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Protocol of a double blind, phase II randomized controlled trial to compare Docosahexaenoic acid (DHA) concomitant with neoadjuvant chemotherapy versus neoadjuvant chemotherapy alone in the treatment of breast cancer: DHA WIN

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ABSTRACT

Introduction: Neoadjuvant chemotherapy for breast cancer treatment is prescribed to facilitate surgery, provide confirmation of drug sensitive disease and the achievement of pathological complete response (pCR) predicts improved long-term outcomes. Docosahexaenoic acid (DHA) has been shown to reduce tumor growth in preclinical models when combined with chemotherapy and is known to beneficially modulate systemic immune function. The purpose of this trial is to investigate the benefit of DHA supplementation in combination with neoadjuvant chemotherapy in patients with breast cancer.

Methods and analysis: This is a double blind phase II randomized controlled trial of 52 women prescribed neoadjuvant chemotherapy to test if DHA supplementation enhances chemotherapy efficacy. The DHA supplementation group will take 5g/day DHA orally and the placebo group will take equal fat supplement of vegetable oil. The primary outcome will be change in Ki67 labelling index from pre-chemotherapy core needle biopsy to definitive surgical specimen.

Secondary endpoints include assessment of: i) DHA plasma phospholipid content; ii) systemic immune cell types, plasma cytokines and inflammatory markers iii) tumor markers for apoptosis and tumor infiltrating lymphocytes iv) rate of pCR in breast and in axillary nodes v) frequency of grade 3 and 4 chemotherapy associated toxicities and vi) patient perceived quality of life.

The trial has 81% power to detect a significant between-group difference in Ki67 index with a two-sided t-test of less than 0.0497, and accounts for 10% drop-out rate.

Ethics and dissemination: This study has full approval from the Health Research Ethics Board of Alberta – Cancer Committee (Protocol #: HREBA.CC -18-0381). We expect to present the findings of this study to the scientific community in peer-reviewed journals and at conferences. The results of this study will provide evidence for supplementing with DHA during neoadjuvant chemotherapy treatment for breast cancer.

Clinical Trial Registration No: NCT03831178

KEYWORDS

Ki67, phospholipids, fatty acids, omega-3, apoptosis, proliferation, immune function

STRENGTHS AND LIMITATIONS OF THIS STUDY

- This study is the first phase II randomized controlled trial to evaluate DHA supplementation concomitant with neoadjuvant chemotherapy to treat non-metastatic breast cancer.
- The intervention is minimally invasive and side effects from the supplementation are not expected.
- This study is powered to examine the key clinical outcome of changes in Ki67 index from pre-chemotherapy biopsy to surgical excision based on group sample sizes of 23 patients in group one and 23 patients in group two in order to achieve 81% power to detect a difference between the group proportions of 0.4.
- This study will measure clinically relevant intermediate outcomes including rate of pCR in breast and in axillary nodes, rate of grade 3 and 4 chemotherapy associated toxicities and hospitalizations as well as additional outcomes including plasma phospholipid content of DHA, markers of immune function (plasma cytokines, chemokines,

inflammatory markers and lymphocyte function), tumor markers for apoptosis and tumor infiltrating lymphocytes and patient perceived quality of life.

- The study will include all subtypes of breast cancer patients undergoing neoadjuvant chemotherapy but is not powered to assess differences between subtypes.

INTRODUCTION

Despite improvements in early diagnosis and treatment, breast cancer remains the second leading cause of cancer related death in women [1]. While neoadjuvant chemotherapy aims to improve surgical resection outcomes and reduce / eliminate micrometastases [2,3], pathological complete response (pCR) is not achieved by all patients [3]. Increasing the efficacy of neoadjuvant treatment without adding additional side-effects would benefit this population.

DHA is an omega-3 long chain polyunsaturated fatty acid (n-3 LCPUFA). The majority of n-3 fatty acids are in the form of the 18-carbon fatty acid alpha-linolenic acid (ALA). While DHA can be synthesized from ALA and other n-3 LCPUFA in the body, endogenous synthesis is low [4,5]. Consequently, the direct consumption of this fatty acid is the only way to significantly increase levels of DHA in tissues [6]. Supplementation can increase blood plasma DHA concentration by 2-fold (500 μM), which can lead to plasma membrane lipid enrichment [7]. Incorporation of DHA into tumor membrane phospholipids has been shown to reduce breast cancer cell proliferation [8,9] and increase apoptosis [10-15] in vitro and decreases tumor growth in animal models [13,15-17]. Additionally, providing/feeding DHA has been shown to increase the efficacy of different chemotherapeutic drugs in animal models of breast cancer [11,12,18,19]. While there is limited clinical evidence, it has been shown that increased dietary intake of n-3 LCPUFA, including DHA, results in increased DHA incorporation in breast adipose tissue [20] and this correlates with improved response to chemotherapy [21]. In an

open label trial with advanced metastatic breast cancer patients, DHA supplementation and enrichment into plasma phospholipids was associated with improved outcomes [22]. Other clinical trials have reported that supplementation with n-3 LCPUFA at a wide range of doses (0.6g-8.6 g/day) increased tolerability of chemotherapeutic drugs in a range of malignancies at other sites, include lung, pancreatic and colorectal (reviewed in [23]). Consequently, we hypothesize that the therapeutic index (efficacy: toxicity ratio) of neoadjuvant breast cancer chemotherapy will be improved with the addition of DHA to the treatment.

Breast cancer proliferation can be assessed by immunohistochemical (IHC) analysis of cells staining positive for the nuclear antigen Ki67 [24], as it is expressed in all phases of the cell cycle, G_1, S, G_2, and M, but not in G_0 [25,26]. The proportion of cells staining for Ki67 is frequently used as a primary endpoint to measure efficacy of neoadjuvant therapy in clinical trials. The Ki67 index, defining the change between pre- and post-treatment Ki67, has been reported to be an independent prognostic factor in luminal A, luminal B, triple-negative, and HER2+ breast cancer, and has been reported to be a useful surrogate marker of relapse free survival in luminal B, triple- negative, and HER2+ breast cancer [27,28].

OBJECTIVES

The objective of this RCT is to assess the efficacy of supplemental DHA combined with neoadjuvant chemotherapy in treatment naïve women with breast cancer measured by changes in Ki67 index from biopsy to surgical excision. We hypothesize that DHA supplementation will increase plasma phospholipid DHA and improve response to neoadjuvant chemotherapy assessed by decrease in Ki67 index.

This protocol follows the Standard Protocol Items for Randomized Trials (SPIRIT) guideline (Spirit Checklist Supplementary Table 1, WHO Checklist Supplementary Table 2) [29,30].

Study Design

The DHA-WIN trial will be a two-arm, double blind phase II randomized controlled trial comparing DHA supplementation and placebo (vegetable oil). The proposed study design with outcomes depicted is shown in **Figure 1** and SPIRIT participant flow chart is shown in **Figure 2**.

METHODS AND ANALYSIS

Study Population

Eligible women have invasive breast cancer (clinical stage I, II or III) for whom systemic chemotherapy [31] is recommended prior to surgery. The study will occur at the Cross Cancer Institute, with central laboratory and clinical analyses occurring the University of Alberta, both in Edmonton, Alberta, Canada. Inclusion and exclusion criteria are listed in **Table 1**

Table 1: Inclusion and Exclusion Criteria for DHAWIN

Inclusion Criteria
1) ECOG Performance status of 0 or 1
2) Hematology and biochemistry assessments [CBC and differential, partial thromboplastin time (PTT), prothrombin time/ international normalized ratio (PT/INR), aspartate aminotransferase **(**AST**),** alkaline phosphatase (ALP), bilirubin, and creatinine] within normal range unless determined not clinically significant by the qualified investigator
3) Ability to take oral medications
4) Adequate tissue specimen for diagnosis, biomarkers, and endpoint Ki67 assays
Exclusion Criteria
1. Patients undergoing surgery prior to chemotherapy
2. Current or previous (within 2 months) daily use (>1 day/week) use of omega-3, fish oil, or other supplements or foods containing DHA (at daily doses > 200 mg)
3. Known allergy to soy or corn
4. Continued intake of supplements containing Vitamin C, Vitamin E or β-carotene exceeding the DRI, or other anti-oxidant supplements
5. Symptomatic but untreated cholelithiasis

6. History of deep venous thrombosis, active thrombophlebitis, pulmonary embolism, stroke, acute myocardial infarction, congestive cardiac failure, untreated hypertension, known inherited hypercoagulable disorder
7. Diagnosis of any other malignancy within the previous year except for adequately treated basal cell or squamous cell skin cancer
8. Medically documented history of a psychiatric disorder that would preclude consent
9. Partial or complete loss of vision or diplopia, from ophthalmic vascular disease
10. Hypersensitivity to any component of the container

Intervention

Patients will be prescribed either 5 g/day DHA (in 11- 1g capsules), in the form of DHA enriched algae-sourced triglyceride oil capsules (life'sDHA™ S40-O400) or 11g placebo (corn/soy oil blend) per day (capsules from DSM Nutritional Products, Columbia, MD), to be taken orally throughout the day as tolerated. The intervention will occur for 12-18 weeks (84-126 days) beginning at the start of the first cycle of chemotherapy and continued through 4-6 cycles of chemotherapy (3 weeks/ cycle). DHA/placebo will be discontinued 21 days after the last administration of cytotoxic chemotherapy.

All patients will be dispensed an additional bottle of DHA/placebo capsules at the beginning of the study to account for circumstances where their treatment is delayed due to treatment associated toxicities (including but not limited to vomiting, diarrhea, abnormalities in blood work, fatigue or severe mouth sores). The patients will be requested to continue taking the DHA or placebo as tolerated and will be dispensed additional capsules as necessary. The extra capsules will remain with the patient until the end of the study.

Patients will be encouraged to take the supplements as tolerated (throughout the day). Treatment adherence will be monitored by review of patient dosing diary and recording the number of any remaining capsules returned at the end of study visit following the last dose of

DHA/placebo. Non-compliance will be assessed as consuming less than 50% of the weekly dose for 2 consecutive cycles. No additional natural health product is permitted beyond a daily multi-vitamin.

Outcome Measurements

Study outcome timelines are summarized in **Table 2**. Briefly, outcomes will be measured at baseline, within ± 3 days of chemotherapy and/ or post-intervention (surgical excision). Electronic medical record and or paper chart review of local control, relapse free survival and overall survival will occur at 3, 5, and 10 years to explore possible effects on long-term outcome.

Primary Outcome

The primary outcome of this study is change in Ki67 from pre-treatment core needle biopsy to surgical excision. It will be calculated by image analysis and will follow analytical and pre-analytical recommendations of Dowsett et al.[24]. The percent change in Ki67 index at experimental end (surgical excision) from baseline will be determined on a log scale and the mean percent change in Ki67 level from baseline will be calculated. Ki67 assays will be performed and reported as part of the routine diagnostic services. A semi-automated computer algorithm scoring system will be employed as previously described [32] using the platform QuPath [33]. It is expected that 5g DHA/day will result in a clinically relevant decrease in Ki67.

Secondary Outcomes

1) DHA incorporation into phospholipids: The changes in level of DHA incorporation in plasma phospholipids will be assessed at baseline and at day 20 (±3 days) of each cycle of chemotherapy to identify the range of DHA incorporation in this patient population. From our hypothesis and previously published data [22]. It is expected that supplementing with DHA will

result in a significant increase in DHA incorporation. If possible, with the small study size, we will also assess difference in DHA incorporation in patients with different breast cancer subtypes and if subtype or disease stage affects DHA incorporation into plasma, controlling for the reported dose taken by the patient. The goal is to determine if plasma phospholipid DHA content can be used to predict treatment outcome.

2) Systemic immune function: Systemic immune function will be assessed on blood samples obtained at baseline, end of chemotherapy cycle 3 (day 20± 3 days) and at the end of chemotherapy treatment. Changes in markers of systemic immune cell type and function will be assessed following supplementation compared to baseline and the change from baseline compared to patients receiving the placebo. We will also examine the relationship between changes in activation markers and the level of DHA incorporation, changes in systemic inflammation (CRP, IL-6, TNFα) and immune function (ability to produce IL-2 after stimulation in vitro) following DHA supplementation.

3) Identify factors that may affect DHA incorporation into plasma phospholipids: If incorporation of DHA in plasma phospholipids is variable within the DHA treatment arm, possible factors that may influence incorporation will be assessed between high and low incorporators. These parameters will be assessed at end of study from data collected throughout the study.

4) Examine changes in markers for apoptosis: Caspase-3 presence in the excised tumor, as percent positive cells, will be calculated by image analysis and a comparison of expression levels at experimental end (surgical excision) to baseline will be determined in patients receiving DHA supplementation and compared to patients receiving placebo. Proportions of negative cells, weakly positive cells and strongly positive cells will be scored by two pathologists and the staining intensity assessed by QuPath [33] will be recorded independently.

A 95% confidence interval for the mean percent change in Caspase-3 will be calculated. Increased apoptosis measured by Caspase-3 is a clinically relevant marker of cell death.

5) Examine changes in tumor infiltrating lymphocytes (TILs): CD4+ and CD8+ in the excised tumor, as number of positive cells for a given area, will be calculated by image analysis and a comparison of expression levels at experimental end (surgical excision) to baseline will be determined in patients receiving DHA supplementation and compared to patients receiving placebo will be made post-treatment. The differences will be compared between treatments and within the treatment group, related to plasma DHA concentrations. Increased infiltration of TILs is potential marker that could be used to predict treatment patient outcomes.

6) Pathological complete response rate (pCR): pCR in resected breast tissue and all sampled axillary nodes will be assessed as absence of invasive cancer on haematoxylin and eosin evaluation as per standard of care. Pathologic complete response will be classified as ypT0/is ypN0 and will be determined at end of study after surgical resection as part of standard of care assessment.

7) Comparison of rate of chemotherapy associated grade 3 and 4 toxicities: Rate of chemotherapy associated grade 3 and 4 toxicities, and chemotherapy-associated hospitalizations will be compared between DHA and placebo arms. Any changes will then be examined in regards to level of supplementation and DHA incorporation. These analyses will be completed at end of study after surgical resection.

Exploratory outcomes

1) Quality of life: Assessment in changes in quality of life will be determined by questionnaire employed at baseline and end of treatment. Comparisons will be assessed from end of treatment to baseline within and between treatment groups.

2) Exercise behavior: Assessment of exercise behavior will be determined by questionnaire employed at baseline, each cycle of chemotherapy and end of treatment. Comparisons will be assessed from end of treatment to baseline within and between treatment groups.

3) Breast conservation: The rate of breast conservation, specifically the rate of lumpectomy and mastectomy, will be determined by review of surgical and pathologic reports at end of study after surgical resection.

4) Volume of surgical blood loss: High intakes of n-3 LCPUFA (that contain some DHA) have been studied to determine if they increase bleeding time [34,35]. We will review surgical report estimates of blood loss to see if there is a qualitative or quantitative difference between placebo and treatment arms, once adjusted for the magnitude of surgery (lumpectomy vs. mastectomy vs. mastectomy + immediate reconstruction; sentinel node dissection vs. full axillary dissection). It is not expected that we will see a difference as it is eicosapentaenoic acid (EPA, the precursor to DHA) that has antithrombotic and antiplatelet properties [36].

5) Local control, relapse free survival and overall survival: Local control, relapse free survival and overall survival will be analyzed by review of electronic medical records, registry reports, and / or paper medical charts at 3, 5, and 10 years to explore possible effects on long-term outcome.

Participant timeline

Breast cancer patients receiving neoadjuvant chemotherapy account for approximately 20% of newly diagnosed breast cancer patients, approximately 10-12/month at the Cross Cancer Institute. Assuming a conservative accrual rate of 30%, accrual is estimated to be completed in 14-18 months with 3-4 patients recruited per month. Each patient will be enrolled for the duration of their individual chemotherapy regimen, an estimated 12-18 weeks (84-126 days)

beginning at the start of the first cycle of chemotherapy and continued through 4-6 cycles of chemotherapy (3 weeks/ cycle). The intervention will be discontinued 21 days after the last administration of cytotoxic chemotherapy. See Figure 1 for schematic of the participant timeline.

Sample Size

Fifty-two women prescribed neoadjuvant breast cancer chemotherapy will be enrolled in a 2-arm trial with 26 participants/arm. The sample size calculation is based on the primary objective, which is to determine the efficacy of supplemental DHA provided with standard neoadjuvant as measured by change in Ki67 index from biopsy to surgical excision. Group sample sizes of 23 patients in each group achieve 81% power to detect a difference between the group proportions of 0.4. The proportion in group one is assumed to be 0.3 under the null hypothesis and 0.7 under the alternate hypothesis. The proportion in group two which is control group is 0.3. The test statistic used is the two-sided t-test. The significance level of the test was targeted at 0.05 and the significance level actually achieved by this design is about 0.0497. Assuming a dropout rate estimated at approximately 10% for this patient population which is approximately 5 patients, a total of 52 patients (26 patients DHA supplementation, and 26 in placebo) is required for the study.

Recruitment

Oncologists and clinical trial nurses at the Cross Cancer Institute in Edmonton, Canada will recruit newly diagnosed breast cancer patients. Patients will be screened for eligibility by the clinical trial nurses and eligible, interested patients will receive detailed explanation of the study by the study coordinators and written informed consent will be obtained (**Supplementary File 1**).

Randomization and Blinding

A biostatistician will generate a patient randomization list by covariate-adaptive randomization (block randomization). Patients will be stratified by histological subtype and grade and send it to an unblinded Clinical Trials Coordinator (Clinical Trials Unit), who will be alerted when a patient is ready to be randomized and will be responsible for assigning the treatment number from the applicable arm (based on pharmacy stock) and providing the information to the blinded study staff (Clinical Trials Nurse/ Clinical Trials Coordinator) confirming the unique study identifier and treatment number. The Clinical Trials Nurse will then notify Pharmacy staff, who will dispense the appropriate treatment at day 1 of each chemotherapy cycle. Following each randomization, the unblinded Clinical Trials Coordinator will keep details on patient E#, Study ID # and Randomization group, covered and placed inside a sealed envelope for the PI to use in case there is an urgent need for breaking of the blind. PIs will store the blind codes in sealed envelopes in their office. Blinding codes should only be broken in emergency situations for patient safety. When a blinding code is broken, the date and reason for unblinding must be fully documented in source documents and entered on case report form. Every effort should be made by site staff to ensure that the treatment arm in which the unblinded patient is assigned is communicated only to those site staff that require the information for treatment purposes. Patients, pathologists, physicians, and researchers will be blinded to patient enrolment in the study and throughout trial. Blinding will only be dropped after analysis of fatty acids, systemic immune function and Ki67 is complete.

Data Collection, Management and Analysis

Study methods are summarized in **Table 3**. Briefly, data will be collected and measured at baseline, within ± 3 days of chemotherapy and/ or post-intervention (surgical excision).

Electronic medical record and or paper chart review of local control, relapse free survival and overall survival will occur at 3, 5, and 10 years. All data will be entered and maintained in REDCap trial database. Baseline measurements will be analyzed once all participants have been enrolled and all other analyses will occur at completion of trial.

Primary Outcome

Ki67 will be tested by immunohistochemistry (IHC) by the diagnostic biomarker laboratory at the Cross Cancer Institute using the MIB1 antibody on 4 µm sections from formalin fixed paraffin embedded (FFPE) needle core biopsy surgical specimens. At final analyses, Ki67 staining will be repeated as single IHC stain and interpreted by image analysis. At time of Ki67 interpretation, slides will be de-identified and coded to ensure the pathologist is blinded to the experimental group. In addition, the original single stained slides will be interpreted visually by research staff. All Ki67 values (routine and image analysis) will be recorded as absolute percentage and as log-transformation in REDCap trial database and participant's case report form. The Ki67 index is validated and used in clinic as marker of proliferation. The Ki67 index (absolute %, log transformed and H-score [37] of biopsy and surgical resection (after chemotherapy) will be compared on each participant and between participants receiving DHA compared to placebo.

