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Altering Lipids for Tolerance of Aromatase Inhibitor Therapy

Omega-3 Fatty Acids, Oxylipins, and Tolerance of Aromatase Inhibitor Therapy

Protocol Number: UMCC 2019.125

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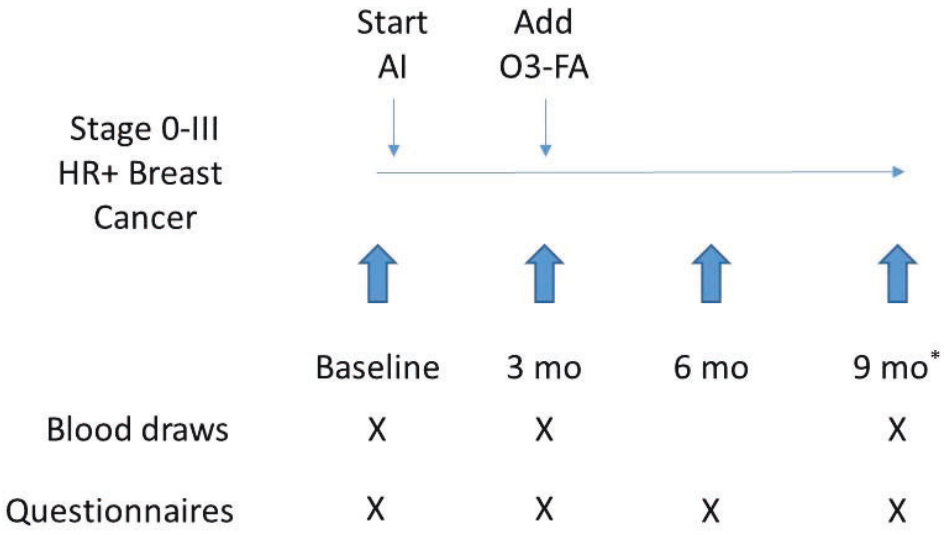
STUDY SUMMARY

Title	Omega-3 Fatty Acids (O3-FA), Oxylipins, and Tolerance of Aromatase Inhibitor Therapy
Short Title	Altering Lipids for Tolerance of AI (ALTA)
Protocol Identifiers (IRB – internal)	HUM00171150
IND number	Exempt
Phase	Phase 2
Design	Single arm phase II interventional trial
Study Duration	3 years
Study Center(s)	Single-center
Objectives	Primary Objective: To investigate the effects of O3-FA supplementation in the setting of concomitant AI therapy on oxylipin profiles Secondary Objectives: To investigate the effects of AI therapy with or without O3-FA supplementation on oxylipin profiles. To examine the effects of omega-3 fatty acids (O3-FA) supplementation on the proportion of patients who develop AIMSS within 9 months of AI initiation To examine the effects of O3-FA supplementation on the proportion of patients who discontinue AI therapy because of musculoskeletal toxicity within 9 months of AI initiation. To examine the effects of O3-FA supplementation on the proportion of patients who discontinue AI therapy because of toxicity within 9 months of AI initiation. Exploratory objectives: To examine the impact of estrogen deprivation with aromatase inhibition on stimulated inflammatory responses.

	To analyze associations between change in inflammatory responses and patient-reported symptoms, including pain measures. To explore changes in gene expression in inflammatory cells with change in symptoms.
Number of Subjects	78 (goal 68 evaluable patients)
Diagnosis and Main Eligibility Criteria (see section 5 for complete eligibility worksheet)	Inclusion Criteria: 1. Female subject aged ≥ 18 years who are postmenopausal according to standard clinical criteria or who have been receiving LHRH agonist therapy for at least 28 days prior to AI initiation 2. Stage 0-3 estrogen receptor positive (≥1%) and/or progesterone receptor positive (≥1%) breast cancer, or planned initiation of AI therapy for breast cancer chemoprevention 3. Planned initiation of aromatase inhibitor therapy (anastrozole, exemestane, or letrozole) for adjuvant treatment of breast cancer up to 30 days following baseline visit (ok to initiate screening up to 2 months before planned baseline visit) 4. Completion of surgery (mastectomy or lumpectomy/partial mastectomy) for treatment of breast cancer. 5. Completion of chemotherapy, if indicated. Concurrent use of radiation therapy, LHRHa therapy, anti-HER2 therapy, and CDK4/6 inhibitor therapy is permitted. Prior tamoxifen is permitted. 6. Agree to avoid taking omega-3 fatty acid supplements from sources outside the trial during study participation 7. Able to complete questionnaires in English. Exclusion Criteria: 1. Prior use of AI therapy for treatment or prevention of breast cancer 2. Use of omega-3 fatty acid supplementation during the 3 months prior to enrollment. Consumption of O3-FA through diet is permitted.

	3. Use of warfarin, enoxaparin, or direct oral anticoagulants during the 7 days prior to enrollment. 4. Known chronic liver disease. 5. Known symptomatic paroxysmal atrial fibrillation or persistent atrial fibrillation 6. History of pancreatitis 7. Hypersensitivity to fish and/or shellfish 8. Unable to take oral medications 9. Any medical condition that would interfere with the absorption of study medication capsules. 10. Patients with a prior or concurrent malignancy whose natural history or treatment does not have the potential to interfere with the safety or efficacy assessment of the investigational regimen are eligible for this trial.
Study Product, Dose, Route, Regimen	Omega-3 ethyl esters orally daily (containing 465mg eicosapentaenoic acid [EPA] and 375mg docosahexaenoic acid [DHA] per capsule, supplied as 4 x 1gm capsule)
Duration of administration	6 months
Reference therapy	N/A
Statistical Methodology	The primary endpoint is change in oxylipin profiles after 3 months of AI + O3-FA supplementation compared to baseline. 78 patients will provide 80% power (paired t-test) for detecting increases/decreases in lipid profiles of actual magnitude 0.34 standard deviations (of the baseline to follow-up changes) or larger, assuming <10% loss to follow-up (for 68 completing patients).

SCHEMA


Start AI
Add O3-FA
Stage 0-III HR+ Breast Cancer
Baseline
3 mo
6 mo
9 mo*
Blood draws
X
X
X
Questionnaires
X
X
X
X

1 OBJECTIVES

1.1 Primary Objective

To investigate the effects of O3-FA supplementation in the setting of concomitant AI therapy on oxylipin profiles.

Primary Endpoint: Oxylipin profiles at after 3 months of AI + O3-FA supplementation compared to after 3 months of AI therapy

1.2 Secondary Objectives

1. To investigate the effects of AI therapy with or without O3-FA supplementation on oxylipin profiles.

Secondary Endpoint 1: Oxylipin profiles at after 3 months of AI + O3-FA supplementation compared to baseline

Secondary Endpoint 2: Oxylipin profiles after 3 months of AI therapy compared to baseline

2. To examine the effects of omega-3 fatty acids (O3-FA) supplementation on the proportion of patients who develop AIMSS within 9 months of AI initiation

Secondary Endpoint 3: Incidence of AI-associated musculoskeletal symptoms (AIMSS) by 9 months of AI initiation

3. To examine the effects of omega-3 fatty acids (O3-FA) supplementation on the proportion of patients who discontinue AI therapy because of musculoskeletal symptoms within 9 months of AI initiation

Secondary Endpoint 4: Discontinuation of AI therapy because of musculoskeletal toxicity by 9 months

4. To examine the effects of omega-3 fatty acids (O3-FA) supplementation on the proportion of patients who discontinue AI therapy because of toxicity within 9 months of AI initiation

Secondary Endpoint 5: Discontinuation of AI therapy because of toxicity by 9 months

1.3 Exploratory Objectives

1. To examine the impact of estrogen deprivation with aromatase inhibition on stimulated inflammatory responses.

Exploratory Endpoint 1: Inflammatory cytokine and chemokine concentrations after 3 months of AI compared to baseline

2. To analyze associations between change in inflammatory responses and patient-reported symptoms, including pain measures.

