A Phase II Study of Tetrathiomolybdate (TM) in patients with breast cancer at moderate to high risk of recurrence

PROTOCOL FACE PAGE FOR
MSK THERAPEUTIC/DIAGNOSTIC PROTOCOL

Principal Investigator/Department:	Linda Vahdat, MD MBA	Medicine- Breast
Co-Principal Investigator(s)/Department:	Tiffany Traina, MD	Medicine- Breast
Investigator(s)/Department:	Ting Bao, MD, DABMA, MS	Medicine- Breast
	Victoria Blinder, MD	Medicine- Breast
	Jacqueline Bromberg, MD, PhD	Medicine- Breast
	Sarat Chandarlapaty, MD, PhD	Medicine- Breast
	Elizabeth Comen, MD	Medicine- Breast
	Gabriella D'Andrea, MD	Medicine- Breast
	Chau Dang, MD	Medicine- Breast
	Carlos Dos Anjos, MD	Medicine- Breast
	Pamela Drullinsky, MD	Medicine- Breast
	Monica Fornier, MD	Medicine- Breast
	Devika Gajria, MD, MPH	Medicine- Breast
	Shari Goldfarb, MD	Medicine- Breast
	Ayca Gucalp, MD	Medicine- Breast
	Neil Iyengar, MD	Medicine- Breast
	Komal Jhaveri, MD	Medicine- Breast
	Diana Lake, MD	Medicine- Breast
	Tomas Lyons, MD	Medicine- Breast
	Shanu Modi, MD	Medicine- Breast
	Larry Norton, MD	Medicine- Breast
	Pedram Razavi, MD, PhD	Medicine- Breast
	Mark Robson, MD	Medicine- Breast
	Rachel Sanford, MD	Medicine- Breast
	Andrew Seidman, MD	Medicine- Breast
	Nancy Sklarin, MD	Medicine- Breast
	Neil Vasan, MD	Medicine- Breast
	Esthefanie Giordano, RN	Nursing
	Renee Mercado, RN	Nursing
	Dariana Espinal, RN	Nursing
	Karen Suarez, RN	Nursing
	Maureen Cannon, NP	Nursing
	Karen Drucker, NP	Nursing
	Odessa Williams, NP	Nursing
	Danielle Alleva, NP	Nursing
	Nicole Jimenez, NP	Nursing
	Melissa Emerzian, NP	Nursing
	Mikel Ross, NP	Nursing
	Alexa Segna, NP	Nursing
	Britni Rizzolo, NP	Nursing

Consenting Professional(s)/Department:	Ting Bao, MD, DABMA, MS	Medicine- Breast
	Victoria Blinder, MD	Medicine- Breast
	Jacqueline Bromberg, MD, PhD	Medicine- Breast
	Sarat Chandarlapaty, MD, PhD	Medicine- Breast
	Elizabeth Comen, MD	Medicine- Breast
	Gabriella D'Andrea, MD	Medicine- Breast
	Pamela Drullinsky, MD	Medicine- Breast
	Chau Dang, MD	Medicine- Breast
	Carlos Dos Anjos, MD	Medicine- Breast
	Monica Fornier, MD	Medicine- Breast
	Devika Gajria, MD, MPH	Medicine- Breast
	Shari Goldfarb, MD	Medicine- Breast
	Ayca Gucalp, MD	Medicine- Breast
	Neil Iyengar, MD	Medicine- Breast
	Komal Jhaveri, MD	Medicine- Breast
	Diana Lake, MD	Medicine- Breast
	Tomas Lyons, MD	Medicine- Breast
	Shanu Modi, MD	Medicine- Breast
	Larry Norton, MD	Medicine- Breast
	Pedram Razavi, MD, PhD	Medicine- Breast
	Mark Robson, MD	Medicine- Breast
	Rachel Sanford, MD	Medicine- Breast
	Andrew Seidman, MD	Medicine- Breast
	Nancy Sklarin, MD	Medicine- Breast
	Tiffany Traina, MD	Medicine- Breast
	Linda Vahdat, MD	Medicine- Breast
	Neil Vasan, MD	Medicine- Breast

Please Note: A Consenting Professional must have completed the mandatory Human Subjects Education and Certification Program.

OneMSK Sites
Manhattan

Participating Institutions – If multicenter study coordinated by MSK:	**PI's Name**	**Site's Role**
Weill Cornell Medical College	Eleni Andreopoulou, MD	Specimen Analysis

Memorial Sloan Kettering Cancer Center
1275 York Avenue
New York, New York 10065

Table of Contents

1.0 PROTOCOL SUMMARY AND/OR SCHEMA

Patients with moderate to high risk primary breast cancer -Stage III, Triple negative (T ≥ 4 cm N0, any N+), Stage IV (without evidence of disease) will take tetrathiomolybdate (TM) pills for two years*.

Breast Cancer without any evidence of disease

Informed Consent:

- Correlatives (serum, plasma)
- Tumor block acquisition (primary, metastases, or both)
- DNA sequencing (tumor, germline for copper related SNPs and ctDNA

Induction Phase (TM dose 180 mg a day): TM 40 mg TID + 60 mg QHS
Cp target: 10-18 mg/dL.
CBC and Cp level q2W until stable TM dose

Maintenance Phase (TM dose 100 mg a day): TM 40 mg BID + 20 mg QHS
Cp target: 10-18 mg/dL and TM dosed according to Cp level in 20mg increments
CBC and Cp level
primary study: q4weeks; cycles 25-48: q 8 weeks; cycles 49-144*: q12 weeks
CMP, Mg, Phos,LDH: Q 12 weeks

Study Duration: TM administered for 2 years in primary study and in 2 year increments on Extension studies

- Correlatives at baseline and Q4 weeks or at study visit
- Disease Evaluation Q6 months.

*Patients who are stage 4 NED, 10 involved LNs or Triple Negative molecular subtype will be given the option to continue on Extension Studies #1-2 for months 25-48 and 49-72 respectively. Extension Study #3 will be open to patients who are stage 4 NED and will be candidates to continue for months 73-96.

Extension Study #4 will be open to patients who are stage 4 NED and will be candidates to continue for months 97-120. Extension Study #5 will be open to patients who are stage 4 NED and will be candidates to continue for months 121-144.

2.0 OBJECTIVES AND SCIENTIFIC AIMS

Note: This study has completed accrual in August 2014. This protocol outlines the ongoing management of patients enrolled on this study.

2.1 To evaluate the effect of tetrathiomolybdate on the tumor microenvironment including VEGFR1+ and VEGFR2+ bone marrow derived progenitor cells and, lysyl oxidase 2

2.2 To assess the safety and tolerability of tetrathiomolybdate in patients with breast cancer at high risk of tumor recurrence.

2.3 To observe the event-free and overall survival of patients enrolled in this trial.

2.4 Exploratory aims:

- To assess single nucleotide polymorphisms of the ceruloplasmin gene and correlate it to the ability to be copper depleted with tetrathiomolybdate.
- To perform whole exome sequencing of tumor and normal tissue from copper depleted patients and then identify mutations in circulating tumor DNA and serially monitor.
- To investigate predictors of response to copper depletion as a therapeutic strategy including assessing ATOX 1 in tumor samples.

3.0 BACKGROUND AND RATIONALE

3.1 Breast Cancer and risk of recurrence: Mortality from breast cancer continues to slowly decrease in the US with a recent improvement of 1.6% [1]. However, despite the advances in the adjuvant therapy of breast cancer, patients with 4 or more positive lymph nodes still have at least a 50% chance of relapse within the next 5 to 10 years. This number increases as the number of positive lymph nodes increases. Those with inflammatory or Stage 3 breast cancer have at least a 75% chance of systemic relapse within the ensuing 5 years despite aggressive adjuvant therapy. The theory is that there is the continued presence of sub-clinical occult disease that contributes to relapse.

Stage IV breast cancer is considered an incurable disease with a median overall survival ranging from 12 months to 4 years [2, 3]. There is no standard approach to HER 2 neu negative disease and treatment includes various combinations of hormonal manipulation, chemotherapy, radiation therapy and for some, biologic therapy [4].The natural history is for a patient to be treated with a first line agent, develop resistance and then switch to a second line agent. This process is repeated until complete resistance of the tumor to chemotherapy is observed. To date only a few modalities increase survival by 3 to 5 months. (2, 3)

Defining risk based on molecular subtype: It is now well established that risk of recurrence correlates with the molecular subtype of breast cancer. Molecular subtypes have been identified as a result of hierarchical clustering from microarray data. These are luminal A, B and normal breast like, HER 2 neu + and basal-like breast cancer. It has further been validated across multiple data sets that molecular subtype correlates with clinical outcome (8, 9) Since there are limitations of the broad application of microarrays in clinical practice, Carey and colleagues correlated these molecular subpopulations with immunohistochemical markers(10).

Triple negative breast cancer: TNBC has no effective targeted therapy. It is characterized by a lack of estrogen and progesterone receptors and is negative for HER2 neu. It often features an aggressive clinical course even in patients with early stage disease with the peak in relapse at 3 years after diagnosis [5-11]. The only treatment advances in the past 5 years have been the addition of carboplatin to standard chemotherapy to improve the pathologic complete response rate in the neoadjuvant setting, with the hope of improved survival and capecitabine for patients with residual disease in the neoadjuvant setting [12-14]. Hence, new strategies are desperately needed to reduce mortality from TNBC.

Therefore, a triple negative breast cancer has a higher a risk of recurrence compared to a non-triple negative when matched for tumor size or number of involved lymph nodes. Therefore, it is probably not fully accurate to only classify risk of recurrence for this subgroup based on traditional staging methods. Using the risk assessment program Adjuvant on line (www.adjuvantonline.com), the prognosis for relapse and mortality of a triple negative small (<2cm) node negative breast cancer is similar to that of a node positive ER + breast cancer. In fact, the risk of small tumors, with 1-3 positive lymph nodes or node negative tumors > 3 cm is similar to that of a ≥4+ nodal patient. It is for these reasons that this subgroup is considered high risk for metastases. Metastases are accountable for >90% of deaths due to breast cancer [15].

3.2 Tumor microenvironment and bone marrow derived progenitor cells: The tumor microenvironment has emerged as a critical component in the establishment, progression and metastatic dissemination of cancer [16-19]. Notably, stromal fibroblasts, endothelial cells and inflammatory cells of the microenvironment provide growth factors, proteases and pro-angiogenic factors to support tumor growth [19, 20].

We know that tumors co-opt infrastructure, including immune cells for initiation and progression [21-23]. Bone marrow (BM)-derived progenitor cells are one component of host infrastructure co-opted by the tumor to initiate and support tumor progression [24-26]. Using pre-clinical models of BC that metastasize to the lungs, we have previously shown that the primary tumor generated "pre-metastatic niche" in distal metastatic organs is comprised of many recruited BM-derived cells including endothelial progenitor cells (EPCs; $CD45^{dim}$, CD133+, VEGFR2+) that regulate the "angiogenic switch" during progression of micrometastases to macrometastases [26, 27]. We demonstrated that EPC deficiency results in impaired metastasis as a result of severe angiogenesis inhibition [24]. We extended these analyses to include a large cohort of BC patients where a >1000-fold surge in EPCs was observed immediately (one month) prior to overt relapse of BC suggesting that EPCs are a critical component for propagation of macrometastases [28]. This EPC surge in the peripheral blood has the potential to serve as a predictive biomarker of impending relapse in TNBC patients.

3.3 Copper and cancer progression: Metastasis requires a tumor cell to acquire the ability to migrate, intravasate, and survive in blood and lymphatic systems, then extravasate into a secondary organ to form a metastatic nodule. A key process at the terminal end is for the tumor cell to form a stable adhesion to the extracellular matrix, followed by angiogenesis and growth. Each of these processes involves rate limiting steps that are influenced by non-malignant cells in the microenvironment [17]. Copper plays key roles both within the tumor cells and in the tumor microenvironment, supporting the notion that copper depletion is an attractive therapeutic strategy [29-38]. Although we seek to elucidate the biologic processes surrounding the tumor microenvironment that promotes tumor metastasis, we acknowledge that this is just the "tip of the iceberg" of the multiple processes that copper can potentially influence within the tumor itself and its microenvironment. For example copper regulates energy balance via oxidative phosphorylation within the mitochondria [39], cell polarity and transitions between EMT and MET within the tumor [40], cell migration and invasion [41], and tumor growth through hypoxia related genes including HIF1 □ [20, 39, 42, 43]. Copper is a critical component of a host of metalloenzymes including superoxide dismutase-1 (SOD1), vascular adhesion protein-1 (VAP-1), MMP-9 and lysyl oxidase (LOX), which were shown by others to be integral to the metastatic process itself [38, 44-47]. Recently copper has been shown to be a necessary component of BRAF signaling in melanoma [48]. Angiogenesis is also dependent on copper as it is a cofactor for molecules such as basic fibroblast growth factor (bFGF), vascular endothelial growth factor (VEGF) and angiogenic [38, 44-46, 49-51]. While copper is important for normal immune function (phagocytic against microbes), copper depletion has been associated with conversion of co-opted tumor associated macrophages (TAMs) from immunosuppressive

to pro-immunogenic in tumor bearing mice by modifying the TAMs cytokine profile and promoting an anti-tumor response of T cells [52].

