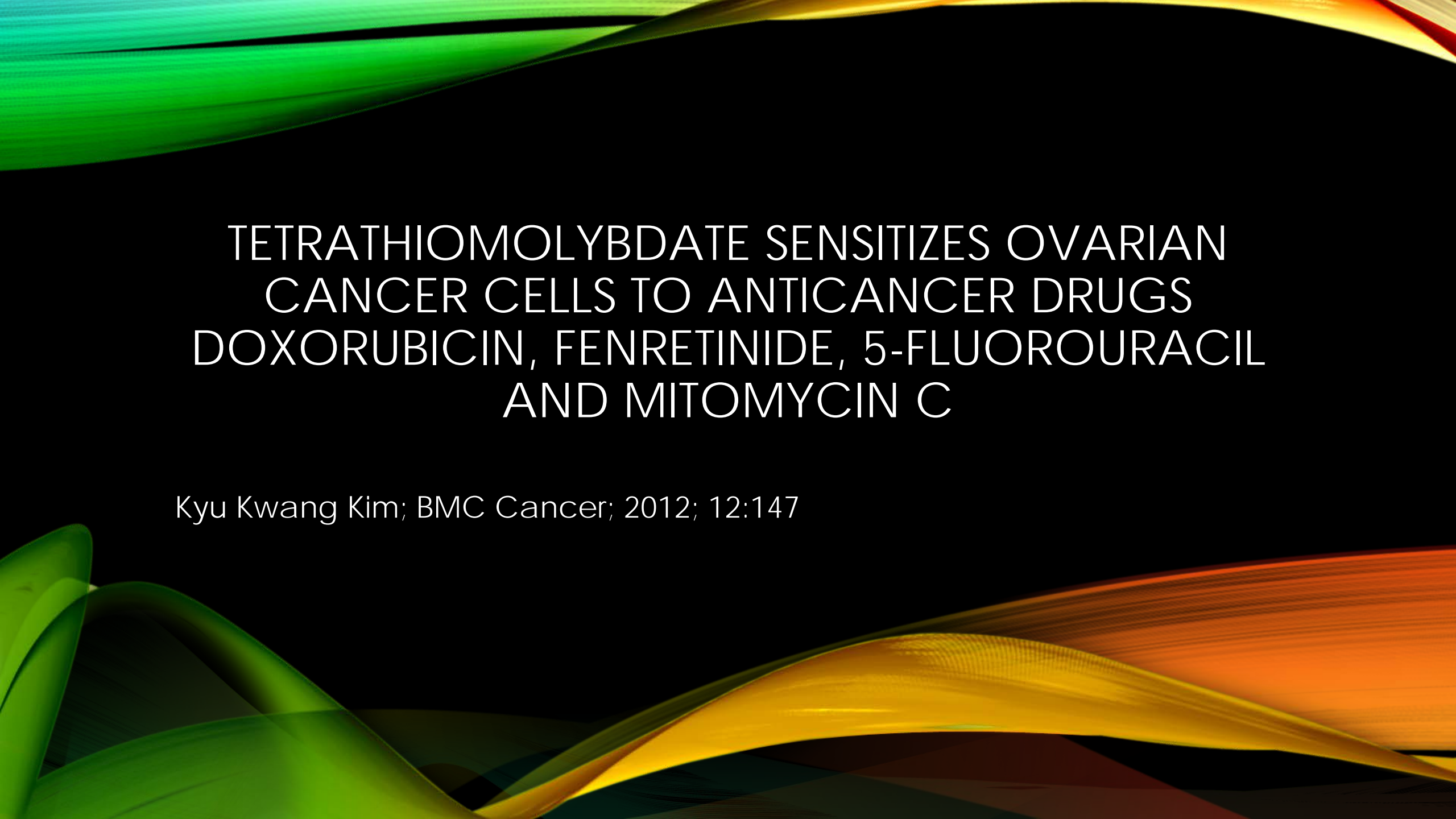
- Cisplatin is an effective breast cancer drug but resistance often develops over prolonged chemotherapy.
- ATP7A has been identified as a copper ATPase transporter responsible for intercellular movement and sequestering of cisplatin, conferring improved cancer cell survival.
- Pharmaceutical replacement for ATP7A by TM enhanced cisplatin treatment in breast cancer cells.
- Allograft and xenograft models in athymic nude mice treated with cisplatin/TM exhibited retarded tumor growth, reduced accumulation of cancer stem cells and decreased cell proliferation as compared to mono-treatment with cisplatin or TM.

