Title: Randomized Placebo Controlled Study of Minocycline for Amelioration of Chemotherapy Induced Affective Disorders

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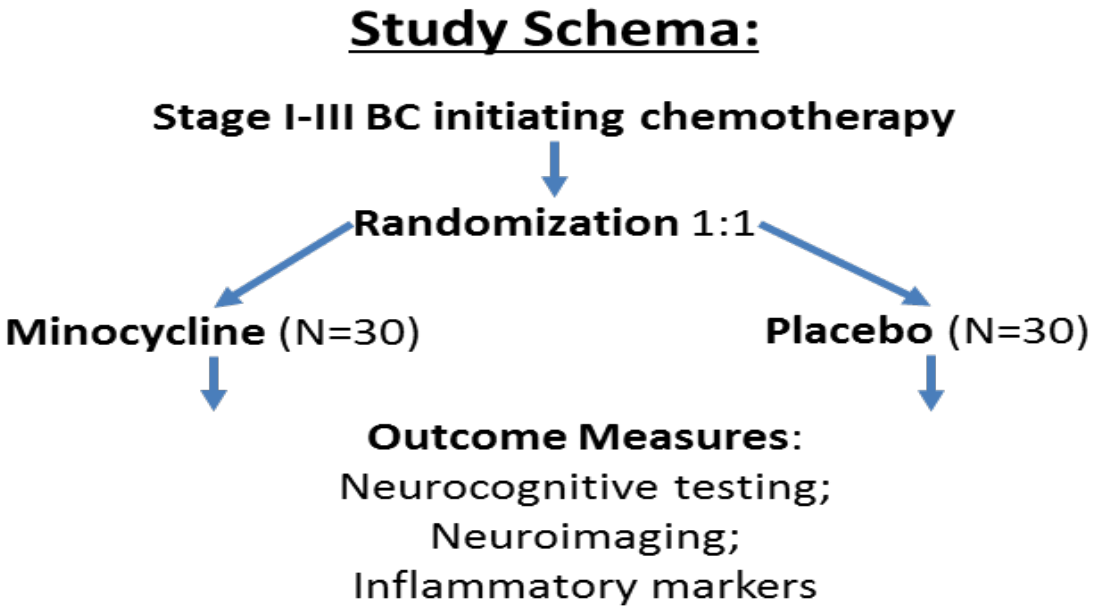
Study Schema



2.0 Introduction

Increased utilization of mammographic screening and adjuvant therapy improves the long-term survival of women with breast cancer (BC), who comprise the largest group of cancer survivors in the United States. The incidence of depression and anxiety is approximately 3-5 times higher among BC patients than healthy women in the general population (20-30% versus 6%, respectively), [1] and is present in a greater proportion of BC survivors who receive chemotherapy than those who do not.[2] Furthermore, depression can persist for years following treatment[3,4] and is associated with reduced survival following treatment.[5-8] Therefore, alleviating anxiety and depression among BC survivors is important for quality of life (QOL) as well as long-term health outcomes.

Despite the high incidence of affective disorders among women being treated for BC, very little is known about the etiology of affective disorders that develop during adjuvant chemotherapy. Current strategies for BC patients include anti-depressants and counseling. Compliance with anti-depressants is poor, discontinuations are frequent during the first month of therapy and approximately 25% of patients do not inform their physician about stopping their antidepressant medication.[9,10] This is due to multiple factors such stigma attached to anti-depressant use, side-effects, and lack of efficacy.[11-13] The consequences of affective disorders impact overall health outcomes. For example, non-depressed patients are 3 times as likely to comply with treatment as depressed patients.[14] Furthermore, some antidepressants potentially inhibit cytochrome P450 2D6, required to activate tamoxifen, a commonly prescribed anti-estrogen treatment. [15] [16,17]

Chemotherapy induced cognitive changes

Chemotherapy may adversely affect the brain, leading to deficits in memory, concentration, processing time, verbal fluency, and mental fatigue in a sub-set of patients.[18,19] Indeed, significant alterations in the functional and structural architecture of the brain have also been reported in individuals undergoing chemotherapy, and these changes can persist for up to 10 years post-chemotherapy.[19,20] Specifically, increased age and reduced cognitive reserve have been associated with decreased processing speed among breast cancer patients following chemotherapy [2]. Perceived and real cognitive deficits have broad implications for quality of life (QOL) among survivors[18]; women report that post-chemotherapy cognitive deficits are frustrating and detrimental to their self-confidence, social relationships, and their ability to perform as effectively at work as before treatment. Despite several studies documenting chemotherapy-related cognitive deficits and concerns raised by patients over the impact of perceived cognitive deficits on their QOL, the etiology remains unknown. Likewise, there are no known preventative or therapeutic treatments for chemotherapy's cognitive effects.

Inflammation and Affective Disorders

Causal links between inflammatory mediators and the development of a constellation of behaviors referred to as "sickness behaviors" have been established in animal models. [21] Sickness behaviors include depressive-like behavior, anorexia, allodynia and lethargy. Based on these observations, elevated concentrations of proinflammatory cytokines and chemokines are hypothesized to underlie the development of depression and anxiety among cancer patients.[22,23] Indeed, doxorubicin (DOX) + cyclophosphamide chemotherapy is among the most commonly used and most effective regimens for treating BC. However, it appears to be more pro-inflammatory than some other regimens, which could lead to increased incidence of "sickness behaviors." Specifically, DOX + cyclophosphamide has been shown to cause an increase in interleukin-6 (IL-6), IL-8, monocyte chemotactic protein-1 (MCP-1), tumor necrosis factor

alpha(TNF-α) and its related receptors in women and mice, and IL-1β, and granulocyte colony stimulating factor in mice.[24-26] Furthermore, studies in IL-1 receptor-deficient mice suggest that IL-1β mediates, at least in part, the proinflammatory cascade that develops after DOX treatment,[25] while, peripheral immune-neutralization of TNF-α prevents DOX-induced increases in brain TNF-α in animal models. [26]

DOX also increases several measures of oxidative stress in serum and brains of rodents within days of the first chemotherapy treatment[27-30] [31] DOX also causes an increase in anxiety-like behavior within an hour of the first injection, and that the anxiety-like behavior persists for at least 3 days.[32] Thus, oxidative stress and neuroinflammation are two interrelated processes through which chemotherapy may be affecting the function and survival of neurons.