Secondary Outcomes

DHA incorporation into plasma phospholipids will be measured in venous blood from patients at baseline (time of enrolment in trial), and at day 20 (± 3 days) of each chemotherapy cycle by a technician blinded to the treatment group. Venous blood will be collected in coated EDTA tubes and centrifuged at 750x g for 10 min to obtain plasma. Plasma will be separated in

6 aliquots, and immediately frozen at -70°C for storage. Plasma (concentration and relative percent) will be extracted by Folch procedure [38,39], phospholipids separated by thin layer chromatography and fatty acid content measured by gas-liquid chromatography as previously described [40]. The percentage change in DHA from baseline will be compared in each patient and a 95% t-confidence interval for the mean percent change in the DHA from baseline will be compared to patients receiving placebo. An internal standard is used to identify and quantify the fatty acid. This is a standard measure for fatty acid status has coefficient of variation <5% and individual GC peaks are validated against phospholipid standards (GLC-502 and GLC-643) from NuChek (Elysian, MN).

Phenotyping of immune cell subsets will be measured using whole blood (collected in EDTA tubes). The various cell types will be identified using specific fluorescently labelled monoclonal antibodies (mAb) to surface receptors (See Supplementary Table 1 for list of antibodies). These will be quantified by flow cytometry, as previously described [41]. With the remaining blood, peripheral mononuclear cells will be isolated and purified on a Ficoll density gradient of Histopaque 1077 as previously described [41,42]. To measure cytokine production in isolated lymphocytes, cells will be cultured in media with or without the mitogens, Phytohemagglutinin (PHA) or Lipopolysaccharide (LPS) for 48 h as previously described [43]. Supernatant will be collected and stored at -80°C for *ex vivo* measures of immune function (ability and pattern of cytokines produced after stimulation). IL-1 β, IL-2, IL-6, IL-10, TNF α, and IFN-γ (pg/ml) cytokines will be measured using electrochemiluminescent multiplex assays (MesoScale Discovery) or by individual ELISA assays. Cytokines listed above and inflammatory markers including C-reactive protein (CRP) in plasma will be measured electrochemiluminescent multiplex assays (MesoScale Discovery) as previously

described [44]. Cytokines and inflammatory markers in plasma and cytokines from cultured lymphocytes will be analyzed when all samples have been collected. Changes in systemic immune function will be assessed in patients compared to baseline and compared between groups. The data analysis will occur at completion of trial. Cytokines are done in duplicate and the coefficient of variance is <15%. Phenotypes will be collected as a relative percent of total cells and the change compared between treatments.

If DHA incorporation in plasma phospholipids and/or tumor tissue is significantly different within DHA supplementation arm, factors that may influence incorporation will be compared in low vs high incorporators, to identify possible factors that predict incorporation, including, including weight (BMI), age, the usual diet and composition of dietary fat of the women (estimated from the FFQ), histology of the tumor (provided from the biopsy) and amount of DHA consumed (adherence to the supplement).

Caspase-3 changes and changes in CD4 and CD8 will be tested by immunohistochemistry (IHC) by the diagnostic biomarker laboratory at the Cross Cancer Institute on 4 μm sections from formalin fixed paraffin embedded (FFPE) surgical specimens. At final analyses, IHC staining will be interpreted by image analysis. At time of interpretation, slides will be de-identified and coded to maintain the blind. All values (routine and image analysis) will be recorded as absolute percentage and as log-transformation. Caspase-3 is a validated marker of apoptosis and CD4 and CD8 are validated marker for lymphocytes. The calculated indices (absolute %, log transformed and H-score) of biopsy and surgical resection will be compared on each participant and between participants receiving DHA compared to placebo.

Pathological complete response in resected breast tissue and axillary nodes will be assessed in hematoxylin and eosin stained tissue for evidence of invasive disease according to standard of care. Pathological complete response will be assessed following breast surgery as per standard of care and recorded in patient's case report form. The rate of pathological complete response in breast tissue and axillary nodes after surgical resection will be compared between participants receiving DHA supplementation compared to placebo.

Grade 3 and 4 toxicities will be assessed and recorded by the clinical trial nurse. Toxicities will be assessed on day 20 (± 3 days) of each chemotherapy cycle. Dates of hospitalization will be recorded in patient's case report form. Rates of chemotherapy-associated grade 3/4 toxicities, all grade neuropathy and hospitalizations will compared between DHA supplementation and placebo arms as scored by a medical oncologist in a standardized toxicity/ neuropathy form for each cycle of chemotherapy.

Exploratory outcomes:

The quality of life questionnaire is a validated questionnaire from European Organization for Research and Treatment of Cancer- Quality of Life Questionnaire-C30 (EORTC-QLQ-C30) [45]. Exercise behavior will be assessed using the modified Godin Leisure-Time Exercise Questionnaire (GLTEQ) [46]. Assessment in changes in quality of life and exercise behavior will be assessed from timepoints collected to baseline within and between treatment groups.

The rate of breast conservation, specifically the rate of lumpectomy and modified radical mastectomy, will be determined by surgical and pathologic reports at time of surgical resection. Volume estimates of blood loss will be assessed by review of surgical report estimates of blood loss to see if there is a qualitative or quantitative difference between placebo and treatment arms, once adjusted for the type of surgery (lumpectomy vs. mastectomy vs. mastectomy + immediate

reconstruction; sentinel node dissection vs. full axillary dissection). Local control, relapse free survival and overall survival will be analyzed by electronic medical record and / or paper medical chart review at 3, 5, and 10 years. Data will be validated by a medical oncologist.

Data Management

All data will be entered and maintained in REDCap trial database. Direct access to clinical and laboratory information on the enrolled trial patients will be limited to the principal investigator, co-investigators, trainees/staff who have had the appropriate training and approval and study nurses and study coordinators who will have access to the source documents through the electronic medical record and laboratory information system at the Cross Cancer Institute. All patients will have biopsy and tumor samples for analysis and we do not expect any missing data for the primary endpoint (Ki67). If supplement compliance is below 50% for more than two consecutive cycles, or participants do not complete chemotherapy (to a minimum of 4 cycles), they will be excluded from final analysis of the primary end point. If patients do not have sufficient blood samples for the secondary analyses (DHA incorporation, systemic immune function), analysis will be performed using data from the remaining patients.

Statistical Methods

Primary Outcome:

The percent change in Ki67 will be determined as an absolute percentage and H-score. The number of patients showing a decrease and the 95% confidence interval for the mean percent change in the Ki67 level from baseline in patients receiving DHA supplementation will be compared to patients receiving placebo. Test of proportions will be used to compare the proportions between the two groups.

Secondary Outcomes:

The Wilcoxon signed rank test will be used to compare the plasma DHA level after each cycle of chemotherapy with baseline. The difference in plasma phospholipid DHA from baseline and between DHA supplementation and placebo arms will be calculated, and the 95% confidence interval for the mean percent change in DHA level from baseline and groups will be assessed.

If systemic immune function data is not normally distributed, it will be log transformed prior to analysis and the normality assumptions will be tested again. Repeated measures ANOVA with post hoc analysis will be used to determine if there is an effect of treatment on immune function.

Factors affecting DHA incorporation will be examined by multivariate analysis. The outcome of interest is binary (DHA vs. Placebo); hence binary logistic regression will be used to determine the factors associated with the outcome variable. Factors significant at the univariate analysis will be entered into the multivariate model. Odds ratio and the corresponding 95% confidence interval will be reported.

The 95% confidence interval for the mean percent change in apoptosis, tumor infiltrating lymphocyte markers in pathological complete response and rates of grade 3 and 4 chemotherapy associated toxicities and hospitalization in patients receiving DHA supplementation will be compared to patients receiving placebo. Test of proportions will be used to compare the proportions between the two groups.

Exploratory outcomes:

Analyses of covariance will be assessed to compare differences between treatment arms for the quality of life and exercise questionnaires. Chi-square tests will be used to compare the degree of breast conservation and volume of surgical blood loss between the two study arms. Rate of local control will be compared between treatment arms using t-test of proportions. Recurrence-

free survival and survival will be analyzed using the log rank test on Kaplan-Meier survival curves.

SAS software, version 9.4 (SAS Institute Inc., Cary, NC), will be used for statistical analysis. A p-value <0.05 level will be used for all statistical significance. Two-sided tests will be used for all statistical tests.

Data Monitoring

The trial activities performed at the Cross Cancer Institute will be monitored by the Cross Cancer Institute, Investigator Initiated Trials Data Safety Monitoring Board (DSMB). The DSMB is independent of the investigator and is composed of representatives from both medical and radiation oncology.

The investigator will assess the relationship between protocol treatment and the occurrence of adverse events (AEs) and this assessment will be recorded in the database for adverse events. This study will use the International Common Terminology Criteria for Adverse Events (CTCAE), version 5.0, for adverse event reporting. The reporting period for adverse events will start at the time the patient takes the first dose of DHA/placebo through and including 28 calendar days after last administration of study agent. If serious adverse reaction to treatment occurs, the Natural and Non-prescription Health Products Directorate (NNHPD), Clinical Trial Unit, Health Ethics Research Board of Alberta, Cancer Committee (HREBA.CC) and DSM will be notified as per guidelines. After 25 evaluable patients, all data and results will be submitted to the DSMB for review.

Auditing

As per the SPIRIT guidelines, the investigators, Cross Cancer Institute and the University of Alberta will permit trial-related monitoring, audits, REB, DSMB review, and regulatory inspection(s), providing direct access to paper and/or electronic documentation pertaining to the clinical study (e.g. CRFs, source documents such as hospital patient charts and investigator study files). All site facilities related to the study conduct could be visited during an audit (e.g. pharmacy, laboratory, outpatient department) and are agreed to co-operate and provide assistance at reasonable times and places with respect to any auditing activity.

Patient and Public Involvement

Patients were not involved in the protocol development or study design. However, oncologists and clinical trial nurses who work in the breast tumor group are involved in patient screening to assess eligibility for the study. The HREBA-CC approved informed consent will be obtained from patients prior to their involvement in the study and it informs patients of their right to withdraw at anytime. At the end of the trial, results will be disseminated to the public through seminars, public talks and in peer-reviewed journals.

Ethics and dissemination

DHAWIN has received Health Canada approval (#HC6-24-c220167), full ethical approval from the Health Research Ethics Board of Alberta – Cancer Committee (Protocol #: HREBA.CC- 18-0381) and is registered at clinicaltrials.gov (Identifier: NCT03831178). Protocol amendments will be submitted to HREBA.CC, Health Canada and the clinical trial registry prior to study implementation according to regulatory requirements. The formal consent of a participant, using the HREBA-CC-approved consent form (Supplementary File 1), will be obtained by a clinical trial nurse before the participant is enrolled in the study and will be signed by the patient, and the principle investigator. A voluntary optional consent form for use of

participant data and biological specimens (Supplementary File 2), will be offered at time of enrollment. Patient confidentiality and anonymity will be maintained and identities protected from unauthorized parties.

Access to data will be restricted to the primary investigators and statistician. They will grant access to other team members as governed and approved by ethics. Ancillary care post-trial will occur as routine standard of care for all participants. Our objective is to determine the efficacy of using DHA supplementation concomitant with chemotherapy and as such our results will be disseminated to clinicians for implementation in future treatment paradigms. The results will be submitted to peer-reviewed journals and presented at national and international conferences.

Funding Statement

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Competing Interests Statement

There are no financial or competing interests or conflicts to declare.

Author Contributions

MN and CJF designed and wrote the manuscript. All authors contributed to the study design and reviewed the manuscript drafts. MAC obtained all regulatory approvals (Health Canada, HREBA and Clinical Trials registration). Authors of the data manuscripts will include at least the Principal Investigator, medical director (J.

Mackey) and any co-investigators who have i) included eligible patients in the trial (by order of inclusion) and/or ii) contributed significantly to the design, conduct and data interpretation regarding companion basic science studies.

Appendices:

Supplemental File 1: Informed consent

Supplemental File 2: Optional consent

Supplemental Table 1: List of Antibodies for Immune cell subset identification

FIGURE LEGENDS

Figure 1 Flowchart of Trial Design with Endpoints and Proposed Experimental Analyses

Figure 2 SPIRIT patient flow diagram of the DHA WIN trial

Table 2: DHA WIN assessment schedule based on the Standard Protocol Items: Recommendations for Interventional Trials (SPIRIT)

	STUDY PERIOD												
		Chemotherapy											
	Enrolment	**Cycle 1**		**Cycle 2**		**Cycle 3**		**Cycle 4**		**Cycle 5**		**Cycle 6**	**Surgery**
TIMEPOINT**	$-t_1$	***Day 1*** [1]	***Day 20***	***Day 1***	***Day 20***	***Day 1***	***Day 20***	***Day 1***	***Day 20***	***Day 1***	***Day 20***	***Day 1***	t_x
ENROLMENT:													
Eligibility screen	X												
Informed consent	X												
Randomization	X												
INTERVENTIONS: [2]													
DHA 5 grams / day (11 - 1g capsules)		—	—	—	—	—	—	—	—	—	—	—	
Vegetable oil placebo (11 -1g capsules /day)		—	—	—	—	—	—	—	—	—	—	—	
Medication Diary		—	—	—	—	—	—	—	—	—	—	—	
Assessment of Compliance				X		X		X		X		X	X
ASSESSMENTS:													
BASELINE / ONGOING													

Demographic data collection	X												
Tumor analysis for Grade / ER/PR/HER2[3]	X												
Physical Exam / anthropometric measurements	X	X		X		X		X		X		X	X
Relevant medical history / current medical conditions	X			X		X		X		X		X	X
ESAS questionnaire	X	X		X		X		X		X		X	X
Blood Chemistry	X						X					X [4]	
CBC and differential	X											X [4]	
Adverse Events		X		X		X		X		X		X	X
Assessment of Relevant Toxicities		X		X		X		X		X		X	
Primary Outcome													
Tumor analysis of Ki67	X												X
Secondary Outcome													
Assessment of immune function:	X							X				X [4]	
Assessment of DHA incorporation	X			X		X		X		X		X	

Tumor analysis of apoptosis and TILs	X												X
Exploratory Outcomes													
Grade 1, 2 neuropathy assessment		X		X		X		X		X		X	X	
Pathological complete response														X
Breast conservation														X
Assessment of surgical blood loss														X
Study Associated Questionnaires														
Quality of life questionnaire	X												X	
Godin Exercise Questionnaire	X	X		X		X		X		X		X	X	
Food frequency questionnaire[5]	X													

ESAS: Edmonton Symptom Assessment System

(1) Day 1 is the day 1 of chemotherapy cycle.

(2) If patient's chemotherapy is delayed due to associated toxicities, they will be encouraged to continue taking the DHA/placebo capsules as tolerated.

(3) From previously collected biopsy.

(4) Tests required at the end of the last round of chemotherapy (i.e., end of cycle 4, 5 or 6 as per patients' individual treatment plan).

(5) Food frequency questionnaire can be completed anytime within the first cycle (21 days) of chemotherapy.

Table 3: Variables, Measures and methods of analysis

VARIABLE / OUTCOME	OUTCOME MEASURE	METHOD	STATISTICAL ANALYSIS
PRIMARY:			
Efficacy of supplemental DHA provided with standard neoadjuvant chemotherapy as measured by change in Ki67	Ki67 labelling index	Immunohistochemistry	95% t-confidence interval for mean percent change in Ki67 Test of proportions to compare the two groups [32]
Secondary:			
1. DHA incorporation into plasma phospholipids	Fatty acid composition of plasma phospholipids	Gas chromatography	Wilcoxon signed rank test to compare plasma DHA level after each cycle of chemotherapy with baseline. 95% t-confidence interval for the mean percent change in the DHA from baseline will be compared to patients receiving placebo
2. Systemic immune function	a) Immune cell subset identification b) Plasma cytokine c) Ex vivo stimulated immune cell response	a) Flow cytometry b and c) ELISA and MesoScale	Repeated Measures ANOVA with post hoc analysis
3. Identify factors that may affect DHA incorporation into tumor	Factors assessed after calculating high and low DHA incorporators:		Binary logistic regression; odds ratio and corresponding 95% confidence interval

tissue and plasma phospholipids.	a) Weight (BMI) b) Age c) The usual diet estimated from the FFQ d) Composition of dietary fat estimated from the FFQ e) Histology of the tumor (provided from the biopsy) f) Amount of DHA consumed (Adherence to the supplement)		
4. Examine changes in markers for apoptosis	Caspase -3	Immunohistochemistry	95% t-confidence interval for mean percent change within and between treatment groups
5. Examine changes in markers for tumor infiltrating lymphocytes	CD4+/CD8+	Immunohistochemistry	95% t-confidence interval for mean percent change within and between treatment groups
6. Describe the rate of pathological complete response in breast and in axillary nodes	Absence of invasive cancer on haematoxylin and eosin evaluation	Immunohistochemistry	pCR= ypT0/is ypN0 95% t-confidence interval for mean percent change between treatment groups
7. Describe the rate of grade 3 and 4 chemotherapy associated toxicities.	Rate of grade 3 /4 toxicities and chemotherapy associated hospitalizations	Chart review	95% t-confidence interval for mean percent change in events between treatment groups

Exploratory Outcomes			
1. Quality of Life	Baseline and Endpoint questionnaires	Questionnaire	Analyses of covariance
2. Exercise	Godin Exercise questionnaire	Questionnaire	Analyses of covariance
3. Assess the rate of breast conservation	Rate of lumpectomy and mastectomy.	Chart review	Chi-square tests
4. Assess the volume of surgical blood loss.	Review surgical reports for quantitative / qualitative loss of blood	Chart review	Chi-square tests
5. Analyze local control, relapse free survival and overall survival	Electronic medical record and / or paper medical chart review at.3, 5, and 10 years to explore possible effects on long-term outcome	Chart review	Kaplan-Meier survival curves

