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STUDY TITLE: Pilot Trial to Evaluate Blood and Imaging Based Biomarkers for Aromatase Inhibitor Induced Musculoskeletal Syndrome

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Protocol Synopsis

Title of Study: Pilot Trial to Evaluate Blood and Imaging Based Biomarkers for Aromatase Inhibitor Induced Musculoskeletal Syndrome (AIMSS)

Hypotheses: Baseline plasma oxylipin profiles can be used to predict who is at risk for developing AIMSS.

Primary Objectives:

- To compare baseline oxylipin levels in women that do vs. those that do not develop AIMSS

Secondary Objectives:

- To correlate changes in oxylipin panels with changes in tendon stiffness
- To correlate changes in levels of oxylipins with changes in pain scores through 6-month AI treatment.

Study design: This is a prospective single arm study enrolling patients (n = 35) with breast cancer scheduled to start adjuvant hormonal therapy. Patients would have completed all their primary treatments (surgery± radiation therapy) and are scheduled to start their adjuvant hormonal therapy. They get baseline blood drawn for oxylipins. They start adjuvant anastrozole and have blood drawn at 3mths and 6mths for measurement of oxylipins.. This is a pilot trial to evaluate blood biomarkers. Once pilot data is analyzed, goal is to apply for funding for a larger trial to further evaluate and validate these biomarkers

Study Center: Banner – University Medical Center, University of Arizona Department of Medical Imaging, University of Arizona Cancer Center-North Campus, University of Arizona Cancer Center-Orange Grove Campus, Tucson, AZ

Number of Patients: We plan to accrue a total of 35 patients into this prospective single arm trial to have 20 evaluable patients for analysis

Main Criteria for Inclusion/Exclusion:

Inclusion Criteria

To be eligible for this trial patient must

1. Be capable of understanding the investigational nature of the study and all pertinent aspects of the study
2. Be capable of signing and providing written consent in accordance with institutional and federal guidelines
3. Have a histologically-confirmed diagnosis of breast cancer
4. Be willing and able to comply with scheduled visits, treatment plan

5. Age ≥ 21 years
6. Post-menopausal women with 1st event of ER+ early stage breast cancer (0-3)
7. Completed definitive therapy (surgery ± radiation)
8. Candidates for adjuvant AI therapy

Exclusion criteria

Patients who fulfill any of the following criteria will be excluded from the study:

1. Have received adjuvant or neo-adjuvant chemotherapy
2. Prior endocrine therapy (AI or tamoxifen)
3. History of rheumatoid arthritis or other autoimmune arthritis
4. Daily non-steroidal anti-inflammatory drug (NSAID) use (except for daily aspirin use)
5. Current use of daily corticosteroids or immunosuppressive therapies

Intervention:

Statistical analysis

Statistical Considerations: This is a pilot study to estimate the values needed to design a larger study. The primary objectives are to estimate the baseline oxylipin levels in patients who develop with AIMSS vs. those who do not develop AIMSS. Analysis will be based on descriptive statistics and 95% confidence intervals for each group. Although we will not formally test for differences between the two groups, we will estimate the difference with a 95% confidence interval to inform the design of a larger study.

The secondary objectives are to correlate changes in oxylipin levels with changes in pain scores. These analyses will be based on all 20 women, irrespective of whether they develop AIMSS.

Feasibility Issues: From 2015 to 2016, ~40 patients were started on adjuvant anastrozole therapy who meet our eligibility criteria. Thus, we anticipate accrual of 2-3 patients per month and completion of accrual this pilot study within 8-12 months of study initiation. Based on previously published studies, it is expected that 50% of women initiating AI will develop AIMSS within one year, with most women experiencing symptoms by 2 months.[17] Women who discontinue use of anastrozole before the 6 month visit will complete all measurements at the time that they switch to another AI and come off study.

Dr. Chalasani and the research personnel will be conducting monthly meetings to review patient accrual data, address any barriers to accruals and resolve them in a timely manner. Dr.Martinez will be overseeing the oxylipin data analysis.

STUDY CALENDAR

			Treatment -Anastrozole	
	Screening	Pre-Treatment	3mths	6mths
Informed Consent	X [a]			
History and Physical Exam	X [a]			
Collection of Blood for biomarker analysis		X [b,d]	X[c,d]	X[c,d]
Questionnaires		X [b]	X[c]	X[c]

A- Performed within 28 days of trial entry
B- May be collected/performed any time prior to starting anastrozole (can be on the same day)
C- Can be done within 28 day window (±) of the time point
D- Labs have to be fasting