Inflammation and cognitive changes

Several researchers have proposed that elevated concentrations of proinflammatory cytokines and chemokines underlie the development of the cognitive and affective side-effects of chemotherapy.[22,23,33] Indeed, DOX + cyclophosphamide chemotherapy is among the most commonly used and most effective regimens for treating breast cancer, however, it appears to be more pro-inflammatory than some other regimens, which could increase the incidence of "sickness behaviors", including cognitive deficits; specifically Dox + cyclophosphamide increases interleukin-6 (IL-6) , IL-8, monocyte chemotactic protein-1 (MCP-1), tumor necrosis factor alpha(TNF-α) and its related receptors in women and mice, and IL-1β, and granulocyte colony stimulating factor in mice.[25,34] Furthermore, studies in IL-1 receptor-deficient mice, suggest that IL-1β mediates, at least in part, the proinflammatory cascade that develops after Dox treatment[25], whereas, peripheral immunoneutralization of TNF-α in rats prevents doxorubicin-induced increases in brain TNF-α.[26] Very few rodent studies have examined chemotherapy-induced changes in behavior, although a recent paper reported that anxiety-like behavior is apparent within an hour of Dox injection and persists for at least 3 days.[32] However, despite increased incidence of cognitive deficits among cancer patients undergoing chemotherapy, and numerous hypothesis papers proposing a role of chemotherapy-induced neuroinflammation in the onset of cognitive disorders, no published studies have assessed whether a causal relationship exists between proinflammatory cytokines and cognitive deficits following chemotherapy; the best clinical evidence to date is correlative.

In addition to DOX, we know clinically that other types of chemotherapy including all agents received in the adjuvant and neoadjuvant settings are also associated with affective and cognitive changes. Since this is a preliminary feasibility study, it will be important to include all utilized agents in order for the study results to have broader applicability.

Minocycline Mechanism of Action

Minocycline, a member of second generation tetracycline analogs, is a highly lipophilic oral antibiotic, and currently approved by the US Food and Drug Administration (FDA) for the treatment of susceptible bacterial infections caused by gram-negative, gram-positive organism and atypical organisms. This drug is well-tolerated for long term use for up to 200 mg a day, with most common toxicity being mild gastrointestinal effects. Of most relevance to this proposal, minocycline readily crosses the blood brain barrier to suppress inflammation by inhibiting microglial activation in a variety of CNS disease models including cerebral ischemia, CNS inflammation, traumatic brain injury and spinal cord injury.[35-38] Minocycline and other tetracyclines are routinely administered during chemotherapy as part of standard of care for treatment of

infections. There are no known drug interactions between minocycline, doxorubicin, cyclophosphamide or other supportive care drugs commonly utilized during chemotherapy. Further, minocycline is not an antibiotic that is routinely used to treat active infections during chemotherapy and does not interact with standard antibiotics used. Therefore, it will not matter whether patients are on placebo or minocycline. Both groups will receive standard antibiotics if needed.

Rationale and feasibility

The biologic mechanism underlying development of anxiety and depression during chemotherapy, however has not been previously demonstrated. Preliminary studies by our group in ovariectomized mice indicate a causal relationship between increased microglial activation and anxiety-like and depressive-like behavior following chemotherapy. Microglia are resident immune cells of the brain, which release proinflammatory cytokines, including IL-1β and TNF-α, and reactive oxygen species when they become activated. Doxorubicin chemotherapy induces microglial activation in the brain within several days of the first administration, which is associated with increased depressive-like behavior in mice. Minocycline is well-tolerated and has no adverse pharmacologic interactions with chemotherapy. ***We hypothesize that chemotherapy activates microglia in the brains of women being treated for breast cancer, which in turn can precipitate or exacerbate depression and anxiety and cognitive changes.*** Minocycline is an immunomodulater that attenuates activation of microglia. Administration of minocycline orally during neoadjuvant or adjuvant chemotherapy will prevent chemotherapy-induced microglial activation will reduce measures of depression and anxiety in breast cancer patients.

3.0 Objectives

The objectives of this study are to:

Objective 1- To evaluate symptoms related to anxiety and depression and cognitive changes in women with stages I-III breast cancer during doxorubicin-based or other chemotherapy regimens for breast cancer randomized to receive either minocycline or placebo

Objective 2- To evaluate markers of neuroinflammation as assessed by blood based inflammatory cytokines and C11-choline PET in in women with stages I-III breast cancer during doxorubicin-based or other chemotherapy regimens for breast cancer randomized to receive either minocycline or placebo.

4.0 Study Design

This is a double blinded randomized study of minocycline (100 mg BID) vs placebo BID women with BC receiving DOX-based chemotherapy or other chemotherapy for breast cancer. Eligible participants will be women with BC who will be undergoing treatment at the Stefanie Spielman Comprehensive Breast Center. Informed consent will be obtained from those who meet the study criteria (see inclusion/exclusion criteria) and are interested in participating. Typically, women begin chemotherapy within 8 weeks of breast surgery provided there are no surgical complications. Participation in the study will not affect the dose or the timing of subsequent chemotherapy treatments. Consented participants will be randomized to either oral administration of minocycline or a placebo with matched appearance for up to a 1 week loading period plus chemotherapy treatment period and an optional subsequent two week period.

An additional cohort will be assessed of women with BC who received DOX-based chemotherapy or other chemotherapy for breast cancer with self-reporting chemotherapy brain symptoms while on chemotherapy or within 18 months of completing the chemotherapy. These patients will then be assigned minocycline (100 mg BID) for 6 months. At baseline, 3 month and 6 month intervals they will undergo questionnaires and imaging.

5.0 Eligibility Criteria

Inclusion criteria

- Women diagnosed with breast cancer stages I-III initiating first line adjuvant or neoadjuvant DOX chemotherapy or other chemotherapy for breast cancer
- Age ≥18 years.
- Ability to understand English and read and write at the 8th grade level and give a written informed consent document.
- For additional cohort, women with breast cancer stages I-III who currently on or within 18 months of completing first line adjuvant or neoadjuvant DOX chemotherapy or other chemotherapy for breast cancer.
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Exclusion Criteria

- Rheumatoid arthritis and other types of autoimmune and inflammatory joint disease, with the exception of osteoarthritis and fibromyalgia.
- Concurrent other malignancy or metastatic malignancy of any kind
- Known bleeding disorders.
- Current use of warfarin or other anticoagulants.
- Uncontrolled intercurrent illness including, but not limited to, ongoing or active infection, symptomatic congestive heart failure, unstable angina pectoris, cardiac arrhythmia, or psychiatric illness/social situation that would limit compliance with study requirements.
- Pregnant or nursing women.
- Unable to give informed consent.
- Tetracycline allergy
- Patient is able to opt out of MRI/PET examinations if there are any contraindications to examinations including, but not limited to, refusal to participate, ferromagnetic metal in the body, pacemaker or severe claustrophobia.

6.0 Registration, stratification and data submission

Subjects, who have given informed consent, will be registered by the research coordinator and given a study number. Each subject will be randomized to either minocycline capsules or placebo capsules using a fixed-block 1:1 randomization. A randomization table will be used to define which randomized group each enrolled subject will fall under. Only the pharmaceutical compounding company (SBH Medical, Worthington, Ohio) will be aware of the study group assignment so the appropriate supplement can be dispensed. Size of the randomized block will be known only by the statistician to help preserve blinding by study investigators.