Lysyl oxidase (LOX) is a copper dependent amine oxidase (secreted by tumor and fibroblasts) that specifically promotes and facilitates metastases. Among many other activities, it promotes tumor cell migration and invasiveness via the activation of FAK [53]. Conversely, inhibition of LOXL2 in tumors not only reduces tumor cell invasion, but also has been demonstrated to attenuate the activation of host cells within the tumor microenvironment including cancer -associated fibroblasts (CAFs) [42]. CAFs partly remodel the extracellular matrix via collagen [42]. LOX was recently shown to be a novel regulator NFATc1- driven osteoclastogenesis that disrupatients bone homeostasis in an ER negative BC model and allows formation of focal pre-metastatic lesions. Those lesions formed a platform for circulating tumor cells to colonize and form bone metastases, which could be a target for therapy [47]. These studies add to the body of evidence that suggests that LOX (secreted by the primary tumor), accumulates at pre-metastatic sites, where it crosslinks collagen forming a scaffold for recruited BM-derived CD11b+ myeloid cells. This scaffold acts as a "pre-metastatic niche" and promotes tumor outgrowth of disseminated metastatic tumor cells [45, 46, 54-57]. Thus, VEGFR1+ hematopoietic progenitor cells (HPCs) and CD11b+ myeloid progenitor cells contribute to the pre-metastatic niche and its colonization of the pre-metastatic niche by EPCs, among other cells, activating the angiogenic switch leading to macrometastases formation [25, 54, 58].

3.4 Tetrathiomolybdate (TM): Tetrathiomolybdate (TM), an oral copper lowering agent, has been established as safe in patients with Wilson's disease (characterized by excessive copper accumulation) and advanced cancer [35, 59-63]. TM forms stable copper-molybdenum clusters sequestering copper and thereby limiting its availability, for the proper functioning of angiogenic factors, including secreted metalloenzymes [64]. TM may also modulate angiogenesis through alternative pathways, including effects on NF-kB, HIF-1 alpha, and by affecting copper-containing enzymes (superoxide dismutase-1 (SOD1), vascular adhesion protein-1 (VAP-1), and MMP-9). Both pre-clinical and phase 1 data suggest that TM may effectively reduce both overt and sub-clinical tumor load. ATN 224 or WTX 101 is the choline salt of TM (another formulation of the same drug). It is fast acting and stable, and several trials have established that its safety profile is similar to TM [65]. Trials with ATN 224 in patients with prostate cancer and a rising PSA did not show any benefit to copper depletion [66]. Our detailed work in breast cancer metastasis suggests that altering the microenvironment of the metastatic organ to prevent tumor recurrence will only be effective if metastatic colonization and outgrowth has not occurred.

Therefore, we hypothesized that the use of TM in a minimal residual disease state would modify the tumor microenvironment promoting tumor dormancy in breast cancer patients at moderate to high risk of relapse.

3.5 Preliminary Results: Our approach to investigate the effect of copper depletion on the tumor microenvironment has been two-fold: (1) to conduct a phase 2 trial of TM in BC patients at high risk of relapse to assess safety and efficacy in the clinic; and (2) to then go back to the bench to understand the molecular basis of how this process is affected in the microenvironment using a copper depletion strategy to focus our interrogation of clinical samples. We have published our findings in Clinical Cancer Research 2017 (63).

Our goal was to copper deplete patients to the level where normal cell function could take place but tumor progression/metastases could not.

3.5.1 Preclinical Studies of TM

Prior to embarking on the pre-clinical studies in our lab (Mittal lab, Weill Cornell Medicine), this was a summary of available data.

***In vitro* studies**: Experiments carried out with TM have demonstrated that the chelation of copper down-regulates the expression of VEGF and IL-8 in SUM-149 inflammatory breast cancer cells *in vitro* as determined by Northern blot (23).TM also had a destabilizing effect on the tumor cell itself by shutting down the production of NF-kB, a transcription factor present in tumor cells that protects the cells from apoptosis and makes them resistant to chemotherapy and radiation as well regulating the expression of multiple factors involved in tumor invasion and metastases including VEGF, IL-8 and matrix metalloproteinases (MMPs) (24)

***In vivo* studies**: The effect of copper chelation on tumor growth has been evaluated in several animal models using TM. Initial experiments at the University of Michigan with TM were conducted in transgenic Her-2/*neu* mice (23). Treatment was initiated in the thirteenth week after birth. By the end of 270 days, nearly 80 percent of the control group developed tumors while no tumor development was noted in the treated animals.TM also inhibited tumor growth in a head and neck cancer xenograft model, in which squamous cell carcinoma tumor cells were implanted into mice and allowed to grow to a substantial size (100 mm^3) prior to the initiation of treatment (25). While untreated mice experienced substantial tumor growth, the tumors in treated mice appeared to decrease in size. Tumors in animals treated with TM also had a 50 percent lower density of blood microvessels, indicating inhibition of angiogenesis.

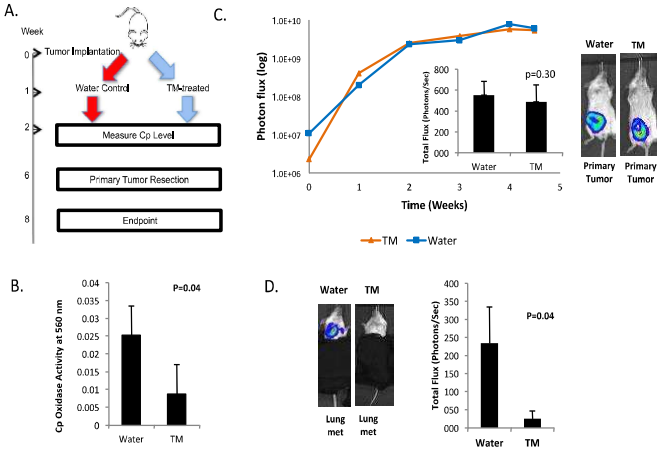
In vivo, TM has also been shown to have anti-tumor and anti-angiogenic effects in 3LL and SUM- 149 models. In the 3LL model, TM's effects were additive with radiation, suggesting that TM may enhance the therapeutic effects of standard treatments (26).

Pre-clinical data from Mittal Lab: In summary, we found that TM reduced lung metastases, abrogated lysyls oxidase and affected collagen fibril length and crosslinking in the pre-metastatic lungs using the MDA-LM2 breast cancer metastasis model [67].

-TM suppresses lung metastases but has no effect on primary tumor: The investigational schema is shown in Figure 1A. TM therapy reduced Cp oxidase levels in the metastatic lungs of mice treated with TM vs. water to acceptable levels (64% below baseline) (Figure 1B, p=0.04). TM-mediated copper depletion decreased secondary lung metastases as demonstrated by bioluminescence imaging (BLI) (Figure 1D, p=0.04), but did not have significant effects on the primary tumor (Figure 1C, p=0.30).

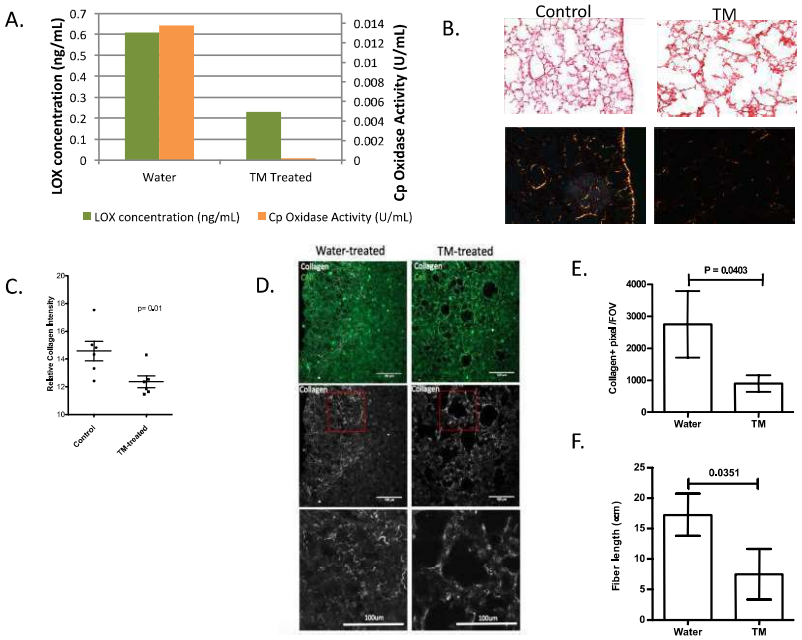
-Lysyl oxidase (LOX) activity and levels in are reduced in TM-treated pre-metastatic lungs: In mice treated with TM, serum Cp levels, primary tumor growth and lung metastasis were monitored as described above. TM therapy significantly reduced Cp oxidase levels in the lungs of TM-treated mice vs water (Figure 2A, p=0.0045)

FIGURE 1 :



Collagen deposition is decreased in TM-treated pre-metastatic lungs: TM therapy diminished collagen deposition as revealed by picrosirius staining and visualization for fibrillar collagen under polarized light in TM and water-treated pre- metastatic lungs and quantified by Image J software (Figure 2B and 2C, p=0.01). Accordingly, Second Harmonic Generation (SHG) imaging analysis confirmed that less collagen was deposited in lungs with TM treatment (Figure 2D and 2E, p=0.04) and that the collagen fibers formed with TM therapy were shorter relative to those formed in control animals (Figure 2F, p=0.04).

FIGURE 2:



3.6 Clinical trials of TM

3.6.1 Phase I trials: Given the fact that copper is an essential cofactor in the development of angiogenesis, Brewer and colleagues at the University of Michigan initiated a Phase I study in which varying doses of TM were administered to 18 patients with late-stage (metastatic) advanced cancer. In the first phase of this trial, the patients, all of whom had solid tumors, were enrolled at three dose levels of oral TM (90, 105, and 120 mg/day) given in six divided doses (three with meals, three between meals). These doses were chosen to achieve copper depletion and maintain patients within a window of plasma copper levels between 10 and 30 percent of normal levels, which appeared to be a therapeutic window based on the pre-clinical studies carried out at the University of Michigan. After achieving this chemical copper deficiency, doses were individually titrated to keep each patient's copper level within the therapeutic window. Serum ceruloplasmin was used as a surrogate marker for copper levels. At the doses studied, the induction of copper deficiency into a therapeutic range took approximately eight weeks. Four patients were removed due to disease progression prior to achieving the target copper levels. Of the 14 patients who achieved target copper levels, eight patients progressed within 30 days or had stable disease for less than 90 days and were removed from the protocol. The remaining patients achieved stable disease of ≥ 90 days' duration (five patients) or progression of disease at only a single site (one patient) (27). **(Table 1)**.

Table 1: Summary of disease progression in patients treated with TM

Outcome	Number	Percentage
Progressed before reaching Cp target	13	32.5
Progressed within 90 days of reaching Cp target	12	30
Stable Disease, >90 days (mean=11.7 months	15	37.5

These promising initial results led to an expansion of the trial. One of the goals of the expansion was to optimize the induction of copper deficiency. In the initial trial, the induction of copper deficiency (and thus any therapeutic effect) took 8 weeks to achieve, during which time many patients' cancers were progressing. The dose and dosing regimen were optimized in the extended phase I trial such that copper depletion could be achieved within three to four weeks (Merajver, unpublished results). In this phase of the trial, daily doses of 120, 140, 160 and 180 mg given QD were tested. For the Phase II dose and schedule, 180 mg/day given QD (with meals and at bedtime) was chosen because it was the dose at which copper deficiency was achieved faster and with the minimal incidence of sulfur-smelling eructation. Of the 40 patients treated in the combined Phase I trials, 15 demonstrated stable disease ≥ 90 days **(Table 2)**.

Table 2. Summary of disease progression in patients treated with TM by tumor type.