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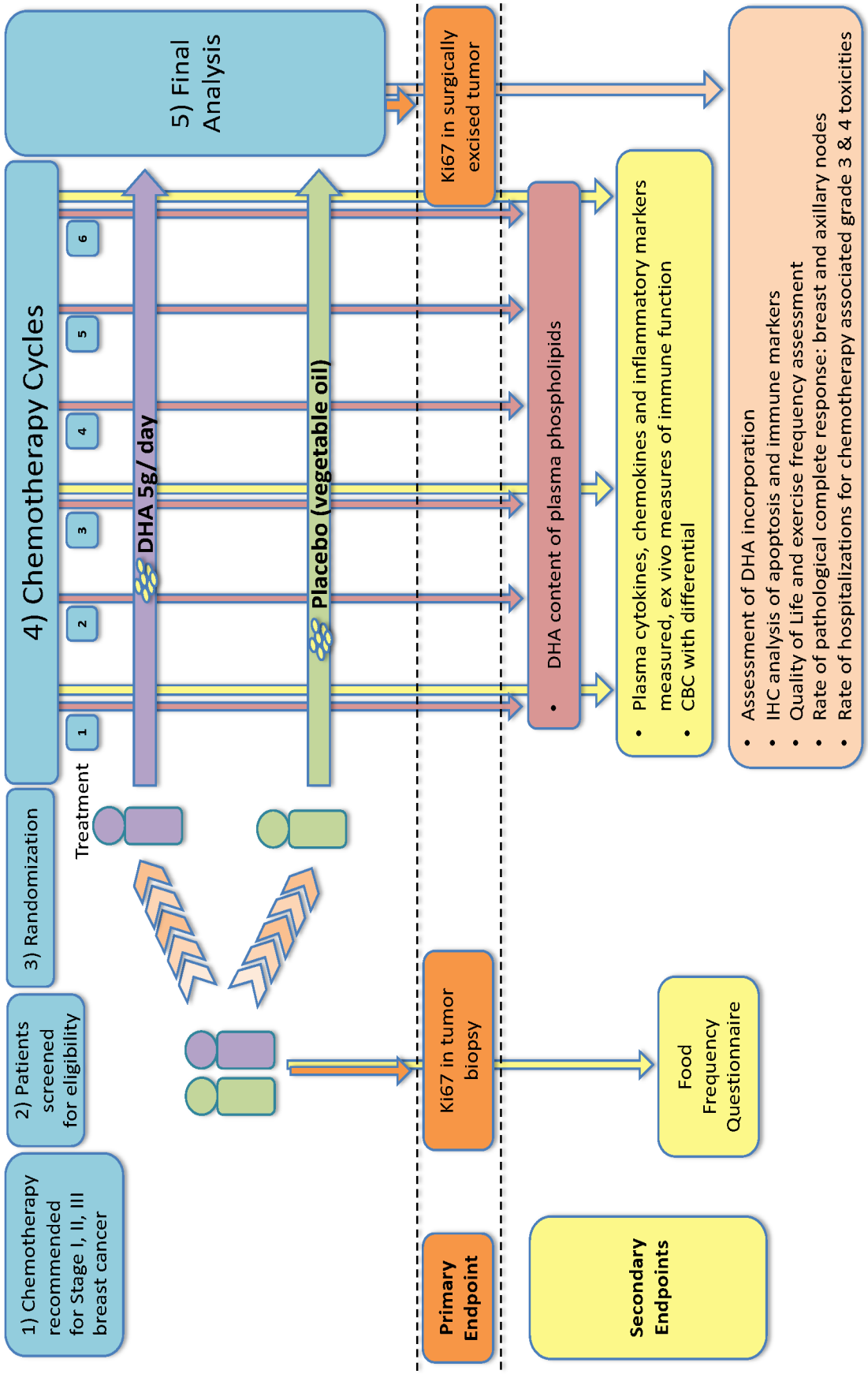
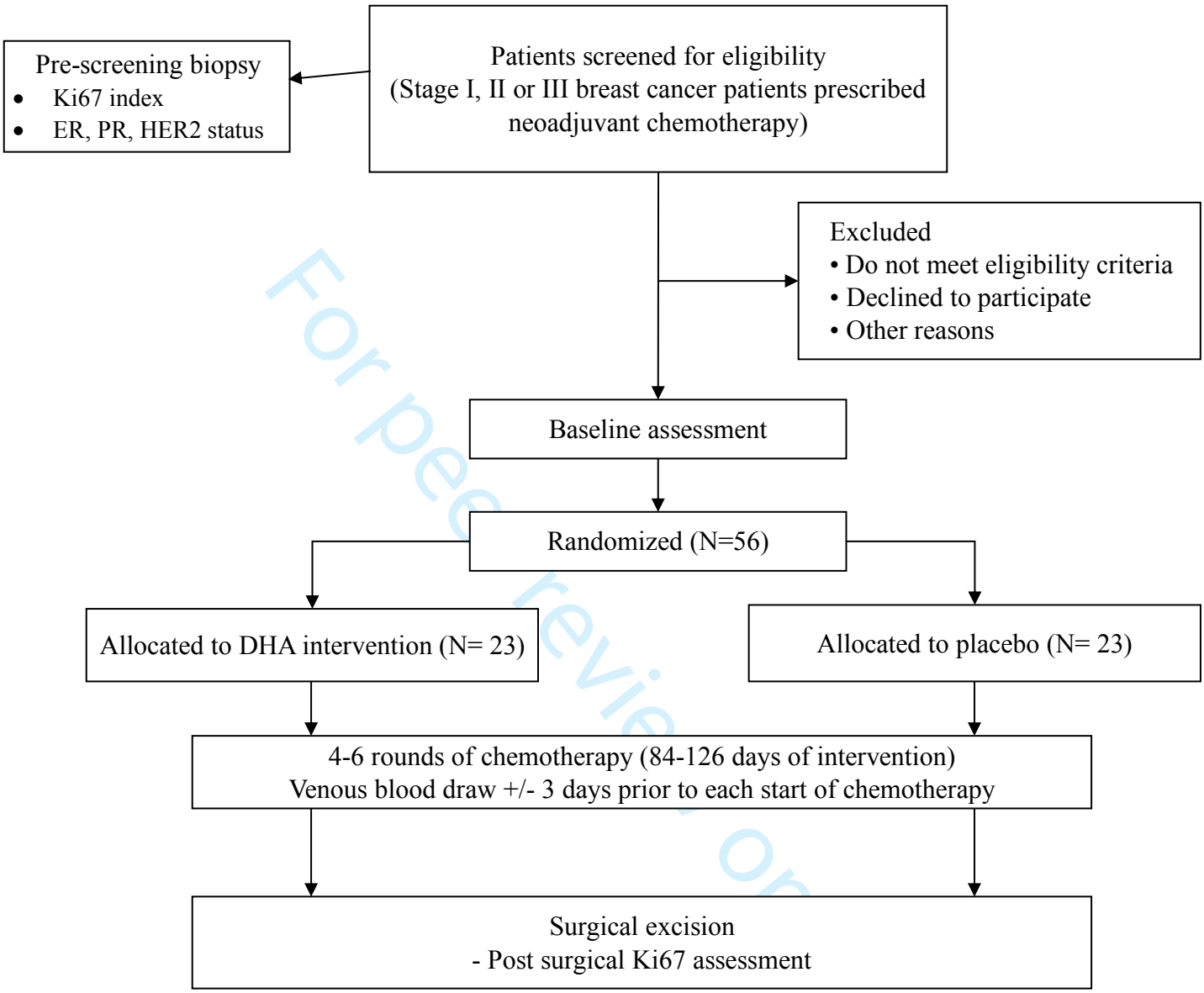


Figure 1

Figure 2



Supplemental Table 2: World Health Organization Trial Registration Data Set DHA WIN Summary

Data Category	**Information**
Primary registry and trial identifying number	ClinicalTrials.gov: NCT03831178
Date of registration in primary registry	February 5, 2019
Secondary identifying numbers	IIT-0005
Sources of monetary or material support	Canadian Institutes of Health Research (CIHR), AHS Cancer Control Alberta, Butler Family Foundation
Primary sponsor	AHS Cancer Control Alberta
Secondary sponsors	University of Alberta
Contact for public queries	Deborah Miede: Deborah.Miede@albertahealthservices.ca
Contact for scientific queries	Catherine Field: Catherine.field@ualberta.ca
Public title	DHA WIN
Scientific title	Docosahexaenoic acid (DHA) for Women with breast cancer in the neoadjuvant setting
Country of recruitment	Canada
Health condition or problems studied	Breast cancer
Interventions	DHA supplementation (5 g/ day) or equal amount of vegetable oil placebo for the duration of the participants chemotherapy treatment
Key inclusion and exclusion criteria	Inclusion: ECOG Performance status of 0 or 1; Hematology and biochemistry assessments within normal range; ability to take oral medication; adequate tissue specimen for diagnosis, biomarkers and endpoint Ki67 assays Exclusion: Patients undergoing surgery prior to chemotherapy; Current or previous (within 2 months) daily use (>1 day/week) use of omega-3, fish oil, or other supplements or foods containing DHA (at daily doses > 200 mg); Known allergy to soy or corn; Continued intake of supplements containing Vitamin C, Vitamin E or β-carotene exceeding the DRI, or other anti-oxidant supplements; History of deep venous thrombosis, active thrombophlebitis, pulmonary embolism, stroke, acute myocardial infarction, congestive cardiac failure, untreated

	hypertension, known inherited hypercoagulable disorder; Diagnosis of any other malignancy within the previous year except for adequately treated basal cell or squamous cell skin cancer
Study type	Randomized controlled trial
Date of first enrolment	Expected April 2019
Target sample size	52
Recruitment status	Not yet recruiting
Primary outcomes	Percent change in Ki67 index from baseline to surgical excision
Key secondary outcomes	Percent of DHA in plasma phospholipids; systemic immune function; Identify factors that may affect DHA incorporation into plasma phospholipids; Examine changes in markers for apoptosis and tumor infiltrating lymphocytes; pathological complete response; Comparison of rate of chemotherapy associated grade 3 and 4 toxicities

36/bmjopen-2019-030552 on 17 September 2019.

Informed Consent Form for Participation in a Research Study

DHA for Women with Breast Cancer in the Neoadjuvant Setting

DHA to improve effectiveness of Chemotherapy in Breast Cancer

Protocol ID: *IIT-0005*

Study Doctor: *Dr. John Mackey*
Department of Medical Oncology
Cross Cancer Institute
780-432-8221

Sponsor/Funder(s): *Alberta Health Services- Cross Cancer Institute*

Emergency Contact Number (24 hours / 7 days a week): *780-965-8824*

Non-Emergency contact numbers are noted at the end of this document under the section heading "WHO DO I CONTACT FOR QUESTIONS?".

For assistance with terminology within this consent form, please refer to the Canadian Cancer Society Glossary of Terms at http://info.cancer.ca/e/glossary/glossary.html.

You are being invited to participate in a research study because you have stage I, II or III breast cancer which has not spread to distant parts of the body and will be receiving chemotherapy prior to surgery. This consent form provides detailed information about the study to assist you with making an informed decision. Please read this document carefully and ask any questions you may have. All questions should be answered to your satisfaction before you decide whether to participate.

The study staff will tell you about timelines for making your decision. You may find it helpful to discuss the study with family and friends so that you can make the best possible decision within the given timelines.

Taking part in this study is voluntary. You may choose not to take part or, if you choose to participate, you may leave the study at any time without giving a reason. Deciding not to take part or deciding to leave the study will not result in any penalty or any loss of medical or health-related benefits to which you are entitled.

The study doctor, who is one of the researchers, will discuss this study with you and will answer any questions you may have. If you do consent to participate in this study, you will need to sign and date this consent form. You will receive a copy of the signed form.

WHAT IS THE BACKGROUND INFORMATION FOR THIS STUDY?

Docosahexaenoic acid (DHA) is an omega-3 fatty acid commonly found in fish and fish oil. In the body, DHA is found in the membranes of cells. DHA is important for brain development, and in the immune system. DHA is also beneficial in heart disease. A diet high in DHA can reduce the incidence of breast cancer.

Incubating breast cancer cells with DHA in cell culture (cells in a dish in a laboratory) decreases the growth of the breast cancer cells, and increases the death of these cells. This is specific to cancer cells, since DHA has no effect on normal breast cells. When breast cancer cells are treated with chemotherapy drugs and DHA, DHA increases the effectiveness of chemotherapy resulting in increased death of the cancer cells.

When mice with breast tumors are fed DHA and treated with chemotherapy their tumors are much smaller than mice who are not fed DHA. In a previous clinical trial, women with metastatic breast cancer were given DHA supplements and treated with chemotherapy. DHA supplements appeared to improve the response to chemotherapy for some women.

Taking DHA may also reduce some side effects of chemotherapy in women with breast cancer. In these previous trials, no side-effects of taking DHA supplements were found.

Health Canada, the regulatory body that oversees the use of natural health products, drugs and devices in Canada, has not approved the sale or use of this DHA supplement to treat this kind of cancer, although they have allowed its use in this study.

The Health Research Ethics Board of Alberta – Cancer Committee (HREBA-CC), which oversees the ethical acceptability of research involving humans, has reviewed and granted ethics approval for this study.

WHY IS THIS STUDY BEING DONE?

This study will test if taking a DHA supplement during chemotherapy for breast cancer increases the effectiveness of the chemotherapy. The purpose of this study is to find out what effects a new agent, DHA supplementation, has on you and your breast cancer.

The investigators of this study are also interested in exploring the factors that may affect DHA incorporation in your blood, such as your weight and height, usual food intake (including amount and type of fat eaten), tumor type and the amount of DHA supplement consumed in the study.

WHAT ARE OTHER OPTIONS IF I DECIDE NOT TO PARTICIPATE IN THIS STUDY?

You do not have to take part in this study, in order to receive continued medical care. Other alternatives in addition to standard care may include:

- Other experimental studies may be available if you decide not take part in this study.
- Continuing regular observation and routine follow-up care e.g., symptom management

Please talk to the study doctor or your care doctor about the known benefits and risks of these other options before you decide to take part in this study. Your study or care doctor can also discuss with you what will happen if you decide not to undertake any treatment at this time.

HOW MANY PEOPLE WILL TAKE PART IN THIS STUDY?

Up to 52 people will take part in this study.

WHAT WILL HAPPEN DURING THIS STUDY?

ASSIGNMENT TO A GROUP

If you decide to participate then you will be "randomized" into one of the groups described below. Randomization means that you are put into a group by chance (like flipping a coin). There is no way to predict which group you will be assigned to. You will have an equal chance of being placed in either DHA supplementation or placebo group. Neither you, the study staff, nor the study doctor can choose what group you will be in.

This is a double-blinded study, which means that neither you nor the study doctor or study staff will know which group you are in. This is done so that you and the study doctor will not be influenced by expectations of the effects of the study agent. Your treatment will be identified if medically necessary by a process referred to as unblinding. Requests to reveal your assignment for your information or participation in other research studies will not be considered until the study has been completed and the results are known.

STUDY INTERVENTION

Group 1 (Experimental intervention): standard intervention of neoadjuvant chemotherapy plus experimental intervention of DHA supplementation.
If you are randomized into this group, you will take DHA capsules by mouth every day during chemotherapy treatment (4-6 cycles of chemotherapy, which would last approximately12-18 weeks)

Group 2 (Non-experimental intervention): standard intervention of neoadjuvant chemotherapy

If you are randomized into this group you will take placebo capsules containing corn/soy oil by mouth every day during chemotherapy treatment (4-6 cycles of chemotherapy, which would last approximately *12-18 weeks).*

Other important information on study intervention:
If you have side effects while you are on this study, the study doctor may make changes to the intervention.

STUDY PROCEDURES

Established Procedures

The following established procedures will be done as part of this study. Some of these procedures may be done as part of your standard care, in which case the results may be used. Some may be done more frequently than if you were not taking part in this study. Some of these procedures may be done solely for the purpose of the study. If the results show that you are not able to continue participating in the study, the study doctor will let you know.

Screening:

- Signed Informed Consent
- Review of inclusion / exclusion criteria
- Confirmation of no known allergies to soybean or corn oil (participants with allergies to soy or corn will be excluded from the study).
- Demographic data
- Physical examination
- You will be asked about your ability to carry out daily activities
- Body height and weight
- Vital signs
- Documentation of the diagnosis and disease stage
- Confirmation of no previous or concomitant treatment
- Complete medical / oncological history and consultation
- Questionnaire about your symptoms and well-being (ESAS questionnaire)
- Quality of Life questionnaire
- Exercise questionnaire
- Food frequency questionnaire (to be completed before the end of the first cycle of chemotherapy)
- Blood sample
- Your biopsy sample will be analyzed for standard tumor analysis: Grade; ER/PR/HER2; Ki67 to be requested if not already performed and other disease-related biomarkers.
- Adverse events before start of treatment

Chemotherapy Cycles (will take place prior to each chemotherapy administration):

- Physical exam
- You will be asked about your ability to carry out daily activities (cycle 1 and upon completion of your chemotherapy).
- Weight
- Vital signs
- Adverse events
- Blood sample
- You will take the DHA/placebo capsules by mouth every day during chemotherapy treatment (4-6 cycles of chemotherapy, which would last approximately 12-18 weeks)
- Quality of Life questionnaire (only at end of 6th cycle)
- Exercise questionnaire

Upon completion of chemotherapy:
If you undergo a surgical procedure to remove the tumor after chemotherapy, we will collect information from your records regarding the extent of the surgical procedure and amount of blood loss. In addition, your tumor sample will be reassessed for Ki67 and other disease-related biomarkers.

Questionnaires

You will be provided with a questionnaire about food intake by research staff during cycle 1 of this study. The purpose of the questionnaire is to determine the amount of DHA in your diet, and other foods that can affect DHA in the body. The questionnaire will take about 1 hr. to complete.

You will also be asked to complete questionnaires about your symptoms and well-being (ESAS questionnaire and exercise questionnaire) at the beginning of each chemotherapy cycle. It may take you 15-20 minutes to complete both questionnaires.

The information you provide is for research purposes only and will remain strictly confidential. Some of the questions are personal; you may choose not to answer them.

Participant Diaries

You will be asked to keep a diary to record *your study supplement capsules intake.* Please record *the times and number of capsules when you take the capsules each day*. You will be asked to return the diary to *the Cross Cancer Institute at the end of each cycle*.

MANDATORY SAMPLE COLLECTION

The researchers doing this study need to do tests on samples as described below. *The biopsy sample will be examined to make sure you have the type of cancer that is being studied in the research study. The surgical resection will be examined and compared to the biopsy sample to see how the cancer cells respond to DHA supplementation. Blood samples will be examined to see how DHA supplementation affects the amount of DHA in these samples, and if DHA alters immune cells.*

The collection of these samples is a necessary part of this study and will be used only for this purpose. The samples will not be sold.

Once these tests have been completed, any leftover samples will be returned to the facility from which they were obtained if needed or destroyed, unless you wish to give permission for other future research purposes, in which case you will be given a separate optional consent form to sign.

Hereditary genetic testing (to look at whether cancer runs in your family) will not be done on these samples.

Reports about research tests done with your samples will be given to the study doctor(s). If you would like to learn the results of this research, please let them know.

Tissue Collection (Mandatory)

A small sample of your tissue that has already been removed by a previous surgery or biopsy will be obtained by the researchers doing this study. No further surgeries or biopsies are required of you for this purpose.

As part of your standard of care and necessary for this study, you will have had a tissue biopsy. Upon completion of your chemotherapy treatment and as part of your standard of care, you may

undergo a surgical procedure to remove the tumor from your breast. The amount of tissue to be removed will depend on the size and location of the tumor. Your doctor will give you more details regarding this procedure.

A sample of the tissues obtained from the initial biopsy and from the subsequent breast surgery will be sent to a laboratory at the *Cross Cancer Institute, and at the University of Alberta in Edmonton, Alberta, Canada,* where they will be examined to confirm your diagnosis *and examine how DHA alters tumour growth, and the amount of DHA in tumour cells*.

Blood Collection (Mandatory)

Blood samples will be taken by inserting a needle into a vein in your arm. These will be taken at the same time as your study related tests whenever possible upon *entry to the study, at the beginning of every cycle of chemotherapy (every three weeks), on day 20 of cycle 3 and before surgery. One tablespoon of blood will be collected for this study at those times*. These blood samples will be sent to a laboratory at the *Cross Cancer Institute and the University of Alberta in Edmonton, Alberta, Canada* where they will be examined to *measure the different cells in your blood, and the amount of DHA in these cells.*

Identification of Samples

To protect your identity, the information that will be on your samples will be limited to the *pathology identification number, and an identification number for the study.*
Despite protections being in place, there is a risk of unintentional release of information that could lead to loss of privacy. Due to technological advances in genetics, there is also a risk of unintentional release of genetic information from the samples. This information can be linked back to you and can lead to possible future discrimination in employment or insurance, against you or your biological relatives.

Withdrawal of Samples

If you no longer want your samples to be used in this research, you should tell the study doctor. The study doctor will ensure the samples are returned to the hospital from which they were obtained, if needed, or destroyed.