Subjects of additional cohort, who have given informed consent, will be registered by the research coordinator and given a study number. Each subject will receive minocycline capsules.

7.0 Minocycline and Placebo Pharmaceutical Information

The same dose of minocycline that we propose to use is the FDA approved human dose and has been shown to reduce microglial activation in the brain in individuals with movement disorders[39]. Participants will be prescribed oral minocycline twice daily (100 mg BID) or the placebo (one capsule BID) beginning up to 7 days prior to chemotherapy, through four cycles of chemotherapy and optionally continuing through two weeks after their fourth cycle of chemotherapy treatment. Drug can be started on same day as chemotherapy start. The matched placebo is being specifically manufactured and will be indistinguishable from the active capsule (SBH Medical, Worthington, Ohio). No known interactions exist between minocycline and chemotherapy/pre-medications used. Also there are no data to suggest that minocycline is antagonistic with the anti-tumor effects of chemotherapy and it is currently in use with patients undergoing chemotherapy who need minocycline for other indications.[40] Compliance will be monitored by patient report through pill diaries and pill counts. Absorption of minocycline is potentially impaired by laxatives and antacids containing aluminum, calcium, or magnesium, and iron-containing preparations, so patients will be instructed to avoid products that contain these ingredients for 2 hours before and 2 hours after minocycline ingestion. Study drug will be initiated up to one week prior to cycle 1 of chemotherapy and continue for up to two weeks after their fourth cycle of chemotherapy, optionally continuing through two weeks after their fourth cycle. All of the measures assessed by the study objectives will be evaluated by providers and research staff blinded to patient's treatment assignment. Active drug and placebo pills will be dispensed by a staff pharmacist who will be blinded to patient's randomized status. Only the pharmaceutical compounding company (SBH Medical, Worthington, Ohio) will be aware of the study group assignment. Unblinding will take place only in the event of concerns regarding toxicity or an adverse event.

During the study, patients are allowed to follow standard of care recommendations for NSAIDs and steroids as needed for management of chemotherapy induced nausea and pain syndromes from chemotherapy and pegfilgrastrim. The study coordinator will make notes of the use and amounts used of these drugs during each visit. If antibiotics are needed during treatment for treatment of infection, study drug will be held during antibiotic therapy. Patients with > 14 day delay in restart of study drug will be taken off study.

8.0 Evaluation of Affective Symptoms During Chemotherapy

Primary endpoints are changes in Center for Epidemiological Studies Depression Scale (CES-D) and State Trait Anxiety Index (STAI) from baseline to end of study after minocycline vs. placebo intervention (Aim 1). The CES-D and STAI will be administrated serially every cycle start on protocol during clinic visits (Patients will self-administer forms given out by research coordinator). These instruments have been reliably used with BC patients/survivors and are well-characterized. The internal consistency for the STAI is .95; higher scores indicate greater anxiety.[41] The internal consistency for the CES-D is approximately .85 among BC patients,[42] and an important benefit of using this scale in medical studies is that it is relatively unaffected by physical symptoms. Total scores range from 0-60 with higher scores reflecting greater depressive symptoms. Although we expect severe depression and suicidal ideation to be rare in this population[43,44], there is a screening and safety plan. At baseline, depressive symptom severity will be assessed using the CES-D instrument. Dr. Cheavens (Co-I, Clinical Psychologist) and/or social work will be available to evaluate the patient's assessments if suicidal ideation is reported at baseline. The subject will be withdrawn from the study and treatment referrals will be provided. During the trial, an increase on the CES-D above a score of 20, will trigger a risk assessment. Dr. Cheavens and/or social work will be available to help manage patients' symptoms. In the case of significant depressive

severity or suicide risk (as determined by Dr. Cheavens and/or social work), the subject will be referred to psychotropic or psychotherapeutic intervention immediately, and removed from the study.

8.1 Evaluation of cognitive changes during chemotherapy

Secondary endpoints are changes in cognitive function during chemotherapy. The cognitive battery will include standardized neuropsychological tests that have been previously demonstrated in prospective studies to detect neurocognitive changes during breast cancer chemotherapy. (See table on page 9). Testing will be done at baseline, within two weeks after the fourth cycle of chemo, and again 6 months after completion of chemotherapy. The MRI N-Back Test is optional and therefore may not be completed during these time points. These will be administered by the research coordinator assigned to the study as well as graduate student in Dr. Courtney DeVries' Laboratory.

Task	Domain	Time to Complete (min)	Representative Reference
Multifactorial Memory Questionnaire Ability scale	Self-appraisal of memory capabilities		[46,47]
Behavioral Rating Inventory of Executive Function*	Assesses executive function	5-10	[46]
N-Back Test	Working memory task during fMRI	8	[48]
Revised UCLA Loneliness Scale	Loneliness	5	[49]

*Completed during MRI procedure, no patient forms needed

9.0 TREATMENT PLAN

9.1 Overview

After obtaining informed written consent, participants will be randomized to either the intervention of minocycline or placebo. One pill will be taken twice a day with or without food. No premedication is required.

The study will require visits concurrent with chemotherapy visits during study intervention. At each visit, self-report diaries (adverse events, record of study drug doses taken/missed) will be collected, and pill count will be recorded by the research coordinator. Medications and supplements will be reviewed with participants and a history and physical examination will be performed at each visit. Study drugs will be dispensed once at the initiation of study protocol with enough pills for the optional one week prior to chemotherapy start, throughout four cycles of chemotherapy and continuing optionally for an additional two weeks after their fourth cycle. Pill compliance will be conducted with the start of each cycle through pill counts and pill diaries to ensure proper reconciliation and adherence.

If the patient's chemotherapy is held, the patients should continue taking study medication. During this hold, the patient will dose from the optional supply provided to them.

In the additional cohort, after obtaining informed written consent, participants will be assigned minocycline (100 mg BID) for 6 months. Questionnaires and imaging will be assessed at baseline, 3 month and 6 month intervals of minocycline treatment.