Tumor Type	Freedom From Progression (Months)	Tumor Type	Freedom From Progression (Months)
Stable Disease		**Stable Disease (continued)**	
Angiosarcoma	6.4	NSCLC	2.9
Renal cell carcinoma	3.7	Angiosarcoma	13.3
Melanoma	6.3	Hemangioendothelioma	20.2
Renal cell carcinoma	18.4	Non-small cell lung cancer	7.0+
Mesothelioma	3.5	Breast cancer*	30.2+
Pancreatic cancer	2.6	Sarcoma	32.1+
Renal cell carcinoma	9.6		
Breast cancer	5.9	**Objective Response**	
NSCLC	3.9	Ovarian cancer	5.9+
Osteosarcoma	2.6	NSCLC	8.5+
		Mean Time to Progression	10.2+

3.6.2 Phase II Clinical Trials in cancer: Four single-arm Phase II studies with TM have been conducted. Patients with renal cell carcinoma (RCC), hepatocellular carcinoma (HCC) or malignant pleural mesothelioma (MPM) were treated with single-agent TM 180 mg/day, taken three times a day with meals and once at bedtime. Patients with colorectal carcinoma (CRC) received the same dose of TM given in combination with 5- FU/LV/CPT-11. Using this dose and schedule, target Cp levels were usually attained in less than 30 days. There were patients in the RCC, HCC and MPM studies who achieved stable disease ≥ 90 days and a subset of these patients who had long-term stable disease (> 6 months). Commonly occurring adverse events (no causality implied) in patients in all four studies included fatigue, anemia and leukopenia (neutropenia). Other frequently occurring adverse events were anorexia, loss of appetite, diarrhea, sulfur burps, peripheral edema, ascites and abdominal pain. Multiple dose adjustments during the maintenance phase of treatment (after reaching target Cp level) were usually necessary.

Other ongoing trials include mesothelioma (Stage II to IV) at Wayne State University and the University of Michigan. Trials in diseases such as hepatocellular carcinoma, head and neck cancer, prostate and colorectal are also underway.

In summary, phase I and II studies in advanced malignancies showed that TM was safe and well tolerated but with limited efficacy in patients with advanced cancer (32-36).

3.7 Study Rationale: TM as a minimal residual disease strategy in breast cancer patients at high risk of relapse.

Our detailed work in breast cancer metastasis suggests that altering the microenvironment of the metastatic organ to prevent tumor recurrence will only be effective if metastatic colonization and outgrowth has not occurred. Therefore, we hypothesized that the use of TM in a minimal residual disease state would modify the tumor microenvironment promoting tumor dormancy in breast cancer patients at moderate to high risk of relapse. At the time this study was designed (2005), high risk was defined as 4+ involved lymph nodes and patients who were stage 4 NED. As the study progressed, new data emerged that patients with a node positive triple negative

breast cancer had the same level of risk as a stage 3 non- triple negative breast cancer so the protocol was amended to include this group of patients as eligible for enrollment.

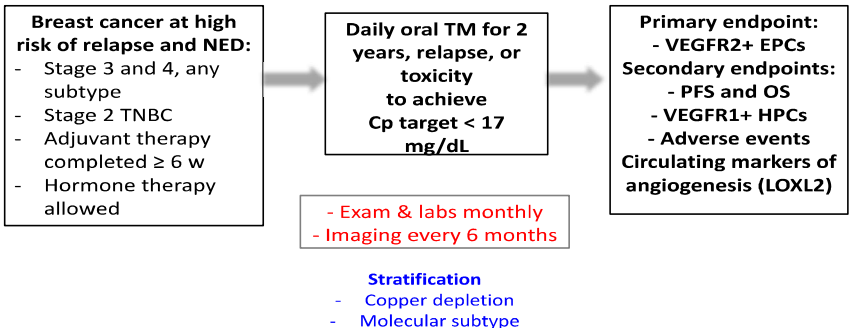
3.7.1 Preliminary Phase II clinical trial in breast cancer patients:

To date we have enrolled 75 patients on this clinical trial completing accrual in August 2014 and results were published in October 2016 [68]

The treatment schema is shown in Figure 3.

FIGURE 3:

Open-label, single-arm phase II trial of TM



Enrolled patient population: The majority of patients were at high risk of relapse, including 55% of patients with stage III disease, and 40% of patients with stage 4 NED. The prior disease sites for stage 4 NED patients include bone, chest wall, axilla and visceral sites such as liver, brain, lung and the peritoneum. Of note, 48% (36/75) of the patients had TNBC. The entire cohort received standard chemotherapy either in the adjuvant or metastatic setting prior to enrolling in this study (Table 3)

Study drug administration: Patients ingested tetrathiomolybdate (TM) pills daily to achieve copper depletion defined as a ceruloplasmin (Cp) level < 17 mg/dL. TM dose was then titrated monthly to maintain the Cp level between 8 and 16 mg/dL. Patients were scheduled to receive TM for 24 months on the primary study.

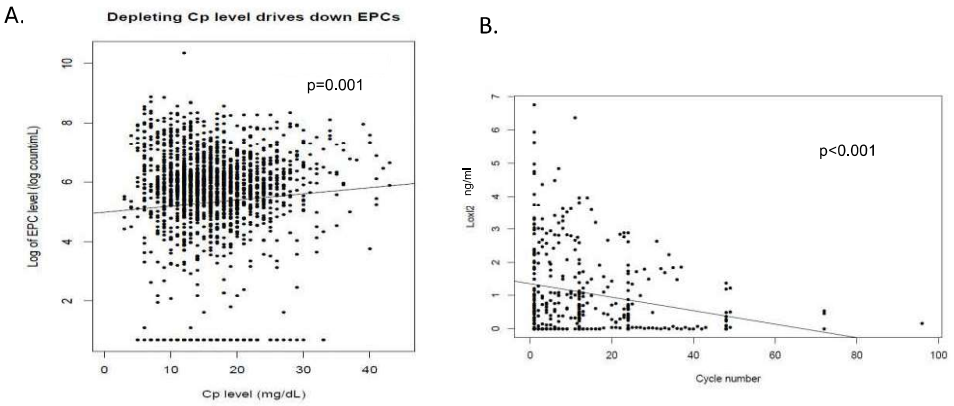
Table 3: Demographics of patients at Baseline	No. of patients (75)
Median Age, (range)	51 years (29-66)
AJCC Stage at Study entry, n (%)	
Stage 2	4 (5)
Stage 3	41 (55)
Stage 4 NED	30 (40)
Median tumor size in Stage 2/3 adjuvant pts, cm (range)	2.3 (1.2-7)
Median no. of positive lymph nodes in Stage 2/3 adjuvant pts, n (range)	6 (1-42)
Sites of Stage 4 disease, n	13/4
•Chest wall/liver	2/3
•Brain/bone only	1/3
•Peritoneum/lung	
Luminal A or B/Her2neu/Triple Negative (%)	40/12/48
Prior Neoadjuvant Therapy, n	10
•Achieved complete pathologic response	2
Prior Adjuvant Therapy (%)	
• Anthracycline +/- taxane-based	80
• Trastuzumab-based	14
• Non anthracyline-based	7

Evaluation on study: PatientsPatients were evaluated by physical exam and laboratory studies (CBC, CMP (Q4mos) Cp, tumor markers) every 28 days (one cycle) and by imaging (CTs or PET/CT) every 6 months.

Results: We depleted copper to target level in 80% of patients within 4 cycles. Copper depletion was most efficient in TNBC patients compared to other molecular subtypes with 91% depleted by the second cycle. Notably, a mixed effects model showed that lowering Cp levels decreased EPC levels ($p<0.001$)(**Figure 4A**). After observing that lysyl oxidase LOXL2, a copper dependent amine oxidase that is critical for modeling the pre-metastatic niche, was abrogated in the pre-clinical model, we set out to interrogate banked specimens from patients enrolled in this trial. We found that LOXL2 decreased by >50% ($p<0.001$) in the serum of our BC patients enrolled in this trial when they were copper depleted to target Cp (**Figure 4B**).

Adverse Events: TM therapy is safe with long-term toxicity data and well tolerated with 2.3% of cycles complicated by grade 3/4 reversible neutropenia, 0.04% of cycles complicated by grade 3/4 anemia, 1.2% of cycles complicated by grade 3/4 leukopenia, and 0.09% of cycles complicated by grade 3/4 fatigue (**Table 4**).

FIGURE 4:

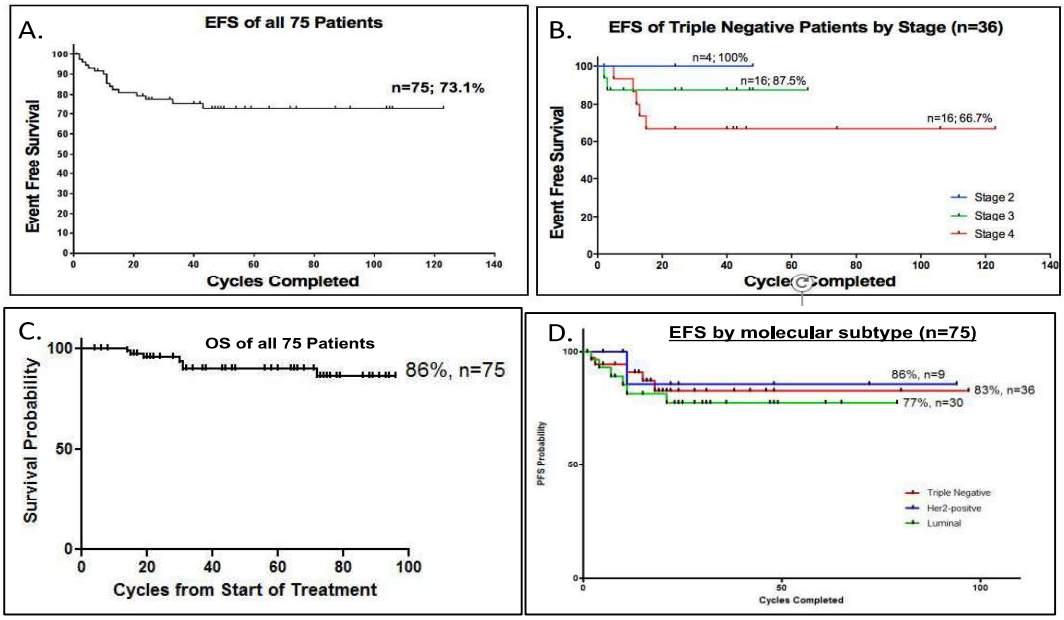


At a median follow-up of 7.1 years, the progression-free survival for all 75 patients is 73%,(**Figure 6A**) including a progression-free survival of 90% for all stage 2/3 patients with TNBC (**Figure 6B**). The overall survival is 86% (**Figure 6C**). Relapse after two years is a rare event. There is no difference between the triple negative breast cancer and non- triple negative breast cancer (**Figure 6D**). The median survival of a patient with stage 4 triple negative breast cancer is less than one year.

Table 4: Number of cycles complicated by adverse events (total cycles = 2650)

Adverse Event		N (%)	N (%)
		All Grades	Grade 3/4
Hematologic			
	Anemia	336 (12.7)	1 (0.04)
	Neutropenia	430 (16.2)	62 (2.3)
	Febrile Neutropenia	1 (0.04)	1 (0.04)
	Leukopenia	406 (15.3)	29 (1.1)
	Thrombocytopenia	32 (1.2)	0 (0)
Gastrointestinal			
	Sulfur Burps	801 (30.2)	0 (0)
	Nausea	75 (2.8)	0 (0)
	Vomiting	7 (0.3)	0 (0)
	Diarrhea	48 (1.8)	0 (0)
	Constipation	7 (0.3)	0 (0)
	Abdominal Pain	1 (0.04)	0 (0)
General			
	Fatigue	605 (22.8)	5 (0.2)
Neurologic			
	Dizziness	23 (0.9)	0 (0)
	Neuropathy	432 (16.3)	2 (0.08)

Figure 6:



Extension studies: In order to study prolonged copper depletion, a series of extension studies in 2 year increments have been conducted in selected groups of patients:

- Extension study#1 is available to the very high-risk patients (TNBC with 10+ LNs and Stage 4 NEDs) from month 25-48.
- Extension study #2 is available to the very high-risk patients (TNBC with 10+ LNs and Stage 4 NEDs) from month 49-72.
- Extension study #3 is available to the Stage 4 NEDs from month 73-96
- Extension study #4 is available to the Stage 4 NEDs from month 97-120.
- Extension study #5 is available to the Stage 4 NEDs from months 121 to 144

Profiles of the 19 patients who continue in the extension studies are shown in **Table 5**.