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1. Introduction

Aromatase Inhibitors (AIs), Adherence, Musculoskeletal Symptoms and Mortality. Adjuvant aromatase inhibitor (AI) therapy is the recommended first-line adjuvant treatment for postmenopausal women with estrogen receptor positive (ER+) breast cancer based on improved disease-free survival (DFS) compared to tamoxifen in randomized control trials. More recently extended adjuvant AI therapy (for 10 years) has been shown to improve DFS when compared to placebo in women who completed 5 years of standard AI therapy.[1,2] However, AI therapy has been associated with significant, activity-limiting musculoskeletal symptoms including arthralgia, myalgia and joint stiffness or AI-induced musculoskeletal syndrome (AIMSS).[3] AIs block estrogen synthesis and induce menopausal symptoms that are particularly severe in younger patients.[4,5] Musculoskeletal complaints are among the most commonly reported and debilitating side effects of adjuvant AI therapy;[6] findings that are supported by observations in aromatase knockout mice which exhibit increased pain responses.[7] Among women with arthralgia syndromes at initiation of AI therapy, half report symptom exacerbation. Joint-related symptoms manifest early after initiation of AI therapy with worsening of symptoms up to 1 year post-initiation of AI.[8,9] AIMSS impairs ability to perform activities of daily living as well as work-related tasks as the most commonly affected joints are hands, knees and hips.[10,11] In a survey conducted by Breast Cancer Action, 27% of respondents reported that they had discontinued adjuvant AI use because of adverse effects (AE), with AIMSS being the leading AE.[12] In a cohort study in California, 61% of patients on AIs developed joint and/or bone pain resulting in discontinuation of therapy in 20% of patients.[13] In a recent study, full adherence, defined as full duration and optimal schedule, was observed for only 49% of breast cancer (BC) patients prescribed any hormone therapy (HT) and was related to higher mortality in those who discontinued [Hazards Ratio (HR) = 1.26 (Confidence interval (CI) 1.09–1.46)] or were non-adherent to HT [HR = 1.49 (CI 1.23–1.81)].[14] This study is concerning and consistent with clinical trial evidence from prior tamoxifen studies showing higher recurrence rates and worse outcomes with < 5 years or suboptimal HT use.[15-17] Clearly, there is a need to maximize efficacy and improve tolerance to AIs. Approaches to study interventions that enhance both adherence and response rates for AIs are important research and clinical gaps that must be addressed to reduce death from breast cancer.[18,19] **A critical first step is to identify those who are at risk of developing AIMSS by developing predictive biomarkers that will provide physicians opportunity for early intervention, thereby improving adherence to AIs. Currently there are no predictive biomarkers available to identify those at risk of developing AIMSS.**

Mechanisms of Aromatase Inhibitor-Associated Pain. The mechanism of AIMSS is not well understood and is grossly understudied. Based on observational data and animal data, it is likely that AIMSS is directly related, at least in part, to a) loss of the anti-nociceptive action of estradiol - resulting in greater sensitivity to stimuli and b) presence of underlying inflammation and the nociceptive activity or pain-inducing response of PGE_2. In aromatase-knockout mice, which lack endogenous estrogen production and serve as an animal model of AI therapy, estrogen deprivation results in a dominance of pro-nociceptive (pain) over anti-nociceptive response mechanisms following noxious stimuli such as formalin injection.[20] Results from experimental models to determine the role of estradiol in inflammation and specifically in cyclooxygenase (COX)-mediated PGE_2 are inconsistent and model dependent. Hunter *et al* demonstrated that ovariectomized rats exhibited greater sensitivity to a pain stimulus, as

measured by latency to paw withdrawal following an inflammatory insult.[21] Serum levels of PGE_2 were increased in these rats, indicating an increase in inflammation. Pain sensitivity was reversed with estradiol replacement but serum PGE_2 levels did not change, indicating a separate mechanism for inflammatory response. The addition of a COX2 inhibitor increased latency to paw withdrawal compared to estradiol replacement alone, indicating a reduced pain response for these animals, and again indicating separate roles for inflammation and estradiol in pain response.[22] Using this same model, ibuprofen (a non-steroidal anti-inflammatory drug, NSAID; a COX inhibitor) + estradiol replacement was shown to be more effective in reducing pain than either estradiol or ibuprofen alone.[22] Collectively, these results indicate that the loss of estrogen alone does not increase pain by inducing PGE_2; but in the presence of PGE_2-mediated inflammation, estrogen deprivation increases sensitivity to inflammatory pain. **Therefore, if PGE_2 is elevated at baseline, this can predispose women to experience pain upon the depletion of estradiol with the addition of an AI.**

In healthy women, joint and muscle pain worsen with age with greatest increases between 50 - 59 years.[23,24] New onset of rheumatoid arthritis is also highest in the decade following menopause. Such observations, in combination with extensive experimental studies in animal models, have established estradiol as having both anti-inflammatory and anti-nociceptive properties.[24] Increased inflammation with aging, mediated by bradykinin and the pro-inflammatory prostaglandins (PGs), including PGE_2, has been mechanistically linked to hyperalgesia (increased sensitivity to painful stimuli) and allodynia (painful response to what would normally be an innocuous stimulus). Thus, inflammation and injury that stimulate PG release are thought to result in a heightened nociception and allodynia with the decline in estradiol levels with menopause.[25]

Management of AIMSS in AI Patients. While all 3 third-generation AIs in routine clinical use cause different levels of estradiol suppression,[26] toxicity profiles are similar for all of them.[27-29] Further, many studies show that there is no correlation with degree of estrogen depletion and pain in women on AI, regardless of AI type.[30,31] Differences between agents in terms of quality of life (QOL) and tolerability are very important when selecting the optimal therapy for individual patients. Randomized clinical trials comparing tolerability and QOL between letrozole and anastrozole did not show any significant differences between the QOL scores. They did report that subjects who were intolerant to one AI were nonetheless able to tolerate another agent.[32,33] Other studies have also demonstrated that switching AIs can result in improvements of AIMSS.[33,34] This is a significant finding given that—despite AIs having similar toxicity profiles—AIMSS can sometimes be managed by switching AIs.