9.2 Correlative/special studies

Inflammatory biomarker analysis:

Correlative blood levels for cortisol, high sensitivity c-reactive protein (hs-CRP), inflammatory factors including but not limited to IL-6, TNF-α, IL-1β, and MCP-1 IL-6, TNF-α, IL-1β, and MCP-1 will be obtained every cycle during study period(Aim 2). Cytokine single nucleotide polymorphisms will be checked within the same blood specimens. The primary comparison will be in changes from baseline to the end of treatment. The blood samples (10 ml collected in a plain red top tube with no gel separator) collected will be centrifuged to obtain serum, and then aliquoted and stored at -80^{o}C (completed at SSCBC; see appendix page 25). These aliquots will then be batch analyzed in DeVries' (PI) laboratory for cortisol, high sensitivity c-reactive protein(hs-CRP), IL-6, TNF-α, IL-1β, and MCP-1 using ELISA; cortisol will be measured because it is a hormone often used as an index of stress and high levels are associated with depression.[50,51] Serum C-reactive protein, IL-6, IL-1β, and TNF-α will be measured as indicators of inflammation. Participants will be scheduled for the first appointment of the day to ensure that the blood samples are all collected within a 2 hour window.

Neuro/PET imaging:

Each enrolled patient will have the opportunity to undergo an optional neuro PET imaging to evaluate CNS levels of inflammation. (PET will mandatory for patients in the additional cohort, who are already experiencing chemotherapy brain symptoms). In addition to F^{18}FDG PET, when available, we will utilize PK11195 labeled with ^{11}C for PET imaging studies. Labeling and imaging will take place under the direction of Dr. Michael Knopp (Co-I), Director of the OSU Wright Center for Imaging (see letter of support). PK11195 ligand targets the translocator protein-18 kDa (TSPO) located on the outer mitochondrial membrane of microglia. TSPO expression is increased in neuroinflammation, and has been used as an index of microglial activation in several neuroinflammatory disease states.[52,53]

Neuro MRI imaging:

In addition to neuro PET imaging, patients will also have the option to undergo a neuro MRI imaging. To investigate cognitive changes associated with our intervention, we will use a multi-modal imaging protocol at 7 Tesla. Functional MRI (fMRI; scan time 6 min) will assess group differences in cortical activation during the N-back test. Resting State Functional MRI (rs-fMRI; scan time 7 min), will explore the brain networks. Structural MRI (total scan time 24 min): a) T1 weighted magnetization prepared rapid gradient echo 3D image will be acquired with a voxel resolution of 1mm x 1mm 0.5 x 0.5 x 2 mm3, TR/TE 23/12 ms; this approach allows registration to a standard brain atlas and measurement of white and gray matter volume and percent brain volume change. b) T2 fluid attenuated inversion recovery image will also be acquired. c) Diffusion

weighted imaging will be used to explore the white matter connectivity and changes in its microstructure. The increase in sensitivity at 7 T to the BOLD effect coupled with the increase in image resolution significantly improves the functional sensitivity compared to lower magnetic field imaging.[54] Importantly, the regions of the brain we are examining are minimally affected by 7 T image distortion artifacts. (2 (3) Co-registration of PET and MRI: Images from all modalities will be co-registered to the high resolution T1 weighted MR image for each subject. Also, the T1 images will be co-registered to the MNI template [76] using FSL12 [77] and AFNI tools. fMRI images will be corrected for distortion and then statistical parametric maps will be created. For each task based fMRI, group analysis will be performed to find spatial brain activation differences between the treated and placebo groups where the z-scores are greater than 2.3. For the rs-fMRI studies, group level independent component analysis will be implemented to identify 100 components. Then, functional parcellation of the cortical and subcortical components will be created. For the patient group, functional correlation between different neural networks will examined chronically and compared with that of the placebo group. The total maximum whole body exposure for all three scan time points is 2111 mrem, which is within the acceptable range by human safety IRB standards.

9.3 Clinical assessments

Clinic visits

Participants will be evaluated during routine clinic/chemotherapy encounters approximately every 2-3 weeks (with each cycle of chemotherapy). Blood draws and study questionnaires will be obtained at the same time. Participants will be requested to keep a diary to record of their intake of study pills as well as any signs or symptoms considered related to the study intervention. The diaries will be collected and reviewed during the clinic visits.

9.4 Adherence

Participant adherence with the study intervention will be encouraged and monitored in several ways. Subjects will maintain a daily diary in which to check off the doses of study drug/placebo when taken. At study visits, participants will return their pill containers to study personnel, who will count and record leftover pills.

9.5 Data and records

Primary source documents will include forms routinely used at the Stefanie Spielman Comprehensive Breast Center, namely the Breast Patient Information Form, clinic and office notes as well as laboratory and radiology reports, including documentation found in the electronic medical record (EPIC).

9.6 Safety monitoring

Adverse events will be monitored by self-reporting of signs and symptoms. Patients will maintain a daily diary of time of drug/placebo intake and any possible ill effects, with instructions to contact the PI or Research Nurse to discuss and manage any possible side effects.

9.7 Accountability

Study drugs (both minocycline and placebo) will be provided at no cost to the patient. Pill bottles will be provided to the patient, with the start date and number of pills recorded. The drugs will be provided in sufficient supply for one optional week prior to chemotherapy, four cycles of chemotherapy and +/- two optional additional weeks after chemotherapy. Pill bottles will be collected at each cycle's follow-up visits, and any unused capsules will be documented and counted to ensure proper adherence. The pill bottle will be returned to the patient after pill reconciliation until the end of study protocol. Additionally, there will be no charge for research related blood work and imaging.

10.0 PROCEDURES FOR PATIENT ENTRY ON STUDY

This study will be open for accrual at the Stefanie Spielman James Comprehensive Breast Center. This trial will be conducted under the auspices of The Ohio State University. Patient eligibility will be determined according to the eligibility criteria listed.

Eligible patients will be approached by study personnel in the clinics at Stefanie Spielman Comprehensive Breast Center. There will be monetary compensation for completion of various study precedures to account for patient's time. The person obtaining informed consent will tell the patient that 1) participation is voluntary, 2) participation or non-participation will not affect their usual care and management, and 3) patient confidentiality will be maintained in the event that the results of the study are published. The potential toxicities associated with minocycline will be explained fully to the patient. Patients will be informed of the need for, blood tests, physical examinations,questionnaires prior to entry into the study and at several specified intervals during study period. Patients will be provided with a consent form to review, and all questions answered.

After signed informed consent has been obtained, a study identification number will be assigned to the patient for use on all data collection forms and samples. A request for randomization will be given to the SBH Compounding Pharmacy who will enter study number into a randomization spreadsheet designed by statistician at the OSU Center for Biostatistics. By entering patient number, the randomization assignment will be given to the staff at SBJ who will then dispense the drug which will be shipped to OSU to be given to the patient by IDS pharmacy. Only SBH will have access to randomized assignments until study closure or if there is a serious adverse event necessitating unblinding of study investigators or if patient withdraws from study.

11.0 POTENTIAL TOXICITY, DOSE MODIFICATIONS, AND MANAGEMENT

The severity of adverse reactions is categorized as grade 1 to grade 5 in increasing severity. These will be attributed separately to both chemotherapy and study drugs.