Table 5: Profiles of 19 patients on extension study:					
Pt ID	**Age at start of study**	**Start of Treatment date**	**Molecular Subtype**	**Stage at study entry**	**Prior disease sites for stage NED 4 patients**
TM02	50	06/13/2007	Triple Negative	Stage 4	Liver
TM18	50	10/15/2008	Triple Negative	Stage 4	Axilla; Skin
TM19	36	10/29/2008	Luminal B	Stage 4	Bone only
TM36	52	11/11/2009	Luminal A/B	Stage 4	Liver
TM39	65	03/24/2010	Luminal B	Stage 4	Chest wall
TM42	54	03/24/2011	Triple Negative	Stage 4	Skin
TM44	47	06/15/2011	Triple Negative	Stage 4	Brain; Lung
TM46	56	12/07/2011	Triple Negative	Locally Advanced/Neoadjuvant-10+ LN	
TM50	51	06/08/2012	Luminal A/B	Stage 3A+ primary peritoneal MMMT	
TM52	60	07/13/2012	Luminal B	Stage 4	Chest wall
TM55	50	09/26/2012	Luminal A/B	Stage 4	Skin
TM63	49	05/01/2013	Triple Negative	Locally Advanced/Neoadjuvant vs Stage 4	IMLN
TM65	56	05/15/2013	Triple Negative	Stage 4	Axilla
TM66	59	08/01/2013	Triple Negative	Stage 4	Skin
TM67	55	08/21/2013	Triple Negative	Stage 4	Lung
TM68	63	08/21/2013	Triple Negative	Locally Advanced/Neoadjuvant	
TM69	29	09/19/2013	Luminal B	Stage 4	Skin
TM70	42	10/23/2013	Triple Negative	Stage 4	Chest wall
TM71	45	10/30/2013	Triple Negative	Stage 4	Skin

3.8 Correlatives

3.8.1 Bone Marrow derived progenitor cells.

There is an increasing body of evidence that endothelial progenitor cells (EPCs. CD 133+/VEGFR2+) are critical in transitioning tumors from micro-metastatic to macro-metastatic (angiogenic switch). Preliminary evidence from this trial suggests that copper depletion modulates VEGFR2+ EPCs [24, 28].

3.8.2: Exploratory markers:

Exploratory markers of interest are the following: LOXL2, Collagen breakdown markers (COL1A, Pro C3, C6M) and ATOX 1.

3.8.2.1 Lysyl Oxidase: Tumor cell metastasis is facilitated by "premetastatic niches" formed in destination organs by invading bone marrow-derived cells (BMDCs). Lysyl oxidase (LOX) is critical for premetastatic niche formation. LOX is secreted by hypoxic breast tumor cells which accumulates at premetastatic sites, crosslinking collagen IV in the basement membrane, which is essential for CD11b+ myeloid cell recruitment. CD11b+ cells adhere to crosslinked collagen IV and produce matrix metalloproteinase-2, which cleaves collagen, enhancing the invasion and recruitment of BMDCs and metastasizing tumor cells. LOX inhibition prevents CD11b+ cell recruitment andmetastatic growth. Erler et al demonstrate a critical role for LOX in premetastatic niche formation and support targeting LOX for the treatment and prevention of metastatic disease[46, 54].

3.8.2.2 Collagen-TMrelated biomarkers: During cancer progression, the homeostasis of the extracellular matrix becomes imbalanced with an excessive collagen remodeling by matrix metalloproteinases. As a consequence, small protein fragments of degraded collagens are released into the circulation. Specific fragments of degraded type I, III and IV collagen (C1M, C3M, C4M) and type III collagen formation (Pro-C3) were assessed in serum from colorectal cancer patients, subjects with adenomas and matched healthy controls using well-characterized and validated ELISAs. Serum levels of the biomarkers were significantly elevated in colorectal cancer patients compared to subjects with adenomas (C1M, Pro-C3, C3M) and controls (C1M, Pro-C3) [69]. Pilot data (Vahdat, unpublished) from a cohort of breast cancer patients undergoing therapy identified C1M, Pro-C3, C6M as significantly associated with tumor progression.

3.8.2.3: ATOX 1: Copper (Cu) is an essential transition metal ion required as cofactor in many key enzymes and is vital for many steps of cancer progression. Atox1 was recently suggested to have additional functionality as a nuclear transcription factor. Recently, Atox1 was found to accumulate at lamellipodia borders of migrating cancer cells and Atox1 silencing resulted in migration defects [70]. We will assess IHC or immunoflourescence staining of ATOX 1 in primary/metastatic tumors of patients enrolled on this trial.

3.9 Proposed next study:

This study will serve as the basis for a future, randomized, placebo controlled phase 2 trial of TM/WTX101 for 3 years in patients with node positive TNBC who have completed standard adjuvant therapy or neo-adjuvant therapy with residual disease.

4.0 OVERVIEW OF STUDY DESIGN/INTERVENTION

4.1 Design

This is a phase II study of Tetrathiomolybdate treatment in triple negative breast cancer patients with moderate to high risk primary breast cancer, Stage III, and Stage IV without

evidence of disease. As of September 2017, we have completed patient enrollment. 19 patients are currently receiving treatment. Based on our preliminary data, we plan to extend the duration of copper depletion for a subsets of patients enrolled within this trial for up to 144 months in selected patients (extension studies). Correlatives are being collected and analyzed to assess possible markers of anti-angiogenic agents.

4.2 Intervention

There will be two treatment phases: induction and maintenance. During the induction phase TM will be administered according to a pre-specified dose and schedule until serum Cp reaches a target range of 5-17 mg/dL. Depending on what extension study, patients will come to clinic every 8 weeks or 12 weeks. A physical exam, review of toxicities and collection of research labs will be collected at each visit as indicated in the Study Calendar (Table 8).

As of February 2018, 19 patients remain on active treatment and are enrolled to the extension studies as noted below:

Extension study 2(Yrs 4-6): 14 patients

Extension study 3 (yrs 6-8) : 4 patients

Extension study 4 (Yrs 8-10): 1 patient

5.0 THERAPEUTIC/DIAGNOSTIC AGENTS

The investigational agent in this study is Tetrathiomolybdate, for oral administration.

5.1 Availability

TM is an investigational agent and will be used according to the information in this IND.

5.2 Agent Ordering/Preparation

TM drug substance is manufactured and tested under GMP by Sigma Aldrich Chemical Company, Milwaukee, WI, as described in the Chemistry, Manufacturing and Controls section of this IND. TM will be compounded and further tested by the Prescription Center (1907 W Avenue South La Crosse, WI 54601-6206; Phone: 800-203-9066; Fax: 608-788-4501; Email: rxcentermed@gmail.com) and mailed to the Investigational Drug Service (IDS) at MSKCC (MSKCC Investigational Drug Service attn: Irfan Hoque; 1275 York Ave Room: C-1087, New York, NY 10065. Phone: 212-639-8074, Fax: 646-227-2459). Prescription Center will serve as the dispensing pharmacy. MSKCC IDS/Pharmacy will serve as the agent of research and coordinating center. Please refer to Appendix B for details.

The final drug product (capsules for oral administration, lactose monohydrate as an excipient) will be further tested for uniformity, identity and purity as described in the Chemistry, Manufacturing and Controls section of this IND.

A prescription is written by the treating physician, the research pharmacists will dispense the appropriate dose of TM to the research clinic. The research nurse and team will dispense the drug to the patient. It was shown previously that in such capsules TM retains its potency for 8 weeks (47). Thus, TM will be dispensed to each patient in 4 to 8 week installments throughout the trial.

5.3 Agent Accountability

The Research Pharmacist in the MSK IDS will maintain a careful record of the inventory and disposition of TM using the NCI Drug Accountability Record Form.

5.4 Bioavailability

Tetrathiomolybdate is a copper chelating agent that forms a stable tripartite complex with copper and protein. If given with food, it complexes food copper with food protein and prevents absorption of copper from the GI tract. It also prevents copper reabsorption of the endogenous secreted copper in saliva and gastric secretions with the same mechanism. If given in-between meals, it is absorbed into the blood where it complexes either free or loosely bound copper with serum albumin. This TM-bound copper fraction is no longer available for cellular uptake, has no known biological activity, and is slowly cleared in the bile and urine. Based on our evaluation of our data, more efficient copper depletion is observed when given concomitantly with a proton pump inhibitor.

5.5 Drug shipment

Due to the lengthy travel distance from home to the study site, two participants (one who lives in California and the other in Canada), will be unable to return to clinic every two months as per protocol for follow up and drug pick up. These participants will return to clinic every three months instead. In order to ensure adequate drug supply, a one month supply of the study drug will be mailed to their home. MSKCC Investigational Drug Pharmacy will be responsible for the the shipment of the study drug. Given that these will be regular shipments for these two participants while on treatment, we will not request prospective deviation approvals from the IRB.

All remaining participants will pick up study drug on site at their clinic visit.

6.0 CRITERIA FOR SUBJECT ELIGIBILITY

This study has completed accrual in September 2017 and eligibility criteria was as noted below. All patients who enrolled to this study previously at Weill Cornell will be eligible for enrollment at Memoral Sloan Kettering Cancer Center. A reassessment of eligibility will not be required for enrollment at MSKCC as there are no changes in eligibility and these patients are currently on study.

6.1 Subject Inclusion Criteria

- Patients must have histologically confirmed breast malignancy that is:
  - 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).
  - Triple negative tumors (ER/PR/HER2/neu negative): Any nodal positivity, node negative and ≥ 4 cm.
- Eligibility for extension study #1 and #2 (months 25 to 72):
  - Stage 2 TNBC
  - Stage 3C breast cancer
  - Stage 4 NED
  - Triple negative breast cancer
- Eligibility for extension study #3 (months 72 to 96)

  - Stage 4 NED
- Eligibility for extension study #4 (months 97 to 120)
  - Stage 4 NED
- Eligibility for extension study #5 (months 121 to 144)
  - Stage 4 NED
- The patient must have completed what is considered standard adjuvant systemic therapy that may include chemotherapy, hormonal therapy and radiation therapy. They may have undergone high dose chemotherapy with stem cell support as part of their therapy in the adjuvant or metastatic setting. The patient is allowed to continue to take adjuvant hormonal therapy (for high risk adjuvant patients) and may be allowed to be on hormonal therapy if they are Stage 4 and without evidence of disease. The patient cannot be actively receiving chemotherapy or any biologic agent to treat their breast cancer.
- Six weeks must elapse from last chemotherapy or radiation therapy.
- The patient must have had definitive surgical therapy for their breast cancer. This includes lumpectomy and axillary dissection or mastectomy.
- No clinical or radiologic evidence of disease after surgery and/or systemic treatment (by CT scan of chest, abdomen and pelvis and bone scan or PET scan prior to enrollment).
- Because no dosing or adverse event data are currently available on the use of TM in patients <18 years of age, children are excluded from this study.
- KPS 90 or 100.
- Life expectancy of greater than 3 months.
- Patients must have normal organ and marrow function as defined below:
  - hemoglobin $\geq$10mg/dL
  - absolute neutrophil count $\geq$1,500/ µL
  - platelets $\geq$100,000/µL
  - total bilirubin $\leq$1.5 x normal institutional limits
  - AST (SGOT)/ALT (SGPT) $\leq$1.5 X institutional upper limit of normal
- Erythropoietin alpha is allowed, as indicated.
- Bisphosphonates may be administered.
- Patients must be on stable medical therapy for at least 2 weeks if they are being treated medically for their peripheral neuropathy.
- Concurrent trastuzumab is not allowed in the adjuvant setting. In the metastatic setting, up to 4 patients will be allowed to receive concurrent TM and trastuzumab in order to assess whether there are any pharmacokinetic interactions.
- The effects of TM on the developing human fetus are unknown. For this reason, women of childbearing potential and men must agree to use adequate contraception (hormonal or barrier method of birth control; abstinence) prior to study entry and for the duration of study participation. Should a woman become pregnant or suspect she is pregnant while participating in this study, she should inform her treating physician immediately.
- Ability to understand and the willingness to sign a written informed consent document.
- Normal B12 and folate levels

6.2 Subject Exclusion Criteria

- Patients who have had chemotherapy or radiotherapy within 4 weeks prior to entering the study.
- Objective evidence of breast cancer.
- Carcinomatous meningitis or active parenchymal brain metastases.
- Serum creatinine >1.5 x normal.
- History of allergic reactions attributed to compounds of similar chemical or biologic

composition to TM.

- Pregnant women are excluded from this study because TM has the potential to have teratogenic or abortifacient effects. Because there is an unknown but potential risk for adverse events in nursing infants secondary to treatment of the mother with TM, breastfeeding should be discontinued if the mother is treated with TM.
- Because patients with immune deficiency are at increased risk of lethal infections when treated with marrow-suppressive therapy, HIV-positive patients receiving combination anti- retroviral therapy are excluded from the study because of possible pharmacokinetic interactions with TM.
- Inclusion of Women and Minorities
    - Both men and women and members of all ethnic groups are eligible for this trial. The proposed study population is illustrated in **Table 6** below.