   Exploratory Endpoint 2: Inflammatory cytokine and chemokine concentrations, pain severity, and AI discontinuation after 3 months of AI compared to baseline

3. To explore changes in gene expression in inflammatory cells with change in symptoms.

   Exploratory Endpoint 3: Gene expression levels, pain severity, and AI discontinuation after 3 months of AI compared to baseline

2 BACKGROUND

2.1 Hypothesis

Use of O3-FA supplementation in postmenopausal women with breast cancer who recently started treatment with AI therapy will result in increased tolerance of AI therapy, as evidenced by decreased development of AI-associated musculoskeletal pain and stiffness and decreased rates of AI treatment discontinuation due to toxicity.

2.2 Rationale and Background:

Aromatase inhibitors (AI), which significantly decrease circulating estrogen concentrations, are a key treatment for the >200,000 postmenopausal women diagnosed annually with hormone receptor-positive early stage breast cancer in the United States.[1, 2] Treatment with an AI for 5-10 years decreases 10-year breast cancer mortality by ~40%.[3] However, ~25% of AI-treated women discontinue therapy within the first year and half or stop treatment before 5 years because of intolerable joint and muscular pain and stiffness, or AI-associated musculoskeletal symptoms (AIMSS).[4] Premature discontinuation of therapy increases cancer recurrence and mortality.[5-7] Interventions to increase tolerance of AI therapy are needed.

The etiology of AIMSS remains poorly understood[4, 8-11] and there are few effective management options.[12-14] Postulated mechanisms include reductions in naturally nociceptive properties of estrogen and inflammation.[15] Risk factors include prior chemotherapy, younger age, and obesity.[16, 17] An exploratory analysis of a randomized, placebo-controlled trial (N=249) demonstrated that ~60% of patients with existing AIMSS treated with omega-3 fatty acid (O3-FA) supplementation experienced improvement in pain; obese patients obtained the most benefit (Figure 1).[18, 19] These findings suggest there may be underlying differences in AIMSS in obese patients, possibly related to the known association with systemic inflammation.[20]

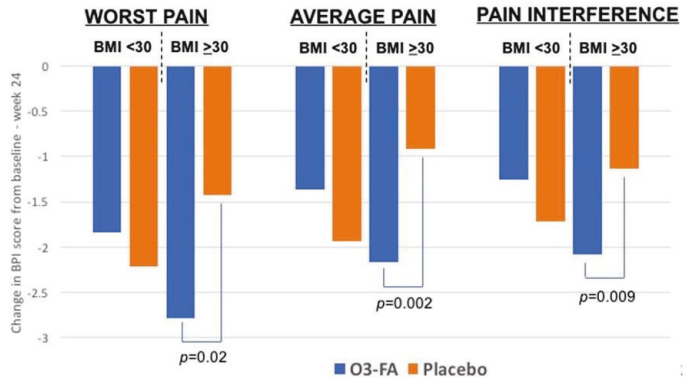


Figure 1. Analysis of SWOG S0927, omega-3 fatty acid (O3-FA) vs placebo for aromatase inhibitor-associated arthralgias, by body mass index (BMI). Results show change in Brief Pain Inventory (BPI) Worst Pain, Average Pain, and Pain Interference between baseline and 24 weeks of therapy, by study arm and BMI.

Quantitative profiles of plasma regulatory lipids can yield tremendous insights into health and medication toxicity, similar to the measurement of cytokines. Of particular interest, lipid species such as oxylipins (oxygenated FA metabolites) derived from dietary polyunsaturated fatty acids (PUFA) including omega-6 (O6)- and O3-FA are known to be pro- or anti-inflammatory mediators (Figure 2).[21-23] Oxylipins have been implicated in inflammation-related pain and pain signaling.[24-26] Additionally, estrogens can influence the systemic PUFA pool, in part through modulating the activity of enzymes that metabolize lipids.[27-30] The diversity of circulating oxylipins have rarely been evaluated with regards to AIMSS, and how dietary modification might influence these pathways is unclear.

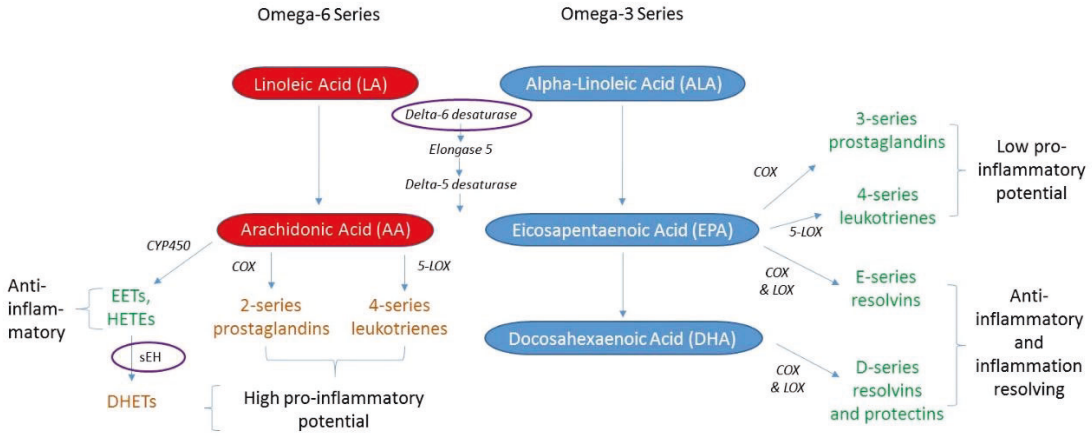


Figure 2. Overview of omega-3 and omega-6 fatty acid metabolism into pro- and anti-inflammatory metabolites. Delta-6 desaturase and soluble epoxide hydrolase (sEH) are both known to be modulated by estrogen. Anti-inflammatory mediators are shown in green, and pro-inflammatory mediators are shown in orange. COX: cyclooxygenase; CYP450: cytochrome P450; DHET: dihydroxyeicosatrienoic acid; EET: epoxyeicosatrienoic acid; HETE: hydroxyeicosatetraenoic acid; LOX: lipoxygenase.

We previously conducted a pilot case-control study of 50 patients with and without AIMSS using untargeted lipidomics, and identified quantitative differences in serum PUFA (Figure 3; unpublished data). This could reflect dietary differences between study groups or a differential response in PUFA metabolism related to estrogen effects,

inherited genetic variants or baseline levels of inflammation. Other researchers have identified differences in O6-derived oxylipin species in patients with and without AIMSS.[31] Taken together, these data suggest that oxylipins may play a role in the development of AIMSS, and importantly, may represent a modifiable risk factor for treatment-related toxicity.

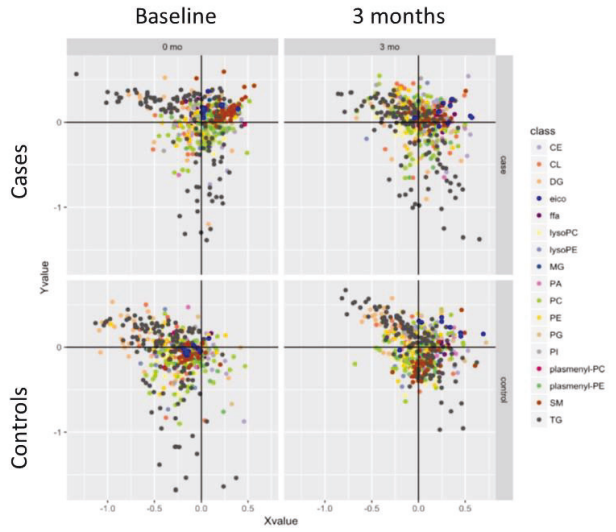


Figure 3. Pilot lipidomics analysis of aromatase inhibitor (AI)-treated patients with AIMSS (cases, n=25) and patients without (controls, n=25) at baseline and after 3 months. Assessed lipidomics at baseline and after 3 months of AI therapy. Controls had notably lower concentrations of polyunsaturated fatty acids (PUFA) at 3 months compared with baseline, possibly due to differences in estrogen-modulated PUFA metabolism or to inherited genetic differences between patients.

Our overall goal is to obtain a greater understanding of the etiology of AIMSS, and to identify interventions that can increase tolerance of and persistence with AI therapy. We hypothesize that oxylipins play a key role in the development of AIMSS, and that increasing O3-FA intake will alter O3- and O6-derived oxylipin production and reduce the incidence of this detrimental toxicity. To address this hypothesis, we will conduct a single-arm phase 2 trial of O3-FA in women who recently started AI therapy to see if O3-FA supplementation will improve tolerance of AI therapy. Through this study we will learn the effect of O3-FA on tolerance of AI therapy, defined as reduced incidence of AIMSS and AI discontinuation, the impact of AI therapy on oxylipins, and the influence of O3-FA on oxylipins in AI-treated women. These important insights into the etiology of AIMSS can enable personalized patient care.