You can request withdrawal of your sample(s) until *you have received your blinded capsules* when the samples will be made anonymous. It won't be possible to return samples after this because the researchers will not know which samples are yours.

You will not be able to continue to participate in this study if required samples are withdrawn.

Assessments	Screening (within 21 days before chemotherapy)	Chemotherapy Cycle 1		Chemotherapy Cycle 2		Chemotherapy Cycle 3		Chemotherapy Cycle 4		Chemotherapy Cycle 5		Chemotherapy Cycle 6		End of Treatment	Surgery
		Day 1[2]	Day 20 (+/- 3 days)	Day 1	Day 20 (+/- 3 days)	Day 1	Day 20 (+/- 3 days)	Day 1	Day 20 (+/- 3 days)	Day 1	Day 20 (+/-3 days	Day 1	Day 20 (+/-3 days	Within 30 days after last dose	
Informed Consent	X														
Confirmation of previous or current medications	X	X		X		X		X		X		X		X	
Demographic data collection	X														
Physical Exam	X	X		X		X		X		X		X		X	
You will be asked about your ability to carry out daily activities	X	X												X	
Height	X														
Weight	X	X												X	
Vital Signs	X	X		X		X		X		X		X		X	
You will be asked about your medical history or current medical conditions	X	X		X		X		X		X		X		X	
You will be asked to complete questionnaires about your symptoms and well-being (ESAS questionnaire)	X	X		X		X		X		X		X		X	
You will be asked to complete questionnaire about your quality of life	X													X	
Exercise questionnaire	X	X		X		X		X		X		X		X	

Food frequency questionnaire		X (anytime within the first cycle)												
Blood will be taken for routine tests to monitor your health	X	X		X		X	X	X		X		X	X		
A sample of your tumour will be analyzed for disease-related biomarkers (signs related to your disease)	X														X
Blood will be collected to measure signs of immune function	X							X					X		
Blood will be collected to measure the level of study treatment in your blood lipids	X			X		X		X		X		X	X		
Treatment: DHA/Placebo		Days 1-21		Days 1-21		Days 1-21		Days 1-21		Days 1-21		Days 1-21			
You will complete a diary with your capsule intake		Days 1 -21		Days 1 -21		Days 1 -21		Days 1 -21		Days 1 -21		Days 1 -21			
You will be asked about any side effects which may or not be related to the study treatment	X	X		X		X		X		X		X		X	
We will collect results from your surgery report															X

OPTIONAL RESEARCH

The researchers doing this study are interested in doing additional optional research. You will be given a separate optional study consent form(s) to read and sign if you wish to give permission to this. You may decide not to participate in the "optional" study and still participate in this main study.

WHAT ARE THE POTENTIAL SIDE EFFECTS FROM PARTICIPATING IN THIS STUDY?

You may experience side effects from participating in this study. Some side effects are known and are listed below, but there may be side effects that are not expected. You should discuss these with the study doctor.

There are no known side effects of this omega 3 (DHA) supplement. A non-medicinal ingredient in this nutritional supplement that may cause an allergic reaction includes gelatin.

The risks and side-effects of the standard or usual treatment will be explained to you as part of your standard care. These risks are not included in this consent form.

A Data and Safety Monitoring Board (DSMB), an independent group of experts, will be reviewing the data throughout the conduct of the study to ensure continuing participant safety as well as scientific validity and quality of the research.

WHAT ARE THE REPRODUCTIVE RISKS?

There appears to be no effect of the nutritional product on the human reproductive system.

WHAT ARE THE BENEFITS OF PARTICIPATING IN THIS STUDY?

Participation in this study may or may not be of personal benefit to you. However, based on the results of this study, it is hoped that in the long-term, patient care can be improved.

WHAT ARE MY RESPONSIBILITIES AS A STUDY PARTICIPANT?

If you choose to participate in this study, you will be expected to:

- Tell the study doctor about your current medical conditions;
- Tell the study doctor about all prescription and non-prescription medications and supplements, including vitamins and herbals, that you may be taking and check with the study doctor before starting, stopping or changing any of these. This is for your safety as these may interact with the intervention you receive on this study;
- Tell the study doctor if you are thinking about participating on another research study;

- Attend all scheduled study visits and undergo all of the procedures described above;
- Return any unused DHA / placebo products;
- Return any *diaries and food frequency questionnaires* taken home to complete;
- Tell the study doctor if you become pregnant while participating on this study;
- Avoid taking fish oil supplements, or any supplements containing DHA.
- Stop taking other *supplements of vitamin C, vitamin E, or β-carotene exceeding the DRI (daily recommended intake), or other anti-oxidant supplements. A multivitamin with vitamin C, E, and β-carotene below the DRI are permitted (75 mg/day vitamin C, 15 mg/day vitamin E, and 700 µg/day β-carotene). A member of the research staff will go through the details of multivitamin intake to ensure it is within the guidelines.*
- *DHA supplement/ placebo capsules are* meant for you alone, and must not be shared with others. If someone accidently takes the capsules, the *intake should be recorded in medication diary, and the study staff should be informed.*

HOW LONG WILL I BE PARTICIPATING IN THIS STUDY?

The study intervention will last as long as it takes for you to receive your chemotherapy (about *12-18 weeks)*.
You may be seen more often if the study doctor determines that this is necessary or if your cancer *gets worse*.

WILL THERE BE ANY LONG-TERM FOLLOW-UP INVOLVED WITH THIS STUDY?

No matter which group you are randomized to, and even if you stop receiving the study intervention early, we would like to keep track of your health for *10 years to look at the long-term effects of your participation on the study*. We would do this *by accessing electronic or paper medical chart review at 3, 5 and 10 years after treatment.*

In the event it is necessary to further evaluate the safety or efficacy of the *DHA supplement*, it may be necessary to have access to additional information about your health status. The study team may attempt to obtain study-related information about your health from you or from other private sources, including your care physician and *electronic or paper medical chart review*. This may include contacting you again by phone or letter, but only if you have not withdrawn your consent for future contact. However, contacting you, your care physician or using other private sources of information, is optional, please indicate your decision using the check boxes below.

You give permission to the study doctor or member of the study team to attempt to obtain study-related information about your health status to further evaluate the safety or efficacy of *DHA supplementation*. This may include contacting your care physician, or by contacting you by phone or letter (i.e., future contact).

☐ Yes ☐ No Participant's Initials: _____

Name/phone number of care physician: _____

In addition, the study team may also attempt to obtain study-relevant information about your health information from public sources such as national patient registries (e.g., cancer registries)

If the study doctor needs to follow up with you but cannot locate you, either because you have moved and not updated your contact information or if, for some reason, your contact information is no longer accurate, the study doctor would like to obtain your new contact information (e.g., address, telephone number) by calling or writing to the persons you've named as your secondary contacts. This is optional, please indicate your decision using the check boxes below.

You give permission to the study doctor or member of the study team to contact your secondary contacts if the study doctor or study team no longer have accurate contact information for you.

☐ Yes ☐ No Participant's Initials: _____

Name/phone number of secondary contacts: _____

If the study doctor cannot obtain information through your secondary contacts, he/she would like to ask for assistance of a third party that specializes in locating persons. The study doctor may only share limited information about you (name and last known address) with a third party locator. None of your personal health or study-related information will be shared with the third party locator. The third party locator will consult public sources and databases to obtain your current contact information but will not contact you. The third party locator will only share this information with the study doctor or study team to help complete the follow-up stage of the study. Only the study doctor or a member of the study team will attempt to contact you directly. This is optional, please indicate your decision using the check boxes below.

If the study doctor is not able to obtain your contact information from your secondary contacts, you give permission for the study doctor to provide your name and last location to a third party that specializes in locating persons.

☐ Yes ☐ No Participant's Initials: _____

CAN I CHOOSE TO LEAVE THIS STUDY EARLY?

You can choose to end your participation in this research (called early withdrawal) at any time without having to provide a reason. If you choose to withdraw early from the study without finishing the intervention, procedure or follow-up, you are encouraged to contact the study doctor or study staff.

You may be asked questions about your experience with the study intervention, and to have laboratory tests and physical examinations considered necessary to safely stop your study involvement.

You may withdraw your permission to use information that was collected about you for this study at any time by letting the study doctor know. However, this would also mean that you withdraw from the study.

Information that was recorded before you withdrew will be used by the researchers for the purposes of the study, but no additional information will be collected or sent to the sponsor after you withdraw your permission.

CAN MY PARTICIPATION IN THIS STUDY END EARLY?

The study doctor may stop your participation in the study early, and without your consent, for reasons such as:

- The intervention does not work for you;
- You are unable to tolerate the study intervention;
- You are unable to complete all required study procedures;
- New information shows that the study intervention is no longer in your best interest;
- The study doctor no longer feels this is the best treatment for you;
- A regulatory authority (for example, Health Canada) or the research ethics board withdraws permission for the study to continue;
- Your treatment assignment becomes known to others (the study doctor or study staff);

If you are removed from the study, the study doctor will discuss the reasons with you and plans will be made for your continued care outside of the study.

HOW WILL MY PERSONAL INFORMATION BE KEPT CONFIDENTIAL?

If you decide to participate in this study, the study doctor and study staff will only collect the information they need for this study.

Records identifying you, including information collect from your medical files/records, such as your Electronic Medical Records (EMR), Netcare, charts, etc., will be kept confidential to the extent permitted by the applicable laws, will not be disclosed or made publicly available, except as described in this consent document.

Authorized representatives of the following organizations may look at your identifiable medical/clinical study records at the site where these records are held for quality assurance purposes and/or to verify that the information collected for the study is correct and follows proper laws and guidelines:

- Members of the Regulatory/Audit team at *Cross Cancer Institute,* for quality assurance purposes;
- The Health Research Ethics Board of Alberta – Cancer Committee, which oversees the ethical conduct of this study;
- Health Canada, which oversees the use of natural health products/drugs/devices in Canada and the conduct of clinical trials;

Authorized representatives of the above organizations and of the University of Alberta may **receive** information related to the study from your medical/clinical study records that will be kept confidential in a secure location and may be used in current or future relevant health research. Your name or other information that may identify you will not be provided (i.e., the information will be de-identified). The records received by these organizations will be coded with a number. The key that indicates what number you have been assigned will be kept secure by the researchers directly involved with your study and will not be released.

Any disclosure of your identifiable health information will be done in accordance with federal and provincial laws including the Alberta Health Information Act (HIA). The organizations listed above are required to have organizational policies and procedures to protect the information they see or receive about you, except where disclosure may be required by law. The study doctor will ensure that any personal health information collected for this study is kept in a secure and confidential location at the *Cross Cancer Institute, Edmonton Alberta* as also required by law.

If the results of this study are published, your identity will remain confidential. It is expected that the information collected during the study will be *used in analyses and will be published/presented to the scientific community at meetings and in journals*. This information may also be used as part of a submission to regulatory authorities around the world to support the approval of this intervention.

Even though the likelihood that someone may identify you from the study data is very small, it can never be completely eliminated. Every effort will be made to keep your identifiable information confidential, and to follow the ethical and legal rules about collecting, using and disclosing this information.

WILL MY HEALTHCARE PROVIDER(S) BE INFORMED OF MY PARTICIPATION IN THIS STUDY?

Your family doctor/health care provider will not be informed by the study team that you are taking part in the study. You can choose to let your family doctor/health care provider know, if you like. If you are undecided, the study doctor can discuss this with you.

WILL THERE BE ANY COSTS INVOLVED WITH PARTICIPATING IN THIS STUDY?

The DHA supplement/ placebo will be given to you free of charge while you take part in this study.

Taking part in this study may result in added costs to you. For example:

- There may be costs associated with hospital visits. For instance, parking, transportation, or snacks/meals during the study.

Possible Costs After the Study is Complete

You may not be able to receive the study intervention after your participation in the study is completed. There are several possible reasons for this, some of which are:

- The intervention may not turn out to be effective or safe;
- The intervention may not be approved for use in Canada;
- Your caregivers may not feel it is the best option for you;
- You may decide it is too expensive and insurance coverage may not be available;
- The intervention, even if approved in Canada, may not be available free of charge.

The study doctor will discuss these options with you.

WILL I BE COMPENSATED FOR PARTICIPATING IN THIS STUDY?

You will not be paid for taking part in this study.

It is possible that the research conducted using your samples and/or study data may eventually lead to the development of new diagnostic tests, new drugs or devices, or other commercial products. There are no plans to provide payment to you if this happens.

In the case of research-related side effects or injury, as a direct result of participating in this research, you will receive all medical treatments or services recommended by your doctors.

Although no funds have been set aside to compensate you in the event of injury or illness related to the study treatment or procedures, you do not give up any of your legal rights for compensation by signing this form.

WHAT ARE MY RIGHTS AS A PARTICIPANT IN THIS STUDY?

You will be told, in a timely manner, about new information that may be relevant to your willingness to stay in this study.

You have the right to be informed of the results of this study once the entire study is complete. If you would like to be informed of these results, please contact the study doctor.

The results of this study will be available on a clinical registry; refer to the section titled “Where can I find online information about this study?”.

Your rights to privacy are legally protected by federal and provincial laws that require safeguards to ensure that your privacy is respected.

By signing this form you do not give up any of your legal rights against the hospital, investigators, sponsor, involved institutions for compensation or their agents, nor does this form relieve these parties from their legal and professional responsibilities.

IS THERE CONFLICT OF INTEREST RELATED TO THIS STUDY?

There are no conflicts of interest declared between the study doctor and sponsor of this study.

WHAT IF RESEARCHERS DISCOVER SOMETHING ABOUT ME AS A RESEARCH PARTICIPANT?

During the study, the researchers may learn something about you that they didn’t expect. For example, the researchers may *find out that you have another medical condition*.

If any clinically important information about your health is obtained as a result of your participation in this study, you will be given the opportunity at that time to decide whether you wish to be made aware of that information.

WHERE CAN I FIND ONLINE INFORMATION ABOUT THIS STUDY?

A description of this clinical trial will be available on *http://www.ClinicalTrials.gov*.

The study registration number to use this website is: NCT03831178

This website will not include information that can identify you. You can search for this website at any time.

WHO DO I CONTACT FOR QUESTIONS?

If you have questions about taking part in this study, or if you suffer a research-related injury, you should talk to the study doctor, co-investigator or study nurse. These person(s) are:

Dr. John Mackey	780-432-8221
Name	Telephone
Name	Telephone
Name	Telephone

He can also be paged through the Cross Cancer Institute switchboard at (780) 432-8771.

If you have questions about your rights as a participant or about ethical issues related to this study and you would like to talk to someone who is not involved in the conduct of the study, please contact the Office of the Health Research Ethics Board of Alberta – Cancer Committee at:

Telephone: 780-423-5727 Toll Free: 1-877-423-5727

SIGNATURES

Part 1 - to be completed by the potential participant.

	Yes	No
Do you understand that you have been asked to take part in a research study?	☐	☐
Do you understand why this study is being done?	☐	☐
Do you understand the potential benefits of taking part in this study?	☐	☐
Do you understand the risks of taking part in this study and the risks of becoming pregnant or fathering a child during this study?	☐	☐
Do you understand what you will be asked to do should you decide to take part in this study?	☐	☐
Do you understand the alternatives to participating in this study?	☐	☐
Do you understand that you are free to leave the study at any time, without out having to give reason and without affecting your future health care?	☐	☐
Do you understand who will see your records, including health information that identifies you?	☐	☐
Do you understand that by signing this consent form you are giving us permission to access your health information and specimens if applicable?	☐	☐
Do you understand that by signing this consent form that you do not give up any of your legal rights?	☐	☐
Have you had enough opportunity to ask questions and discuss this study?	☐	☐

By signing this form I agree, to participate in this study.

Signature of Participant	PRINTED NAME	Date

Part 2 - to be completed by the study doctor or designee who conducted the informed consent discussion. Only compete this section if the potential participant has **agreed** to participate.

I believe that the person signing this form understands what is involved in the study and has freely decided to participate.

Signature of Person Conducting the Consent Discussion	PRINTED NAME	Date

Part 3 - to be completed only if the participant is unable to read or requires assistance of an oral translator/interpreter.

- The informed consent form was accurately explained to, and apparently understood by the participant.
- Informed consent was freely given by *or on behalf of* the participant.

Signature of Impartial Witness/Interpreter	PRINTED NAME	Date

You will be given a copy of this signed and dated consent form prior to participating in this study.

Informed Consent Form for Participation in Optional Research

DHA for Women with Breast Cancer in the Neoadjuvant Setting (DHA WIN)

DHA to improve effectiveness of Chemotherapy in Breast Cancer

Protocol ID: IIT-0005

Researcher: Dr. John Mackey
Department of Medical Oncology
Cross Cancer Institute
780-432-8221

Funder(s)/Sponsor: Alberta Health Services- Cross Cancer Institute

INTRODUCTION

In addition to the main study, you also are being invited to take part in optional research. Although it is optional, the study of human samples and data focusing on the prevention, diagnosis and treatment of cancer and other diseases is an important part of research. Taking part in this optional research is voluntary. You still can take part in the main study, and will continue to receive treatment and care even if you say “no” to any or all of this optional research now or later. This form and your discussion with the researcher/study staff will give you the information you need to make your decision.

WHY IS THIS OPTIONAL RESEARCH BEING DONE?

The researchers conducting this research are interested in doing the following:

- Biomarker research for the main study using tumour tissue / blood already collected
- Bio-banking for use in future research using tumour tissue / blood already collected

As part of this optional research, the researchers would like to examine your tumour tissue/blood samples to look for any **biomarkers** (small “signature” molecules or indicators) in your cancer cells or circulating in your blood. These biomarkers might help predict which patients are most likely to be affected by the study drug. This is called biomarker research.

Bio-banking is the collection, storage, and use of human body samples and related health information for future research. It provides an important resource for health research

locally, across Canada, and around the world. The researchers doing the main study are also interested in storing your tissue/blood samples for future research. The research that may be done on your samples in the future is unknown at this time. It may be related to your condition or it may be used to address research questions that are unrelated.

Some of this research may be about genes. Genes carry information about features, such as hair or eye colour. This research may include looking at changes in genes found in you and in people who are related to you. These changes may be inherited (passed on in families). This is called hereditary genetic testing. Researchers also may be interested in the way that genes affect health and disease, or how your body responds to treatment.

WHAT WILL HAPPEN DURING THIS OPTIONAL RESEARCH?

You may take part in all or some of the optional research described here, it is your choice. If you agree to take part:

- the samples used for this optional research have already been collected as part of your standard of care. No further biopsies or surgeries are needed for this purpose.
- the blood samples used for this optional research will be those left over or remaining from your participation in the main study. No further biopsies or surgeries are needed for this purpose.

HOW WILL MY SAMPLES BE HANDLED?

Your sample(s) and some related health information already collected from your participation in the main study will be sent to the Nutritional Immunology laboratory at the University of Alberta, Edmonton, AB, for analysis. The samples and data will be kept indefinitely or until they are used up, destroyed or returned to the hospital where you had your surgery or biopsy.

Qualified researchers can submit a request to use the materials stored at the University of Alberta. Your samples and related health information will be used only by researchers whose requests have been accepted by the sponsor and who have met regulatory requirements and secured ethics approval for their research. The samples and data may be sent to other countries. Your name or any other information that could directly identify you will not be given to these researchers.

The results of research done on your samples will not be added to your personal health records and you or the researcher will not know the results.

WHAT ARE THE RISKS OF PARTICIPATING IN THIS OPTIONAL RESEARCH?

Risks related to sample collection:

- Since the tissue sample(s) already have been collected for the main study or as part of your standard of care, no additional physical risks are expected.