Table 6: Patient Demographics

	Race/Ethnicity					
Gender	White, not of Hispanic Origin	Black, not of Hispanic Origin	Hispanic	Asian or Pacific Islander	Unknown	Total
Male	0%	0%	0%	0%	0%	0%
Female	85%	5%	10%	0%	0%	100%
Total	85%	5%	10%	0%	0%	100%

7.0 RECRUITMENT PLAN

N/A. We have completed enrollment for this study.

8.0 PRETREATMENT EVALUATION

Please refer to the study calendar under Section 10.0, Table 8. We have completed enrollment for this study as of September 2017.

9.0 TREATMENT/INTERVENTION PLAN

9.1 TM Administration

Treatment will be administered on an outpatient basis. Reported adverse events and potential risks are described in Section 11. Appropriate dose modifications for TM are described in Section 9.10. No investigational or commercial agents or therapies other than those described below may be administered with the intent to treat the patient's malignancy.

Data analysis shows that absorption of TM is much improved with the addition of a proton pump inhibitor. The use of a PPI should be left up to Investigator discretion however if it will be used, it should be given with first dose of TM.

9.2 Induction with Tetrathiomolybdate (TM)

Tetrathiomolybdate 40 mg. p.o. TID with meals and tetrathiomolybdate 60 mg at bedtime for a total of 4 doses (180 mg) per day. If ceruloplasmin (Cp) level is < 19g/dL at baseline or the ANC

is between 1.5/μL and 1.8/μL with a total WBC < 3.0/μL then, tetrathiomolybdate 40 mg. p.o. in the morning, 20 mg in the afternoon and 40 mg at bedtime for a total of 3 doses (100 mg) per day.

9.3 Induction goal
Total tetrathiomolybdate dose per day = 180 mg or 100 mg until serum Cp level decreases to 10-18mg/dL.

When target Cp window is reached, then the maintenance phase begins.

9.4 Maintenance with Tetrathiomolybdate
Total tetrathiomolybdate dose per day will be in 20 mg increments to tailor the therapy to individualized patient needs to maintain the Cp level at 10-18mg/dL. Thus all dose modifications will be dependent on individual patient Cp levels.

Tetrathiomolybdate 40 mg p.o. BID with meals and tetrathiomolybdate 20 mg at bedtime.

9.5 Dose increases
Intra-patient dose escalations are not allowed except to maintain target Cp levels. If a patient's Cp level exceeds 19 mg/dL (lower limits of normal at ARUP lab), the TM dose may be increased in increments of 20 mg/day or every other day in the absence of ≥Grade 2 treatment-related toxicity. Dose increases may not occur more frequently than once every 2 weeks. Only the PI or the CO-PI, will make dose changes.

9.6 Definition of Dose-Limiting Toxicity
In our study a dose limiting toxicity will be defined as any toxicity, other than myelosuppression, that is life threatening (Grade IV). If a patient experiences a dose limiting toxicity before reaching the specified serum Cu^{2+} target range, he/she will be taken off the study. Management and dose modifications associated with the above adverse events are outlined in Section 9.10.

9.7 Supportive Care Guidelines/ Prohibited and Allowed Concurrent Medications
Medications used to alleviate toxicity symptoms associated with TM may be prescribed at the discretion of the patient's physician. Physicians must adhere to the following guidelines:

9.7.1 Concurrent anti-cancer therapy is not allowed with the exception of hormonal manipulation and the stage 4 NEDs on trastuzumab.

9.7.2 Patients may receive hormonal replacement therapy for thyroid, or glucocorticoid deficiency.

9.7.3 Any disease progression requiring anti-tumor therapy other than TM will be cause for discontinuation from the study

9.7.4 Copper- or zinc- containing vitamins or supplements are not allowed.

9.7.5 Colony-stimulating factors (i.e. G-CSF, etc.) should not be administered on a prophylactic basis.

9.7.6 Use of erythropoietin alpha is permitted.

9.8 Duration of Therapy
In the absence of treatment delays due to adverse events, TM treatment may continue for 2 years on the primary study. When originally designed, this was an arbitrary number as the ideal duration of treatment with a minimal residual disease strategy was unknown. After the safety and potential

efficacy was observed after completion of 2 years, patients had the opportunity to continue for an additional 2 years on a series of extension studies (1-5).

Of note is that the optimal duration of minimal residual disease therapy is still unknown. Treatment with TM will continue until patients experience recurrent or progressive disease. Treatment may also be discontinued for other reasons including if it is considered to be in the best interests of the patient. Reasons for discontinuation include:

- Disease progression,
- Intercurrent illness that prevents further administration of treatment,
- Unacceptable adverse event(s),
- Patient decides to withdraw from the study, or general or specific changes in the patient's condition render the patient unacceptable for further treatment in the judgment of the investigator.
- Non-compliance
- Loss to Follow Up

9.9 TM extension study #3, #4 and #5

The data from this trial is being continually evaluated (46). The first paper of our experience with TM in the first 40 patients who have completed one year of therapy has been published (30). Interesting patterns have continued to emerge with regard to the high-risk molecular subgroups and the majority of the Stage 4 NEDs continue to be disease free. While a randomized trial is the gold standard to truly assess efficacy, these results continue to be encouraging and a randomized phase 2 trial is being planned. We propose that in selected patients (triple negatives and breast cancers that are Stage 4 NED) that there is a provision to continue TM for additional years up to 12 years if the patient and treating physician have reason to believe that it would benefit the patient.

9.10 Dosing Delays/Dose Modifications

This study will use the NCI CTC Version 3.0 for toxicity and adverse event reporting. In the event of Grade 3 or 4 treatment-related toxicity (excluding alopecia), dosing will be held until recovery to at least Grade 1 (**Table 7**). However, if recovery has not occurred within *2 weeks*, the patient will be discontinued. In the event of Grade 2 toxicity involving vital organs (hepatic, pulmonary, renal, neurologic, gastrointestinal or cardiotoxicity), the dose of TM will be held until recovery to Grade 0-1 before a new cycle is initiated at 100%. If the Grade 2 toxicity recurs, dosing will be held until recovery to Grade 0-1 and the next cycle will resume at a dose deemed appropriate by an investigator. Since each individual patient will be receiving a unique TM dose, selected specifically to achieve the target Cu2+ serum levels, exact TM doses cannot be given at this time. Dr. Linda Vahdat must approve all dose modifications. In the event of hematologic-related toxicity, dosing will be held until recovery, with repeat CBC approximately every two to three days. TM will be resumed at a lower dose after recovery.

Basic Principles of TM management:

- The goal is to have the Cp level between 10 and 18 mg/dL (Using a lower limit of normal of 19mg/dL)
- Reflections of change in Cp level take 2 weeks from change of TM dose
- A PPI markedly increases TM absorption and can drive the Cp level down to the copper depleted range within 2 weeks.
- Changes in TM dose should be made in 20 mg increments (1 pill)

- Think of dosing as total number of pills (or mg) per week. Strategies for dosing can include taking a certain number of pills during the week and different number on the weekend.
- The effect of copper depletion is cumulative so keep that in mind when you see Cp levels decreasing over time when you have not made a change in dose from one month to the next. You might need to cut back on the total number of pills per day (or week) to avoid over copper depleting patients.
- The first sign of over copper depletion is neutropenia. Sometimes fatigue can precede neutropenia.

Table 7: Dose Modifications

Cp Level	ANC	TM dose
> 19	WNL	Can increase by 20 mg/day
>19	1.5 to 2.0	Pt is close to being copper depleted. Might want to recheck in 2 wks
>19	1.0 to 1.4	Probably copper depleted and their Cp level if driven below 19 might make them neutropenic. Might want to decrease dose by 20 mg or hold TM for a day or two if ANC close to 1.0
<19	≥1.5	Keep on current dose
<19	1.0 to 1.4	Just about over copper depleted. Might want to decrease dose by 20 mg or hold TM for a day or two if ANC close to 1.0
<19	<1.0	Hold TM. Recheck CBC in 3 days. Restart TM at 20 mg lower dose when ANC1.5

9.11 Correlative/special Studies

Currently, there is no method to predict response to therapy for an anti- cancer agent or toxicity of therapy. Nevertheless, several interesting avenues are being explored to address these issues. These correlative studies will be performed in the hematology/oncology Translational Core Laboratory at Weill Medical College of Cornell University. Approximately 23 ml of blood will be collected in 3 purple top and one red top tubes and delivered to the Translational Core laboratory at to isolate plasma and mononuclear cells for analysis. Samples should be shipped to the following address:

Weill Medical College of Cornell University
Translational Core Laboratory
Attn: Maureen Ward
1300 York Avenue, Room C-635
Tel: 212-746-3123
Email: mmw2003@med.cornell.edu

Research Bloods: This is done monthly for up to cycle 24 (primary study), at least every other month up to cycle 48 (completion of extension study #1) and then research bloods only will be done at least every 3 months up to cycle 144 (completion of extension study #5), on a stable TM dose. If patient is not on a stable TM dose or per the discretion of Dr. Vahdat at any time during extension #1-5, all study procedures will be completed every month.

Specimens will be de-identified using a unique patient identification number. An associated lab requisition detailing the tubes, the timepoint of collection and processing will be included. Samples will be sent via dry ice. Shipments should be received during business hours, Monday- Friday, 9am-5pm. An email will be sent to the above noted contact prior to the shipment for notification. The study team will transport the research samples to Weill Cornell.

Correlative studies:

9.11.1 Archived tumor samples will be collected for analysis (WES, SNPs).
The following will be performed in the TCL and will be assessed at each time point (1) ceruloplasmin, (2) hemangiogenic progenitor cells VEGFR1+ HPCs and VEGFR2+ EPCs), (3) serum lysyl oxidase (LOXL2) and (4) other exploratory biomarkers to be identified from banked specimens. The IPM will assess the ctDNA at the same time points.

9.11.2 VEGFR1+ and VEGFR2+ bone marrow derived progenitor cells:
Ten to 20 mL of venous blood is collected in EDTA-containing tubes and processed within 12 hours. Peripheral blood mononuclear cells are isolated by Ficoll density-gradient centrifugation. To quantitate circulating EPCs, cells are stained with CD133-PE (Miltenyi Biotec, Auburn, CA), VEGFR2-APC (R&D Systems, Minneapolis, MN), and CD45-PerCP (BD Biosciences, Franklin Lakes, NJ). To quantitate HPCs, cells are stained with CD34-FITC (BD Biosciences), VEGFR1-APC (R&D Systems), and CD45-PerCP (BD Biosciences). Multi-color flow cytometry is performed as previously described [28].

9.11.3 Lysyl Oxidase 2 (LOXL-2):
Methods for Quantification of LOXL2 levels in Patient Serum: Pt serum samples will be collected in blood vacutainer tubes containing no anticoagulant. Blood is left at room temperature for at least 30 minutes to allow clotting. Blood samples are spun at 1500 g for 10 minutes at 4^0C. Serum is removed and stored at -80^0C until assayed.

Quantitation of Blood Serum LOXL2 Levels: Pt LOXL2 serum levels are quantitated using an ELISA kit from US Biologicals (Swampscott, MA) following the manufacturer's protocol. One hundred microliters of patient serum is added in duplicate to the wells of a microtiter plate coated with a biotin-conjugated antibody specific to LOXL2, along with concentration standards. After a two-hour incubation, Avidin conjugated to Horseradish Peroxidase (HRP) is added to each well and incubated. TMB substrate solution is added and wells containing LOXL2 biotin-conjugated antibody and HRP enzyme conjugated Avidin exhibited a color change. The degree of color change is then measured with a spectrophotometric plate reader at 450nm. Concentrations are calculated based on the standard curve.

9.11.4 Genomics:
Genetic factors appear to contribute to virtually every human disease, conferring susceptibility or resistance, affecting the severity or progression of disease, and interacting with environmental influences. Emerging data suggest single nucleotide polymorphisms (SNPs) may have a strong impact on the ability of a gene to function and may contribute to

differences in responses between individuals. These differences may affect an individual's ability to respond to drug and predict toxicity.

CP SNP: In this small exploratory study, we propose to analyze polymorphisms of ceruloplasmin, the main copper-carrying protein. Several polymorphisms in the ceruloplasmin gene have already been identified, some of which are associated with human disease including disorders of copper transport and iron transport (1, 48).

CTR1 SNP: We will explore whether other polymorphisms in the CTR1 gene affect an individual's response to copper-lowering anti-angiogenic therapy. Similarly, we will correlate surrogate marker response as a function of single nucleotide polymorphism of the ceruloplasmin gene by PCR analysis. This will be done through the Translational Core Facility of Cornell. The methods and amount of blood needed to do these studies are as yet to be determined.