Stimulated inflammatory responses. As noted above, both estrogen suppression and inflammation are thought to contribute to AIMSS development, although the actual mechanism remains unclear and may be due to an interaction of these factors.[4, 9-11, 32] Preclinical models of AI therapy have suggested potential estrogen-modulated effects on cytokine production leading to progression of inflammatory arthritis,[33] but cytokine analyses of previously collected samples from studies of AI-treated patients have demonstrated inconsistent results.[10, 34, 35] In addition, a genome-wide association study revealed a single nucleotide polymorphism linking AIMSS and T-cell maturation.[36] Studies have questioned whether there is a shared inflammatory mechanism underlying development of common symptoms such as fatigue, insomnia, pain, and mood changes in breast cancer survivors,[35, 37] although it is uncertain how estrogen deprivation directly impacts these symptoms.[35, 37] Interestingly, a similar symptom cluster has been associated with multiple chronic pain conditions.[38]

Studies of AIMSS reported to date have measured inflammatory markers in blood in their basal state. However, research in the field of chronic pain has demonstrated that measuring the inflammatory response when isolated immune cells or whole blood are challenged with noxious substances like lipopolysaccharide (LPS) can assess the degree to which circulating immune cells are primed to be pro-inflammatory. LPS activates immune activity along the toll-like receptor (TLR)-4 pathway, a highly-conserved element of the innate immune system.[39] Animal models have shown that this activation is critical for pain sensitization ,[40] and the stimulated immune response appears to be distinctly elevated in multiple chronic pain conditions compared to healthy controls.[41-44] Therefore, in this study we will also collect samples for AI-treated patients to further explore the inflammatory mechanisms underlying development of AIMSS with a particular focus on pro-inflammatory and chemotactic cytokines.

Assessment of AIMSS

No published scales have been established or validated to specifically measure the effects of treatments or preventative agents for AI-induced joint pain and stiffness. Therefore we have chosen to make sure of a combination of scales to assess the effect of O3-FA for improving tolerance of AI therapy. Patients will complete self-reported questionnaires including the Health Assessment Questionnaire (HAQ)[45] to assess functional status and pain and the Brief Pain Inventory-Short Form (BPI-SF)[46] to assess severity of joint pain/stiffness, and the Global Ratings of Change (GRC) scale to identify overall change in pain/stiffness since starting AI therapy. All of these measures have been used numerous times in prior studies of AIMSS incidence and treatment. To assess other symptoms related to endocrine therapy including functional well-being, patients will complete the Functional Assessment of Cancer Therapy-Endocrine Symptoms (FACT-ES).[47] Patients will also complete the Voils medication adherence questionnaire[48] to assess AI adherence in addition to the medication logs for the study drug. To assess amount of omega-3 and omega-6 dietary intake, patients will complete the Diet History Questionnaire III. Finally, patients will complete the Mao Expectation of Side Effects of Therapies (MESET) to assess expectation of side effects from AI therapy and O3-FA supplementation prior to initiation of each therapy.[49]

3 DRUG INFORMATION

3.1 Omega-3 fatty acids

3.1.1 Pharmacology

The mechanism of action of O3-FA to reduce pain is not completely understood. O3-FA have anti-inflammatory effects. They seem to suppress COX-2 expression and the inflammatory cytokines interleukin (IL)-1 alpha and tumor necrosis factor (TNF)-alpha. Other clinical research suggests O3-FA decrease endothelial activation by reducing intercellular adhesion molecule 1 (ICAM-1) and thrombomodulin levels, indicating a reduction in inflammation. O3-FA seem to be beneficial in rheumatoid arthritis due to anti-inflammatory effects, and

epidemiological data suggest EPA levels are decreased in total plasma fatty acids and synovial fluid, and DHA is decreased in the synovial fluid of patients with rheumatoid arthritis.

3.1.2 Physical and Chemical Properties

O3-FA are predominantly a combination of ethyl esters of eicosapentaenoid acid (EPA) and docosahexaenoid acid (DHA) used as add on treatment for patients with persistently high triglycerides. Taking O3-DA orally, alone or in combination with naproxen, seems to significantly decrease the duration of morning stiffness in patients with rheumatoid arthritis.

3.1.3 Pharmaceutical Properties and Formulation

Each 1-gram capsule of generic Lovaza contains at least 900 mg of the ethyl esters of O3-FA sourced from fish oils. These are predominantly a combination of ethyl esters of EPA (approximately 465 mg) and DHA (approximately 375 mg). The empirical formula of EPA ethyl ester is $C_{22}H_{34}O_2$, and the molecular weight of EPA ethyl ester is 330.51. The empirical formula of DHA ethyl ester is $C_{24}H_{36}O_2$, and the molecular weight of DHA ethyl ester is 356.55.

Pharmacokinetics: O3-FA are well absorbed orally and stored primarily in adipose tissue. Serum concentrations of EPA and DHA have been found to increase as dietary consumption increases. Elimination of O3-FA occurs primarily through oxidative catabolism to carbon dioxide and water though small quantities are eliminated when skin and digestive cells are sloughed. In healthy volunteers and in subjects with hypertriglyceridemia, EPA and DHA were absorbed when administered as ethyl esters orally. O3-FA administered as ethyl esters induced significant, dose-dependent increases in serum phospholipid EPA content, though increases in DHA content were less marked and not dose-dependent when administered as ethyl esters.

3.1.4 Clinical Safety

Adverse events reported in at least 3% and at a greater rate than placebo include: eructation (4%), dyspepsia (3%), taste perversion (4%). Additional adverse reactions include:

Digestive system: constipation, gastrointestinal disorder, and vomiting

Metabolic and nutritional disorders: increased ALT and AST

Skin: pruritus and rash

Drug Interactions – Some trials with O3-FA demonstrated prolongation of bleeding time. It has not exceeded normal limits and did not produce clinically significant bleeding episodes. No drug-drug interactions were identified between O3-FA and simvastatin, atorvastatin, or rosuvastatin. Clinically significant cytochrome P450-mediated inhibition by EPA/DHA combinations are not expected in humans.

4 STUDY DESIGN

4.1 Description

This will be a single arm phase 2 trial of O3-FA to examine the impact of the supplement on tolerance of AI therapy. In addition, there are correlative studies designed to examine the effect of AI therapy and the combination of AI and O3-FA on inflammatory lipid mediators and cytokines/chemokines.

4.2 Number of Patients

A total of 78 patients will be enrolled, assuming a 10% dropout rate for reasons other than AI toxicity, for a total of 68 evaluable patients. Patients who discontinue AI therapy prior to the 12 week assessment and who do not initiate O3-FA supplementation will be replaced.

4.3 Number of Study Centers

This will be a single center trial run at the Rogel Cancer Center at the University of Michigan.

4.4 Study Duration

The study is anticipated to be open for 3 years.

5 ELIGIBILITY CRITERIA

5.1 Inclusion Criteria
Yes/No (Response of "no" = patient ineligible)

5.1.1 _____ Female subject aged ≥ 18 years who are postmenopausal according to standard clinical criteria (see section 8.3) or who will have been receiving LHRH agonist therapy for at least 28 days prior to AI initiation.

5.1.2 _____ Stage 0-3 estrogen receptor positive (≥1%) and/or progesterone receptor positive (≥1%) breast cancer, or patients at high risk of developing breast cancer who are planning to initiate AI therapy for chemoprevention (see 5.1.3).

5.1.3 _____ Planned initiation of aromatase inhibitor therapy (anastrozole, exemestane, or letrozole) for adjuvant treatment of breast cancer or for chemoprevention up to 30 days following baseline visit (ok to initiate screening up to 2 months before planned baseline visit). Concurrent LHRH agonist, anti-HER2 directed therapy (e.g., trastuzumab, pertuzumab, ado-trastuzumab emtansine), and/or CDK4/6 inhibitor therapy (e.g., palbociclib, ribociclib, abemaciclib) is permitted. Prior tamoxifen and/or toremifene is permitted.