TM AND ATP7A II

- Cisplatin/TM treatment of cisplatin-resistant tumors reduced ATP7A protein levels, attenuated cisplatin sequestering by ATP7A, increased nuclear availability of cisplatin, and subsequently enhanced DNA damage and apoptosis.

TM AND ATP7A III

- The authors have previously shown that prolonged treatment with cisplatin triggers accumulation of cisplatin resistant CSCs.
- In this study, the authors found that the combination of TM and cisplatin could prevent accumulation of CSCs, accompanied by widespread DNA damage and apoptosis.
- To uncover the mechanism underlying the synergistic actions between cisplatin and TM, microarray analysis was performed. It revealed that combined treatment of cisplatin and TM induced changes both in gene expression level and the number of genes that are involved in DNA damage repair, cell cycle checkpoint, and apoptosis at much higher frequency than the treatment of either drug alone. This finding provides the molecular basis for the synergy between these two drugs.



TETRATHIOMOLYBDATE SENSITIZES OVARIAN
CANCER CELLS TO ANTICANCER DRUGS
DOXORUBICIN, FENRETINIDE, 5-FLUOROURACIL
AND MITOMYCIN C

Kyu Kwang Kim; BMC Cancer; 2012; 12:147

TM SENSITIZES OVARIAN CANCER CELLS

- The author's recent study showed that TM can sensitize drug-resistant endometrial cancer cells to reactive oxygen species (ROS)-generating anticancer drug doxorubicin.
- The present study explores the ability of TM to potentiate the effect of doxorubicin and several other anticancer drugs including mitomycin C (MMC), fenretinide (retinoid derivative) and 5-fluorouracil (5-FU) in ovarian cancer cells.
 - These drugs were chosen for this study since they share one common target, the cellular oxidative defense system. Treatment with any of these drugs can lead to the elevation of oxidative stress promoting cell death in cancer cells.
 - In the present study the authors describe the effect of TM combination treatment with doxorubicin, 4-HPR, 5-FU, and MMC on various cellular apoptotic markers and determine the correlation between ROS activity and cell death in ovarian cancer cells.

TM AND DOXORUBICIN

- Platinum resistant SKOV-3 ovarian cancer cells were treated with TM (0, 30 μM) for 24 h, after which the cells were treated with doxorubicin (0, 2.5, 5, 10 μM) for another 24 h.
- Cells treated solely with doxorubicin (following vehicle pre-treatment for 24 h) at all concentrations tested reduced cell proliferation by 29% as compared to untreated controls.
- Cells treated solely with TM (30 μM) reduced cell viability by 5%.
- Pre-treatment with TM followed by treatment with doxorubicin revealed significant sensitization with viability inhibited by 42.1% (2.5 μM doxorubicin), 62.0% (5 μM) and 79.1% (10 μM).

TM AND DOXORUBICIN II

- To verify TM-mediated sensitization of ovarian cancer cells to doxorubicin, platinum sensitive A2780 cells were treated as described above with the exception that TM was applied at the concentration of 7.5 μM .
- Treatment with TM alone resulted in 14% reduction of viability while doxorubicin alone exerted effects in a dose-dependent manner (41.7% viability reduction at 2.5 μM ; 50% at 5 μM ; 58.7% at 10 μM doxorubicin).
- The TM/doxorubicin combination revealed significant sensitization of A2780 cells with viability inhibited by 74.8% (2.5 μM doxorubicin), 87.5% (5 μM) or 97.1% (10 μM).