AIMSS management is an area of active research interest. For patients developing AIMSS, there is evidence that acupuncture[35,36] high dose vitamin D[37] or exercise[38] might help in alleviating their symptoms. Yet results are not always consistent and are limited by small numbers of participants. Recently, a randomized, placebo-controlled study reported a 50% decrease in AIMSS symptoms with ω-3 fatty acid supplementation; however, this change was not significantly different when compared to the placebo arm.[39] Retrospective clinical data reported a significantly decreased recurrence and longer disease-free survival in obese women with ER+ tumors who take NSAIDs.[40] Anti-inflammatory medications like NSAIDs or COX2 inhibitors might alleviate arthralgia and myalgia but have gastrointestinal and cardiac side effects. Currently, for a patient who develops AIMSS, routine management involves i) pain control with NSAID's or other pain medicines, ii) stopping and switching to a different AI or tamoxifen, iii) stopping adjuvant hormonal therapy completely or iv) referral to orthopedic surgeon based on

symptom severity. It is important to realize **not all patients who take AIs develop AIMSS**. It is crucial to determine which patients are at risk so that preventative strategies can be implemented early on to improve adherence to AI therapy and eventually decrease breast cancer relapse and death. **Identifying at-risk subjects by developing predictive biomarkers is a critical unmet need in AIMSS.**

Pain and Oxylipin Cascade. While PGE_2 is the best-studied metabolite of arachidonic acid metabolism, arachidonic acid and other polyunsaturated fatty acids (i.e., ω-3 and ω-6) are also metabolized by the lipoxygenases (LOX) and cytochrome P450 (CYP) enzymes, yielding distinct sets of metabolites with their own biological activities. Collectively, these COX, LOX and CYP metabolites are called oxylipins. The oxylipin metabolites of ω-3 and ω-6 fatty acids comprise over 100 metabolites, each of which exhibit potent biological effects, including acting as modulators of inflammation (pro- and anti-) as well as activators of angiogenesis and mediators of hyperalgesia and allodynia.[41-43] Recently, it has been demonstrated that there is cross-talk between the estrogen receptor and LOX-mediated oxylipins,[44] and that AIs can upregulate the CYP450 pathway and downstream epoxyeicosatrienoic acids *in vitro*.[45] It is well established that downregulation of PGE_2 production through inhibition of COX2 alleviates inflammatory pain. Preliminary evidence implicates both the CYP450 and LOX pathways in the development of tendinopathy.[46,47] Two earlier clinical trials showed that 12-hydroxyeicosatetraenoic acid (12-HETE) was significantly correlated with pain in menstruating women,[48,49] suggesting interplay between 12-HETE and hormonal changes. Our preliminary data in AI-treated women participating in an intervention with sulindac (a non-selective COX2 inhibitor) suggests involvement of 12-HETE and other LOX metabolites in AIMSS (see **Approach**). If we successfully show that oxylipins are pathogenic in the development of AIMSS, there are already pharmacologic agents available (COX and LOX inhibitors) or in development (CYP pathway inhibitors) that can be implemented to target these pathways[50] and alleviate symptoms, with the ultimate goal of improving AI adherence in clinical practice. As cited above, interventional clinical trials have been inconsistent, and there have been no studies that have determined predictive blood-based biomarkers for AIMSS in order to guide optimal subject selection to study appropriate interventions.

Table 2: Relationship between oxylipins and pain in 10 breast cancer patients on AI[a]

Enzyme	Oxylipin	How much pain interferes with walking ability	
		R^2	*P*-value
COX	PGE_2[c]	0.11[d]	0.36
LOX	12-HETE[c]	**0.44**	**0.04**
LOX	15-HETE	0.25	0.15
LOX	11-HETE	0.21	0.18
LOX	9-HETE	0.25	0.14
LOX	8-HETE	0.21	0.19
LOX	5-HETE	0.09	0.41

[a]Oxylipins quantified in plasma drawn after a 3 month observation period
[b]Question from the Brief Pain Inventory Short Form (BPI-SF) on a scale of 1-10.
[c]Target oxylipins: Prostaglandin E_2 (PGE_2) and 12-Hydroxyeicosatetraenoic acid (12-HETE)
[d]All R^2 values are positive correlations between indicated oxylipin level in plasma and score from BPI question 'Walking'

Preliminary Data:

Oxylipins:

Dr. Chalasani is institutional PI of an ongoing clinical trial to determine the effect of sulindac (an NSAID) on breast density and pain in women that have been stable on AI for 6 months. Participants receive sulindac (150 mg BID) for 12 months.