Alberta Health Services

Risks related to the disclosure of personal health information:

- There is a risk that someone could get access to the personal information in your personal health records or other information researchers have stored about you.
- There is a risk that someone could trace the information in a central or public database back to you. Even without your name or other identifiers, your genetic information is unique to you. The researchers believe the chance that someone will identify you is very small, but the risk may change in the future as people come up with new ways of tracing information.
- New health information about inherited traits that might affect you or your blood relatives could be found during a study. The researchers believe the chance these things will happen is very small, but cannot promise that they will not occur.
- Due to the rapid pace of technological advances, the potential future use of genetic information is unknown and therefore the potential future risks also are unknown.
- There may be risks to eligibility for employment or insurance if the results of genetic testing were inadvertently disclosed to certain parties.
- Genetic information cannot be protected from court-ordered disclosure.

WHAT ARE THE POTENTIAL BENEFITS OF PARTICIPATING IN THIS OPTIONAL RESEARCH?

You will not benefit directly from taking part in this optional research. However, research done with your donated samples or health information may benefit other patients with your condition or other similar or related condition(s).

HOW WILL MY PERSONAL INFORMATION BE KEPT PRIVATE?

Your privacy is very important to the researchers and they will make every effort to protect it. Here are the steps they will take:

- When your sample(s) are sent to the laboratory, no information identifying you (such as your name, date of birth, health insurance number) will be provided or shared. Samples may be identified by your study code.
- The samples that are provided to researchers by the Cross Cancer Institute are identified only by that biobank code; researchers will not know who you are.
- The list that links the samples to your personal identifiers (i.e., name) will be kept separate from your sample(s) and health information in a secure and confidential location at the main study site. If you change your mind about participating in this optional research, this list will be used to locate and return or destroy your samples. Decoding can only be done by the researcher or an individual authorized by the researcher.
- Study records will be kept for 25 years.
- A record of your participation in this optional study will be kept with your main study records and may be monitored for quality assurance.

Information that identifies you, will be kept confidential and, to the extent permitted by the applicable laws, will not be disclosed or made publicly available except as described in this document. If research results are published, your name and other personal information will not be used.

Qualified representatives of the sponsor will make sure the study has been done properly by checking your records at the researcher's site. Regulatory authorities, such as Health Canada and the applicable Research Ethics Board also may wish to check that the study has been done properly, and may also have direct access to your personal health information. Except as expressly stated in this section, all of the information provided in the main study consent form about confidentiality and direct access to your personal health information applies to this optional research consent form.

WHAT IF RESEARCHERS DISCOVER SOMETHING ABOUT ME DURING THE STUDY?

During the study, the researchers may learn something about you that they didn't expect. For example, the researchers may find out that you have another medical condition.

If any new clinically important information about your health is obtained as a result of your participation in this optional research, you will be given the opportunity to decide whether you wish to be made aware of that information.

WILL THERE BE ANY COSTS OR COMPENSATION INVOLVED WITH THS RESEARCH?

There are no costs to you. You will not be paid for taking part. No samples or information/data will be sold.

It is possible that the research conducted using your samples and/or my data may eventually lead to the development of new diagnostic tests, new drugs or other commercial products. There are no plans to provide payment to you if this happens.

WHAT ARE MY RIGHTS AS A PARTICIPANT IN THIS OPTIONAL RESEARCH?

You will be told, in a timely manner, about new information that may be relevant to your willingness to stay in this study.

If you decide you no longer want your samples or related health information to be used, you should tell the researcher. Any sample(s) that remain(s) in the laboratory will be destroyed (if blood) or returned to the hospital where you had your original biopsy or surgery (if tumour block). If tests have already been done on your sample and included in an analysis or publication, it will not be possible to withdraw these results.

You will be given a copy of this signed and dated consent form prior to participating in this

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study.

IS THERE ANY CONFLICT OF INTEREST RELATED TO THIS OPTIONAL RESEARCH?
There are no current or potential conflicts of interest concerning the optional research study.

WHO DO I CONTACT FOR QUESTIONS RELATED TO THIS OPTIONAL RESEARCH?

If you have questions about the use of your samples/data for optional research, or if you suffer a research-related injury, contact the researcher of this optional study:

Catherine J Field	780-492-5297
Name	Telephone Number

If you have questions about your rights as a participant or about ethical issues related to this optional research and you would like to speak to someone not involved in its conduct, please contact the Office of the Health Research Ethics Board of Alberta – Cancer Committee at: 780-423-5727 or toll-free 1-877-423-5727.

UNDERSTANDING AND SIGNATURES PAGE

Please circle your answer to show whether or not you would like to take part in the optional research:

I agree that samples which were already collected and related health information may be used for the optional research described above.

YES NO

I agree that my samples and related health information may be kept in a biobank for use in future health research related to my condition or may be used to address research questions that are unrelated.

YES NO

I agree that the researcher, or their representative, may contact me or my physician to see if I wish to learn about results from this research.

YES NO

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SIGNATURES

PARTICIPANT ACKNOWLEDGEMENT

- I understand the information within this optional consent form.
- All of my questions have been answered to my satisfaction.
- I am aware of the risks and potential benefits to me of participating in this optional research.
- I allow access to my personal health information and samples as explained in this form.
- I understand that I do not give up any of my legal rights by signing this consent form.
- I agree to take part in this optional research as described and where "YES" above has been circled.

_____	_____	_____
Signature of Participant	Printed Name	Date

STUDY TEAM ACKNOWLEDGEMENT

I believe that the person signing this form understands what is involved in this optional research and has freely decided to participate.

_____	_____	_____
Signature of Person Conducting the Consent Discussion	Printed Name	Date

PARTICIPANT ASSISTANCE (IMPARTIAL WITNESS)

This section is to be completed only if the participant is unable to read the consent document. The individual assisting the participant must be impartial.

- The informed consent form was accurately explained to, and apparently understood by the research participant.
- Informed consent was freely given by the participant.

_____	_____	_____
Signature of Impartial Witness	Printed Name	Date

TRANSLATOR/INTERPRETER ACKNOWLEDGEMENT

This section is to be completed only if the participant requires the assistance of a qualified oral translator/interpreter. The interpreter must be impartial.

- The informed consent discussion was accurately explained to, and apparently

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understood by the research participant.

- A sight translation of the consent document was provided by the interpreter as directed by the research staff conducting the consent process.

Signature of Interpreter	Printed Name	Date

You will be given a copy of this signed and dated consent form prior to participating in this optional research.

Supplementary Table 1: List of Antibodies used for immune cell phenotyping

CD1a	FITC	300104
CD1c	BV421	331526
CD3	FITC	300306
CD4	APC	357408
CD8	PerCP/Cy5.5	344710
CD11b	PE	301306
CD11c	APC	301614
CD14	APC	367118
CD16	PE	302008
CD20	FITC	302304
CD25	PE	302606
CD27	PECy7	356412
CD28	APC	302912
CD45RA	PE	304108
CD45RO	FITC	304204
CD56	APC	362504
CD86	PCP	374210
CD95	BV421	305624
CD103	PECy7	350212
CD107	PE	328608
CD141	PECy7	344110
CD152	PE	369604
CD183	PerCP/Cy5.5	353720
CD196	PE	353410
CD279	APC	329908
FOXP3	FITC	320106
HLADR	PerCP/Cy5.5	307630

SPIRIT
STANDARD PROTOCOL ITEMS: RECOMMENDATIONS FOR INTERVENTIONAL TRIALS

SPIRIT 2013 Checklist: Recommended items to address in a clinical trial protocol and related documents*

Section/item	Item No	Description	Reported on Page No
Administrative information			
Title	1	Descriptive title identifying the study design, population, interventions, and, if applicable, trial acronym	1
Trial registration	2a	Trial identifier and registry name. If not yet registered, name of intended registry	a
	2b	All items from the World Health Organization Trial Registration Data Set	Supp. files
Protocol version	3	Date and version identifier	1
Funding	4	Sources and types of financial, material, and other support	22
Roles and responsibilities	5a	Names, affiliations, and roles of protocol contributors	1
	5b	Name and contact information for the trial sponsor	1
	5c	Role of study sponsor and funders, if any, in study design; collection, management, analysis, and interpretation of data; writing of the report; and the decision to submit the report for publication, including whether they will have ultimate authority over any of these activities	22
	5d	Composition, roles, and responsibilities of the coordinating centre, steering committee, endpoint adjudication committee, data management team, and other individuals or groups overseeing the trial, if applicable (see Item 21a for data monitoring committee)	20-21
Introduction			
Background and rationale	6a	Description of research question and justification for undertaking the trial, including summary of relevant studies (published and unpublished) examining benefits and harms for each intervention	4-5

	6b	Explanation for choice of comparators	7
Objectives	7	Specific objectives or hypotheses	5
Trial design	8	Description of trial design including type of trial (eg, parallel group, crossover, factorial, single group), allocation ratio, and framework (eg, superiority, equivalence, noninferiority, exploratory)	6, Fig 1, Fig 2
Methods: Participants, interventions, and outcomes			
Study setting	9	Description of study settings (eg, community clinic, academic hospital) and list of countries where data will be collected. Reference to where list of study sites can be obtained	6
Eligibility criteria	10	Inclusion and exclusion criteria for participants. If applicable, eligibility criteria for study centres and individuals who will perform the interventions (eg, surgeons, psychotherapists)	Table 1 (page 1,2)
Interventions	11a	Interventions for each group with sufficient detail to allow replication, including how and when they will be administered	7
	11b	Criteria for discontinuing or modifying allocated interventions for a given trial participant (eg, drug dose change in response to harms, participant request, or improving/worsening disease)	8
	11c	Strategies to improve adherence to intervention protocols, and any procedures for monitoring adherence (eg, drug tablet return, laboratory tests)	8
	11d	Relevant concomitant care and interventions that are permitted or prohibited during the trial	-
Outcomes	12	Primary, secondary, and other outcomes, including the specific measurement variable (eg, systolic blood pressure), analysis metric (eg, change from baseline, final value, time to event), method of aggregation (eg, median, proportion), and time point for each outcome. Explanation of the clinical relevance of chosen efficacy and harm outcomes is strongly recommended	8-11 Table 3
Participant timeline	13	Time schedule of enrolment, interventions (including any run-ins and washouts), assessments, and visits for participants. A schematic diagram is highly recommended (see Figure)	12, Table 2

Sample size	14	Estimated number of participants needed to achieve study objectives and how it was determined, including clinical and statistical assumptions supporting any sample size calculations	12
Recruitment	15	Strategies for achieving adequate participant enrolment to reach target sample size	13
Methods: Assignment of interventions (for controlled trials)			
Allocation:			
Sequence generation	16a	Method of generating the allocation sequence (eg, computer-generated random numbers), and list of any factors for stratification. To reduce predictability of a random sequence, details of any planned restriction (eg, blocking) should be provided in a separate document that is unavailable to those who enrol participants or assign interventions	13
Allocation concealment mechanism	16b	Mechanism of implementing the allocation sequence (eg, central telephone; sequentially numbered, opaque, sealed envelopes), describing any steps to conceal the sequence until interventions are assigned	13
Implementation	16c	Who will generate the allocation sequence, who will enrol participants, and who will assign participants to interventions	13
Blinding (masking)	17a	Who will be blinded after assignment to interventions (eg, trial participants, care providers, outcome assessors, data analysts), and how	13
	17b	If blinded, circumstances under which unblinding is permissible, and procedure for revealing a participant's allocated intervention during the trial	13
Methods: Data collection, management, and analysis			
Data collection methods	18a	Plans for assessment and collection of outcome, baseline, and other trial data, including any related processes to promote data quality (eg, duplicate measurements, training of assessors) and a description of study instruments (eg, questionnaires, laboratory tests) along with their reliability and validity, if known. Reference to where data collection forms can be found, if not in the protocol	14-18 Table 3

	18b	Plans to promote participant retention and complete follow-up, including list of any outcome data to be collected for participants who discontinue or deviate from intervention protocols	-
Data management	19	Plans for data entry, coding, security, and storage, including any related processes to promote data quality (eg, double data entry; range checks for data values). Reference to where details of data management procedures can be found, if not in the protocol	18
Statistical methods	20a	Statistical methods for analysing primary and secondary outcomes. Reference to where other details of the statistical analysis plan can be found, if not in the protocol	19-20 Table 3
	20b	Methods for any additional analyses (eg, subgroup and adjusted analyses)	19-20
	20c	Definition of analysis population relating to protocol non-adherence (eg, as randomised analysis), and any statistical methods to handle missing data (eg, multiple imputation)	-
Methods: Monitoring			
Data monitoring	21a	Composition of data monitoring committee (DMC); summary of its role and reporting structure; statement of whether it is independent from the sponsor and competing interests; and reference to where further details about its charter can be found, if not in the protocol. Alternatively, an explanation of why a DMC is not needed	20
	21b	Description of any interim analyses and stopping guidelines, including who will have access to these interim results and make the final decision to terminate the trial	21
Harms	22	Plans for collecting, assessing, reporting, and managing solicited and spontaneously reported adverse events and other unintended effects of trial interventions or trial conduct	20
Auditing	23	Frequency and procedures for auditing trial conduct, if any, and whether the process will be independent from investigators and the sponsor	21
Ethics and dissemination			

Research ethics approval	24	Plans for seeking research ethics committee/institutional review board (REC/IRB) approval	21
Protocol amendments	25	Plans for communicating important protocol modifications (eg, changes to eligibility criteria, outcomes, analyses) to relevant parties (eg, investigators, REC/IRBs, trial participants, trial registries, journals, regulators)	21
Consent or assent	26a	Who will obtain informed consent or assent from potential trial participants or authorised surrogates, and how (see Item 32)	21 Supp file
	26b	Additional consent provisions for collection and use of participant data and biological specimens in ancillary studies, if applicable	21 Supp file
Confidentiality	27	How personal information about potential and enrolled participants will be collected, shared, and maintained in order to protect confidentiality before, during, and after the trial	22
Declaration of interests	28	Financial and other competing interests for principal investigators for the overall trial and each study site	22
Access to data	29	Statement of who will have access to the final trial dataset, and disclosure of contractual agreements that limit such access for investigators	22
Ancillary and post-trial care	30	Provisions, if any, for ancillary and post-trial care, and for compensation to those who suffer harm from trial participation	22
Dissemination policy	31a	Plans for investigators and sponsor to communicate trial results to participants, healthcare professionals, the public, and other relevant groups (eg, via publication, reporting in results databases, or other data sharing arrangements), including any publication restrictions	22
	31b	Authorship eligibility guidelines and any intended use of professional writers	22
	31c	Plans, if any, for granting public access to the full protocol, participant-level dataset, and statistical code	-
Appendices			
Informed consent materials	32	Model consent form and other related documentation given to participants and authorised surrogates	Supp file

Biological specimens	33	Plans for collection, laboratory evaluation, and storage of biological specimens for genetic or molecular analysis in the current trial and for future use in ancillary studies, if applicable	Supp file

*It is strongly recommended that this checklist be read in conjunction with the SPIRIT 2013 Explanation & Elaboration for important clarification on the items. Amendments to the protocol should be tracked and dated. The SPIRIT checklist is copyrighted by the SPIRIT Group under the Creative Commons "Attribution-NonCommercial-NoDerivs 3.0 Unported" license.

BMJ Open

Protocol of a double blind, phase II randomized controlled trial to compare Docosahexaenoic acid (DHA) concomitant with neoadjuvant chemotherapy versus neoadjuvant chemotherapy alone in the treatment of breast cancer: DHA WIN

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Primary Subject Heading:	Oncology
Secondary Subject Heading:	Nutrition and metabolism, Immunology (including allergy)

Keywords:	ki67, phospholipids, omega-3, apoptosis, proliferation, immune function

Protocol of a double blind, phase II randomized controlled trial to compare Docosahexaenoic acid (DHA) concomitant with neoadjuvant chemotherapy versus neoadjuvant chemotherapy alone in the treatment of breast cancer: DHA WIN

Protocol Number: IIT-0005

Version Date: March 17, 2019

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Word Count: 5804

ABSTRACT

Introduction: Neoadjuvant chemotherapy for breast cancer treatment is prescribed to facilitate surgery, provide confirmation of drug sensitive disease and the achievement of pathological complete response (pCR) predicts improved long-term outcomes. Docosahexaenoic acid (DHA) has been shown to reduce tumor growth in preclinical models when combined with chemotherapy and is known to beneficially modulate systemic immune function. The purpose of this trial is to investigate the benefit of DHA supplementation in combination with neoadjuvant chemotherapy in patients with breast cancer.

Methods and analysis: This is a double blind phase II randomized controlled trial of 52 women prescribed neoadjuvant chemotherapy to test if DHA supplementation enhances chemotherapy efficacy. The DHA supplementation group will take 5g/day DHA orally and the placebo group will take equal fat supplement of vegetable oil. The primary outcome will be change in Ki67 labelling index from pre-chemotherapy core needle biopsy to definitive surgical specimen.

Secondary endpoints include assessment of: i) DHA plasma phospholipid content; ii) systemic immune cell types, plasma cytokines and inflammatory markers iii) tumor markers for apoptosis and tumor infiltrating lymphocytes iv) rate of pCR in breast and in axillary nodes v) frequency of grade 3 and 4 chemotherapy associated toxicities and vi) patient perceived quality of life.

The trial has 81% power to detect a significant between-group difference in Ki67 index with a two-sided t-test of less than 0.0497, and accounts for 10% drop-out rate.

Ethics and dissemination: This study has full approval from the Health Research Ethics Board of Alberta – Cancer Committee (Protocol #: HREBA.CC -18-0381). We expect to present the findings of this study to the scientific community in peer-reviewed journals and at conferences. The results of this study will provide evidence for supplementing with DHA during neoadjuvant chemotherapy treatment for breast cancer.

Clinical Trial Registration No: NCT03831178

KEYWORDS

Ki67, phospholipids, fatty acids, omega-3, apoptosis, proliferation, immune function

STRENGTHS AND LIMITATIONS OF THIS STUDY

- This study is the first phase II randomized controlled trial to evaluate DHA supplementation concomitant with neoadjuvant chemotherapy to treat non-metastatic breast cancer.
- The intervention is minimally invasive and side effects from the supplementation are not expected.
- This study is powered to examine the key clinical outcome of changes in Ki67 index from pre-chemotherapy biopsy to surgical excision based on group sample sizes of 23 patients in group one and 23 patients in group two in order to achieve 81% power to detect a difference between the group proportions of 0.4.
- This study will measure clinically relevant intermediate outcomes including rate of pCR in breast and in axillary nodes, rate of grade 3 and 4 chemotherapy associated toxicities and hospitalizations as well as additional outcomes including plasma phospholipid content of DHA, markers of immune function (plasma cytokines, inflammatory markers

and lymphocyte function), tumor markers for apoptosis and tumor infiltrating lymphocytes and patient perceived quality of life.

- The study will include all subtypes of breast cancer patients undergoing neoadjuvant chemotherapy but is not powered to assess differences between subtypes.

INTRODUCTION

Despite improvements in early diagnosis and treatment, breast cancer remains the second leading cause of cancer related death in women [1]. While neoadjuvant chemotherapy aims to improve surgical resection outcomes and reduce / eliminate micrometastases [2,3], pathological complete response (pCR) is not achieved by all patients [3]. Increasing the efficacy of neoadjuvant treatment without adding additional side-effects would benefit this population.