Whole Exome Sequencing and ctDNA: In the era of personalized medicine, technology has provided the ability to perform whole exome sequencing of tumor DNA in an effort to provide future targeted therapies. We will perform DNA extraction and targeted genetic sequencing from FFPE (formalin fixed paraffin embedded) tumors from this cohort of patients, which will identify biologic pathways and genes that are specifically altered. Once the FFPE tumor tissue is sequenced and we are able to identify tumor specific mutations, we will then isolate circulating tumor DNA from the peripheral blood. Mutations within circulating tumor DNA can then be identified. These mutations can be monitored serially through targeted sequencing over time, and evaluated during times of response and relapse. We will use plasma samples that are drawn as part of the study, and are frozen and stored. This will allow us to gain insight into mutations and genetic alterations which may be resistant or sensitive to copper depletion over time, and help to determine the optimal duration of copper depletion for the metastatic patients and patients on adjuvant treatment.

9.11.5 Other exploratory correlatives of interest

Pre-clinical work has been initiated to aid in biomarker development to predict sensitivity, resistance and markers of effect of copper chelation on the tumor microenvironment. This includes but is not limited to evaluation of lysyl oxidase, matrix metalloproteinases, superoxide dismutase, ATOX 1 and ROS.

9.11.16 Trastuzumab Pharmacokinetics:

For patients enrolled onto the study who are receiving maintenance trastuzumab, peak and trough levels will be performed in the Translational core lab according to published methods(49). Blood (10 cc) will be drawn and sent to the Translational Core Facility of Cornell for analysis. As of February 2018, the trastuzumab pharmacokinetics collection has been completed.

10.0 EVALUATION DURING TREATMENT/INTERVENTION

Please see Table 8: Study Calendar noted below:

Table 8: Study Calendar							
Tests/ Observations	**Screening/ Baseline**	**Induction Day 1** until Cp10-18 mg/dL	**Induction Day 15** Cp10-18 mg/dL	**Stable Maintenance, primary study** (Q 4 weeks)[5]	**Stable Maintenance, Extension #1** (Q 8 weeks)[5]	**Stable Maintenance, Extension #2, 3, 4, 5** (Q 12 weeks)[5]	**Follow-up Phase**
Signed informed consent	X						
History/Interval History	X	X		X	X	X	
Physical Exam	X	X		X	X	X	
Vitals (Pulse, BP, Weight)	X	X		X	X	X	
Medication review	X	X		X	X	X	
Drug toxicity review	X	X	X	X	X	X	
Imaging							
CT scan; PET/CT scan	X			X[1]	X[1]	X[1]	
Bone scan	X			X[1]	X[1]	X[1]	
Echocardiogram	X					X[1]	
Labs							
CBC w/ diff. [2]	X	X	X	X	X	X	
COMP, Mg, Phos, LDH	X	X		X[3]	X	X	
Tumor markers (CEA, CA15-3)	X	X		X	X	X	
Ceruloplasmin (Cp)	X	X	X	X	X	X	
Serum B12	X				X[4]	X[4]	
Correlatives							
BM derived Progenitors by Flow Cytometry	x	x		x	x	x	x
ctDNA	x	x		x	x	x	x
Bank blood specimen for future use (LOXL2)	x	x		x	x	x	x
primary breast tumor block/unstained slides	x						

[1]If chest x-ray is negative, it is not necessary for the patient to have a CT scan. If the neurological exam is normal, it is not necessary for the patient to have a brain CT scan. Imaging will be done once a year for patients who are on study ≥ 5 years. For patients who are <5 years, imaging should be done every 6 months± 2 months.

[2]During induction will have CBC checked every 2 weeks until maintenance dose established. Early maintenance is defined as the 4 week window of a stable TM dose after the induction phase. During this early maintenance phase, check CBC every 2 weeks for one month to establish stable dose of TM. When this is established, then check a CBC once every 4 weeks for the duration of the study. If there is a hematologic toxicity, check CBC every two days until it resolves to at least grade 1.

[3] Serum chemistries and hepatic profile will be checked every 3 cycles for a total of 4 times a year. Mg, Phosphorus, LDH may be checked at every cycle at the discretion of the physician.

[4]Will be done at baseline, every 12 months, and if patient comes off study (or at disease progression).

[5]On a stable TM dose. If patient is not on a stable TM dose at any time during extension #1-5, all study procedures will be completed every month.

10.1 Patient monitoring

10.1.1 Induction phase:

Monitor Cp and CBC every 2 weeks until Cp≤ 19 mg/dL, at which point the patient enters the maintenance phase

10.1.2 Maintenance phase:

Monitor every two weeks for the first four weeks and subsequently repeat every 4 weeks for the duration of the primary study, every 8 weeks for extension #1, and every 12 weeks for extension #2-5, or more frequently per physician's discretion.

- Patients should be examined
- Imaging studies should be performed every 6 months during the primary study and extension #2 and every 6 months to a year with extension #2 on, at the physician's discretion, and should include a CT scan of the chest, abdomen and pelvis and a bone scan or a PET/CT scan.
- Blood tests should include CBC, and tumor markers (including CA 15-3 and CEA), and Cp
- Chemistries and hepatic profile, Mg, Phos, and LDH will be performed every 3 cycles for a total of 4 times a year.
- B12 will be measured by methylmalonic acid during the timepoints noted in the study calendar above.
- An Echocardiogram will be performed prior to and at the completion of extension studies #4 and 5

10.1.3 Follow-up phase

Should a patient discontinue TM for any reason specified in Section 9.8, the patient will be followed for a period of ten years. During this follow-up period, we will document survival status and research samples will be collected once a year concurrently with standard of care blood draws at the discretion of the treating investigator. All clinic visits during the follow-up period will be at the discretion of the treating investigator.

Evaluating response to therapy:

Since TM is hypothesized to modulate copper dependent processes important in the process of metastases, we will collect plasma and serum for analysis at a later date. Specific analysis are as yet undetermined.

11.0 TOXICITIES/SIDE EFFECTS

The descriptions and grading scales found in the revised NCI Common Toxicity Criteria (CTC) version 3.0 will be utilized for adverse event reporting. All appropriate treatment areas should have access to a copy of the CTC version 3.0. A list of **expected** adverse events specific for TM is found below (Section 11.2).

11.1 Adverse Events

Adverse events include adverse drug reactions, any unfavorable sign or symptom, illnesses with onset during the study, or exacerbations of preexisting illnesses or conditions. Progression of the patient's malignancy should not be considered an adverse event, unless, in the investigator's opinion, study treatment resulted in an exacerbation of the patient's condition. Exceptions are if disease progression results in death or hospitalization while on study or within 30 days of the last dose. If either occurs, progressive disease will be considered a serious adverse event (see Section 17.2) and will also be documented on the adverse event case report form. All observed

or volunteered adverse events, regardless of treatment group or suspected causal relationship to study drug, will be recorded on the adverse event case report form.

Clinically significant changes in physical examination findings and abnormal objective test findings (i.e. laboratory, x-ray, ECG) should also be recorded as adverse events. The criteria for determining whether an abnormal objective test finding should be reported as an adverse event are as follows:

- Associated with accompanying symptoms,
- Requires additional diagnostic testing or medical/surgical intervention,
- Leads to a change in study dosing or discontinuation from the study,
- Requires additional concomitant drug treatment or other therapy, or
- Considered clinically significant by the investigator or sponsor.

11.2 Agent-Specific Expected Adverse Events List

The following lists the expected adverse events:

- Fatigue
- Anemia
- Neutropenia

12.0 CRITERIA FOR THERAPEUTIC RESPONSE/OUTCOME ASSESSMENT

Recurrence and progression will be evaluated in this study using the new international criteria proposed by the Response Evaluation Criteria in Solid Tumors (RECIST) Committee.

12.1 Progression-Free Survival

Time to progression (TTP) will be assessed using RECIST guidelines if objective progression is measurable and can be documented by radiologic or physical exam.

13.0 CRITERIA FOR REMOVAL FROM STUDY

Patients will be removed from study due to:

- Progression of disease and/or death
- Severe or unexpected toxicities/side effects
- Non-compliance with the defined treatment plan
- Subject's right to withdraw consent for continued participation.

14.0 BIOSTATISTICS

14.1 Study Design/Endpoints

Endpoints: The following outcomes will be recorded:
The primary endpoint is the effect of TM on: (a) VEGR1+ and VEGFR2+ bone marrow derived progenitor cells and serum markers of reflective of copper dependent processes (CTP) in the tumor microenvironment measured at baseline and over time. Secondary endpoints are (a) incidence of toxicity due to TM; and (b) relapse of disease (TTP) (c) polymorphisms of the Ceruloplasmin genes (d) genetic sequencing of tumor and circulating tumor DNA

14.1.1

Descriptive statistics for demographic and CTP variables were calculated for all patients on study. The intent-to-treat (ITT) population consists of all patientspatients who receive at least two doses of TM. Incidence of adverse events and their associated 95% confidence

intervals were estimated using standard methods for proportions. The following outcomes are recorded: toxicity attributable to TM, time to progression of disease (TTP), number of circulating hemangiogenic progenitor cells, serum markers of angiogenesis. Comparisons of continuous variables are made using paired test if data are skewed or Wilcoxon rank test otherwise. Incidence of adverse events (appropriately aggregated according to Theradex, MedDRA or COSTART criteria) and their associated 95% confidence intervals are estimated using standard methods for proportions. TTP is analyzed using survival analysis techniques. The mixed model approach to repeated measures analysis of variance (RMANOVA) will be carried out to determine significant changes over time in the serum markers of the microenvironment (LOX, MMPs and other markers of interest that emerge from pre- clinical studies) . RFS was evaluated using survival analysis techniques. Baseline Cp and EPC values were compared to subsequent time points by the Wilcoxon signed-rank or Mann-Whitney, as appropriate. To assess the association between Cp and EPC over time, three independent mixed effects linear models with subject as a random effect were used to account for the correlation between observations on the same subject. A sample size of 35 achieves a 90% power to detect a difference of 0.5 between EPC/ml at baseline and at last time point, with an estimated standard deviation of 1.1 and two-sided alpha level of 0.05. All analyses were performed in R: A Language and Environment for Statistical Computing, R Development Core Team, Vienna, Austria, 2011.

14.2 Sample Size/Accrual Rate

Sample size: The sample size will change from 50 patients to a sample size of 80 patients which then achieves an 80% power to detect a difference of 0.2 between EPC at baseline and EPC Last dose with an estimated standard deviation of 0.5 and with a significance level (alpha) of 0.10 using a two-sided Wilcoxon test. Accrual was completed at 75 patients because patients were on study for much longer than was originally planned and we thought we would get all the information needed without accruing all 80 patients. There was also a concern about having sufficient funding to have 5 additional patients on study since the patients were on continued extension studies.

For extensions study #1: We expect 30 patients to continue treatment and enroll on extension study #1. For this group, incidence rates of adverse events can be estimated with a precision of at worst, ±18% using a 95% confidence interval. Using a paired t-test approach to approximate statistical power for a within subjects analysis (i.e., detecting differences between two time points) there will be 80% power for detecting an effect size of 0.50 using a two-tailed paired t-test with alpha=0.05

For extension study #2: We expect 15 patients to continue treatment and enroll on the extension study #2. For this group, incidence rates of adverse events can be estimated with a precision of at worst, ±25% using a 95% confidence interval. Using a paired t-test approach to approximate statistical power for a within subjects analysis (i.e., detecting differences between two time points) there will be 80% power for detecting an effect size of 0.75 using a two-tailed paired t-test with alpha=0.05.

For extension study #3: We expect 15 patients to continue treatment and enroll on the extension study #3. For this group, incidence rates of adverse events can be estimated with a precision of at worst, ±25% using a 95% confidence interval. Using a paired t-test approach to approximate statistical power for a within subjects analysis (i.e., detecting differences between two time points) there will be 80% power for detecting an effect size of 0.75 using a two-tailed paired t-test with alpha=0.05.

For extension study #4: We expect 15 patients to continue treatment and enroll on the extension study #4. For this group, incidence rates of adverse events can be estimated with a precision of at worst, ±25% using a 95% confidence interval. Using a paired t-test approach to

approximate statistical power for a within subjects analysis (i.e., detecting differences between two time points) there will be 80% power for detecting an effect size of 0.75 using a two-tailed paired t-test with alpha=0.05.

For extension study #5: We expect 15 patients to continue treatment and enroll on the extension study #5. For this group, incidence rates of adverse events can be estimated with a precision of at worst, ±25% using a 95% confidence interval. Using a paired t-test approach to approximate statistical power for a within subjects analysis (i.e., detecting differences between two time points) there will be 80% power for detecting an effect size of 0.75 using a two-tailed paired t-test with alpha=0.05.

14.3 Analysis of Secondary Endpoints

In order to determine whether specific polymorphisms correlate with reduction (or a trend toward reduction) in a given marker of angiogenesis, we will apply the RMANOVA model (see Aim 2) with the addition of indicator functions at baseline for the given polymorphism in that patient.