Table 1: Plasma levels of target oxylipins in 10 breast cancer patients on AI[a]

	Plasma (nM)[b]
PGE_2[c]	0.35 (±0.47)
12-HETE[d]	65.2 (±53.4)

[a]Oxylipins measured in plasma collected after a 3-month observation period
[b]Data presented as: mean (±SD)
[c]PGE_2: prostaglandin E_2
[d]12-HETE: 12-Hydroxyeicosatetraenoic acid

Plasma and urine samples are collected before and after a 3-month observation period (months -3 and 0), and after 3, 6, 9, and 12 months on sulindac.

Participant demographics. This study is currently conducted in post-menopausal women diagnosed with ER+ breast cancer. Inclusion and exclusion criteria for this study regarding breast cancer history are the similar as the proposed work except that participants in the Sulindac trial must have been stable on AI for 6 months and exclusion criteria for this trial also includes active smokers, history of diabetes mellitus, uncontrolled hypertension, GI ulcers and bleeding diathesis .

Oxylipins in women on AI. We have conducted oxylipin profiling in the plasma and urine in 10 women after the 3 month observation period (selected based on availability of samples). We quantified 92 total oxylipins in plasma and 87 in urine with good reproducibility. PGE_2 levels were similar to the ranges previously reported in plasma of other breast cancer patients.[86-88] Levels of target oxylipins after a 3-month observation period (PGE_2 and 12-HETE) in women on AI are presented in **Table 1**.

Pain and oxylipins. How much pain interferes with daily activities such as walking ability was evaluated using the Brief Pain Inventory Short Form (BPI-SF). Oxylipin levels after a 3 month observation period were correlated with questionnaire items at the same time-point. **Table 2** presents the results from linear regression comparing how much pain interferes with walking ability and selected plasma oxylipin levels in 10 women stable on AI. PGE_2 levels were not correlated with how much pain interfered with walking ability. However, plasma levels of the LOX metabolite, 12-HETE, were significantly correlated. While not statistically significant, a similar pattern was observed across multiple LOX metabolites. This trend could still be observed in only 10 participants with overall low scores for how much pain interferes with walking ability (mean ± SD; 2.48 ± 2.45), which suggests a role for the LOX arm of the oxylipin pathway in the development of AIMSS. There was no correlation (positive or negative) with CYP450-derived metabolites and pain scores (data not shown).

While these results are promising and suggest that the LOX pathway may be related to AIMSS, samples from this clinical trial have several limitations. First, participants in this study were stable on AI for at least 6 months. Therefore, there is no true “baseline” to quantify oxylipins levels. Second, hand-specific symptoms, common in AIMSS, were not evaluated. Third, there is no BPI-SF score that specifically addresses AIMSS development. Fourth, while we have presented our preliminary data from the baseline (post-NSAID washout and 3 month observation period) only, women in this study initiate intervention with sulindac (a non-specific COX2 inhibitor). Thus, it is likely that the oxylipin cascade will be modulated by the intervention, and stored samples from this trial will not be appropriate to determine changes in oxylipins related to the natural history of AIMSS. Despite these limitations, these data demonstrate that oxylipins are quantifiable in the plasma of women currently taking an AI, and that they are present in a wide enough range of levels to relate to pain scores.

2. Study Objectives

Primary Objectives:

- To compare baseline oxylipin levels in women that do vs. those that do not develop AIMSS

Secondary Objectives:

- To correlate changes in levels of oxylipins with changes in pain scores through 6-month AI treatment.

3. Study design:

This is a prospective single arm pilot study enrolling patients (n = 35) with breast cancer scheduled to start adjuvant hormonal therapy. Patients would have completed all their primary treatments (surgery±radiation therapy) and are scheduled to start their adjuvant hormonal therapy. At baseline, patients will undergo a blood draw. They will start adjuvant anastrozole and have blood drawn at 3 and 6 months for oxylipins. This is a pilot trial to evaluate oxylipins as blood biomarkers of AIMSS.

4. Selection of patients

Number of Patients: We plan to accrue a total of 35 patients with breast cancer into this prospective single arm pilot trial with the goal of having data from 20 evaluable patients.

Main Criteria for Inclusion/Exclusion:

4.1 Inclusion criteria

To be eligible for this trial patient must

1. Be capable of understanding the investigational nature of the study and all pertinent aspects of the study
2. Be capable of signing and providing written consent in accordance with institutional and federal guidelines
3. Have a histologically-confirmed diagnosis of breast cancer
4. Be willing and able to comply with scheduled visits, treatment plan
5. Age ≥ 21 years
6. Post-menopausal women with 1st event of ER+ early stage breast cancer (0-3)
7. Completed definitive therapy (surgery ± radiation)
8. Candidates for adjuvant AI therapy

4.2 Exclusion criteria

Patients who fulfill any of the following criteria will be excluded from the study

1. Have received adjuvant or neo-adjuvant chemotherapy

2. Prior endocrine therapy (AI or tamoxifen)
3. History of rheumatoid arthritis or other autoimmune arthritis
4. Daily NSAID use (except daily aspirin use)
5. Current use of daily corticosteroids or immunosuppressive therapies

4.3 Patient identification

Patients who have been consented and are undergoing study screening will be identified on study-related documentation and forms by their initials (first/middle and last name initials). All patients treated on this trial will be identified by their study initials and a unique study identification number. A unique study number will only be assigned to patients who meet the eligibility requirements and have completed the screening visit and are registered for treatment. The unique number will begin with the following prefix: AI. The prefix will be followed by the patient identification number beginning with # 001 in each cohort. These numbers will be issued to patients sequentially and no patient identification numbers will be re-assigned in the event that the subject withdraws from protocol treatment.