General descriptors for the toxicity grades range from none to fatal:
Grade 1 – Mild (The adverse reaction does not interfere in a significant manner with the subject's normal functioning level. It may be an annoyance.)
Grade 2 – Moderate (The adverse reaction produces some impairment of functioning but is not hazardous to health. It is uncomfortable and/or an embarrassment)
Grade 3 – Severe (The adverse reaction produces significant impairment of funcitoning or incapacitation and is a definite hazard to the subject's health)
Grade 4 – Adverse reactions that include or lead to either a) a life-threatening event, though acute and without permanent effect, b) prolonged inability to resume usual life pattern, or c) impairment of ability to adequately deal with future medical problems

Grade 5 – Death related to AE

Toxicity will be monitored during study visits and telephone calls using the National Cancer Institute Common Terminology Criteria for Adverse Events v4.0 (CTCAE) of the National Cancer Institute will be used (http://evs.nci.nih.gov/ftp1/CTCAE/CTCAE_4.02_2998-09-15_QuickReference_5x7.pdf). Grade 3, 4 and 5 toxicities will be reported as adverse events.

Patients with Grade 3-4 adverse reactions that are related to the study will be removed from the study. No dose modifications are permitted for study drug.

Listed below are specific categories for potential adverse effects associated minocycline. No dose modifications will be made. Patients with grade 3 or 4 adverse events attributed to minocycline will be removed from the study.

Due to oral minocycline's virtually complete absorption, side effects to the lower bowel, particularly diarrhea, have been infrequent. The following adverse reactions have been observed in patients receiving tetracyclines.

Body as a Whole: Fever, and discoloration of secretions.

Gastrointestinal: Anorexia, nausea, vomiting, diarrhea, dyspepsia, stomatitis, glossitis, dysphagia, enamel hypoplasia, enterocolitis, pseudomembranous colitis, pancreatitis, inflammatory lesions (with monilial overgrowth) in the oral and anogenital regions. Instances of esophagitis and esophageal ulcerations have been reported in patients taking the tetracycline-class antibiotics in capsule and tablet form. Most of these patients took the medication immediately before going to bed

Genitourinary: Vulvovaginitis.

Hepatic Toxicity: Hyperbilirubinemia, hepatic cholestasis, increases in liver enzymes, fatal hepatic failure, and jaundice. Hepatitis, including autoimmune hepatitis, and liver failure have been reported.

Skin: Alopecia, erythema nodosum, hyperpigmentation of nails, pruritus, toxic epidermal necrolysis, and vasculitis. Maculopapular and erythematous rashes. Exfoliative dermatitis has been reported. Fixed drug eruptions have been reported. Lesions occurring on the glans penis have caused balanitis. Erythema multiforme and Stevens-Johnson syndrome have been reported. Photosensitivity. Pigmentation of the skin and mucous membranes has been reported.

Respiratory: Cough, dyspnea, bronchospasm, exacerbation of asthma, and pneumonitis.

Renal Toxicity: Interstitial nephritis. Elevations in BUN have been reported and are apparently dose relatated. Reversible acute renal failure has been reported.

Musculoskeletal: Arthralgia, arthritis, bone discoloration, myalgia, joint stiffness, and joint swelling.

Hypersensitivity Reactions: Urticaria, angioneurotic edema, polyarthralgia, anaphylaxis/anaphylactoid reaction (including shock and fatalities), anaphylactoid purpura, myocarditis, pericarditis, exacerbation of systemic lupus erythematosus and pulmonary infiltrates with eosinophilia have been reported. A transient lupus-like syndrome and serum sickness-like reactions also have been reported.

Blood: Agranulocytosis, hemolytic anemia, thrombocytopenia, leukopenia, neutropenia, pancytopenia, and eosinophilia have been reported.

Central Nervous System: Convulsions, dizziness, hypesthesia, paresthesia, sedation, and vertigo. Bulging fontanels in infants and benign intracranial hypertension (pseudotumor cerebri) in adults have been reported. Headache has also been reported.

Other: Thyroid cancer has been reported in the post-marketing setting in association with minocycline products. When minocycline therapy is given over prolonged periods, monitoring for signs of thyroid cancer should be considered. When given over prolonged periods, tetracyclines have been reported to produce brown-black microscopic discoloration of the thyroid gland. Cases of abnormal thyroid function have been reported.

Tooth discoloration in children less than 8 years of age and also, in adults has been reported.

Oral cavity discoloration (including tongue, lip, and gum) have been reported.

Tinnitus and decreased hearing have been reported in patients on minocycline hydrochloride.

The following syndromes have been reported. In some cases involving these syndromes, death has been reported. As with other serious adverse reactions, if any of these syndromes are recognized, the drug should be discontinued immediately:

Hypersensitivity syndrome consisting of cutaneous reaction (such as rash or exfoliative dermatitis), eosinophilia, and one or more of the following: hepatitis, pneumonitis, nephritis, myocarditis, and pericarditis. Fever and lymphadenopathy may be present.

Lupus-like syndrome consisting of positive antinuclear antibody; arthralgia, arthritis, joint stiffness, or joint swelling; and one or more of the following: fever, myalgia, hepatitis, rash, and vasculitis.

Serum sickness-like syndrome consisting of fever; urticaria or rash; and arthralgia, arthritis, joint stiffness, or joint swelling. Eosinophilia may be present.

Contraindications

This drug is contraindicated in persons who have shown hypersensitivity to any of the tetracyclines or to any of the components of the product formulation.

Warnings

Minocycline hydrochloride capsules, like other tetracycline-class antibiotics, can cause fetal harm when administered to a pregnant woman. If any tetracycline is used during pregnancy or if the patient becomes pregnant while taking these drugs, the patient should be apprised of the potential hazard to the fetus. The use of drugs of the tetracycline class during tooth development (last half of pregnancy, infancy, and childhood to the age of 8 years) may cause permanent discoloration of the teeth (yellow-gray-brown).

This adverse reaction is more common during long-term use of the drug but has been observed following repeated short-term courses. Enamel hypoplasia has also been reported. Tetracycline drugs, therefore, should not be used during tooth development unless other drugs are not likely to be effective or are contraindicated.

All tetracyclines form a stable calcium complex in any bone-forming tissue. A decrease in the fibula growth rate has been observed in premature human infants given oral tetracycline in doses of 25 mg/kg every six hours. This reaction was shown to be reversible when the drug was discontinued.

Results of animal studies indicate that tetracyclines cross the placenta, are found in fetal tissues, and can have toxic effects on the developing fetus (often related to retardation of skeletal development). Evidence of embryotoxicity has been noted in animals treated early in pregnancy.

Drug Rash with Eosinophilia and Systemic Symptoms (DRESS) including fatal cases have been reported with minocycline use. If this syndrome is recognized, the drug should be discontinued immediately.