5.1.4 _____ Completion of surgery (mastectomy or lumpectomy/partial mastectomy) for treatment of breast cancer. Completion of axillary surgery as indicated (not required). For patients at high risk of breast cancer who have not been diagnosed with breast cancer, no surgery is required.

5.1.5 _____ Completion of chemotherapy, if indicated. Concurrent use of radiation therapy, LHRHa therapy, anti-HER2 therapy, PARP inhibitor, and CDK4/6 inhibitor therapy is permitted. Prior tamoxifen is permitted.

5.1.6 _____ Agree to avoid taking omega-3 fatty acid supplements from sources outside the trial during study participation.

5.1.7 _____ ECOG Performance Status ≤ 3.

5.1.8 _____ Able to complete questionnaires in English.

5.1.9 _____ Able to provide informed consent and willing to sign an approved consent form that conforms to federal and institutional guidelines.

5.2 Exclusion Criteria
Yes/No (Response of "yes" = patient ineligible)

5.2.1 _____ Prior use of AI therapy for treatment or prevention of breast cancer.

5.2.2 _____ Use of omega-3 fatty acid supplementation during the 3 months prior to enrollment. Consumption of O3-FA through diet is permitted.

5.2.3 _____ Use of warfarin, enoxaparin, or direct oral anticoagulants within 7 days prior to registration.

5.2.4 _____ Known chronic liver disease (laboratory studies will not be assessed). Patients with hepatosteatosis, viral hepatitis, or other liver disorders who have adequate liver function according to the treating physician are permitted to enroll.

5.2.5 _____ Known symptomatic paroxysmal atrial fibrillation or persistent atrial fibrillation (EKGs will not be performed).

5.2.6 _____ History of pancreatitis.

5.2.7 _____ Hypersensitivity to fish and/or shellfish.

5.2.8 _____ Unable to take oral medications.

5.2.9 _____ Any medical condition that would interfere with the absorption of study medication capsules.

5.2.10 _____ Patients with a prior or concurrent malignancy whose natural history or treatment does not, in the opinion of the treating investigator, have the potential to interfere with the safety or efficacy assessment of the investigational regimen are eligible for this trial.

6 STRATIFICATION FACTORS

N/A

7 TREATMENT PLAN

7.1 Omega-3 Fatty Acid (O3-FA) Supplements

7.1.1 How Supplied, Stored, Packaged and Labeled

Omega-3 fatty acid supplements (1 gm, generic for Lovaza) will be purchased from commercial supply and stored in the Investigational Drug Services pharmacy. The investigational pharmacy will dispense sufficient quantity for the dosing window at each visit.

7.1.2 Preparation and Administration

Patients should be instructed to take 4 capsules daily, either 4 capsules once daily at approximately the same time each day, or 2 capsules twice daily approximately 12 hours apart. Patients should take the capsules whole; do not chew, crush, dissolve, or open the capsule. If a dose is missed the patient should take it as soon as she remembers, although if it isn't until the next day she should not double up on dosing. If the patient is usually taking 2 capsules twice a day it is ok to take all 4 capsules at one time in the evening if she forgets to take the morning dose.

7.1.3 Accountability and Compliance

Study drug compliance during any period will be closely monitored by counting the number of capsules dispensed and returned. Every effort will be made to collect all unused study drug before dispensing new study drug at each relevant visit.

7.2 Concomitant Medications and Therapies

7.2.1 Allowed Therapy

Patients are permitted to take concomitant LHRH agonist therapy, anti-HER2 directed therapy, PARP inhibitor therapy, CDK4/6 inhibitor therapy, and/or anti-osteoclast therapy.

7.2.2 Prohibited Therapy

Patients must not take anticoagulants (warfarin, heparin, direct oral anticoagulants) during study treatment. Aspirin and NSAIDs are permitted.

Patients must refrain from taking over-the-counter omega-3 fatty acid supplements during study participation.

Patients with a history of hormone receptor positive breast cancer should avoid taking systemic or transdermal estrogen products. Vaginal estrogen preparations are permitted.

7.3 Duration of Therapy

Patients will be treated with the study drug for 24 weeks (starting at the week 12 visit).

7.3.1 Criteria for discontinuation of treatment ("off treatment")

The following will result in study treatment discontinuation:

- Discontinuation of AI therapy, defined as:
  - Discontinuation of initial AI therapy BEFORE the 12 week visit without restarting the same or a different AI medication at least 4 weeks before the 12 week study visit, or
  - Delay of 6 weeks or more of AI therapy starting after the 12 week visit
- Completion of 24 weeks of study drug
- Evidence of new cancer or cancer recurrence
- Unacceptable toxicity (see section 8.1)
- Delay of 30 consecutive days of study treatment (O3-FA) due to any reason
- The participant may discontinue study treatment (O3-FA) at any time for any reason

7.3.2 Criteria for discontinuation of study ("off study")

Subjects will be taken off study for the following:

- Participant requests to be withdrawn from study
- Non-compliance with study protocol activities
- Death

8 TOXICITIES AND DOSEAGE MODIFICATION

This study will utilize the CTCAE (NCI Common Terminology Criteria for Adverse Events) Version 5.0 for adverse event and serious adverse event reporting.

8.1 Dose Modifications and Guidelines for Adverse Event Management

8.1.1 Dose Modifications

Grade 1 and Grade 2 Toxicity

If grade 1 or 2 toxicities occur that are felt to be possibly, probably, or definitely related to study drug, monitor as needed until the problem has resolved (i.e., grade 0), stabilized (i.e., remains as grade 1 or grade 2), or is otherwise explained. If Grade 1 or Grade 2 symptoms persist, the PI or Co-I will assess whether symptom management should be initiated or whether the dose should be reduced from 4 capsules per day to 3 capsules per day. Follow-up will be conducted at the intervals specified in the protocol.

Grade 3 or Grade 4 Toxicity

If the patient develops any Grade 3 toxicity possibly, probably, or definitely related to the study drug, the patient should go on a drug holiday.

- If the symptoms resolve or improve to grade 1 or 2 within 7 days, the dose should be reduced from 4 capsules per day to 3 capsules per day. This dose is to be maintained if no further Grade 3 toxicity is noted.
- If the symptoms do not resolve or improve to grade 1 or 2 within 7 days, the patient can continue to hold study drug for another 7 days. If the symptoms resolve or improve to grade 1 or 2, the dose should be reduced from 4 capsules per day to 3 capsules per day. This dose is to be maintained if no further Grade 3 toxicity is noted. If the symptoms remain Grade 3 possibly, probably, or definitely related to the study drug, the patient should be permanently removed from protocol treatment. Follow-up will continue at the intervals specified in the protocol.

If at the 3 capsules per day dose the patient again experiences Grade 3 toxicity, the patient can be given a 1-week drug holiday from the study drug. Subsequently the patient may be rechallenged at the 3 capsule per day dose. If symptoms do not recur (i.e., if symptoms resolve to grade 0), the dose should be maintained. Alternatively, if Grade 1 or Grade 2 symptoms occur, the PI or Co-I will assess whether symptom management should be initiated or whether the patient should be removed from protocol treatment. Follow-up will continue at the intervals specified in the protocol.

If the patient develops any Grade 4 toxicity possibly, probably, or definitely related to the study drug, the patient should be permanently removed from protocol treatment. Follow-up will continue at the intervals specified in the protocol.

8.2 Supportive Care

All supportive measures consistent with optimal patient care may be given throughout the study.