4.4 Study exit

Patients will exit the study upon completion of the last collection of blood for biomarkers and completing study questionnaires. Patients will not be followed further for purposes of this study.

5. Study Procedures

5.1 Pretreatment

Patients will be consented and evaluated for participation based on the following procedures before being registered and starting trial:

- History and physical exam (should be performed within 28 days of trial entry)
- Eligibility as determined by inclusion and exclusion criteria

5.2 Patient Registration

Patients must be registered prior to initiation of treatment. Patients will be registered through a Breast Team Clinical Research Coordinator (CRC) from 8:00 a.m. to 5:00 p.m., Mountain Standard Time, Monday through Friday, (excluding holidays)

Registration Guidelines

Before a subject participates in the trial, the investigator or delegate is responsible for obtaining written informed consent after adequate explanation of the aims, methods, anticipated benefits, subject responsibilities and potential hazards of the study and before any protocol-specific screening or study medications are administered. All patients must be registered before the start of treatment.

5.3 Research Blood Draw

Currently there are no blood based biomarkers evaluating AIMSS. We propose to prospectively collect blood for this pilot study for initial analysis of oxylipins.
Fasting blood will be drawn at the pre-determined time points (figure 1) and sent to Dr. Martinez's laboratory at the University of Arizona Cancer Center for further processing.

5.4 Self-reported questionnaires

The following questionnaires will be administered for the study to look for potential confounders like depression which can influence reporting of pain. The pain questionnaires will help us classify AIMSS in an objective way.

Pain Assessment. Breast Cancer Prevention Trial–Musculoskeletal Symptom (BCPT-MS) subscale and The Western Ontario and McMaster Osteoarthritis Index (WOMAC) have been validated as the most responsive tools for evaluating the development of AIMSS.[62] These tools will be administered by the study nurse.

BCPT-MS: Individuals with a subscale score of ≥1.5 on the BCPT-MS are considered to have developed AIMSS, and it is expected that 50% of our sample will develop AIMSS.[62,63] Our primary study endpoints will be correlated to three items on the BCPT-MS: general aches and pains, joint pain, and muscle stiffness.[64,65] The BCPT-MS was derived from the original BCPT Symptom Check-list, a 18-item questionnaire validated in breast cancer survivors.[66] The subscale score consists of the mean of responses to three questions addressing general aches and pains, joint pain, and muscle stiffness(questions 8,9,10 of the attached subscale). Scores range from 0–4, with higher scores representing worse symptoms. Changes in the BCPT-MS subscale have been shown to be the most responsive to changes in AIMSS with 54% of women developing AIMSS within 6 months.[62]

WOMAC: The WOMAC is a 24-item instrument developed to assess pain, stiffness, and physical function in participants with hip and/or knee osteoarthritis.[68] This instrument is well validated and has been modified from its original form, and used extensively to examine specific joint pain changes following treatment, including drug-based (NSAID) interventions for back pain, rheumatoid arthritis, and fibromyalgia.[69-78] For our study, we will use the 5-point Likert format.

Depression Questionnaire. It has been reported that up to 50% of newly diagnosed breast cancer patients have symptoms of depression or anxiety[79] and perception of pain may be altered in individuals with symptoms of depression.[80] Therefore, we will also administer the Patient Health Questionnaire (PHQ)-9 questionnaire, which is a validated multipurpose tool used for screening, diagnosing, monitoring, and measuring severity of depression.[81] Additionally, the PHQ-9 has been successfully used in breast cancer patients to evaluate psychometric properties.[82,83]

5.5 Toxicities to be monitored

Monitoring for side effects from anastrozole will be done per their standard of care by patient's treating physician.

5.6 Follow Up

Patients will not be followed for this study after completion of the 6 mths blood draw, study questionnaires. However, they will continue to follow-up with their treating physician per standard of care.

6. Criteria for Evaluation

The primary endpoint is comparing baseline oxylipin levels for patients who develop AIMSS vs. those who do not develop AIMSS. For this pilot trial of 20 evaluable patients we expect 10 patients to develop AIMSS and 10 who do not develop AIMSS. The goal is to prospectively evaluate for any baseline levels in both groups and for changes in oxylipin panels.

7. Statistical Considerations:

This is a pilot study to estimate the values needed to design a larger study. The primary objectives are to estimate the baseline oxylipin levels in patients who develop AIMSS vs. those who do not develop AIMSS. Descriptive statistics and 95% confidence intervals will be computed for two baseline oxylipin levels (PGE_2 and 12-HETE) for each group. Based on clinical observation, it is expected that 50% of the women will develop AIMSS. Ten patients in each group will allow us to estimate the 95% confidence interval with width 1.4 times the observed standard deviation. Although we will not formally test for differences between the two groups, we will estimate the difference between the means with a 95% confidence interval, to inform the sample size justification for a larger study.