DOXORUBICIN-MEDIATED APOPTOSIS IN OVARIAN CANCER CELLS IS ENHANCED BY PRE-TREATMENT WITH TM

- Cells treated with TM alone (30 μM , 48 h) only displayed minimal change in $\Delta\Psi\text{m}$ and remained mostly viable.
- Similarly, the majority of cells treated with doxorubicin alone remained mostly viable with 8.1% of cells being dead and an additional 13.9% displaying a disrupted $\Delta\Psi\text{m}$.
- In contrast, treatment with TM (30 μM , 24 h), followed by additional treatment with doxorubicin (5 μM , 24 h) increased the population of dead cells to 15.2% and also increased the population of cells with a disrupted $\Delta\Psi\text{m}$ to 26.8%.

AN INCREASED GENERATION OF INTRACELLULAR ROS UPON TM/DOXORUBICIN COMBINATION TREATMENT CAUSES OVARIAN CANCER CELL DEATH

- ROS generation was slightly elevated over baseline after treatment with TM alone.
- Doxorubicin alone also caused a peak shift, which was further increased when the cells were first treated with TM followed by doxorubicin treatment.
- To confirm that the generation of ROS by TM/doxorubicin combination treatment is the predominant mechanism of cytotoxic action we performed viability assays with SKOV-3 cells in the absence or presence of antioxidant ascorbic acid.
 - the growth inhibitory effect of TM was not notably affected by ascorbic acid treatment (90.2% viability with ascorbic acid; 88.1% without).
 - Similarly, scavenging of ROS did not reduce the cytotoxic action (70.4% viability with ascorbic acid; 68.9% without) by doxorubicin treatment.
 - In contrast, in TM/doxorubicin combination treatment, ascorbic acid partially restored the viability (69.9% viability with ascorbic acid; 42.6% without)

TM INDUCES SENSITIVITY OF OVARIAN CANCER CELLS TO VARIOUS ANTICANCER DRUGS VIA PROMOTION OF APOPTOTIC SIGNALING AND ROS GENERATION

- The authors investigated if TM potentially can sensitize cancer cells to a panel of drugs including MMC, 4-HPR, and 5-FU.
- SKOV-3 cells were treated with TM (0, 30 μM) for 24 h, after which the cells treated for another 24 h with MMC or another 48 h with 4-HPR or 5-FU.
 - Cells treated solely with MMC (2.5, 5, 10 μM), similarly to doxorubicin, did not show a clear dose response, and reduced cell viability by only 27% as compared to untreated controls.
 - 4-HPR (fenretinide) exerted dose-dependent cytotoxicity on SKOV-3 cells (viability of 83.1% at 5 μM ; 50.4% at 10 μM).
 - 5-FU displayed partial effects (viability of 78.3% at 1 mM). As seen for doxorubicin, treatment with TM potentiated the cytotoxic effect of all three drugs tested. Under these conditions the viability was reduced to 48.2% (TM + 10 μM MMC), 40.3% (TM + 5 μM 4-HPR) and 48.2% (TM + 1 mM 5-FU), respectively.



RADIOTHERAPY AND ANTIANGIOGENIC TM IN LUNG CANCER

Mohamed K. Khan; Neoplasia; Volume 4, Issue 2, 2002, Pages 164-170

RT AND TM

- Using a Lewis lung high metastatic (LLHM) carcinoma mouse tumor model, the authors demonstrate that the combination of TM and RT is more effective than either used as monotherapy.
- They also show that their therapeutic effects are additive, with no additional toxicity. They show that TM has no significant cytotoxicity in vitro against LLHM tumor cells, further supporting the antiangiogenic mechanism for its action.



COMPLETED HUMAN STUDIES WITH TM



ANNALS OF ONCOLOGY

TETRATHIOMOLYBDATE-ASSOCIATED
COPPER DEPLETION DECREASES
CIRCULATING ENDOTHELIAL PROGENITOR
CELLS IN WOMEN WITH BREAST CANCER
AT HIGH RISK OF RELAPSE

ANNALS OF ONCOLOGY II

- Bone marrow-derived endothelial progenitor cells (EPCs) are critical for metastatic progression. This study explores the effect of tetrathiomolybdate (TM) on EPCs in patients at high risk for breast cancer recurrence.
- Phase 2 study enrolled breast cancer patients with stage 3 and stage 4 without evidence of disease (NED), and stage 2 if triple-negative. TM 100 mg orally was administered to maintain ceruloplasmin <17 mg/dl for 2 years or until relapse. The primary end point was change in EPCs.