If feasible, time-to-relapse will be analyzed using survival analysis techniques however this will not be compared to any other population.

14.4 Proposed next study:

This study will serve as the basis for a future, randomized, placebo controlled phase 2 trial of TM/WTX101 for 3 years in patientspatients with node positive TNBC who have completed standard adjuvant therapy or neo-adjuvant therapy with residual disease.

15.0 RESEARCH PARTICIPANT REGISTRATION AND RANDOMIZATION PROCEDURES

15.1 Research Participant Registration

Consenting Professional(s)/Department:
Confirm eligibility as defined in the section entitled Inclusion/Exclusion Criteria. Obtain informed consent, by following procedures defined in section entitled Informed Consent Procedures.

All patients who enrolled to this study previously at Weill Cornell will be eligible for enrollment at Memoral Sloan Kettering Cancer Center. A reassessment of eligibility will not be required for enrollment at MSKCC as there are no changes in eligibility and these patients are currently on study. There will be no eligibility checklist completed for patients who enroll onto this study. Patients will be fully informed of the transfer and fully consented to this protocol. The discussion will be noted in the patient's electronic medical record.

15.2 Randomization

N/A

16.0 DATA MANAGEMENT ISSUES

A study coordinator will be assigned to the study. The responsibilities of the study coordinator include project compliance, data collection, abstraction and entry, data reporting, regulatory monitoring, problem resolution and prioritization and coordinate the activities of the protocol study team.

The data collected for this study will be entered into a secured database (CAISIS) at Memorial Sloan Kettering Cancer Center.

16.1 Quality Assurance

Routine data quality reports will be generated to assess missing data and inconsistencies. Accrual rates and extent and accuracy of evaluations and follow-up will be monitored periodically throughout the study and potential problems will be brought to the attention of the study team for discussion.

Random-Sample data quality and protocol compliance audits may be conducted by the study team, at a minimum of once a year, more frequently if indicated.

16.2 Data and Safety Monitoring

The Data and Safety Monitoring Plan utilized for this study must align with the MSK DSM Plan, where applicable.

The Data and Safety Monitoring (DSM) Plans at Memorial Sloan Kettering were approved by the National Cancer Institute in August 2018. The plans address the new policies set forth by the NCI in the document entitled "Policy of the National Cancer Institute for Data and Safety Monitoring of Clinical Trials."

There are several different mechanisms by which clinical studies are monitored for data, safety and quality. At a departmental/PI level there exists procedures for quality control by the research team(s). Institutional processes in place for quality assurance include protocol monitoring, compliance and data verification audits, staff education on clinical research QA and two institutional committees that are responsible for monitoring the activities of our clinical trials programs. The committees: *Data and Safety Monitoring Committee (DSMC)* for Phase I and II clinical trials, and the *Data and Safety Monitoring Board (DSMB)* for Phase III clinical trials, report to the Deputy Physician-in-Chief, Clinical Research.

During the protocol development and review process, each protocol will be assessed for its level of risk and degree of monitoring required.

The MSK DSMB monitors phase III trials and the DSMC monitors non-phase III trials. The DSMB/C have oversight over the following trials:

- MSK Investigator Initiated Trials (IITs; MSK as sponsor)
- External studies where MSK is the data coordinating center
- Low risk studies identified as requiring DSMB/C review

The DSMC will initiate review following the enrollment of the first participant/or by the end of the year one if no accruals and will continue for the study lifecycle until there are no participants under active therapy and the protocol has closed to accrual. The DSMB will initiate review once the protocol is open to accrual.

16.3 Regulatory Documentation

Prior to implementing this protocol at MSK, the protocol, informed consent form, HIPAA authorization and any other information pertaining to participants must be approved by the

MSK Institutional Review Board/Privacy Board (IRB/PB). There will be one protocol document and each participating site will utilize that document.

Participating sites that are conducting specimen analysis must submit the following documents to MSK before specimens can be shipped to the site:
Participating Site 1572
Conflict of Interest forms for Participating Site Investigators on the 1572

Participating sites that are conducting specimen analysis should submit this protocol to their IRB according to local guidelines. Copies of any site IRB correspondence should be forwarded to MSK.

17.0 PROTECTION OF HUMAN SUBJECTS

Prior to the enrollment of each patient, the risks, benefits and objectives of the study will be reviewed with the participant, including a discussion of the possible side effects. Patients reserve the right to withdraw participation from this study at any time. Patients will be responsible for all standard exams, tests or procedures related to their care while on this study.

Every effort will be made to keep study records private. Neither the patient's name nor anything else that could identify the patient will be used in any reports or publications that result from this study. Trained staff at Memorial Hospital and the Institutional Review Board at Memorial Hospital may review medical records if necessary. The patient may terminate participation in the study at any time during the trial.

17.1 Privacy

MSK's Privacy Office may allow the use and disclosure of protected health information pursuant to a completed and signed Research Authorization form. The use and disclosure of protected health information will be limited to the individuals/entities described in the Research Authorization form. A Research Authorization form must be approved by the IRB and Privacy Board (IRB/PB).

The consent indicates that individualized de identified information collected for the purposes of this study may be shared with other qualified researchers. Only researchers who have received approval from MSK will be allowed to access this information which will not include protected health information, such as the participant's name, except for dates. It is also stated in the Research Authorization that their research data may be shared with others at the time of study publication.

17.2 Serious Adverse Event (SAE) Reporting

An adverse event is considered serious if it results in ANY of the following outcomes:

- Death
- A life-threatening adverse event
- An adverse event that results in inpatient hospitalization or prolongation of existing hospitalization
- A persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions

- A congenital anomaly/birth defect
- Important Medical Events (IME) that may not result in death, be life threatening, or require hospitalization may be considered serious when, based upon medical judgment, they may jeopardize the patient or participant and may require medical or surgical intervention to prevent one of the outcomes listed in this definition

Note: Hospital admission for a planned procedure/disease treatment is not considered an SAE.

SAE reporting is required as soon as the participant starts investigational treatment/intervention. SAE reporting is required for 30-days after the participant's last investigational treatment/intervention. Any event that occur after the 30-day period that is unexpected and at least possibly related to protocol treatment must be reported.

Please note: Any SAE that occurs prior to the start of investigational treatment/intervention and is related to a screening test or procedure (i.e., a screening biopsy) must be reported.

All SAEs must be submitted in PIMS. If an SAE requires submission to the HRPP office per IRB SOP RR-408 'Reporting of Serious Adverse Events', the SAE report must be submitted within 5 calendar days of the event. All other SAEs must be submitted within 30 calendar days of the event.

The report should contain the following information:

- The date the adverse event occurred
- The adverse event
- The grade of the event
- Relationship of the adverse event to the treatment(s)
- If the AE was expected
- Detailed text that includes the following
    - An explanation of how the AE was handled
    - A description of the participant's condition
    - Indication if the participant remains on the study
- If an amendment will need to be made to the protocol and/or consent form
- If the SAE is an Unanticipated Problem

For IND/IDE protocols: The SAE report should be completed as per above instructions. If appropriate, the report will be forwarded to the FDA by the IND Office

17.2.1

N/A

18.0 INFORMED CONSENT PROCEDURES

Before protocol-specified procedures are carried out, consenting professionals will explain full details of the protocol and study procedures as well as the risks involved to participants prior to their inclusion in the study. Participants will also be informed that they are free to withdraw from the study at any time. All participants must sign an IRB/PB-approved consent form indicating their consent to participate. This consent form meets the requirements of the Code of Federal Regulations and the Institutional Review Board/Privacy Board of this Center. The consent form will include the following:

1. The nature and objectives, potential risks and benefits of the intended study.
2. The length of study and the likely follow-up required.
3. Alternatives to the proposed study. (This will include available standard and investigational therapies. In addition, patients will be offered an option of supportive care for therapeutic studies.)
4. The name of the investigator(s) responsible for the protocol.
5. The right of the participant to accept or refuse study interventions/interactions and to withdraw from participation at any time.

Before any protocol-specific procedures can be carried out, the consenting professional will fully explain the aspects of patient privacy concerning research specific information. In addition to signing the IRB Informed Consent, all patients must agree to the Research Authorization component of the informed consent form.

Each participant and consenting professional will sign the consent form. The participant must receive a copy of the signed informed consent form.

Participants who are unable to come on site to consent due to extenuating circumstances (i.e. lengthy distance from home to study site location) will be able to consent to the protocol via a mailed/emailed consent process. A copy of the consent form must be mailed/faxed/emailed to the participant. The consenting professional must conduct the consent discussion over the phone and have the participant sign and date the consent and returned within 30 days. Once the consent is received back from the participant, the consenting professional will sign and date. The consenting professional must mail a copy back to the participant for his/her records. This consenting process must be documented in the participant's electronic medical record.

19.0 REFERENCES

1. Jemal, A., et al., *Annual Report to the Nation on the Status of Cancer, 1975-2014, Featuring Survival.* J Natl Cancer Inst, 2017. **109**(9).
2. Clarke, C.A., et al., *Age-specific incidence of breast cancer subtypes: understanding the black-white crossover.* J Natl Cancer Inst, 2012. **104**(14): p. 1094-101.
3. Siegel, R.L., K.D. Miller, and A. Jemal, *Cancer statistics, 2015.* CA Cancer J Clin, 2015. **65**(1): p. 5-29.
4. Cardoso, F., et al., *3rd ESO-ESMO international consensus guidelines for Advanced Breast Cancer (ABC 3).* Breast, 2017. **31**: p. 244-259.
5. Dent, R., et al., *Time to disease recurrence in basal-type breast cancers: effects of tumor size and lymph node status.* Cancer, 2009. **115**(21): p. 4917-23.
6. Dent, R., et al., *Pattern of metastatic spread in triple-negative breast cancer.* Breast Cancer Res Treat, 2009. **115**(2): p. 423-8.
7. Dent, R., et al., *Triple-negative breast cancer: clinical features and patterns of recurrence.* Clin Cancer Res, 2007. **13**(15 Pt 1): p. 4429-34.
8. Dent, R.A., et al. *Basal-like phenotype of breast cancer predicts for poor prognosis*. in *San Antonio Breast Cancer Symposium*. 2006. San Antonio, Texas.
9. Hernandez-Aya, L.F., et al., *Nodal status and clinical outcomes in a large cohort of patients with triple-negative breast cancer.* J Clin Oncol, 2011. **29**(19): p. 2628-34.
10. Liedtke, C., et al., *The prognostic impact of age in patients with triple-negative breast cancer.* Breast Cancer Res Treat, 2013. **138**(2): p. 591-9.
11. Pal, S.K., B.H. Childs, and M. Pegram, *Triple negative breast cancer: unmet medical needs.* Breast Cancer Res Treat, 2011. **125**(3): p. 627-36.