The secondary objectives are to correlate changes in oxylipin levels with changes in pain scores. These analyses will be based on all 20 women, irrespective of whether they develop AIMSS. We expect that only a small number of women may switch to another AI due to the development of AIMSS. For these women, we will use the values obtained at the time of the switch. Linear regression will be used to estimate the change in pain scores (as measured by the BCPT-MS) versus changes in PGE_2, 12-HETE. Additionally, we will control for changes in depression levels as measured by the PHQ-9. This model will allow us to estimate the effects of each of the oxylipin measures adjusted for depression in a single model.

8.Data and Safety Monitoring Plan

The University of Arizona Cancer Center Data and Safety Monitoring Board (DSMB) has determined that this study falls into the low risk category with regards to DSMB oversight or QA/QC Program monitoring, per the current University of Arizona Cancer Center Data and Safety Monitoring Board (DSMB) Charter

This trial will undergo monitoring as indicated below:

Identification of the DSMB obligated for oversight responsibilities:
The University of Arizona Cancer Center Data and Safety Monitoring Board (DSMB) will provide ongoing oversight for this trial. This study has been assigned a Low Risk level by the DSMB.

Identification of the entity obligated for routine monitoring duties:
Routine monitoring will be provided by the Quality Assurance/Quality Control (QA/QC) Program to ensure that the investigation is conducted according to protocol design and regulatory requirements.

This trial will also undergo real-time monitoring by the PI and study team, including documentation of real-time monitoring of any new or ongoing safety issues. PI will review with the study team on a monthly basis regarding any routine safety or adverse issues. Adverse events and serious adverse events will be dealt right away as per DSMB/IRB requirements. The study team will inform the PI via email regarding any AE's and subsequent notification forms for the AE's will be completed and filed.

Monitoring progress and data review process:
Routine monitoring of subject data will be conducted at least quarterly.
The first routine monitoring visit will include at a minimum:

- Informed consent – 50% of cases enrolled;
- Subject eligibility – 10% of cases, up to two subjects;
- Data review – 10% of cases, up to two subjects.

All subsequent monitoring visits will consist of randomly selected subject cases based on current enrollment and include continuing review of previously selected cases, as applicable.

A monitoring visit report and follow-up letter will be completed approximately two weeks after the routine monitoring visit; a copy will be maintained in the study file. The monitor will request additional source documentation, clarification, information, or corrections to the CRF and/or regulatory records from the Clinical Research Coordinator (CRC) or other applicable staff responsible for the study and resolution of queries/findings. Documentation of such a request will be maintained with a copy of the monitor's visit report for follow-up at the next monitoring visit. Electronic records will be available in the institutional database or provided by the QA/QC Program staff.

The Principal Investigator will ensure the accuracy, completeness, legibility and timeliness of the data reported in the Case Report Form (CRF), or other acceptable data formats. Source documentation supporting the study data should indicate the subject's participation in the trial and should document the dates and details of study procedures, adverse events, and patient status.

Case report forms, which include the inclusion/exclusion criteria form, adverse event forms and serious adverse event forms, etc. should be completed via the institution database or other acceptable data formats. Trials using paper CRFs will have the data entered with a black ball-point pen or typed. Corrections to the forms should not obscure the original entry and should be made by striking the incorrect information with a single line. Each strike should be accompanied by the initials of the corrector and the correction date. All subject forms and study files will be stored in a secure area limited to authorized staff.

Note: Routine monitoring of regulatory documents will be conducted at least annually.

Process to implement study closure when significant risks or benefits are identified:

This study may be prematurely terminated, if in the opinion of the principal investigator there is sufficient reasonable cause.
Circumstances that may warrant termination include, but are not limited to:

* Determination of unexpected, significant, or unacceptable risk to patients
* Failure to enter patients at an acceptable rate
* Insufficient adherence to protocol requirements
* Insufficient complete and/or evaluable data

Description of adverse events and reporting procedures:
This study evaluates changes in oxylipins when patients with breast cancer are treated with anastrozole. Side effects from anastrozole will be managed per standard of care and will not be captured as adverse events for the purpose of this study. Since the other study procedures are blood draws and questionnaires, we do not anticipate to have AE or SAEs.

Plan for assuring data accuracy and protocol compliance:
Routine study activity and safety information will be reported to the DSMB on a quarterly basis, or more frequently if requested. These reports will include:

- Study activity, cumulative and for the period under review;
- Safety (narrative description on non-serious and serious adverse events, protocol pre-determined early stopping rules for safety or treatment-emergent adverse events);
- Predetermined protocol early stopping rules for efficacy/futility;
- Status of study in relationship to stopping rules;
- Current dose level of study agent;
- Routine monitoring and protocol compliance (describe the monitoring process and identify the status of the monitoring);
- Comments;
- Attachments (AE data reviewed by the PI to compile the report, SAE letters and reports, results of any review(s), applicable correspondence with the IRB or other regulatory agencies

Data, safety and study progress will be reported to:

- Human Subjects Protection Program (IRB) at least annually;
- Sponsor (if applicable) at least annually.