8.3 Contraception

Only post-menopausal women (including those rendered postmenopausal with LHRH agonist therapy) are eligible for enrollment on this clinical trial. Postmenopausal is defined as one or more of the following:

- Absence of menses for at least 12 months, without an intervention that could have caused amenorrhea (hysterectomy without bilateral salpingo-oophorectomy, chemotherapy within 12 months of final menstrual cycle, or current treatment with tamoxifen)
- Concurrent use of LHRH agonist therapy
- Prior bilateral salpingo-oophorectomy
- Serum or plasma concentrations of estradiol within postmenopausal range according to local lab values

9 STUDY CALENDAR

Required Studies	Screening [a]	Baseline [b]	Week 12 (+/- 2 wk) [c,g]	Week 24 (+/- 4 wk) [c]	Week 36 (+/- 4 wk) [c]	Off Study (if prior to week 36) [d]
Informed consent	X					
Medical History	X					
Performance status	X					
Vital signs [e]		X	X	X	X	X
Concomitant medications		X	X	X	X	X
Adverse event assessment			X	X	X	X
Patient reported questionnaires [f]						
- HAQ and BPI		X	X	X	X	X
- FACT-ES		X	X	X	X	X
- MESET-AI		X				
- MESET-O3			X			
- BMQ		X			X	X[g]
- DHQIII		X				
- GRC			X	X	X	X
- AI medication questionnaire			X	X	X	X
Phlebotomy [h]						
- Research		X	X	X		X
- LFTs			X		X	X[g]
- Lipid panel			X		X	X[g]
AI therapy (standard of care)[g]		→	→	→	→	
Dispense study medicine			X	X		
O3-FA treatment [i]			→	→	→	
Medication log [k]		X	X	X		
Reasons for AI discontinuation form [j]			X	X	X	X

a. Screening and baseline visits can take place on the same day. History and performance status assessment should take place at the time of baseline visit or up to 2 months before at the in person or virtual screening visit.
b. Baseline visit should take place before AI therapy is started. It can take place up to 30 days prior to planned AI therapy initiation. Patients can be receiving radiation therapy at the time of the baseline visit.
c. Timed based on initiation of AI therapy.
d. This is the in person or virtual visit conducted at the time of permanent AI discontinuation and/or study drug discontinuation. These procedures should be completed within 3 business days of medication discontinuation.

e. Height and weight at baseline visit, weight at all subsequent visits. If patient is seen in a virtual visit, these values can be patient-reported.
f. Questionnaires other than the DHQIII can be completed electronically within 3 days before the scheduled visit, at the time of the visit, or within 7 days after the visit. It is preferable to have patients complete the questionnaires before or at each visit. Emailed reminders will be sent to patients from REDCap, and telephone reminders may also be used. If a scheduled visit is rescheduled after the questionnaires have been completed by the patient, they will not considered to be out of window. The DHQIII can be completed at the baseline visit +/- 4 weeks. The DHQIII must be completed electronically; the remainder can be completed either electronically or on paper.
g. Patients who discontinue AI therapy BEFORE the 12 week visit and do not restart the same or a different AI medication at least 4 weeks before the 12 week visit will discontinue study participation and will not start treatment with the study medication. These patients should complete the off study visit procedures except for BMQ, lipids, and LFTs. These patients will be replaced.
h. It is preferred, but not required, that subjects fast for at least 8 hours prior to all blood collections. Time of blood draw and fasting status should be recorded. Details about research labs are in section 15.2.1. Liver function tests and lipid panel are standard of care.
i. Study medication should start 12 weeks (+/-2 weeks) after AI initiation. Pill counts should be done at each study visit (weeks 24-36).
j. To be completed by study coordinator at the time the patient discontinues AI therapy (if the patient discontinues any AI therapy medication, not just permanently discontinuing all AI therapy).
k. Medication log can be sent electronically to patients either via email or through the portal, or via mail.

10 STUDY PROCEDURES

10.1 Screening Evaluations

A review of inclusion/exclusion criteria will be conducted to determine the patient's eligibility for enrollment. Study procedures will be reviewed with the patient, and documentation of informed consent will be obtained. After signing the informed consent form (ICF), patients will be assigned a unique study ID number in sequential order.

The following medical history elements will be abstracted from the medical record: date of breast cancer diagnosis, tumor information (e.g., tumor histology, receptor status), treatment information (e.g., surgery type and dates, chemotherapy doses and dates, radiation therapy doses and dates), information about other medical co-morbidities. Because the COVID-19 virus has been associated with arthralgias, will abstract information about both COVID-19 infection and vaccination (including dates) from the medical record. A patient's email address and phone number will also be recorded.

10.2 On Treatment Evaluations

See study calendar (section 9) for complete details of on treatment evaluations. The following is only a very brief description of what should take place at each visit.

Baseline Visit:

Baseline Visit (week 0) will be conducted 0 to 2 months after Screening, before initiation of AI therapy. It can occur up to 30 days before planned initiation of AI therapy. At this visit, patients will undergo blood draw, have height and weight measured, and complete questionnaires (other than the DHQIII) if they haven't been completed within 3 days before the visit. The baseline visit can be conducted virtually, as long as the patient can come in to the clinic for a fasting blood draw within 2 days of the baseline visit. If a patient is not seen physically in clinic at the time of the baseline visit, she can self-report height and weight. If a patient hasn't completed the questionnaires and is unwilling to remain in clinic to complete them, she has up to 7 days following the visit to complete them. Email reminders and/or phone calls will be used to remind patients.

The DHQIII can be completed +/- 4 weeks of this visit and should be completed by patients electronically outside of the clinic. Patients will be sent a unique URL to access the DHQIII questionnaire. The only potential PHI included in the DHQIII is patient age. If a patient does not have access to a computer with internet access, failure to complete the questionnaire will not be considered a deviation.

At this visit, patients should be reminded to report all symptoms and limitations on the questionnaires, whether or not they are related to the cancer or its treatment. They should also be told that their treating provider will not be told about symptoms that they report on the questionnaires, and they should mention them during their visit or contact their provider between visits.

- Patients will start taking standard of care AI therapy within 30 days following this visit. Study coordinator should contact the patient by phone to confirm which AI medication she is taking and the date she started AI therapy.

12 Week Visit:

Week 12 Visit will be conducted 12 weeks +/- 2 weeks after AI initiation. At this visit, patients will undergo blood draw, complete questionnaires if they haven't been completed within 3 days before the visit, and be given prescription bottles containing a sufficient supply of study drug for the treatment period, as well as the medication log. If a patient hasn't completed the questionnaires and is unwilling to remain in clinic to complete them, she has up to 7 days following the visit to complete them. Email reminders and/or phone calls will be used to remind patients.

If the patient has stopped taking the initial AI therapy by the time of this visit and did not restart AI therapy at least 4 weeks prior to the visit, she should complete the off study procedures (study calendar, section 9) within 3 business days of stopping the AI medication if possible but then should discontinue study participation and should not start the study medication. (It will not be a deviation if the blood draw and questionnaires are not completed within 3 days.) These patients will be replaced.

- Study coordinator should contact the patient by phone within 3 business days of the visit to confirm that she started the study medication (and the date)

24 Week Visit:

Week 24 Visit will be conducted 24 weeks +/- 4 weeks after AI initiation, or sooner as "End of Treatment Visit" if patient discontinues study medication according to criteria in Section 7.5.1 or permanently discontinues all AI medication (switches to tamoxifen or stops all endocrine therapy). In the setting of AI discontinuation, visit should preferably occur within 1 week of AI discontinuation if possible, and questionnaires should be sent to the patient for completion as soon as notified about AI discontinuation (ok if >3 days before visit).

At this visit, patients will undergo blood draw, complete questionnaires if they haven't been completed within 3 days before the visit, and be given prescription bottles containing a sufficient supply of study drug for the treatment period, as well as the medication log. If a patient hasn't completed the questionnaires and is unwilling to remain in clinic to complete them, she has up to 7 days following the visit to complete them. Email reminders and/or phone calls will be used to remind patients.

- Collect study medication log and perform pill count
- Dispense study medication and new medication log

10.3 End of Treatment Evaluations

Week 36 Visit will be conducted 36 weeks +/- 4 weeks after AI initiation, or sooner as "End of Treatment Visit" if patient discontinues study medication according to criteria in Section

7.5.1 or permanently discontinues all AI medication (switches to tamoxifen or stops all endocrine therapy). In the setting of AI discontinuation, visit should preferably occur within 1 week of AI discontinuation if possible, and questionnaires should be sent to the patient for completion as soon as notified about AI discontinuation (ok if >3 days before visit).

At this visit, patients will complete questionnaires if they haven't been completed within 3 days before the visit and undergo blood draw. If a patient hasn't completed the questionnaires and is unwilling to remain in clinic to complete them, she has up to 7 days following the visit to complete them. Email reminders and/or phone calls will be used to remind patients.