DHA is an omega-3 long chain polyunsaturated fatty acid (n-3 LCPUFA). The majority of n-3 fatty acids are in the form of the 18-carbon fatty acid alpha-linolenic acid (ALA). While DHA can be synthesized from ALA and other n-3 LCPUFA in the body, endogenous synthesis is low [4,5]. Consequently, the direct consumption of this fatty acid is the only way to significantly increase levels of DHA in tissues [6]. Supplementation can increase blood plasma DHA concentration by 2-fold (500 μM), which can lead to plasma membrane lipid enrichment [7]. Incorporation of DHA into tumor membrane phospholipids has been shown to reduce breast cancer cell proliferation [8,9] and increase apoptosis [10-15] in vitro and decreases tumor growth in animal models [13,15-17]. Additionally, providing/feeding DHA has been shown to increase the efficacy of different chemotherapeutic drugs in animal models of breast cancer [11,12,18,19]. While there is limited clinical evidence, it has been shown that increased dietary intake of n-3 LCPUFA, including DHA, results in increased DHA incorporation in breast adipose tissue [20] and this correlates with improved response to chemotherapy [21]. In an

open label trial with advanced metastatic breast cancer patients, DHA supplementation and enrichment into plasma phospholipids was associated with improved outcomes [22]. Other clinical trials have reported that supplementation with n-3 LCPUFA at a wide range of doses (0.6g-8.6 g/day) increased tolerability of chemotherapeutic drugs in a range of malignancies at other sites, include lung, pancreatic and colorectal (reviewed in [23]). Consequently, we hypothesize that the therapeutic index (efficacy: toxicity ratio) of neoadjuvant breast cancer chemotherapy will be improved with the addition of DHA to the treatment.

Breast cancer proliferation can be assessed by immunohistochemical (IHC) analysis of cells staining positive for the nuclear antigen Ki67 [24], as it is expressed in all phases of the cell cycle, G_1, S, G_2, and M, but not in G_0 [25,26]. The proportion of cells staining for Ki67 is frequently used as a primary endpoint to measure efficacy of neoadjuvant therapy in clinical trials. The Ki67 index, defining the change between pre- and post-treatment Ki67, has been reported to be an independent prognostic factor in luminal A, luminal B, triple-negative, and HER2+ breast cancer, and has been reported to be a useful surrogate marker of relapse free survival in luminal B, triple- negative, and HER2+ breast cancer [27,28].

OBJECTIVES

The objective of this RCT is to assess the efficacy of supplemental DHA combined with neoadjuvant chemotherapy in treatment naïve women with breast cancer measured by changes in Ki67 index from biopsy to surgical excision. We hypothesize that DHA supplementation will increase plasma phospholipid DHA and improve response to neoadjuvant chemotherapy assessed by decrease in Ki67 index.

This protocol follows the Standard Protocol Items for Randomized Trials (SPIRIT) guideline (Spirit Checklist: Supplemental Table 1, WHO Checklist: Supplemental Table 2) [29,30].

Study Design

The DHA-WIN trial will be a two-arm, double blind phase II randomized controlled trial comparing DHA supplementation and placebo (vegetable oil). The proposed study design with outcomes depicted is shown in **Figure 1** and SPIRIT participant flow chart is shown in **Figure 2**.

METHODS AND ANALYSIS

Study Population

Eligible women have invasive breast cancer (clinical stage I, II or III) for whom systemic chemotherapy [31] is recommended prior to surgery. The study will occur at the Cross Cancer Institute, with central laboratory and clinical analyses occurring the University of Alberta, both in Edmonton, Alberta, Canada. Inclusion and exclusion criteria are listed in **Table 1**

Table 1: Inclusion and Exclusion Criteria for DHAWIN

Inclusion Criteria
1) ECOG Performance status of 0 or 1
2) Hematology and biochemistry assessments [CBC and differential, partial thromboplastin time (PTT), prothrombin time/ international normalized ratio (PT/INR), aspartate aminotransferase **(**AST**),** alkaline phosphatase (ALP), bilirubin, and creatinine] within normal range unless determined not clinically significant by the qualified investigator
3) Ability to take oral medications
4) Adequate tissue specimen for diagnosis, biomarkers, and endpoint Ki67 assays
Exclusion Criteria
1. Patients undergoing surgery prior to chemotherapy
2. Current or previous (within 2 months) daily use (>1 day/week) use of omega-3, fish oil, or other supplements or foods containing DHA (at daily doses > 200 mg)
3. Known allergy to soy or corn
4. Continued intake of supplements containing Vitamin C, Vitamin E or β-carotene exceeding the DRI, or other anti-oxidant supplements
5. Symptomatic but untreated cholelithiasis

6. History of deep venous thrombosis, active thrombophlebitis, pulmonary embolism, stroke, acute myocardial infarction, congestive cardiac failure, untreated hypertension, known inherited hypercoagulable disorder
7. Diagnosis of any other malignancy within the previous year except for adequately treated basal cell or squamous cell skin cancer
8. Medically documented history of a psychiatric disorder that would preclude consent
9. Partial or complete loss of vision or diplopia, from ophthalmic vascular disease
10. Hypersensitivity to any component of the container

Intervention

All women in this trial will receive standard of care chemotherapy throughout the duration of the trial. Breast cancer chemotherapy is developed in a guideline-coordinated system by a single team residing at the Cross Cancer Institute. Consequently, there are only two chemotherapy regimens that are used for neoadjuvant chemotherapy in this population. Each of the two regimens are six cycles in length and given at three-week intervals with a resultant chemotherapy regimen duration of 18 weeks. Both regimens are docetaxel based. For HER2 negative disease, patients universally receive the FEC-D (fluorouracil, epirubicin, cyclophosphamide; docetaxel) [32] regimen as neoadjuvant therapy, while HER2 positive patients receive the DCH regimen (docetaxel, carboplatinum, trastuzumab) [33].

Patients will be prescribed either 5 g/day DHA (in 11- 1g capsules), in the form of DHA enriched algae-sourced triglyceride oil capsules (life'sDHA™ S40-O400) or 11g placebo (corn/soy oil blend) per day (capsules from DSM Nutritional Products, Columbia, MD, Supplemental Table 3 for the main fatty acid content of DHA and the placebo). The placebo is balanced for PUFA content with linoleic acid to match the DHA treatment. The amount of additional linoleic acid in the diet of this group is not expected to increase inflammation [34] and has not been shown to elicit a tumoral response [35]. The capsules are to be taken orally

throughout the day as tolerated (at anytime, with or without food). Capsules are identical in appearance and composition (other than the oils) to maintain blinding of participants and study staff. As the DHA source is an algae-synthesized triglyceride, there are no differences in texture or taste.

All patients will begin a cytotoxic chemotherapy regimen intended to require 18 weeks for delivery. The intervention (DHA or placebo) will commence at the start of the first cycle of chemotherapy and continue through 4-6 cycles of chemotherapy (3 weeks/ cycle). Should a patient not be able to complete the full six cycles of therapy, the timing of surgery remains 3-5 weeks after completion of the last cycle of chemotherapy delivered. As local guidelines mandate surgery between 3 and 5 weeks from the last round of chemotherapy, DHA/placebo will be continued until this time (21-35 days after the last administration of cytotoxic chemotherapy).

All patients will be dispensed an additional bottle of DHA/placebo capsules at the beginning of the study to account for circumstances where their treatment is delayed due to treatment associated toxicities (including but not limited to vomiting, diarrhea, abnormalities in blood work, fatigue or severe mouth sores). The patients will be requested to continue taking the DHA or placebo as tolerated and will be dispensed additional capsules as necessary. The extra capsules will remain with the patient until the end of the study.

Patients will be encouraged to take the supplements as tolerated (throughout the day at anytime, with or without food). Treatment adherence will be monitored by review of patient dosing diary and recording the number of any remaining capsules returned at the end of study visit following the last dose of DHA/placebo. Non-compliance will be assessed as consuming less than 50% of the weekly dose for 2 consecutive cycles. No additional natural health product is permitted beyond a daily multi-vitamin.

Outcome Measurements

Study outcome timelines are summarized in **Table 2**. Briefly, outcomes will be measured at baseline, within ± 3 days of chemotherapy and/ or post-intervention (surgical excision). Electronic medical record and or paper chart review of local control, relapse free survival and overall survival will occur at 3, 5, and 10 years to explore possible effects on long-term outcome.

Primary Outcome

The primary outcome of this study is change in Ki67 from pre-treatment core needle biopsy to surgical excision. It will be calculated by image analysis and will follow analytical and pre-analytical recommendations of Dowsett et al.[24]. The percent change in Ki67 index at experimental end (surgical excision) from baseline will be determined on a log scale and the mean percent change in Ki67 level from baseline will be calculated. Ki67 assays will be performed and reported as part of the routine diagnostic services. A semi-automated computer algorithm scoring system will be employed as previously described [36] using the platform QuPath [37]. It is expected that 5g DHA/day will result in a clinically relevant decrease in Ki67.

Secondary Outcomes

1) DHA incorporation into phospholipids: The changes in level of DHA incorporation in plasma phospholipids will be assessed at baseline and at day 1 (±3 days) of each cycle of chemotherapy (2-6) and end of cycle 6 to identify the range of DHA incorporation in this patient population. The use plasma rather than red blood cells or whole blood for this study is supported by the recent recommendations for best practices for fatty acids described by Brenna et al [38]. Analysis of the plasma phospholipid rather than plasma total lipids avoids the postprandial fluctuation of the triacylglycerol pool and is believed to adequately represent the cell membrane composition [38]. From our hypothesis and previously published data [22], it is expected that

supplementing with DHA will result in a significant increase in DHA incorporation. If possible, with the small study size, we will also assess difference in DHA incorporation in patients with different breast cancer subtypes and if subtype or disease stage affects DHA incorporation into plasma, controlling for the reported dose taken by the patient. The goal is to determine if plasma phospholipid DHA content can be used to predict treatment outcome. We will also assess incorporation of other essential fatty acids (linoleic, linolenic, arachidonic, eicosapentaenoic, docosapentaenoic) to determine if there are differences between or within treatment groups.

2) Systemic immune function: Systemic immune function will be assessed on blood samples obtained at baseline, beginning of chemotherapy cycle 4 (day 1± 3 days) and at the end of chemotherapy treatment. Changes in markers of systemic immune cell type and function will be assessed following supplementation compared to baseline and the change from baseline compared to patients receiving the placebo. We will also examine the relationship between changes in activation markers and the level of DHA incorporation, changes in systemic inflammation (CRP, IL-6, TNFα) and immune function (ability to produce IL-2 after stimulation in vitro) following DHA supplementation.

3) Identify factors that may affect DHA incorporation into plasma phospholipids: If incorporation of DHA in plasma phospholipids is variable within the DHA treatment arm, possible factors that may influence incorporation will be assessed between high and low incorporators. These parameters will be assessed at end of study from data collected throughout the study.

4) Examine changes in markers for apoptosis: Caspase-3 presence in the excised tumor, as percent positive cells, will be calculated by image analysis and a comparison of expression levels at experimental end (surgical excision) to baseline will be determined in patients

receiving DHA supplementation and compared to patients receiving placebo. Proportions of negative cells, weakly positive cells and strongly positive cells will be scored by two pathologists and the staining intensity assessed by QuPath [37] will be recorded independently. Increased apoptosis measured by Caspase-3 is a clinically relevant marker of cell death.

5) Examine changes in tumor infiltrating lymphocytes (TILs): CD4+ and CD8+ in the excised tumor, as number of positive cells for a given area, will be calculated by image analysis and a comparison of expression levels at experimental end (surgical excision) to baseline will be determined in patients receiving DHA supplementation and compared to patients receiving placebo will be made post-treatment. The differences will be compared between treatments and within the treatment group, related to plasma DHA concentrations. Increased infiltration of TILs is potential marker that could be used to predict treatment patient outcomes.

6) Pathological complete response rate (pCR): pCR in resected breast tissue and all sampled axillary nodes will be assessed as absence of invasive cancer on haematoxylin and eosin evaluation as per standard of care. Pathologic complete response will be classified as ypT0/is ypN0 and will be determined at end of study after surgical resection as part of standard of care assessment.

7) Comparison of rate of chemotherapy associated grade 3 and 4 toxicities: Rate of chemotherapy associated grade 3 and 4 toxicities, and chemotherapy-associated hospitalizations will be compared between DHA and placebo arms. Any changes will then be examined in regards to level of supplementation and DHA incorporation. These analyses will be completed at end of study after surgical resection.

Exploratory outcomes

1) Food frequency questionnaire (FFQ): Assessment of the FFQ to compare the estimated (pre-diagnosis) usual intake of macronutrients on an energy basis (including fat content and composition) between our two groups at baseline. In the future, the overall medians/ means of the subjects in this study will be compared to age-matched women in the Alberta Tomorrow Project.

2) Quality of life: Assessment in changes in quality of life will be determined by questionnaire employed at baseline and end of treatment. Comparisons will be assessed from end of treatment to baseline within and between treatment groups.

3) Exercise behavior: Assessment of exercise behavior will be determined by questionnaire employed at baseline, each cycle of chemotherapy and end of treatment. Comparisons will be assessed from end of treatment to baseline within and between treatment groups.

4) Breast conservation: The rate of breast conservation, specifically the rate of lumpectomy and mastectomy, will be determined by review of surgical and pathologic reports at end of study after surgical resection.

5) Volume of surgical blood loss: High intakes of n-3 LCPUFA (that contain some DHA) have been studied to determine if they increase bleeding time [39,40]. We will review surgical report estimates of blood loss to see if there is a qualitative or quantitative difference between placebo and treatment arms, once adjusted for the magnitude of surgery (lumpectomy vs. mastectomy vs. mastectomy + immediate reconstruction; sentinel node dissection vs. full axillary dissection). It is not expected that we will see a difference as it is eicosapentaenoic acid (EPA, the precursor to DHA) that has antithrombotic and antiplatelet properties [41].

6) Local control, relapse free survival and overall survival: Local control, relapse free survival and overall survival will be analyzed by review of electronic medical records, registry reports,

and / or paper medical charts at 3, 5, and 10 years to explore possible effects on long-term outcome.

Participant timeline

Breast cancer patients receiving neoadjuvant chemotherapy account for approximately 20% of newly diagnosed breast cancer patients, approximately 10-12/month at the Cross Cancer Institute. Assuming a conservative accrual rate of 30%, accrual is estimated to be completed in 14-18 months with 3-4 patients recruited per month. Each patient will be enrolled for the duration of their individual chemotherapy regimen, an estimated 12-18 weeks (84-126 days) beginning at the start of the first cycle of chemotherapy and continued through 4-6 cycles of chemotherapy (3 weeks/ cycle). The intervention will be discontinued 21-35 days after the last administration of cytotoxic chemotherapy when surgery to remove the tumor occurs. See Figure 1 for schematic of the participant timeline.

Sample Size

Fifty-two women prescribed neoadjuvant breast cancer chemotherapy will be enrolled in a 2-arm trial with 26 participants/arm. The sample size calculation is based on the primary objective, which is to determine the efficacy of supplemental DHA provided with standard neoadjuvant as measured by change in Ki67 index from biopsy to surgical excision. Group sample sizes of 23 patients in each group achieve 81% power to detect a difference between the group proportions of 0.4. The proportion in group one is assumed to be 0.3 under the null hypothesis and 0.7 under the alternate hypothesis. The proportion in group two which is control group is 0.3. The test statistic used is the two-sided t-test. The significance level of the test was targeted at 0.05 and the significance level actually achieved by this design is about 0.0497. Assuming a dropout rate estimated at approximately 10% for this patient population which is

approximately 5 patients, a total of 52 patients (26 patients DHA supplementation, and 26 in placebo) is required for the study.

Recruitment

Oncologists and clinical trial nurses at the Cross Cancer Institute in Edmonton, Canada will recruit newly diagnosed breast cancer patients. Patients will be screened for eligibility by the clinical trial nurses and eligible, interested patients will receive detailed explanation of the study by the study coordinators and written informed consent will be obtained (**Supplemental File 1**).

Randomization and Blinding

A biostatistician will generate a patient randomization list and randomized bottle numbers by covariate-adaptive randomization (block randomization). The randomized bottle numbers will be provided to DSM for labeling for both the DHA and placebo groups and the randomized bottle list will also be provided to the unblinded Clinical Trials Coordinator (Clinical Trials Unit) and the unblinded pharmacist. Patients will be stratified by histological subtype and then randomized. The allocation of the study arm (as the study is blinded, hence, the study arm A and B will be used as this will not identify the placebo or intervention arm) and a unique study identifier will be conducted using the REDCap database. The key to the study arm A and B will only be provided to the unblinded CTC, statistician and the pharmacist. The study coordinator will enter the new patient information in REDCap and assign the unique ID and arm. This information will be shared with the unblinded Clinical Trials Coordinator (Clinical Trials Unit) and the unblinded pharmacy staff. The pharmacist staff will assign the correct bottle number based on the study arm at day 1 of each chemotherapy cycle. Following the allocation of the bottle number, this information will then be shared with the study coordinator and the unblinded CTC. The bottle ID will be entered in the REDCap database by the study co-ordinator. All future bottle allocation

with the unique bottle ID will be entered into the REDCap database. The key to the study arm will be kept in password protected computers and will only be shared in an urgent need for breaking of the blind. When a blinding code is broken, the date and reason for unblinding must be fully documented in source documents and entered on case report form. Every effort should be made by site staff to ensure that the treatment arm in which the unblinded patient is assigned is communicated only to those site staff that require the information for treatment purposes. To assist in maintaining the blind of the patients, supplements and placebo are identical in size, shape, color and texture, in addition to identical bottles for dispensing. Patients, pathologists, physicians, and researchers will be blinded to patient enrolment in the study and throughout trial. Blinding will only be dropped after analysis of fatty acids, systemic immune function and Ki67 is complete.

Data Collection, Management and Analysis

Study methods are summarized in **Table 3**. Briefly, data will be collected and measured at baseline, within ± 3 days of chemotherapy and/ or post-intervention (surgical excision). Electronic medical record and or paper chart review of local control, relapse free survival and overall survival will occur at 3, 5, and 10 years. All data will be entered and maintained in REDCap trial database. Baseline measurements will be analyzed once all participants have been enrolled and all other analyses will occur at completion of trial.

Primary Outcome

Ki67 will be tested by immunohistochemistry (IHC) by the diagnostic biomarker laboratory at the Cross Cancer Institute using the MIB1 antibody on 4 μm sections from formalin fixed paraffin embedded (FFPE) needle core biopsy surgical specimens. At final

analyses, Ki67 staining will be repeated as single IHC stain and interpreted by image analysis. At time of Ki67 interpretation, slides will be de-identified and coded to ensure the pathologist is blinded to the experimental group. In addition, the original single stained slides will be interpreted visually by research staff. All Ki67 values (routine and image analysis) will be recorded as absolute percentage and as log-transformation in REDCap trial database and participant's case report form. The Ki67 index is validated and used in clinic as marker of proliferation. The Ki67 index (absolute %, log transformed and H-score [42] of biopsy and surgical resection (after chemotherapy) will be compared on each participant and between participants receiving DHA compared to placebo.

Secondary Outcomes

DHA incorporation into plasma phospholipids will be measured in venous blood from patients at baseline (time of enrolment in trial), and at day 20 (± 3 days) of each chemotherapy cycle by a technician blinded to the treatment group. Venous blood will be collected in coated EDTA tubes and centrifuged at 750x g for 10 min to obtain plasma. Red blood cells will be immediately frozen and banked at -70°C for storage for future secondary analysis. Plasma will be separated in 6 aliquots, and immediately frozen at -70°C for storage. Plasma (concentration and relative percent) will be extracted by Folch procedure [43,44], phospholipids separated by thin layer chromatography and fatty acid content measured by gas-liquid chromatography as previously described [45]. The percentage change in DHA from baseline will be compared in each patient and a 95% t-confidence interval for the mean percent change in the DHA from baseline will be compared to patients receiving placebo. An internal standard is used to identify and quantify the fatty acid. This is a standard measure for fatty acid status has

coefficient of variation <5% and individual GC peaks are validated against phospholipid standards (GLC-502 and GLC-643) from NuChek (Elysian, MN).