12. Sikov, W.M., et al., *Impact of the Addition of Carboplatin and/or Bevacizumab to Neoadjuvant Once-per-Week Paclitaxel Followed by Dose-Dense Doxorubicin and Cyclophosphamide on Pathologic Complete Response Rates in Stage II to III Triple-Negative Breast Cancer: CALGB 40603 (Alliance).* J Clin Oncol, 2015. **33**(1): p. 13-21.
13. von Minckwitz, G., et al., *Neoadjuvant carboplatin in patients with triple-negative and HER2-positive early breast cancer (GeparSixto; GBG 66): a randomised phase 2 trial.* Lancet Oncol, 2014. **15**(7): p. 747-56.
14. Toi, M., et al., *A phase III trial of adjuvant capecitabine in breast cancer patients with HER2-negative pathologic residual invasive disease after neoadjuvant chemotherapy (CREATE-X, JBCRG-04) - Abstract S1-07.* Presented at the Thirty-Eighth Annual CTRC-AACR San Antonio Breast Cancer Symposium, 2015.
15. Park, N.J., et al., *Cardiovascular disease and mortality after breast cancer in postmenopausal women: Results from the Women's Health Initiative.* PLoS One, 2017. **12**(9): p. e0184174.
16. Gao, D. and V. Mittal, *The role of bone-marrow-derived cells in tumor growth, metastasis initiation and progression.* Trends Mol Med, 2009. **15**(8): p. 333-43.
17. Joyce, J.A. and J.W. Pollard, *Microenvironmental regulation of metastasis.* Nat Rev Cancer, 2009. **9**(4): p. 239-52.
18. Kessenbrock, K., V. Plaks, and Z. Werb, *Matrix metalloproteinases: regulators of the tumor microenvironment.* Cell, 2010. **141**(1): p. 52-67.
19. Pitteri, S., et al., *Tumor microenvironment-derived proteins dominate the plasma proteome response during breast cancer induction and progression.* Cancer Res, 2011. **71**: p. 5090-5100.
20. Barry-Hamilton, V., et al., *Allosteric inhibition of lysyl oxidase-like-2 impedes the development of a pathologic microenvironment.* Nat Med, 2010. **16**(9): p. 1009-17.
21. Reiss, K.A., P.M. Forde, and J.R. Brahmer, *Harnessing the power of the immune system via blockade of PD-1 and PD-L1: a promising new anticancer strategy.* Immunotherapy, 2014. **6**(4): p. 459-475.
22. Scherz-Shouval, R., et al., *The Reprogramming of Tumor Stroma by HSF1 Is a Potent Enabler of Malignancy.* Cell, 2014. **158**(3): p. 564-578.
23. Whipple, C.A., *Tumor talk: understanding the conversation between the tumor and its microenvironment.* Cancer Cell Microenviron, 2015. **2**(2): p. e773.
24. Gao, D., et al., *Endothelial progenitor cells control the angiogenic switch in mouse lung metastasis.* Science, 2008. **319**(5860): p. 195-8.
25. Kaplan, R.N., et al., *VEGFR1-positive haematopoietic bone marrow progenitors initiate the pre-metastatic niche.* Nature, 2005. **438**(7069): p. 820-7.
26. Lyden, D., et al., *Impaired recruitment of bone marrow derived endothelial and a hematopoietic precursor cells blocks tumor angiogenesis and growth.* Nature Medicine, 2001. **7**: p. 1194-1201.
27. Gao, D., et al., *Myeloid progenitor cells in the premetastatic lung promote metastases by inducing mesenchymal to epithelial transition.* Cancer Res, 2012. **72**(6): p. 1384-94.
28. Jain, S., et al., *Incremental increase in VEGFR1(+) hematopoietic progenitor cells and VEGFR2(+) endothelial progenitor cells predicts relapse and lack of tumor response in breast cancer patients.* Breast Cancer Res Treat, 2012. **132**(1): p. 235-42.
29. Allensworth, J.L., et al., *Disulfiram (DSF) acts as a copper ionophore to induce copper-dependent oxidative stress and mediate anti-tumor efficacy in inflammatory breast cancer.* Molecular Oncology, 2015. **9**(6): p. 1155-1168.
30. Festa, R.A. and D.J. Thiele, *Copper: An essential metal in biology.* Current Biology, 2011. **21**(21): p. R877-R883.
31. Safi, R., et al., *Copper Signaling Axis as a Target for Prostate Cancer Therapeutics.* Cancer Research, 2014. **74**(20): p. 5819-5831.
32. Tsai, C.Y., et al., *Copper influx transporter 1 is required for FGF, PDGF and EGF-induced MAPK signaling.* Biochemical Pharmacology, 2012. **84**(8): p. 1007-1013.
33. Turski, M.L., et al., *A Novel Role for Copper in Ras/Mitogen-Activated Protein Kinase Signaling.* Molecular and Cellular Biology, 2012. **32**(7): p. 1284-1295.
34. Turski, M.L. and D.J. Thiele, *New Roles for Copper Metabolism in Cell Proliferation, Signaling,*

and Disease. Journal of Biological Chemistry, 2009. **284**(2): p. 717-721.

35. Brewer, G.J., et al., *Treatment of metastatic cancer with tetrathiomolybdate, an anticopper, antiangiogenic agent: Phase I study.* Clin Cancer Res, 2000. **6**(1): p. 1-10.
36. Finney, L., et al., *Copper and angiogenesis: unravelling a relationship key to cancer progression.* Clin Exp Pharmacol Physiol, 2009. **36**(1): p. 88-94.
37. Juarez, J.C., et al., *Copper binding by tetrathiomolybdate attenuates angiogenesis and tumor cell proliferation through the inhibition of superoxide dismutase 1.* Clin Cancer Res, 2006. **12**(16): p. 4974-82.
38. Pan, Q., et al., *Copper deficiency induced by tetrathiomolybdate suppresses tumor growth and angiogenesis.* Cancer Res, 2002. **62**(17): p. 4854-9.
39. Ishida, S., et al., *Bioavailable copper modulates oxidative phosphorylation and growth of tumors.* Proc Natl Acad Sci U S A, 2013. **110**(48): p. 19507-12.
40. Moreno-Bueno, G., et al., *Lysyl oxidase-like 2 (LOXL2), a new regulator of cell polarity required for metastatic dissemination of basal-like breast carcinomas.* EMBO Mol Med, 2011. **3**(9): p. 528-44.
41. Finney, L., et al., *X-ray fluorescence microscopy reveals large-scale relocalization and extracellular translocation of cellular copper during angiogenesis.* Proc Natl Acad Sci U S A, 2007. **104**(7): p. 2247-52.
42. Barker, H.E., et al., *Tumor-secreted LOXL2 activates fibroblasts through FAK signaling.* Mol Cancer Res, 2013. **11**(11): p. 1425-36.
43. Mayorca-Guiliani, A. and J.T. Erler, *The potential for targeting extracellular LOX proteins in human malignancy.* Onco Targets Ther, 2013. **6**: p. 1729-35.
44. Lowndes, S.A. and A.L. Harris, *Copper chelation as an antiangiogenic therapy.* Oncol Res, 2004. **14**(11-12): p. 529-39.
45. Marttila-Ichihara, F., et al., *Vascular adhesion protein-1 enhances tumor growth by supporting recruitment of Gr-1+CD11b+ myeloid cells into tumors.* Cancer Res, 2009. **69**(19): p. 7875-83.
46. Erler, J.T., et al., *Lysyl oxidase is essential for hypoxia-induced metastasis.* Nature, 2006. **440**(7088): p. 1222-6.
47. Cox, T.R., et al., *The hypoxic cancer secretome induces pre-metastatic bone lesions through lysyl oxidase.* Nature, 2015. **522**(7554): p. 106-U279.
48. Brady, D.C., et al., *Copper is required for oncogenic BRAF signalling and tumorigenesis.* Nature, 2014. **509**(7501): p. 492-6.
49. Badet, J., et al., *Specific binding of angiogenin to calf pulmonary artery endothelial cells.* Proc Natl Acad Sci U S A, 1989. **86**(21): p. 8427-31.
50. Brem, S., et al., *Inhibition of angiogenesis and tumor growth in the brain: Suppression of endothelial cell turnover by penicillamine and depletion of copper, an angiogenic cofactor.* Am J Pathol, 1990. **137**: p. 1121-1142.
51. Hassouneh, B., et al., *Tetrathiomolybdate promotes tumor necrosis and prevents distant metastases by suppressing angiogenesis in head and neck cancer.* Mol Cancer Ther, 2007. **6**(3): p. 1039-45.
52. Chatterjee, S., et al., *A Novel Copper Chelate Modulates Tumor Associated Macrophages to Promote Anti-Tumor Response of T Cells.* Plos One, 2009. **4**(9).
53. Payne, S.L., et al., *Lysyl oxidase regulates breast cancer cell migration and adhesion through a hydrogen peroxide-mediated mechanism.* Cancer Research, 2005. **65**(24): p. 11429-11436.
54. Erler, J.T., et al., *Hypoxia-induced lysyl oxidase is a critical mediator of bone marrow cell recruitment to form the premetastatic niche.* Cancer Cell, 2009. **15**(1): p. 35-44.
55. Erler, J.T. and V.M. Weaver, *Three-dimensional context regulation of metastasis.* Clin Exp Metastasis, 2009. **26**(1): p. 35-49.
56. Barker, H.E., et al., *LOXL2-Mediated Matrix Remodeling in Metastasis and Mammary Gland Involution.* Cancer Res, 2011. **71**(5): p. 1561-72.
57. Lopez, K.M. and F.T. Greenaway, *Identification of the copper-binding ligands of lysyl oxidase.* J Neural Transm, 2010.
58. Gao, D., et al., *Bone marrow-derived endothelial progenitor cells contribute to the angiogenic switch in tumor growth and metastatic progression.* Biochim Biophys Acta, 2009. **1796**(1): p. 33-40.

59. Brewer, G.J., et al., *Treatment of Wilson disease with ammonium tetrathiomolybdate. II. Initial therapy in 33 neurologically affected patients and follow-up with zinc therapy.* Arch Neurol, 1996. **53**(10): p. 1017-25.
60. Brewer, G.J., et al., *Treatment of Wilson disease with ammonium tetrathiomolybdate: IV. Comparison of tetrathiomolybdate and trientine in a double-blind study of treatment of the neurologic presentation of Wilson disease.* Arch Neurol, 2006. **63**(4): p. 521-7.
61. Lin, J., et al., *A non-comparative randomized phase II study of 2 doses of ATN-224, a copper/zinc superoxide dismutase inhibitor, in patients with biochemically recurrent hormone-naive prostate cancer.* Urol Oncol, 2011.
62. Pass, H.I., et al., *A phase II trial of tetrathiomolybdate after surgery for malignant mesothelioma: final results.* Ann Thorac Surg, 2008. **86**(2): p. 383-9; discussion 390.
63. Redman, B.G., et al., *Phase II trial of tetrathiomolybdate in patients with advanced kidney cancer.* Clin Cancer Res, 2003. **9**(5): p. 1666-72.
64. Alvarez, H.M., et al., *Tetrathiomolybdate inhibits copper trafficking proteins through metal cluster formation.* Science, 2010. **327**(5963): p. 331-4.
65. Lowndes, S.A., et al., *Phase I study of copper-binding agent ATN-224 in patients with advanced solid tumors.* Clin Cancer Res, 2008. **14**(22): p. 7526-34.
66. Lin, J., et al., *A non-comparative randomized phase II study of 2 doses of ATN-224, a copper/zinc superoxide dismutase inhibitor, in patients with biochemically recurrent hormone-naive prostate cancer.* Urol Oncol, 2013. **31**(5): p. 581-8.
67. Chan, N., et al., *Influencing the tumor microenvironment: A phase II study of copper-depletion using tetrathiomolybdate (TM) in patients with breast cancer at high risk for recurrence and in preclinical models of lung metastases.* Clinical Cancer Research, 2016. **Under Revision.**
68. Chan, N., et al., *Influencing the Tumor Microenvironment: A Phase II Study of Copper Depletion Using Tetrathiomolybdate in Patients with Breast Cancer at High Risk for Recurrence and in Preclinical Models of Lung Metastases.* Clin Cancer Res, 2017. **23**(3): p. 666-676.
69. Kehlet, S.N., et al., *Excessive collagen turnover products are released during colorectal cancer progression and elevated in serum from metastatic colorectal cancer patients.* Sci Rep, 2016. **6**: p. 30599.
70. Blockhuys, S. and P. Wittung-Stafshede, *Copper chaperone Atox1 plays role in breast cancer cell migration.* Biochem Biophys Res Commun, 2017. **483**(1): p. 301-304.
71. Wolff, A.C., et al., *Recommendations for Human Epidermal Growth Factor Receptor 2 Testing in Breast Cancer: American Society of Clinical Oncology/College of American Pathologists Clinical Practice Guideline Update.* Journal of Clinical Oncology, 2013. **31**(31): p. 3997-4013.
72. Denkert, C., et al., *Tumor-Infiltrating Lymphocytes and Response to Neoadjuvant Chemotherapy With or Without Carboplatin in Human Epidermal Growth Factor Receptor 2-Positive and Triple-Negative Primary Breast Cancers.* Journal of Clinical Oncology, 2015. **33**(9): p. 983-+.

20.0 APPENDICES

APPENDIX A: Performance Status Criteria

ECOG Performance Status Scale		**Karnofsky Performance Scale**	
Grade	Descriptions	Percent	Description
0	Normal activity. Fully active, able to carry on all pre-disease performance without restriction.	100	Normal, no complaints, no evidence of disease.
		90	Able to carry on normal activity; minor signs or symptoms of disease.
1	Symptoms, but ambulatory. Restricted in physically strenuous activity, but ambulatory and able to carry out work of a light or sedentary nature (e.g., light housework, office work).	80	Normal activity with effort; some signs or symptoms of disease.
		70	Cares for self, unable to carry on normal activity or to do active work.
2	In bed <50% of the time. Ambulatory and capable of all self-care, but unable to carry out any work activities. Up and about more than 50% of waking hours.	60	Requires occasional assistance, but is able to care for most of his/her needs.
		50	Requires considerable assistance and frequent medical care.
3	In bed >50% of the time. Capable of only limited self-care, confined to bed or chair more than 50% of waking hours.	40	Disabled, requires special care and assistance.
		30	Severely disabled, hospitalization indicated. Death not imminent.
4	100% bedridden. Completely disabled. Cannot carry on any self-care. Totally confined to bed or chair.	20	Very sick, hospitalization indicated. Death not imminent.
		10	Moribund, fatal processes progressing rapidly.
5	Dead.	0	Dead.