- Collect study medication bottles and medication log and perform pill count

10.4 Follow-up Evaluations

Study participation is complete following the end of study visit. Patient data on endocrine therapy use will be abstracted from the medical record 15 months (+/- 3 months) and 27 months (+/- 3 months) following end of study visit.

10.5 Follow-up Evaluations

Health Assessment Questionnaire (HAQ): The HAQ is a well-validated tool that has been used extensively to evaluate patients with rheumatic disorders for the past two decades.[94] The 2-page HAQ includes the HAQ disability index (HAQ-DI) and visual analog scales (VAS) to assess both pain and global health status. In the HAQ-DI, each of eight activities (dressing, rising, eating, walking, grooming, reaching, gripping, and performing errands) is scored as 0 (no difficulty), 1 (some difficulty), 2 (much difficulty), or 3 (unable to do), and the scores are averaged across the eight activities. Average scores for the general population range from 0.25-0.49, whereas for patients with osteoarthritis the average score is 0.80 and for rheumatoid arthritis it is 1.20.[95, 96] Many studies have suggested that an increase of 0.22 is the Minimal Clinically Important Difference.[97] The questionnaire was used in the ELPh trial to assess women with AIMSS. In this study it will be administered at baseline and after 3 and 6 months of treatment with an AI and will be used to provide objective information regarding changes in patients' function and symptoms with AI therapy.

Brief Pain Inventory (BPI): The BPI is a 17-item patient self-rating scale that assessed sensory and reactive components of pain.[46] For sensory components, it addresses severity, location, chronicity, and degree of relief due to therapy. For reactive components, it assesses depression, suffering, and perceived availability of relief. Reliability has been demonstrated over short intervals using test retest item correlation; worst pain, r=0.93, usual pain, r=0.78, pain now r=0.59. It has been validated in patients with both cancer and non-cancer pain.[50, 51] Ratings of pain interference with various activities increased as ratings of pain severity were higher. The proportion of patients receiving opioid analgesics also increased with increased severity rating.

The BPI uses 0 to 10 numeric rating scales for item rating because of its simplicity and lack of ambiguity. Since pain can be variable over a day, the BPI asks patients to rate their pain at the time of completing the questionnaire, and also at its worst, least, and average over the

previous 24 hours. The primary endpoint for this clinical trial will be based on the 24-hour average pain as reported on the BPI. The ratings can be combined to give a composite index of pain severity. Also, using numeric 0 to 10 scales, with 0 being "no interference" and 10 being "interferes completely", the BPI asks for ratings of the degree to which pain interferes with mood, walking and other physical activity, works, social activity, relations with others, and sleep. The mean of these scores can be used as a pain interference score.

Global Ratings of Change (GRC) Scale: This is a single-item measure to assess the overall change in pain and stiffness since stopping AI therapy. It is a 7-point Likert scale from -3 to +3.

Functional Assessment of Cancer Therapy-Endocrine Symptoms (FACT-ES): The FACT-ES Trial Outcomes Index (TOI) will be used to measure ET side effects. The FACT-ES includes the general 27-item FACT-General (FACT-G) questionnaire, which includes the Physical, Functional, Social, and Emotional Well-Being subscales and the 19-item endocrine symptom subscale (ES). The FACT-ES TOI score is the sum of the physical and functional well-being domains as well as ES, with higher scores representing better overall quality of life and fewer symptoms.[47] The FACT-ES has been shown to have acceptable validity and reliability, with alpha coefficients for all subscales of 0.65-0.87 and test-retest reliability of 0.93.[47] Higher scores reflect better HRQoL, and a change of 5 points is considered clinically relevant.[47]

Mao Expectancy of Side Effects of Treatment (MESET): The MESET is a 4-question patient-reported instrument rated on a scale of 1 to 5 (1 is total disagreement, 5 is total agreement) with three normally coded items and one reverse-coded item. The scale assesses a patient's expectation that she will experience side effects during the course of a specific treatment. Patients will be asked about expectancy prior to starting AI therapy and to starting O#-FA therapy. The MESET had adequate internal consistency in a prior study of chamomile (Cronbach's alpha 0.72).[49]

Brief Medication Questionnaire (BMQ)-Specific: Two five-item scales assessing patients' beliefs about the *necessity* of a prescribed medication and their *concerns* about the potential adverse consequences of taking it. The BMQ has been tested in a wide variety of patient populations and is a valid and reliable measure of medication beliefs.[52-55] The BMQ-Specific uses a 1 to 5 Likert-scale from "strongly disagree" to "strongly agree". Scores obtained for the individual items within each scale are summed to give a total score for the *necessity* (α=0.87) and *concerns* (α=0.78) scales each, ranging from 5 to 25.[53] A higher score on specific-necessity indicates stronger beliefs about the necessity of treatment, and a higher score on specific-concerns indicates stronger concerns. The necessity-concerns differential score can be calculated by subtracting the specific-concerns scale from the specific-necessity scale (range -20 to 20).[53-55]

Diet History Questionnaire III (DHQIII): The DHQIII is a patient-reported, web-based, freely available food frequency questionnaire (https://epi.grants.cancer.gov/dhq3/index.html). Patient complete questions asking about consumption of 135 food and beverages and 26 dietary supplements during the prior month. Nutrients, dietary constituents, and food groups are calculated, including intake of omega-3 fatty acids, PUFAs, and polyunsaturated to saturated fatty acid ratio. The Health Eating Index-2015 reflecting diet quality can be

calculated from the data.

Voils Adherence Measure: The Voils self-reported adherence instrument contains 3 items that assess the extent of nonadherence over the past week using a 5-item scale (higher levels indicate greater nonadherence), and additional items to assess reasons for nonadherence using a 5-item scale (ranging from not at all to very much).[48] The extent items produced reliable scores in a sample of patients with hypertension (Cronbach alpha 0.84), and were significantly correlated with blood pressure and had evidence of convergent and discriminant validity.

11 CRITERIA FOR EVALUATION AND ENDPOINT

11.1 Safety

Routine safety and tolerability will be evaluated from the results of reported signs and symptoms and vital sign measurements. More frequent safety evaluations may be performed if clinically indicated or at the discretion of the investigator.

Performance Status Assessment

Performance status assessment at screening will be performed by a licensed physician (or physician's assistant or nurse practitioner).

11.2 Stopping Rules

N/A

12 STATISTICAL CONSIDERATIONS

12.1 Statistical hypothesis

Use of O3-FA supplementation in postmenopausal women with breast cancer who recently started treatment with AI therapy will result in alterations in oxylipin profiles. As a secondary hypothesis, use of O3-FA supplementation and specific oxylipin profiles will be associated with tolerance of AI therapy.

12.2 Sample size determination

Enrollment of 78 patients would provide at least 80% power (paired t-test) for detecting increases/decreases in lipid profiles of actual magnitude 0.34 standard deviations (of the baseline to follow-up changes) or larger, assuming <10% loss to follow-up (for 68 completing patients).

12.3 Statistical Analyses

12.3.1 Primary endpoint

To analyze effects of estrogen deprivation on change in oxylipins, we will compare PUFA composition in baseline (on AI) and follow-up (on combination) samples from AI and O3-FA supplement treated patients. Analyses will be based on multiple

imputation handling of missing data.[56] Only oxylipins which exceed the limit of quantification in ≥95% of each sample set will be analyzed. Differences between time points within groups will be analyzed by *t*-test for paired samples. To compare their relative distribution, oxylipins will be grouped by substrate, and the sum of the median values of the analyzed oxylipins from each long-chain PUFA (18:2/n-6, 18:3/n-3, 20:4/n-6, 20:5/n-3 and 22:6/n-3) will be divided by the sum of the medians for all oxylipins.[57] For this analysis, 78 patients treated with AI will provide 80% power (paired t-test) for detecting increases/decreases in lipid profiles of actual magnitude 0.34 standard deviations (of the baseline to follow-up changes) or larger, assuming <10% loss to follow-up. Since this study is a mechanistic study and the results are hypothesis generating the results will be primarily presented in raw form not adjusted for multiple comparisons, although a post-hoc multiple comparisons adjustment such as Bonferroni or Benjamini-Hochberg may be applied depending on the target interpretation.