Phenotyping of immune cell subsets will be measured using whole blood (collected in EDTA tubes). The various cell types will be identified using specific fluorescently labelled monoclonal antibodies (mAb) to surface receptors (See Supplementary Table 4 for list of antibodies). These will be quantified by flow cytometry, as previously described [46]. With the remaining blood, peripheral mononuclear cells will be isolated and purified on a Ficoll density gradient of Histopaque 1077 as previously described [46,47]. To measure cytokine production in isolated lymphocytes, cells will be cultured in media with or without the mitogens, Phytohemagglutinin (PHA) or Lipopolysaccharide (LPS) for 48 h as previously described [48]. Supernatant will be collected and stored at -80°C for *ex vivo* measures of immune function (ability and pattern of cytokines produced after stimulation). IL-1 β, IL-2, IL-6, IL-10, TNF α, and IFN-γ (pg/ml) cytokines will be measured using electrochemiluminescent multiplex assays (MesoScale Discovery) or by individual ELISA assays. Cytokines listed above and inflammatory markers including C-reactive protein (CRP) in plasma will be measured electrochemiluminescent multiplex assays (MesoScale Discovery) as previously described [49]. Cytokines and inflammatory markers in plasma and cytokines from cultured lymphocytes will be analyzed when all samples have been collected. Changes in systemic immune function will be assessed in patients compared to baseline and compared between groups. The data analysis will occur at completion of trial. Cytokines are done in duplicate and the coefficient of variance is <15%. Phenotypes will be collected as a relative percent of total cells and the change compared between treatments. Additionally, white blood cells that are not used for the immune assays will be assessed for fatty acid composition.

If DHA incorporation in plasma phospholipids is significantly different within the DHA supplementation arm, factors that may influence incorporation will be compared in low vs high incorporators, to identify possible factors that predict incorporation, including, including BMI, age, the estimated macronutrient intake and composition of dietary fat of the women (estimated from the FFQ), histology of the tumor (provided from the biopsy), the amount of DHA consumed (adherence to the supplement) and length of time DHA consumed (if treatment is ended early) . We will also assess incorporation of other fatty acids (palmitic, oleic, linoleic, linolenic, eicosapentaenoic, docosapentaenoic) to determine if there are differences between or within treatment groups

Caspase-3 changes and changes in CD4 and CD8 will be tested by immunohistochemistry (IHC) by the diagnostic biomarker laboratory at the Cross Cancer Institute on 4 μm sections from formalin fixed paraffin embedded (FFPE) surgical specimens. At final analyses, IHC staining will be interpreted by image analysis. At time of interpretation, slides will be de-identified and coded to maintain the blind. All values (routine and image analysis) will be recorded as absolute percentage and as log-transformation. Caspase-3 is a validated marker of apoptosis and CD4 and CD8 are validated marker for lymphocytes. The calculated indices (absolute %, log transformed and H-score) of biopsy and surgical resection will be compared on each participant and between participants receiving DHA compared to placebo.

Pathological complete response in resected breast tissue and axillary nodes will be assessed in hematoxylin and eosin stained tissue for evidence of invasive disease according to

standard of care. Pathological complete response will be assessed following breast surgery as per standard of care and recorded in patient's case report form. The rate of pathological complete response in breast tissue and axillary nodes after surgical resection will be compared between participants receiving DHA supplementation compared to placebo.

Grade 3 and 4 toxicities will be assessed and recorded by the clinical trial nurse. Toxicities will be assessed on day 1 (± 3 days) of each chemotherapy cycle. Dates of hospitalization will be recorded in patient's case report form. Rates of chemotherapy-associated grade 3/4 toxicities, all grade neuropathy and hospitalizations will be compared between DHA supplementation and placebo arms as scored by a medical oncologist in a standardized toxicity/ neuropathy form for each cycle of chemotherapy.

Exploratory outcomes:

The FFQ is a validated questionnaire for macronutrient intake [50-52]. The quality of life questionnaire is a validated questionnaire from European Organization for Research and Treatment of Cancer- Quality of Life Questionnaire-C30 (EORTC-QLQ-C30) [53]. Exercise behavior will be assessed using the modified Godin Leisure-Time Exercise Questionnaire (GLTEQ) [54,55]. Assessment in changes in quality of life and exercise behavior will be assessed from timepoints collected to baseline within and between treatment groups. We do not expect the supplement/ placebo to influence this variable but since exercise alters immune function, quality of life and tumor growth we have included it herein to determine if it changes during therapy.

The rate of breast conservation, specifically the rate of lumpectomy and modified radical mastectomy, will be determined by surgical and pathologic reports at time of surgical resection. Volume estimates of blood loss will be assessed by review of surgical report estimates of blood

loss to see if there is a qualitative or quantitative difference between placebo and treatment arms, once adjusted for the type of surgery (lumpectomy vs. mastectomy vs. mastectomy + immediate reconstruction; sentinel node dissection vs. full axillary dissection). Local control, relapse free survival and overall survival will be analyzed by electronic medical record and / or paper medical chart review at 3, 5, and 10 years. Data will be validated by a medical oncologist.

Data Management

All data will be entered and maintained in REDCap trial database. Direct access to clinical and laboratory information on the enrolled trial patients will be limited to the principal investigator, co-investigators, trainees/staff who have had the appropriate training and approval and study nurses and study coordinators who will have access to the source documents through the electronic medical record and laboratory information system at the Cross Cancer Institute. All patients will have biopsy and tumor samples for analysis and we do not expect any missing data for the primary endpoint (Ki67). If supplement compliance is below 50% for more than two consecutive cycles, or participants do not complete chemotherapy (to a minimum of 4 cycles), they will be excluded from final analysis of the primary end point. If patients do not have sufficient blood samples for the secondary analyses (DHA incorporation, systemic immune function), analysis will be performed using data from the remaining patients.

Statistical Methods

Primary Outcome:

The percent change in Ki67 will be determined as an absolute percentage and H-score. The number of patients showing a decrease and the 95% confidence interval for the mean percent change in the Ki67 level from baseline in patients receiving DHA supplementation will be

compared to patients receiving placebo. The mean change will be measured using independent t-test between the two groups.

Secondary Outcomes:

Paired t-test will be used to compare the mean percent change in the plasma DHA level of the patients after each cycle of chemotherapy with their baseline values. If the data is not normally distributed, the Wilcoxon signed rank test will be used to compare the plasma DHA level after each cycle of chemotherapy with baseline. The difference in plasma phospholipid DHA from baseline and between DHA supplementation and placebo arms will be calculated, and the 95% confidence interval for the mean percent change in DHA level from baseline and groups will be assessed.

If systemic immune function data is not normally distributed, it will be log transformed prior to analysis and the normality assumptions will be tested again. Repeated measures ANOVA with post hoc analysis will be used to determine if there is an effect of treatment on immune function.

Factors affecting DHA incorporation will be examined by independent t-test to compare the mean values between the DHA and placebo groups. Chi-square tests will be conducted to determine correlation between two categorical variables for the outcome measures listed.

The within subject and between subject variability between the two groups for the mean percent change in apoptosis, tumor infiltrating lymphocyte markers will be tested using generalized estimating equation (GEE) method.

The 95% confidence interval using independent t-test will be conducted for the mean percent change in pathological complete response and rates of grade 3 and 4 chemotherapy associated toxicities and hospitalization in patients receiving DHA supplementation compared to patients receiving placebo.

Exploratory outcomes:

Independent t-test for macronutrient and fat content obtained from the food frequency questionnaire will be examined between groups. Paired t-test for continuous variables and McNemar's test for categorical variables will be assessed for mean percent change in events between treatment arms for the quality of life and exercise questionnaires. Chi-square tests will be used to compare the degree of breast conservation and the volume of surgical blood loss will employ an independent t-test between the two study arms. Rate of local control will be compared between treatment arms using t-test of proportions. Recurrence-free survival and survival will be analyzed using the log rank test on Kaplan-Meier survival curves.

SAS software, version 9.4 (SAS Institute Inc., Cary, NC), will be used for statistical analysis. A p-value <0.05 level will be used for all statistical significance. Two-sided tests will be used for all statistical tests.

Data Monitoring

The trial activities performed at the Cross Cancer Institute will be monitored by the Cross Cancer Institute, Investigator Initiated Trials Data Safety Monitoring Board (DSMB). The DSMB is independent of the investigator and is composed of representatives from both medical and radiation oncology.

The investigator will assess the relationship between protocol treatment and the occurrence of adverse events (AEs) and this assessment will be recorded in the database for adverse events. This study will use the International Common Terminology Criteria for Adverse Events (CTCAE), version 5.0, for adverse event reporting. The reporting period for adverse events will start at the time the patient takes the first dose of DHA/placebo through and including 28 calendar days after last administration of study agent. If serious adverse reaction

to treatment occurs, the Natural and Non-prescription Health Products Directorate (NNHPD), Clinical Trial Unit, Health Ethics Research Board of Alberta, Cancer Committee (HREBA.CC) and DSM will be notified as per guidelines. After 25 evaluable patients, all data and results will be submitted to the DSMB for review.

Auditing

As per the SPIRIT guidelines, the investigators, Cross Cancer Institute and the University of Alberta will permit trial-related monitoring, audits, REB, DSMB review, and regulatory inspection(s), providing direct access to paper and/or electronic documentation pertaining to the clinical study (e.g. CRFs, source documents such as hospital patient charts and investigator study files). All site facilities related to the study conduct could be visited during an audit (e.g. pharmacy, laboratory, outpatient department) and are agreed to co-operate and provide assistance at reasonable times and places with respect to any auditing activity.

Patient and Public Involvement

Patients were not involved in the protocol development or study design. However, oncologists and clinical trial nurses who work in the breast tumor group are involved in patient screening to assess eligibility for the study. The HREBA-CC approved informed consent will be obtained from patients prior to their involvement in the study and it informs patients of their right to withdraw at anytime. At the end of the trial, results will be disseminated to the public through seminars, public talks and in peer-reviewed journals.

Ethics and dissemination

DHAWIN has received Health Canada approval (#HC6-24-c220167), full ethical approval from the Health Research Ethics Board of Alberta – Cancer Committee (Protocol #:

HREBA.CC- 18-0381) and is registered at clinicaltrials.gov (Identifier: NCT03831178). Protocol amendments will be submitted to HREBA.CC, Health Canada and the clinical trial registry prior to study implementation according to regulatory requirements. The formal consent of a participant, using the HREBA-CC-approved consent form (Supplemental File 1), will be obtained by a clinical trial nurse before the participant is enrolled in the study and will be signed by the patient, and the principle investigator. A voluntary optional consent form for use of participant data and biological specimens (Supplemental File 2), will be offered at time of enrollment. Patient confidentiality and anonymity will be maintained and identities protected from unauthorized parties.

Access to data will be restricted to the primary investigators and statistician. They will grant access to other team members as governed and approved by ethics. Ancillary care post-trial will occur as routine standard of care for all participants. Our objective is to determine the efficacy of using DHA supplementation concomitant with chemotherapy and as such our results will be disseminated to clinicians for implementation in future treatment paradigms. The results will be submitted to peer-reviewed journals and presented at national and international conferences.

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Competing Interests Statement

There are no financial or competing interests or conflicts to declare.

Author Contributions

MN and CJF wrote the manuscript. JRM, GB, MAC, SG, SG, AS, DM, AC, LP, KB, VM, KSC, RB, WFD, GW, SKB, AAJ, KK, JMJ, and XZ contributed to the study design and reviewed the manuscript drafts. JRM, SKB, AAJ, KK, JMJ, and XZ provided oncological expertise. GB, RB, WFD, and GW provided pathology and immunohistochemistry expertise. SG and KB contributed to the design of the immunologic component of the study; SG designed the statistical models for all components of the study. AS, DM, AC, and MAC obtained all regulatory and operational approvals (Health Canada, HREBA, Clinical Trials registration and site approvals). CJF, LP, VM, KB, JRM were co-applicants on the successful CIHR grant that designed the immune component of the trial. KSC contributed expertise for the QoL and exercise component of the study. All authors reviewed drafts of the manuscript Authors of the data manuscripts will include at least the Principal Investigator, medical director (J. Mackey) and any co-investigators who have i) included eligible patients in the trial (by order of inclusion) and/or ii) contributed significantly to the design, conduct and data interpretation regarding companion basic science studies.

Appendices:

Supplemental File 1: Informed consent

Supplemental File 2: Optional consent

Supplemental Table 1: Spirit Checklist

Supplemental Table 2: WHO Checklist

Supplemental Table 3: Main fatty acid content of DHA supplement and placebo

Supplemental Table 4: List of Antibodies for Immune cell subset identification

FIGURE LEGENDS

Figure 1 Flowchart of Trial Design with Endpoints and Proposed Experimental Analyses

Figure 2 SPIRIT patient flow diagram of the DHA WIN trial

552 **Table 2:** DHA WIN assessment schedule based on the Standard Protocol Items: Recommendations for Interventional Trials (SPIRIT)

	STUDY PERIOD												
		Chemotherapy											
	Enrolment	**Cycle 1**		**Cycle 2**		**Cycle 3**		**Cycle 4**		**Cycle 5**		**Cycle 6**	**Surgery**
TIMEPOINT**	$-t_1$	***Day 1*** [1]	***Day 20***	***Day 1***	***Day 20***	***Day 1***	***Day 20***	***Day 1***	***Day 20***	***Day 1***	***Day 20***	***Day 1***	***Day 20***	t_x
ENROLMENT:														
Eligibility screen	X													
Informed consent	X													
Randomization	X													
INTERVENTIONS: [2]														
DHA 5 grams / day (11 - 1g capsules)		—	—	—	—	—	—	—	—	—	—	—	—	
Vegetable oil placebo (11 -1g capsules /day)		—	—	—	—	—	—	—	—	—	—	—	—	
Medication Diary		—	—	—	—	—	—	—	—	—	—	—	—	
Assessment of Compliance				X		X		X		X		X	X	
ASSESSMENTS:														
BASELINE / ONGOING														

Demographic data collection	X												
Tumor analysis for Grade / ER/PR/HER2[3]	X												
Physical Exam / anthropometric measurements	X	X		X		X		X		X		X	X
Relevant medical history / current medical conditions	X			X		X		X		X		X	X
ESAS questionnaire	X	X		X		X		X		X		X	X
Blood Chemistry	X											X [4]	
CBC and differential	X							X				X [4]	
Adverse Events		X		X		X		X		X		X	X
Assessment of Relevant Toxicities		X		X		X		X		X		X	
Primary Outcome													
Tumor analysis of Ki67	X												X
Secondary Outcome													
Assessment of immune function:	X							X				X [4]	
Assessment of DHA incorporation	X			X		X		X		X		X	

Tumor analysis of apoptosis and TILs	X												X
Exploratory Outcomes													
Grade 1, 2 neuropathy assessment		X		X		X		X		X		X	X	
Pathological complete response														X
Breast conservation														X
Assessment of surgical blood loss														X
Study Associated Questionnaires														
Food frequency questionnaire[5]	X													
Quality of life questionnaire	X												X	
Godin Exercise Questionnaire	X			X		X		X		X		X	X	

ESAS: Edmonton Symptom Assessment System

(1) Day 1 is the day 1 of chemotherapy cycle.

(2) If patient's chemotherapy is delayed due to associated toxicities, they will be encouraged to continue taking the DHA/placebo capsules as tolerated.

(3) From previously collected biopsy.

(4) Tests required at the end of the last round of chemotherapy (i.e., end of cycle 4, 5 or 6 as per patients' individual treatment plan).

(5) Food frequency questionnaire can be completed anytime within the first cycle (21 days) of chemotherapy.

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560 **Table 3: Variables, Measures and methods of analysis**

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VARIABLE / OUTCOME	OUTCOME MEASURE	METHOD	STATISTICAL ANALYSIS
PRIMARY:			
Efficacy of supplemental DHA provided with standard neoadjuvant chemotherapy as measured by change in Ki67	Ki67 labelling index	Immunohistochemistry	95% t-confidence interval for mean percent change in Ki67. Independent t-test to compare change between the study groups [36]
Secondary:			
1. DHA incorporation into plasma phospholipids	Fatty acid composition of plasma phospholipids	Gas chromatography	Paired t-test will be used to compare the mean percent change in the DHA level of patients after each cycle with their baseline values. If the data is not normally distributed, the Wilcoxon signed rank test will be employed for this comparison. A 95% t-confidence interval for the mean percent change in the DHA from baseline will be compared to patients receiving placebo
2. Systemic immune function	a) Immune cell subset identification b) Plasma cytokine	a) Flow cytometry b and c) ELISA and MesoScale	Repeated Measures ANOVA with post hoc analysis

	c) Ex vivo stimulated immune cell response		
3. Identify factors that may affect DHA incorporation into tumor tissue and plasma phospholipids.	Factors assessed after calculating high and low DHA incorporators: a) Weight (BMI) b) Age c) The usual diet estimated from the FFQ d) Composition of dietary fat estimated from the FFQ e) Histology of the tumor (provided from the biopsy) f) Amount of DHA consumed (Adherence to the supplement) g) % incorporation of other fatty acids		Independet t-test will be conducted to compare the mean values between the two study groups. Chi-square test will be conducted to determine correlation between two categorical variables for outcome measures listed
4. Examine changes in markers for apoptosis	Caspase -3	Immunohistochemistry	Within subject and between subject variability between the two groups will be tested using generalized estimating equation (GEE) method.
5. Examine changes in markers for tumor	CD4+/CD8+	Immunohistochemistry	Within subject and between subject variability between the two groups will be

infiltrating lymphocytes			tested using generalized estimating equation (GEE) method.
6. Describe the rate of pathological complete response in breast and in axillary nodes	Absence of invasive cancer on haematoxylin and eosin evaluation	Immunohistochemistry	pCR= ypT0/is ypN0 95% t-confidence interval using independent t-test for mean percent change between treatment groups
7. Describe the rate of grade 3 and 4 chemotherapy associated toxicities.	Rate of grade 3 /4 toxicities and chemotherapy associated hospitalizations	Chart review	95% t-confidence interval using independent t-test for mean percent change in events between treatment groups
Exploratory Outcomes			
1. Food Frequency Questionnaire	DHQ II questionnaire	Questionnaire	Independent t-test of macronutrient and fat content / composition between groups
2. Quality of Life	Baseline and Endpoint questionnaires	Questionnaire	Paired t-test for continuous variables and McNemar's for categorical variables for mean percent change in events between treatment groups
3. Exercise	Godin Exercise questionnaire	Questionnaire	Paired t-test for continuous variables and McNemar's for categorical variables for mean percent change in events between treatment groups
4. Assess the rate of breast conservation	Rate of lumpectomy and mastectomy.	Chart review	Chi-square tests

5. Assess the volume of surgical blood loss.	Review surgical reports for quantitative / qualitative loss of blood	Chart review	Independent t-test
6. Analyze local control, relapse free survival and overall survival	Electronic medical record and / or paper medical chart review at.3, 5, and 10 years to explore possible effects on long-term outcome	Chart review	Kaplan-Meier estimates along with the survival curves, log-rank test will be used for statistical comparison between groups