The anti-anabolic action of the tetracyclines may cause an increase in BUN. While this is not a problem in those with normal renal function, in patients with significantly impaired function, higher serum levels of tetracycline may lead to azotemia, hyperphosphatemia, and acidosis. Under such conditions, monitoring of creatinine and BUN is recommended, and the total daily dosage should not exceed 200 mg in 24 hours (see DOSAGE AND ADMINISTRATION). If renal impairment exists, even usual oral or parenteral doses may lead to systemic accumulation of the drug and possible liver toxicity.

Photosensitivity manifested by an exaggerated sunburn reaction has been observed in some individuals taking tetracyclines. This has been reported with minocycline.

Central nervous system side effects including light-headedness, dizziness, or vertigo have been reported with minocycline therapy. Patients who experience these symptoms should be cautioned about driving vehicles or using hazardous machinery while on minocycline therapy. These symptoms may disappear during therapy and usually disappear rapidly when the drug is discontinued.

Clostridium difficile associated diarrhea (CDAD) has been reported with use of nearly all antibacterial agents, including minocycline hydrochloride, and may range in severity from mild diarrhea to fatal colitis. Treatment with antibacterial agents alters the normal flora of the colon leading to overgrowth of *C. difficile*.

12.0 ADVERSE EVENT REPORTING

12.1 Definition

Adverse event: Any unfavorable and unintended sign (including abnormal laboratory finding), symptom, or disease temporally associated with the use of a medical treatment or procedure, regardless of whether it is considered related to the medical treatment or procedure; also an "unanticipated problem" of any nature (e.g., psychological or social harm)
(designated as unrelated, definitely related, probably related, or possibly related; see below)

Serious adverse event: Any adverse event that is fatal or life threatening, is permanently disabling, requires inpatient hospitalization or prolongs hospitalization, or results in a congenital anomaly or birth defect.

Life-threatening event: Any adverse event in which the subject is at immediate risk of death from the reaction as it occurs; does not include a reaction that, if it were to occur in a more serious form, might cause death.

Unexpected event: Any adverse event that is not identified in nature, severity, or frequency in the investigator brochure, study protocol, consent form, or IND application; or the event was more serious than anticipated.

Association:

Definitely Related: An adverse event that has a timely relationship to the administration of the investigational drug/study procedure and follows a known pattern of response for which no alternative cause is present.

Probably Related: An adverse event that has a timely relationship to the administration of the investigational drug/study procedure and follows a known pattern of response, but for which a potential alternative cause may be present.

Possibly Related: An adverse event that has a timely relationship to the administration of the investigational drug/study procedure, follows no known pattern of response, but a potential alternative cause does not exist.

Unrelated: An adverse event for which there is evidence that it is definitely related to a cause other than the investigational drug/agent; in general, no timely relationship to the administration of the drug/procedure exists, or if so, the event does not follow a pattern of response and an alternative cause is present.

The Common Terminology Criteria for Adverse Events v4.0 (CTCAE) of the National Cancer Institute will be used. The severity of adverse reactions is categorized as grade 1 to grade 5 in increasing severity. Grade 3, 4 and 5 toxicities will be reported as adverse events. General descriptors for the toxicity grades range from mild to fatal:

Grade 1 – Mild (The adverse reaction does not interfere in a significant manner with the subject's normal functioning level. It may be an annoyance.)
Grade 2 – Moderate (The adverse reaction produces some impairment of functioning but is not hazardous to health. It is uncomfortable and/or an embarrassment)
Grade 3 – Severe (The adverse reaction produces significant impairment of functioning or incapacitation and is a definite hazard to the subject's health)
Grade 4 – Adverse reactions that include or lead to either a) a life-threatening event, though acute and without permanent effect, b) prolonged inability to resume usual life pattern, or c) impairment of ability to adequately deal with future medical problems
Grade 5 – Fatal

12.2 Documentation

All adverse events must be documented in detail within the medical record. The patient will be observed and monitored carefully until the condition resolves, stabilizes, or its cause is identified. All adverse events, including laboratory abnormalities, will be followed up according to good medical practices. Information to be recorded includes the following:

a. Specific type of reaction.
b. Duration of reaction.
c. Severity/grade of reaction according to the NCI Common Terminology Criteria for Adverse Events v4.0 (CTCAE).
d. Suspected cause of the reaction (i.e. possibly or probably related to one of the following: study treatment, progression of disease, concurrent medications, concurrent illness, or other factors).
e. Changes made in the administration of the study drugs and other actions taken to alleviate the clinical event.

f. Patient's response to medical interventions.

12.3 Reporting

According to FDA regulations (21 CFR 312.32), IND safety reports shall address "any adverse experience associated with the use of a drug that is both serious and unexpected." The IRB will be notified of any adverse event fulfilling the following criteria:

1. The adverse event is **SERIOUS** (as defined above),
   or
2. The adverse event is not serious, but is **UNEXPECTED** and its association with the study drug, device, or research-related procedure is either **DEFINITELY**, **PROBABLY**, or **POSSIBLY RELATED,** or **UNKNOWN** (as defined above).

Federal policy [45 CFR 46.116(b)(5)] also requires that investigators inform subjects of any important new information that might affect their willingness to continue participating in the research. When an adverse event necessitates changes to the consent/assent form(s) and/or protocol, or that notification is given to currently or previously enrolled subjects, an amendment request will be submitted in conjunction with the adverse event report. The IRB will make a determination whether any new findings, new knowledge, or adverse effects should be communicated to subjects.

In accordance with IRB guidelines, serious adverse events will be reported within 10 days of the investigator's or research staff members' learning of the event to The Ohio State University Institutional Review Board. OSU IRB Event Reports should be submitted through BuckIRB at: http:/orrp.osu.edu/irb/buck-IRB/. Events resulting in temporary or permanent interruption of study activities by the investigator or sponsor to avoid potential harm to subjects should be reported within 48 hours whenever possible.

All events that may represent unanticipated problems involving risks to subjects or others will be promptly reported (as described above), regardless of whether they occur during or after the study, or involve a subject who has withdrawn from or completed study participation. If changes to the research or consent process are proposed as a result of the event, or if additional information will be provided to current and/or past participants, an amendment request will also be submitted for IRB review.

Related events involving risk but not meeting the prompt reporting requirements will be reported to the IRB in summary form at the time of continuing review.

13.0 CRITERIA FOR RESPONSE ASSESSMENT

All randomized patients will be considered evaluable. Participants with only baseline measures will be considered as drop-outs.