ANNALS OF ONCOLOGY III

- Forty patients (28 stage 2/3, 12 stage 4 NED) were enrolled. Seventy-five percent patients achieved the copper depletion target by 1 month.
- In copper-depleted patients only, there was a significant reduction in EPCs/ml by 27. Only six patients relapsed while on study, of which only one patient had EPCs maintained below baseline.
- Two stage-4 NED triple-negative patients remain disease-free at 65 and 49 months on TM therapy, which is encouraging given the dismal median survival of 9 months in metastatic triple-negative patients.

AMMONIUM TETRATHIOMOLYBDATE IV

- 18 patients in hospice with 11 different types of metastatic cancer. The goal of the study was to reduce ceruloplasmin to 20% of baseline for at least 90 days.
- The treatment achieved this goal in 6 patients, and 5 of those patients had seen no tumor growth or new tumors for more than 2 years. The other 12 patients could not achieve the target copper levels.

ONGOING HUMAN STUDIES WITH TM

- A Phase II Study of Tetrathiomolybdate (TM) in Patients With Breast Cancer at Moderate to High Risk of Recurrence
 - High risk stage II breast cancer (≥ 4 positive lymph nodes),
 - Stage III breast cancer, including inflammatory breast cancer
 - Stage IV breast cancer in a complete remission (bone only not allowed unless the bone scan is normal).
- A Phase II Trial of Tetrathiomolybdate in Patients With Hormone Refractory Prostate Cancer
 - Patients must have histologic diagnosis of adenocarcinoma of the prostate with progression following hormonal therapy and antiandrogen withdrawal.
 - Patients must have minimal disease (defined as bone metastasis or visceral disease with no impairment of organ function or pain severe enough to require narcotics for control)

ONGOING HUMAN STUDIES WITH TM II

- Phase I Study of Tetrathiomolybdate in Combination With Carboplatin/Pemetrexed in Metastatic Non-small Cell Lung Cancer
- Phase II Trial; Pre-Operative Chemoradiation Followed by Post-Operative Tetrathiomolybdate (TM) in Patients With Loco-Regional Esophageal Carcinoma (used very low dose at 20 mg/d).
 - Paclitaxel is administered intravenously over 1 hour on Days 1, 8, 15, and 22. Cisplatin will then be administered intravenously over 1 hour on Days 1 and 22. Radiation treatments will be given twice/day, on Days 1-5, 8-12 and 15-19. The subject's esophagus will be surgically removed on approximately Day #50. Approximately 4-6 weeks after surgery, the subject will start taking Tetrathiomolybdate, for 2 years or until treatment is no longer working to control your cancer.
 - Results; Median Recurrence Free Survival – 23.1 months; Median Overall Survival – 31.5 months.
- Reported median survivals from single and multi-institutional trials have varied from 12-29 months.

ONGOING HUMAN STUDIES WITH TM III

- Treatment of Hepatocellular Carcinoma With Tetrathiomolybdate
- Pilot Trial of Irinotecan, 5-Fluorouracil, and Leucovorin Combined With the Anti-Angiogenesis Agent Tetrathiomolybdate in Metastatic Colorectal Carcinoma



COPPER IS REQUIRED FOR ONCOGENIC BRAF SIGNALLING AND TUMORIGENESIS

Donita C. Brady; Nature 509, 492–496 (22 May 2014)

COPPER AND BRAF SIGNALING

- The BRAF kinase is mutated, typically Val 600 to Glu (V600E), to induce an active oncogenic state in a large fraction of melanomas, thyroid cancers, hairy cell leukaemias and, to a smaller extent, a wide spectrum of other cancers.

AMMONIUM TETRATHIOMOLYBDATE PROTOCOL

- Measure baseline CBC and ceruloplasmin
- TM 160-180 mg daily in four divided doses until Cp levels decreased to a target range of 7–15.
- Goal: reduce ceruloplasmin to 10-15; anemia/pancytopenia may limit use prior to optimal reduction