12.3.2 Secondary endpoints

1. To analyze effects of estrogen deprivation on change in oxylipins, we will compare PUFA composition in baseline (before AI) and follow-up (either on AI alone or on combination) samples. Analyses will be based on multiple imputation handling of missing data.[56] Only oxylipins which exceed the limit of quantification in ≥95% of each sample set will be analyzed. Differences between time points within groups will be analyzed by *t*-test for paired samples. To compare their relative distribution, oxylipins will be grouped by substrate, and the sum of the median values of the analyzed oxylipins from each long-chain PUFA (18:2/n-6, 18:3/n-3, 20:4/n-6, 20:5/n-3 and 22:6/n-3) will be divided by the sum of the medians for all oxylipins.[57] For this analysis, 78 patients treated with AI will provide 80% power (paired t-test) for detecting increases/decreases in lipid profiles of actual magnitude 0.34 standard deviations (of the baseline to follow-up changes) or larger, assuming <10% loss to follow-up. Since this study is a mechanistic study and the results are hypothesis generating the results will be primarily presented in raw form not adjusted for multiple comparisons, although a post-hoc multiple comparisons adjustment such as Bonferroni or Benjamini-Hochberg may be applied depending on the target interpretation.

2. Development of AIMSS. The HAQ assesses interference of pain with daily activities (range 0-3), with change of 0.22 defined as a clinically meaningful difference. The BPI assesses average pain over 7 days (range 0-10), with a change of 2.0 defined as a clinically meaningful difference. A patient will be considered to have developed AIMSS if there is (1) a ≥0.22 increase in HAQ score within 9 months, (2) a ≥2.0 increase in BPI average pain within 9 months, or (3) discontinuation of AI therapy within 9 months because of new or worsened musculoskeletal symptoms. From previous studies, it is estimated that 50-60% of patients will develop AIMSS within 1 year of AI initiation.
3. Discontinuation of AI therapy because of AIMSS. If the patient or the provider lists pain as the first or second-ranked reason for discontinuation, this will be considered an event. Patients lost to follow-up or with unknown reason for

discontinuation will be considered event-free. A sensitivity analysis based on multiple imputation, which requires that missing data be missing-at-random, will also be performed.[56] Additionally, results will be examined in obese/non-obese subgroups, as well as by baseline concentrations of EPA and DHA. Data on potential confounders will also be collected for post-hoc analysis of confounder distribution.

4. Discontinuation of AI therapy because of any toxicity. If the patient or the provider lists any toxicity as the first or second-ranked reason for discontinuation, this will be considered an event. Patients lost to follow-up or with unknown reason for discontinuation will be considered event-free. A sensitivity analysis based on multiple imputation, which requires that missing data be missing-at-random, will also be performed.[56] Additionally, results will be examined in obese/non-obese subgroups, as well as by baseline concentrations of EPA and DHA. Data on potential confounders will also be collected for post-hoc analysis of confounder distribution.

12.3.3 Exploratory endpoints

1. To examine the impact of estrogen deprivation with aromatase inhibition on stimulated inflammatory responses. Cytokine/chemokine data often does not follow a normal distribution, which can create issues when using parametric statistical approaches. Therefore, we will evaluate normality with the Shapiro-Wilk test statistic and apply Box-Cox transformations where required. Any values below the lower limit of detection will be set to half that value. In addition to looking at each cytokine/chemokine independently, we will also create a composite score for the NF-κB-mediated pro-inflammatory suite. This will be calculated as the averaged z-scores ((mean-value)/standard deviation) of IL-1β, IL-6, TNF-α).[43, 58] We will create separate composite score for the LPS and null conditions, reflecting the pro-inflammatory response along the TLR4 and control pathways. With 50 patients, assuming a two-sided 0.05 type I error, we have 80% power to detect a medium standardized effect size (units of standard deviation) of 0.4 in the change of cytokines from baseline to 3 months. The level of each cytokine at baseline and 3 months and the change in cytokine from baseline to 3 months will be summarized using descriptive statistics (i.e. mean, standard deviation, median and range). The change in cytokines will be analyzed using a one sample t-test (with corresponding transformation) or a sign test if no normalizing transformation can be achieved. We will perform multi-level models with a random effect for batch if there are significant batch effects. We will not control type I error across the multiple cytokines or PROs in this analysis as it is hypothesis generating.

2. To analyze associations between change in inflammatory responses and patient-reported symptoms, including pain measures. With 50 patients where 10-12 are estimated to have AIMSS assuming a two-sided 0.05 type I error, we have 80% power to detect a large effect size of 0.95-1 standard deviation units difference in cytokines between the two groups. The change in cytokines will be compared between those with and without AIMSS using a two-sample t-test with normalizing

transformation or Wilcoxon rank sum test if no normalizing transformation can be achieved. We will perform multi-level models with a random effect for batch if there are significant batch effects. We will assess the correlation between changes in cytokines with changes in PROs using Spearman correlation or multi-level models to control for batch effects. We will not control type I error across the multiple cytokines or PROs in this analysis as it is hypothesis generating.

3. To explore changes in gene expression in inflammatory cells with change in symptoms, RNA-seq will be performed using the MiSeq system. Sequenced reads will be assessed for quality and cleaned, and then mapped to the human genome with STAR version 2.4.2a. RNA reads will be normalized and differential gene expression will be determined using DESeq2-1.10.1. Correction for multiple testing will be performed using adjustment for a false discovery rare (FDR) of 0.05. We will use qRT-PCR to confirm gene expression changes in 10% of the most mechanistically plausible differentially expressed genes detected by RNA-seq, with emphasis on genes that promote or regulate inflammation. We will then perform extensive bioinformatics analyses to identify common pathways and gene ontologies unifying annotated genes and gene regions that are differentially regulated in women whose do and do not develop pain on AI therapy.

13 REGISTRATION GUIDELINES

Study related screening procedures can only begin once the patient has signed a consent form.

Patients must meet all of the eligibility requirements listed in Section 5 prior to registration.

Patients must be registered before receiving any study treatment and must begin study treatment 12 weeks +/-2 weeks of starting AI therapy (at the time of the 12 week visit). See study calendar (section 9) for details about windows between Screening and Baseline Visits and between Baseline Visit and start of study treatment.

14 DATA SUBMISSION SCHEDULE

The Case Report Forms (CRFs) are a set of (electronic or paper) forms for each patient that provides a record of the data generated according to the protocol. These forms will be completed on an on-going basis during the study. The medical records will be source of verification of the data. The CRFs will be completed by the Investigator or a member of the study team as listed on the Delegation of Duties Log.

15 SPECIAL INSTRUCTIONS

15.1 Patient-reported questionnaires

Patients will complete patient-reported questionnaires within 3 days before the scheduled visit, at the time of each in person or virtual clinic visit, or within 7 days after each clinic visit using RedCap and the DHQIII website (dhq3.org), as per the study calendar. Prior to the first time the patient completes the questionnaires: Patients should be directed to report all

symptoms and limitations whether or not they are related to the cancer or its treatment. They should also be told that their treating provider will not be told about symptoms that they report on the questionnaires, and they should mention them during their visit.

When patients are completing them at the time of the clinic visit, it is permissible to assist patients with completing the questionnaires being careful not to influence the patient's response. Discourage family members from influencing patient responses to the questions.

The study coordinator will review the questionnaires after completion on paper to ensure that no questions were accidentally skipped or that more than one answer was provided. Whenever possible, questionnaires will be completed online, although if a patient states that she is unable to complete the questionnaire online then a paper form will be provided for all except for the DHQIII, which must be completed online. If a patient does not have access to a computer with internet access, failure to complete the DHQIII questionnaire will not be considered a deviation.

If a patient is unwilling to complete the questionnaires at the time of the clinic visit and has not yet done so within 2 working days following the visit, the study coordinator will follow-up with the patient by phone within 5 working days of the visit to remind her to complete the questionnaires. The questionnaires should be completed electronically, or the patient should be given a paper copy to complete at home and return by mail. They should be completed within 7 days after each clinic visit.

15.2 Correlative Studies

15.2.1 Blood correlative studies

The following samples will be collected:

A. 10 cc blood will be collected for isolation of plasma at baseline, 12 week, and 24 week or end of study visits, and stored in 500 ul aliquots.

B. Blood will be collected in TruCulture system 1 ml tubes containing either LPS or media at baseline and 12 weeks, immediately incubated at 37C for 24 hours, then the supernatant will be isolated and stored in 250 ul aliquots.