14.0 STUDY CALENDAR

Tests & observations	Baseline, within 14 days of study start [a]	Pre- chemo^	Cycle 1 chemo	Cycle 2 chemo	Cycle 3 chemo	Cycle 4 chemo *	End of chemo **	6 months post end of chemo
Signed informed consent	x							
History and Physical Exam	x		x	x	x	x		
Height/weight	x		x	x	x	x		
Clinic visit	x		x	x	x	x		x
Review of medications, supplements	x					x	x	
Performance status	x		x	x	x	x	x	
Daily Symptom Logs		x	x	x	x	x	x	
Pill Counts			x	x	x	x	x	
Minocycline/placebo pills (twice daily)		x	x	x	x	x	x	
Neurocognitive testing			x				x	x
STAI and CES-D			x	x	x	x	x	x
Blood Inflammatory markers			x	x	x	x		x
Neuroimging Brain imaging (MRI and PET scans)***	x					x		x

* May occur within 2 weeks of last exposure to cycle 4 of chemotherapy.
** May occur within 3 weeks of last exposure to cycle 4 Dox chemo if patient receives dox, otherwise, patient will not have this optional time point.
*** PET and or MRI scans are optional but strongly recommended
^ Up to one week prior to the start of chemotherapy (optional)
a. Since pre-chemo minocycline is optional, baseline and cycle 1 chemotherapy start dates may coincide.

For additional cohort:

Tests & observations	Baseline, within 14 days of study start [a]	3 months of Minocycline therapy	6 months of Minocycline therapy
Signed informed consent	x		
History and Physical Exam	x	x	x
Height/weight	x	x	x
Clinic visit	x	x	x
Review of medications, supplements	x		
Performance status	x	x	x
Daily Symptom Logs		x	x
Pill Counts		x	x
Minocycline pills (twice daily)		x	x
Neurocognitive testing	x	x	x
STAI and CES-D	x	x	x
Blood Inflammatory markers		x	x
Neuroimging Brain imaging (MRI and PET scans)***	x	x	x

15.0 CRITERIA FOR REMOVAL OF PATIENTS FROM PROTOCOL THERAPY

Study patients may voluntarily withdraw at any time from the protocol. If a treating physician elects to remove a patient from the study, the Prinicpal Investigator must be notified of withdrawal from the protocol. The reasons for discontinuation of the study must be documented in the patient record and data collection forms.

Although we expect severe depression and suicidal ideation to be rare in this population[43,44], there is a screening and safety plan. At baseline, depressive symptom severity will be assessed using the CES-D instrument. Dr. Cheavens (Co-I, Clinical Psychologist) and/or social work will be available to evaluative the patient assessments if suicidal ideation is reported at baseline. The subject will be withdrawn from the study and treatment referrals will be provided. During the trial, an increase on the CES-D above a score of 20 will trigger a risk assessment. Dr. Cheavens and/or social work will be available to help manage patients symptoms. In the case of significant depressive severity or suicide risk (as determined by Dr. Cheavens and/or social work), the subject will be referred to psychotropic or psychotherapeutic intervention immediately, and removed from the study.

Patients experiencing irreversible Grade 3-4 toxicity that is clearly related to the study treatment will be removed from the protocol.

16.0 ETHICAL AND REGULATORY CONSIDERATIONS

This trial will be conducted in compliance with the protocol, Good Clinical Practice guidelines, and all applicable regulatory requirements.

16.1 Institutional Review Board

The Principal Investigator will have obtained written approval to conduct the study from The Ohio State University IRB and the Clinical Scientific Review Committee of the James Cancer Hospital and Solove Research Institute. All amendments must be approved by the Institutional Review Board of The Ohio State University prior to implementation.

16.2 Informed consent

All potential candidates for the study will be given a copy to read of the consent form for the study. The Principal Investigator and/or designee will explain all aspects of the study in lay language and answer all the candidate's questions regarding the study. If the candidate desires to participate in the study, she will be asked to sign the Informed Consent. The study agent will not be released to a subject without a signed Informed Consent.

Elements of informed consent include explanations of 1) the purpose of the trial, 2) what the study entails, 3) alternate treatments, 4) expenses and inconveniences to be incurred, 5) discomfort and risks to the subject, 6) whether she will receive payment for participation in the study, 7) contact person to call in the event of an emergency, 8) subject rights as a result of illness or injury from trial participation, 9) her right to withdraw from the trial at any time without prejudice, 10) confidentiality of trial participation.

16.3 Patient confidentiality

The information obtained during the conduct of this study is considered confidential and will not be released without the written permission of the subject, except as necessary for monitoring by the

FDA or other regulatory agencies. All laboratory specimens will be labeled with coded identifiers in order to maintain confidentiality. Signed consent forms, data sheets, and laboratory notebooks will be kept in locked cabinets in Dr. Bhuvaneswari Ramaswamy's office and/or research laboratories.

16.4 Publication of research findings

Publications of the research findings will present data in a format that will not reveal the identity of the participants.

16.5 Compliance monitoring

In accordance with IRB guidelines, the study program will be reviewed by the IRB every 12 months or less. Deviations from the protocol must be documented in the medical record and reported immediately to the PI. Deviations that meet the criteria for Immediate Event Reporting (http://orrp.osu.edu/irb/event/index.cfm) such as those that increase risks to subjects and/or compromise scientific integrity will be reported immediately to the IRB.

16.6 Biosafety

This project will involve the use and analysis of human cells and tissues. Specific precautions will be taken to protect laboratory personnel and support personnel form possible infective agents from these samples, with the goals of containment of biological materials, proper waste disposal, routine decontamination of equipment and surfaces, and implementation of procedures for accidents.

16.7 Data Safety Monitoring Plan

The data and safety monitoring plan will involve the continuous evaluation of safety, data quality and data timeliness. Investigators will conduct continuous review of data and patient safety at their regular Disease Group meetings (at least monthly) and the discussion will be documented in the minutes. The PI of the trial will review toxicities and responses of the trial where applicable at these disease center meetings and determine if the risk/benefit ratio of the trial changes. Frequency and severity of adverse events will be reviewed by the PI and compared to what is known about the agent/device from other sources; including published literature, scientific meetings and discussions with the sponsors, to determine if the trial should be terminated before completion. Serious adverse events and responses will also be reviewed by the OSUCCC Data and Safety Monitoring Committee (DSMC). The PI will also submit a progress report biannually that will be reviewed by the committee per the DSMC plan. All reportable Serious Adverse Events (SAE) will also be reported to the IRB of record as per the policies of the IRB.

16.8 Compensation for study subjects:

Participants will be compensated for their time. At all visits, they will receive free parking. In addition, they will receive $50 for completing cognitive testing assessments. They will receive $25 for completing any additional questionnaires with each cycle including STAI and CES-D. They will receive $150 for completing neuroimaging at each allocated time point. Therefore, they will have an opportunity to receive up to a total of $750. If they are not able to complete the full day visit, your payment will be pro-rated based on the percentage of the visit that you actually completed. Payment will be sent after completing all visits that will ideally be scheduled within

about a month; if their visits are more than a month apart, a check in the appropriate amount will be mailed to them within 2-4 weeks of each phase of study completion. By law, payments to subjects are considered taxable income.