Identification of the sponsor or funding agency, as applicable:
The PI will immediately notify; in writing, the funding agency, if applicable, any action resulting in a temporary or permanent suspension of the study.
A copy of this correspondence will also be forwarded to the DSMB and the SRC.

9.Data Submission Schedule

Data forms must be completed for all subjects registered to the study.
Electronic case report forms will be completed in the OnCore system.

10.Ethics

The trial will be conducted in accordance with the Declaration of Helsinki for biomedical research involving human subjects and local regulatory requirements.

Ethical Principles

This study will be conducted in accordance with Title 21 of the Code of Federal Regulations (CFR). Specifically, this study will be conducted under a protocol reviewed by an Institutional Review Board; the study is to be conducted by scientifically and medically qualified persons; the benefits of the study are in proportion to the risks; the rights and welfare of the patients will be respected; the physicians conducting the study will ensure that the hazards do not outweigh the potential benefits; the results to be reported will be accurate; patients will give their informed consent and will be competent to do so and not under duress; and the study will comply with the ethical principles in Title 21 of the CFR.

Informed Consent

This study will be conducted in full compliance with the informed consent regulations in 21 CFR 50. The Sponsor-Investigator is responsible for ensuring that written informed consent from potential patients is obtained prior to performing any trial tests or assessments required by the protocol.

A copy of the fully executed informed consent form (PHI authorization form and ancillary consent forms if applicable), is given to the subject. One copy is placed in the subject's medical record, another copy is placed in the research chart, and the originals are filed by the protocol number in room 2111 at UACC North Campus.

Institutional Review Board

This study will be conducted in full compliance with the Institutional Review Board (IRB) regulations in 21 CFR 56, in accordance with the Declaration of Helsinki. This protocol will not be initiated unless it and the informed consent form have been reviewed and approved by, and remains open to continuing review by, an IRB meeting the requirements of 21 CFR 56. The IRB shall review and have the authority to approve, require modification in (to secure approval), or disapprove the protocol. The IRB shall notify the Investigator and the institution in writing of its decision. The IRB shall require that the information given to patients as part of the informed consent is in accordance with 21 CFR 50.25. The IRB shall conduct continuing reviews of the protocol at intervals appropriate to the degree of risk, but not less than once per year. At the completion or early termination of the trial, a final report should be sent to the IRB by the Investigator. The Investigator is obligated to maintain an IRB correspondence file.

Confidentiality of Patient Data

The investigator must ensure that patient confidentiality will be maintained. Patients will be identified by initials and a protocol-assigned patient number as described in section 4.5. Permission for direct access to patient data will be sought in writing for the patient by the investigator as part of the informed consent procedure. The patient will be informed

that all clinical information is confidential, but that the IRB, and regulatory authorities may inspect these records.

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12. Appendix

12.1 BCPT-MS

EVERYDAY PROBLEMS DURING THE PAST 4 WEEKS

We are interested in knowing how much you have been bothered by any of the following problems during the **PAST 4 WEEKS.** *(Circle one number on each line. If you do not have the problem, circle "not at all".)*

During the **past 4 weeks,** how much were you bothered by:	Not at all	Slightly	Moder -ately	Quite a bit	Extremely
1. Hot flashes……..	0	1	2	3	4
2. Nausea…….....	0	1	2	3	4
3. Vomiting…….....	0	1	2	3	4
4. Difficulty with bladder control when laughing or crying.	0	1	2	3	4
5. Difficulty with bladder control at other times…….....	0	1	2	3	4
6. Vaginal dryness……	0	1	2	3	4
7. Pain with intercourse..……...……	0	1	2	3	4
8. General aches and pains..……..…….....	0	1	2	3	4
9. Joint pains……...……...……	0	1	2	3	4
10. Muscle stiffness……...……	0	1	2	3	4
11. Weight gain……..	0	1	2	3	4
12. Unhappy with the appearance of my body...……....	0	1	2	3	4
13. Forgetfulness..……	0	1	2	3	4
14. Night sweats..……	0	1	2	3	4
15. Difficulty concentrating..…….....	0	1	2	3	4
16. Easily distracted..…….....	0	1	2	3	4
17. Arm swelling (lymphedema)..…….....	0	1	2	3	4
18. Decreased range of motion in arm on surgery side..…...	0	1	2	3	4

The BCPT Symptom Scales

Stanton, A. L., Bernaards, C. A., & Ganz, P. A. (2005). The BCPT Symptom Scales: A measure of physical symptoms for women diagnosed with or at risk for breast cancer. Journal of the National Cancer Institute, 97, 448-456.