C. Blood will be collected in PAX gene 2.5 ml tubes at baseline and 12 weeks, for isolation of total RNA.

Patients will fast for at least 8 hours prior to phlebotomy whenever possible. The study coordinator will record time of blood draw and whether or not patient was fasting.

At times of research restrictions due to pandemic or other causes, only the plasma samples will be collected in order to limit the need for complex sample processing. This will not be considered a deviation, and will be recorded on the CRF.

16 ETHICAL AND REGULATORY CONSIDERATIONS

16.1 Informed consent

Informed consent will be obtained from all research participants prior to performing any study procedures using the most recent IRB-approved version.

16.2 Human Subjects Protections

16.2.1 Rationale for Subject Selection

This clinical trial is open to all patients starting AI therapy with minimal restrictions in order to permit inclusion of as broad a population as possible. Only patients who read English will be enrolled because not all of the patient reported questionnaires have been linguistically validated in other languages.

16.2.2 Participation of Children

Patients must be at least 18 years of age to participate.

16.3 Institutional Review

This study will be approved by the Institutional Review Board of the University of Michigan.

16.4 Data and Safety Monitoring Plan

This study will be monitored in accordance with the NCI approved University of Michigan Rogel Cancer Center Data and Safety Monitoring Plan.

The study team will meet every six months or more frequently depending on the activity of the protocol. The discussion will include matters related to the safety of study participants (SAE/UaP reporting), validity and integrity of the data, enrollment rate relative to expectations, characteristics of participants, retention of participants, adherence to the protocol (potential or real protocol deviations) and data completeness. At these regular meetings, the protocol specific Data and Safety Monitoring Report form will be completed and signed by the Principal Investigator or by one of the co-investigators.

Data and Safety Monitoring Reports will be submitted to the University of Michigan Rogel Cancer Center Data and Safety Monitoring Committee (DSMC) every six months for independent review.

16.5 Adverse Events and Serious Adverse Events

This study will utilize the CTCAE (NCI Common Terminology Criteria for Adverse Events) Version 5.0 for AE and SAE reporting.

16.5.1 Adverse Events (AEs)

An adverse event is the appearance or worsening of any undesirable sign, symptom, or medical condition occurring after starting the study drug even if the event is not considered to be related to study drug. For the purposes of this study, the terms toxicity and adverse event are used interchangeably. Medical conditions/diseases present before starting study drug are only considered adverse events if they worsen after starting study drug. Abnormal laboratory values or test results constitute adverse events only if they induce clinical signs or symptoms, are considered clinically significant, or require therapy.

Collection of adverse events will begin after the first dose of study drug and end 30 days after the last dose of study drug.

Information about all adverse events except those listed below, whether volunteered by the subject, discovered by investigator questioning, or detected through physical examination, laboratory test or other means, will be collected and recorded and followed as appropriate. AEs related to the following will not be collected or reported:

1. Symptoms and signs due to AI therapy (will be collected on patient-reported questionnaires), except those possibly due to study drug (nausea, vomiting, diarrhea, constipation, abdominal pain, dyspepsia, change in breath or taste, eructation, rash)
2. Symptoms and signs due to prior treatments (chemotherapy, radiation therapy, surgery)
3. Adverse events related to disease progression
4. Hospitalization or treatment related to breast surgery or breast reconstruction procedures

The occurrence of adverse events should be sought by non-directive questioning of the patient at each visit or phone contact during the study. Adverse events also may be detected when they are volunteered by the patient during or between visits or through physical examination, laboratory test, or other assessments. Adverse events can be collected by the clinical research coordinators, PI, or Co-Is. As much as possible, each adverse event should be evaluated to determine:

1. The severity grade based on CTCAE v5.0
2. Its relationship to the O3-FA (definite, probable, possible, unlikely, not related)
3. Its duration (start and end dates or if continuing at final exam)
4. Action taken (no action taken; study drug dosage adjusted/temporarily interrupted; study drug permanently discontinued due to this adverse event; concomitant medication taken; non-drug therapy given; hospitalization/prolonged hospitalization)
5. Whether it constitutes an SAE

Attribution and relatedness will be determined by the PI or Co-Is. All adverse events will be treated appropriately. Such treatment may include changes in study drug treatment as listed in the dose modification section of this protocol (see section 8 for guidance). Once an adverse event is detected, it should be followed until its resolution, and assessment should be made at each visit (or more frequently, if necessary) of any changes in severity, the suspected relationship to the study drug, the interventions required to treat it, and the outcome.

Information about common side effects already known about O3-FA are described in the Drug Information section (section 3.1.4) and in the current product insert for Lovaza. This information will be included in the patient informed consent and will be discussed with the patient during the study as needed.

All adverse events (except those noted above) will be immediately recorded in the patient research chart.

16.5.2 Serious Adverse Event (SAE)

Information about all serious adverse events will be collected and recorded. A serious adverse event is an undesirable sign, symptom or medical condition which:

- Is fatal or life-threatening
- Results in persistent or significant disability/incapacity
- Is medically significant, i.e., defined as an event that jeopardizes the patient or may require medical or surgical intervention to prevent one of the outcomes listed above
- Causes congenital anomaly or birth defect
- Requires inpatient hospitalization or prolongation of existing hospitalization, unless hospitalization is for:
    - Routine treatment or monitoring of the studied indication, not associated with any deterioration in condition (procedures such as central line placements, paracentesis, pain control)
    - Elective or pre-planned treatment for a pre-existing condition that is unrelated to the indication under study and has not worsened since the start of study drug
    - Treatment on an emergency outpatient basis for an event not fulfilling any of the definitions of a SAE given above and not resulting in hospital admission
    - Social reasons and respite care in the absence of any deterioration in the patient's general condition

Collection of serious adverse events will begin on the day of study drug initiation and end 30 days after the last dose of study treatment or until a new cancer treatment is initiated, whichever happens the soonest.

Any death from any cause while a patient is receiving treatment on this protocol or up to 30 days after the last dose of protocol treatment, or any death which occurs more than 30 days after protocol treatment has ended but which is felt to be treatment related, must be reported.

Toxicities which fall within the definitions listed above must be reported as an SAE regardless if they are felt to be treatment related or not. Toxicities unrelated to treatment that do NOT fall within the definitions above, must simply be documented as AEs in the patient research chart.

16.6 SAE Reporting Requirements

All local serious unexpected and expected adverse events and reactions will be reported to the IRB per current institutional guidelines.

16.7 Reporting of Pregnancy

Although pregnancy is not considered an adverse event, it is the responsibility of investigators or their designees to report any pregnancy or lactation in a subject as an SAE. Pregnancies or lactation that occurs during the course of the trial or with 30 days of completing the trial or starting another new anticancer therapy, whichever is earlier, must be reported to the DSMC and IRB as applicable. All subjects who become pregnant must be followed to the completion/termination of the pregnancy. Pregnancy outcomes of spontaneous abortion, missed abortion, fetal death, intrauterine death, miscarriage and stillbirth must be reported as serious events. Since only postmenopausal women are eligible

to enroll on this trial, it is unlikely that participants will become pregnant during study participation.

16.8 Protocol Amendments

Any amendments or administrative changes in the research protocol during the period, for which the IRB approval has already been given, will not be initiated without submission of an amendment for IRB review and approval.

These requirements for approval will in no way prevent any immediate action from being taken by the investigator in the interests of preserving the safety of all subjects included in the trial.

16.9 Protocol Deviations

A protocol deviation (or violation) is any departure from the defined procedures and treatment plans as outlined in the protocol version submitted and previously approved by the IRB. Protocol deviations have the potential to place participants at risk and can also undermine the scientific integrity of the study thus jeopardizing the justification for the research. Protocol deviations are unplanned and unintentional events. All deviations will be reported to the IRB per current institutional guidelines.

16.10 FDA Annual Reporting

This study is IND exempt therefore there are no annual reporting requirements to the FDA.

16.11 Clinical Trials Data Bank

The study will be registered on **http://clinicaltrials.gov** and the NCI CTRP (Clinical Trials Reporting Program) by the Oncology Clinical Trials Support Unit (O-CTSU).

17 BIBLIOGRAPHY

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