17.0 STATISTICAL CONSIDERATIONS

Data Analysis Plan. Patient demographics information will be summarized for each treatment group. Drug compliance will be evaluated using pill counts and MEMS caps. Adverse events will also be tabulated for each treatment group. Primary analysis for efficacy will be conducted based on intention-to-treat (ITT) principle and sensitivity analysis will be conducted for those patients who received the treatment (per-protocol patients, or PP)). We will use mixed model for repeated measures to account for the association of these weekly measures from the same individual, and to estimate the effect size of the treatment group and test the differences between two groups in terms of the changes of CES-D and STAI scores after 8 weeks. The advantage of using mixed model include that it can handle potential missing data (with missing at random assumption), and use it to study the pattern of weekly changes overtime for each group (include linear change overtime). All patients will be used as ITT for safety and compliance measures analysis.

Primary Outcomes. For each primary outcome measure (CES-D and STAI scores**)**, we will evaluate (1) the change over time from baseline to the end of study for the two groups, and (2) the differences of the changes between the two groups after four cycles of chemotherapy. Mixed models for repeated measures will be used to account for the association of the measures over time from the same patients. The 95% confidence intervals of the change in the primary outcome measures from baseline to the end of study and the differences between the treatment and placebo groups will be estimated based on the models. The influences of covariates, such as disease stage and baseline measures, will be considered in the mixed models as exploratory analyses.

Secondary and correlative outcomes. The same method will also be used to evaluate the changes of these secondary and correlative outcomes (inflammatory blood biomarkers and the PET/MRI measures). We will use scatter plots to explore the pair-wise correlation among the changes of CES-D and STAI scores, blood biomarkers changes, and PET/MR measures. We will use linear regression to explore whether the blood based biomarkers and PET/MRI measures can be used to predict the changes in CES-D and STAI scores, which then could be used as potential surrogate markers in future studies.

The secondary goal of this study is to collect data of the cognitive function changes in breast cancer patients undergoing chemotherapy and gather evidence of the effect of minocycline in this patient population. All variables will be plotted over time (pre vs post-chemotherapy) and summarized for the minocycline treatment group and the placebo group. The changes of the variables for each group and the difference between two groups will be estimated based on a mixed model for repeated measures to account for the association of measurements from the same patient over time. The influence of the biomarkers on the cognitive function changes will also be evaluated visually using pair-wise scatter-plots. The associations among the changes of cognitive function measures, depression score and biomarkers will also be explored.

Sample Size and Power Calculations. Sample Size and Power Calculations. The main objective of this phase II study is to preliminarily evaluate the effect of minocycline on chemotherapy-induced depressive symptoms in terms of changes in CES-D and STAI scores. With a sample size of 23 per group, we will have at least 80% power to detect an effect size of

0.74 standard deviation difference between the two groups at significance level of 0.10 based on a 2 sided two-sample t-test. It will also provide at least 90% power to detect a change of 0.63 effect size for each group. Based on previous studies, an effect size of 0.75 standard deviation of the STAI or CES-D scores is considered a significant difference, and is clinically meaningful. Our goal is to ensure at least 23 patients per group complete the treatment regimen and have measures from baseline to the end of study. From our experience, attrition of less than 15-20% is expected for studies in this patient population in our center, and to account for this, we plan to recruit up to 60 patients.

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19.0 Appendix

Inflammatory Biomarkers Specimen Processing, Storage & Shipping Instructions

Note: To ensure the correct analysis of "Inflammatory Biomarkers" in clinical specimens, these procedures must be followed carefully. Specimens should be ideally centrifuged immediately following clot formation but no longer than **90 minutes** after specimen collection. Specific instructions for collecting, processing, storing and shipping specimens are outlined below:

1. Venous whole blood (**approximately 10 mL**) should be drawn into a **10-mL plain red top tube with no gel separator.**
2. Make sure that the **ACTUAL clock time** that the specimen was drawn (not the expected or targeted time) is recorded on the "Clinical Trials Flow Sheet:" (or other appropriate source document) and on the specimen collection tube label.
3. Mix the vacutainer by **gentle inversion 5 times**
4. **Allow the specimen to clot for 30-60 minutes at room temperature**.
5. **SERUM** should be separated from whole blood**, by centrifugation at 1200 G (RCF) for 10 minutes in a non-refrigerated or refrigerated centrifuge (4°C).** .
6. Immediately after centrifugation, using a disposable pipette, carefully transfer approximately the top two-thirds of the upper serum layer and place **0.5 mL (500 microliters) serum into eight (8) cryovials**.
7. Pour-off tube labels should include the following information:
   - Protocol Number – OSU 13165
   - Subject Number (ID Number)
   - Reference time point relative to protocol (i.e., nominal collection time)
   - Date of actual specimen collection
   - Actual time specimen was collected (military time)
   - Type of Specimen (Inflammatory Biomarkers)
   - Nominal Time Point (Baseline, 3 Months, 6 Months)
8. Immediately place the cryovials containing serum into a storage box and place in a **-70°C** freezer.
9. Specimens should be batched and stored at **-70°C** until the time of transport and analysis.
10. Specimens should be sent to the OSU CRC Core Lab at 6-month intervals.
11. Specimens will be sent via Best Courier to the CRC Core Lab and a completed "specimen log" should accompany each set of samples. **Specimens should only be sent on Monday-Wednesday**.
12. Samples are to be packed in dry ice in styrofoam shippers with enough dry ice to ensure that the samples will not thaw during transport.

    Dry ice should be placed along the bottom of the styrofoam container. Each set of

specimens should be placed in a plastic resealable bag, grouped according to the subject. A paper towel should be wrapped around the samples. Each plastic bag for each patient should be identified with the following information: protocol number, institution name, patient. Before plastic bags are placed into the styrofoam box a layer of paper towels should be placed on top of the dry ice. The plastic bag should then be placed into the box, and additional dry ice should be placed along its sides. A completed specimen log listing all specimens should be included in a plastic resealable bag along with the samples shipped. The styrofoam container should be sealed with strapping tape and placed into a sturdy cardboard mailing box. The box should subsequently be sealed. Samples should be transported to:

DeVries Laboratory
Attn: Courtney Devries Laboratory
Biomedical Research Tower, Suite 614
The Ohio State University
Columbus, Ohio

Please include a contact name, phone number and email address, so that receipt of samples can be acknowledged.

Please send Courtney DeVries an email (Courtney.DeVries@osumc.edu) the day before the specimens are to be shipped to acknowledge that a shipment will be made.