Attached is the instrument we report in Stanton, Bernaards, and Ganz (2005). We calculate scores on each scale by averaging the items designated below:

Hot flash: 1, 14
Nausea: 2, 3
Bladder control: 4, 5
Vaginal problems: 6, 7
Musculoskeletal pain: 8, 9, 10
Cognitive problems: 13, 15, 16
Weight problems: 11, 12
Arm problems: 17, 18

Note that the additional following items also formed a weak factor: Vaginal discharge, Vaginal bleeding or spotting, Genital itching/irritation
As we use this instrument in the future, we plan to add items to provide a preliminary assessment of fatigue (e.g., lack of energy, tiredness).

I would appreciate your sending me your relevant findings, and I am happy to discuss the measure with you.

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12.2 WOMAC

The Western Ontario and McMaster Universities Osteoarthritis Index
(WOMAC)

Name:_____ Date:_____

Instructions: Please rate the activities in each category according to the following scale of difficulty: 0 = None, 1 = Slight, 2 = Moderate, 3 = Very, 4 = Extremely

Circle **one number** for each activity

Pain	1. Walking	0 1 2 3 4
	2. Stair Climbing	0 1 2 3 4
	3. Nocturnal	0 1 2 3 4
	4. Rest	0 1 2 3 4
	5. Weight bearing	0 1 2 3 4
Stiffness	1. Morning stiffness	0 1 2 3 4
	2. Stiffness occurring later in the day	0 1 2 3 4
Physical Function	1. Descending stairs	0 1 2 3 4
	2. Ascending stairs	0 1 2 3 4
	3. Rising from sitting	0 1 2 3 4
	4. Standing	0 1 2 3 4
	5. Bending to floor	0 1 2 3 4
	6. Walking on flat surface	0 1 2 3 4
	7. Getting in / out of car	0 1 2 3 4
	8. Going shopping	0 1 2 3 4
	9. Putting on socks	0 1 2 3 4
	10. Lying in bed	0 1 2 3 4
	11. Taking off socks	0 1 2 3 4
	12. Rising from bed	0 1 2 3 4
	13. Getting in/out of bath	0 1 2 3 4
	14. Sitting	0 1 2 3 4
	15. Getting on/off toilet	0 1 2 3 4
	16. Heavy domestic duties	0 1 2 3 4
	17. Light domestic duties	0 1 2 3 4

Total Score: _____ / 96 = _____%

Comments / Interpretation (to be completed by therapist only):

12.3 PHQ-9

PATIENT HEALTH QUESTIONNAIRE-9 (PHQ-9)

Over the last 2 weeks, how often have you been bothered by any of the following problems? *(Use "✔" to indicate your answer)*	**Not at all**	**Several days**	**More than half the days**	**Nearly every day**
1. Little interest or pleasure in doing things	0	1	2	3
2. Feeling down, depressed, or hopeless	0	1	2	3
3. Trouble falling or staying asleep, or sleeping too much	0	1	2	3
4. Feeling tired or having little energy	0	1	2	3
5. Poor appetite or overeating	0	1	2	3
6. Feeling bad about yourself — or that you are a failure or have let yourself or your family down	0	1	2	3
7. Trouble concentrating on things, such as reading the newspaper or watching television	0	1	2	3
8. Moving or speaking so slowly that other people could have noticed? Or the opposite — being so fidgety or restless that you have been moving around a lot more than usual	0	1	2	3
9. Thoughts that you would be better off dead or of hurting yourself in some way	0	1	2	3

FOR OFFICE CODING 0 + _____ + _____ + _____

=Total Score: _____

If you checked off any problems, how difficult have these problems made it for you to do your work, take care of things at home, or get along with other people?

Not difficult at all	**Somewhat difficult**	**Very difficult**	**Extremely difficult**
☐	☐	☐	☐

Developed by Drs. Robert L. Spitzer, Janet B.W. Williams, Kurt Kroenke and colleagues, with an educational grant from Pfizer Inc. No permission required to reproduce, translate, display or distribute.

13. Protocol Signature Page

Investigator Agreement

STUDY TITLE: Pilot Trial to Evaluate Blood and Imaging Based Biomarkers for Aromatase Inhibitor Induced Musculoskeletal Syndrome

By signing below I agree:

1) That my staff and I have read, understand and will adhere to the protocol as written, and that any changes to the protocol will be agreed to and approved by the Principal Investigator and the Institutional Review Board (IRB)

2) To abide by all obligations stated on the FDA Form 1572 and other documents required by regulation;

3) To conduct this study in accordance with the current International Conference on Harmonization (ICH) guidance, the Good Clinical Practices (GCP) guidance, the Declaration of Helsinki, US FDA regulations and local IRB and legal requirements;

4) To obtain IRB approval of the protocol, any amendments to the protocol, and periodic re-approval as required, and to keep the IRB informed of adverse events as required by their guidelines report the status of the study to them;

5) To ensure that each individual enrolled into the trial, or legally authorized representative, has read, understands, and has signed the Informed Consent form;

6) To ensure that I and all persons assisting me with the study are adequately informed and trained about the study and the possible adverse events associated with the study required medication

7) To make prompt reports of SAEs and deaths to the FDA according to the regulations;

8) To prepare and maintain adequate and accurate case histories to document all observations and other data pertinent to the study for each individual enrolled in the clinical trial.

_____ _____

Investigator Signature **Date**

Investigator Name (Print)

_____