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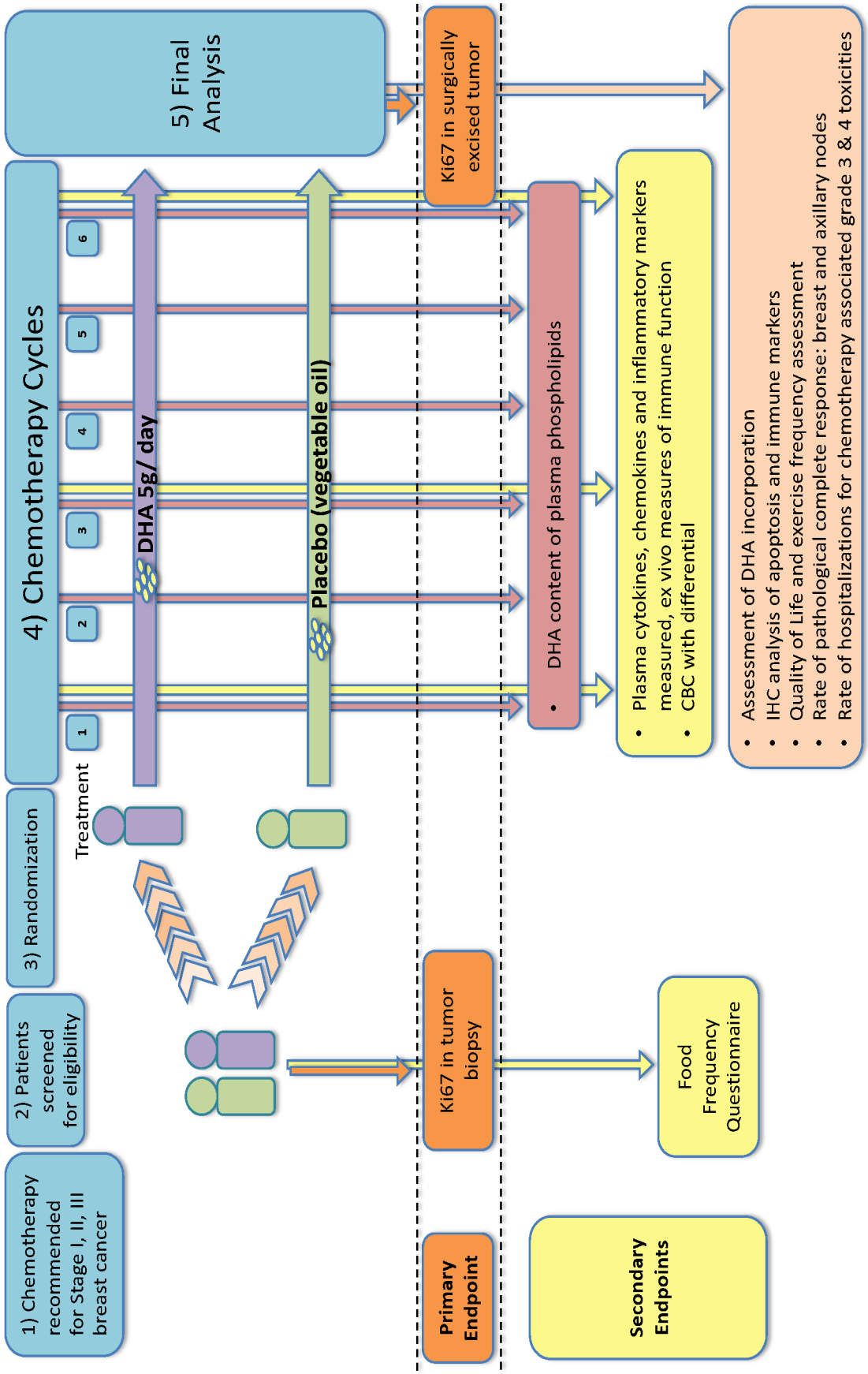
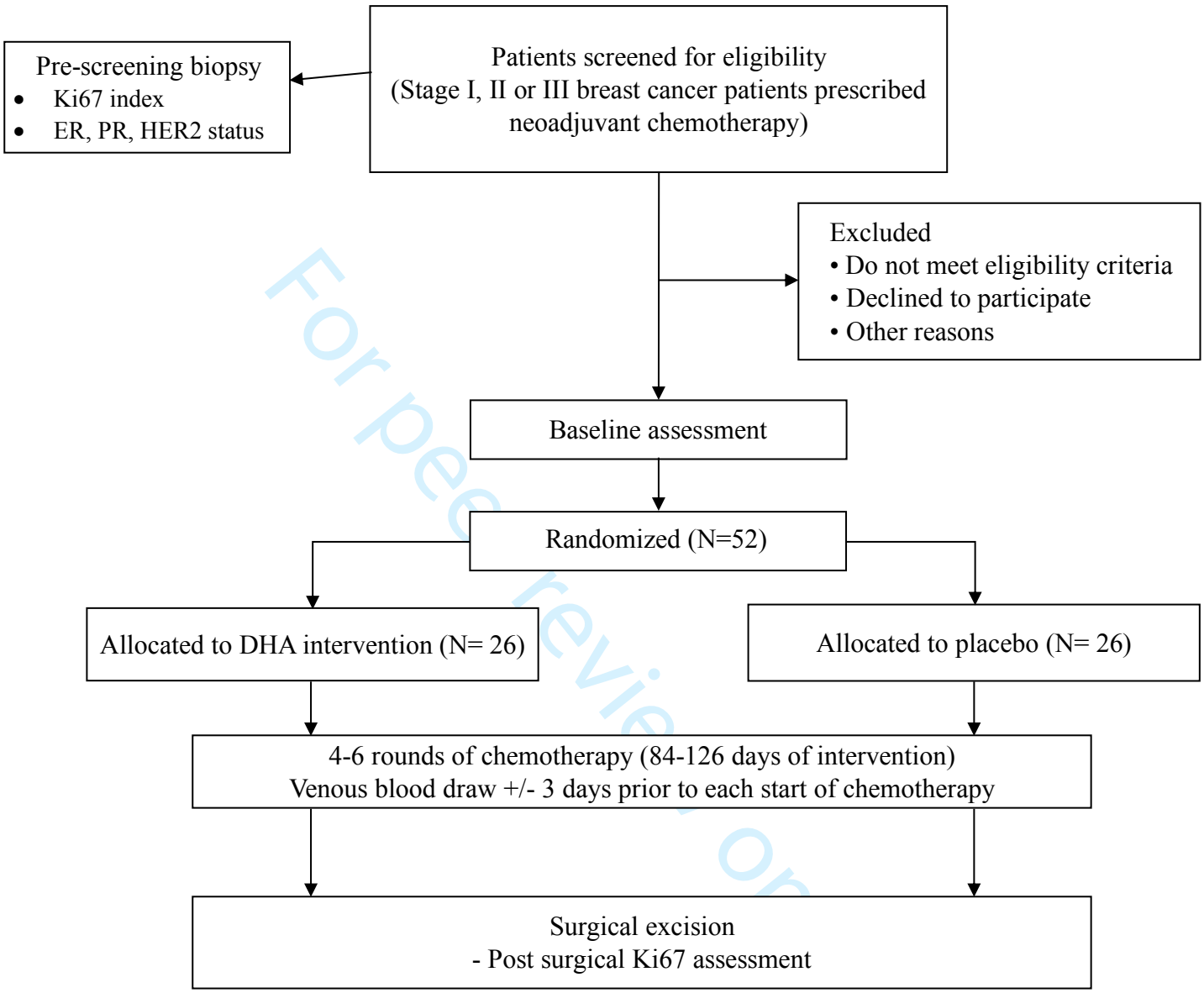


Figure 1

Figure 2



Alberta Health Services

Informed Consent Form for Participation in a Research Study

DHA for Women with Breast Cancer in the Neoadjuvant Setting

DHA to improve effectiveness of Chemotherapy in Breast Cancer

Protocol ID: *IIT-0005*

Study Doctor: *Dr. John Mackey*
Department of Medical Oncology
Cross Cancer Institute
780-432-8221

Sponsor/Funder(s): *Alberta Health Services- Cross Cancer Institute*

Emergency Contact Number (24 hours / 7 days a week): *780-965-8824*

Non-Emergency contact numbers are noted at the end of this document under the section heading "WHO DO I CONTACT FOR QUESTIONS?".

For assistance with terminology within this consent form, please refer to the Canadian Cancer Society Glossary of Terms at http://info.cancer.ca/e/glossary/glossary.html.

You are being invited to participate in a research study because you have stage I, II or III breast cancer which has not spread to distant parts of the body and will be receiving chemotherapy prior to surgery. This consent form provides detailed information about the study to assist you with making an informed decision. Please read this document carefully and ask any questions you may have. All questions should be answered to your satisfaction before you decide whether to participate.

The study staff will tell you about timelines for making your decision. You may find it helpful to discuss the study with family and friends so that you can make the best possible decision within the given timelines.

Taking part in this study is voluntary. You may choose not to take part or, if you choose to participate, you may leave the study at any time without giving a reason. Deciding not to take part or deciding to leave the study will not result in any penalty or any loss of medical or health-related benefits to which you are entitled.

The study doctor, who is one of the researchers, will discuss this study with you and will answer any questions you may have. If you do consent to participate in this study, you will need to sign and date this consent form. You will receive a copy of the signed form.

WHAT IS THE BACKGROUND INFORMATION FOR THIS STUDY?

Docosahexaenoic acid (DHA) is an omega-3 fatty acid commonly found in fish and fish oil. In the body, DHA is found in the membranes of cells. DHA is important for brain development, and in the immune system. DHA is also beneficial in heart disease. A diet high in DHA can reduce the incidence of breast cancer.

Incubating breast cancer cells with DHA in cell culture (cells in a dish in a laboratory) decreases the growth of the breast cancer cells, and increases the death of these cells. This is specific to cancer cells, since DHA has no effect on normal breast cells. When breast cancer cells are treated with chemotherapy drugs and DHA, DHA increases the effectiveness of chemotherapy resulting in increased death of the cancer cells.

When mice with breast tumors are fed DHA and treated with chemotherapy their tumors are much smaller than mice who are not fed DHA. In a previous clinical trial, women with metastatic breast cancer were given DHA supplements and treated with chemotherapy. DHA supplements appeared to improve the response to chemotherapy for some women.

Taking DHA may also reduce some side effects of chemotherapy in women with breast cancer. In these previous trials, no side-effects of taking DHA supplements were found.

Health Canada, the regulatory body that oversees the use of natural health products, drugs and devices in Canada, has not approved the sale or use of this DHA supplement to treat this kind of cancer, although they have allowed its use in this study.

The Health Research Ethics Board of Alberta – Cancer Committee (HREBA-CC), which oversees the ethical acceptability of research involving humans, has reviewed and granted ethics approval for this study.

WHY IS THIS STUDY BEING DONE?

This study will test if taking a DHA supplement during chemotherapy for breast cancer increases the effectiveness of the chemotherapy. The purpose of this study is to find out what effects a new agent, DHA supplementation, has on you and your breast cancer.

The investigators of this study are also interested in exploring the factors that may affect DHA incorporation in your blood, such as your weight and height, usual food intake (including amount and type of fat eaten), tumor type and the amount of DHA supplement consumed in the study.

WHAT ARE OTHER OPTIONS IF I DECIDE NOT TO PARTICIPATE IN THIS STUDY?

You do not have to take part in this study, in order to receive continued medical care. Other alternatives in addition to standard care may include:

- Other experimental studies may be available if you decide not take part in this study.
- Continuing regular observation and routine follow-up care e.g., symptom management

Please talk to the study doctor or your care doctor about the known benefits and risks of these other options before you decide to take part in this study. Your study or care doctor can also discuss with you what will happen if you decide not to undertake any treatment at this time.

HOW MANY PEOPLE WILL TAKE PART IN THIS STUDY?

Up to 52 people will take part in this study.

WHAT WILL HAPPEN DURING THIS STUDY?

ASSIGNMENT TO A GROUP

If you decide to participate then you will be "randomized" into one of the groups described below. Randomization means that you are put into a group by chance (like flipping a coin). There is no way to predict which group you will be assigned to. You will have an equal chance of being placed in either DHA supplementation or placebo group. Neither you, the study staff, nor the study doctor can choose what group you will be in.

This is a double-blinded study, which means that neither you nor the study doctor or study staff will know which group you are in. This is done so that you and the study doctor will not be influenced by expectations of the effects of the study agent. Your treatment will be identified if medically necessary by a process referred to as unblinding. Requests to reveal your assignment for your information or participation in other research studies will not be considered until the study has been completed and the results are known.

STUDY INTERVENTION

Group 1 (Experimental intervention): standard intervention of neoadjuvant chemotherapy plus experimental intervention of DHA supplementation.
If you are randomized into this group, you will take DHA capsules by mouth every day during chemotherapy treatment (4-6 cycles of chemotherapy, which would last approximately12-18 weeks)

Group 2 (Non-experimental intervention): standard intervention of neoadjuvant chemotherapy

If you are randomized into this group you will take placebo capsules containing corn/soy oil by mouth every day during chemotherapy treatment (4-6 cycles of chemotherapy, which would last approximately *12-18 weeks).*

Other important information on study intervention:
If you have side effects while you are on this study, the study doctor may make changes to the intervention.

STUDY PROCEDURES

Established Procedures

The following established procedures will be done as part of this study. Some of these procedures may be done as part of your standard care, in which case the results may be used. Some may be done more frequently than if you were not taking part in this study. Some of these procedures may be done solely for the purpose of the study. If the results show that you are not able to continue participating in the study, the study doctor will let you know.

Screening:

- Signed Informed Consent
- Review of inclusion / exclusion criteria
- Confirmation of no known allergies to soybean or corn oil (participants with allergies to soy or corn will be excluded from the study).
- Demographic data
- Physical examination
- You will be asked about your ability to carry out daily activities
- Body height and weight
- Vital signs
- Documentation of the diagnosis and disease stage
- Confirmation of no previous or concomitant treatment
- Complete medical / oncological history and consultation
- Questionnaire about your symptoms and well-being (ESAS questionnaire)
- Quality of Life questionnaire
- Exercise questionnaire
- Food frequency questionnaire (to be completed before the end of the first cycle of chemotherapy)
- Blood sample
- Your biopsy sample will be analyzed for standard tumor analysis: Grade; ER/PR/HER2; Ki67 to be requested if not already performed and other disease-related biomarkers.
- Adverse events before start of treatment

Chemotherapy Cycles (will take place prior to each chemotherapy administration):

- Physical exam
- You will be asked about your ability to carry out daily activities (cycle 1 and upon completion of your chemotherapy).
- Weight
- Vital signs
- Adverse events
- Blood sample
- You will take the DHA/placebo capsules by mouth every day during chemotherapy treatment (4-6 cycles of chemotherapy, which would last approximately 12-18 weeks)
- Quality of Life questionnaire (only at end of 6th cycle)
- Exercise questionnaire

Upon completion of chemotherapy:

If you undergo a surgical procedure to remove the tumor after chemotherapy, we will collect information from your records regarding the extent of the surgical procedure and amount of blood loss. In addition, your tumor sample will be reassessed for Ki67 and other disease-related biomarkers.

Alberta Health Services

Questionnaires

You will be provided with a questionnaire about food intake by research staff during cycle 1 of this study. The purpose of the questionnaire is to determine the amount of DHA in your diet, and other foods that can affect DHA in the body. The questionnaire will take about 1 hr. to complete and it can be done online or on paper. If you decide to complete it online, you will receive a link and a password to complete the questionnaire at home. If you don't have access to a computer or prefer a paper version, a printed questionnaire will be offered to you.

You will also be asked to complete questionnaires about your symptoms and well-being (ESAS questionnaire and exercise questionnaire) at the beginning of each chemotherapy cycle. It may take you 15-20 minutes to complete both questionnaires.

The information you provide is for research purposes only and will remain strictly confidential. Some of the questions are personal; you may choose not to answer them.

Participant Diaries

You will be asked to keep a diary to record *your study supplement capsules intake.* Please record *the times and number of capsules when you take the capsules each day*. You will be asked to return the diary to *the Cross Cancer Institute at the end of each cycle*.

MANDATORY SAMPLE COLLECTION

The researchers doing this study need to do tests on samples as described below. *The biopsy sample will be examined to make sure you have the type of cancer that is being studied in the research study. The surgical resection will be examined and compared to the biopsy sample to see how the cancer cells respond to DHA supplementation. Blood samples will be examined to see how DHA supplementation affects the amount of DHA in these samples, and if DHA alters immune cells.*

The collection of these samples is a necessary part of this study and will be used only for this purpose. The samples will not be sold.

Once these tests have been completed, any leftover samples will be returned to the facility from which they were obtained if needed or destroyed, unless you wish to give permission for other future research purposes, in which case you will be given a separate optional consent form to sign.

Hereditary genetic testing (to look at whether cancer runs in your family) will not be done on these samples.

Reports about research tests done with your samples will be given to the study doctor(s). If you would like to learn the results of this research, please let them know.

Tissue Collection (Mandatory)

A small sample of your tissue that has already been removed by a previous surgery or biopsy will be obtained by the researchers doing this study. No further surgeries or biopsies are required of you for this purpose.

As part of your standard of care and necessary for this study, you will have had a tissue biopsy. Upon completion of your chemotherapy treatment and as part of your standard of care, you may undergo a surgical procedure to remove the tumor from your breast. The amount of tissue to be removed will depend on the size and location of the tumor. Your doctor will give you more details regarding this procedure.

A sample of the tissues obtained from the initial biopsy and from the subsequent breast surgery will be sent to a laboratory at the *Cross Cancer Institute, and at the University of Alberta in Edmonton, Alberta, Canada,* where they will be examined to confirm your diagnosis *and examine how DHA alters tumour growth, and the amount of DHA in tumour cells*.

Blood Collection (Mandatory)

Blood samples will be taken by inserting a needle into a vein in your arm. These will be taken at the same time as your study related tests whenever possible upon *entry to the study, at the beginning of every cycle of chemotherapy (every three weeks), on day 20 of cycle 3 and before surgery. One tablespoon of blood will be collected for this study at those times*. These blood samples will be sent to a laboratory at the *Cross Cancer Institute and the University of Alberta in Edmonton, Alberta, Canada* where they will be examined to *measure the different cells in your blood, and the amount of DHA in these cells.*

Identification of Samples

To protect your identity, the information that will be on your samples will be limited to the *pathology identification number, and an identification number for the study.*
Despite protections being in place, there is a risk of unintentional release of information that could lead to loss of privacy. Due to technological advances in genetics, there is also a risk of unintentional release of genetic information from the samples. This information can be linked back to you and can lead to possible future discrimination in employment or insurance, against you or your biological relatives.

Withdrawal of Samples

If you no longer want your samples to be used in this research, you should tell the study doctor. The study doctor will ensure the samples are returned to the hospital from which they were obtained, if needed, or destroyed.

You can request withdrawal of your sample(s) until *you have received your blinded capsules* when the samples will be made anonymous. It won't be possible to return samples after this because the researchers will not know which samples are yours.

You will not be able to continue to participate in this study if required samples are withdrawn.

Assessments (Part 1 of 2)	**Screening** (within 21 days before chemotherapy)	**Chemotherapy Cycle 1**		**Chemotherapy Cycle 2**		**Chemotherapy Cycle 3**		**Chemotherapy Cycle 4**		**Chemotherapy Cycle 5**		**Chemotherapy Cycle 6**		**End of Treatment**	**Surgery**
		Day 1[2]	**Day 20 (+/- 3 days)**	**Day 1**	**Day 20 (+/- 3 days)**	**Day 1**	**Day 20 (+/- 3 days)**	**Day 1**	**Day 20 (+/- 3 days)**	**Day 1**	**Day 20 (+/-3 days**	**Day 1**	**Day 20 (+/-3 days**	**Within 28 days after last dose**	
Informed Consent	X														
Demographic data collection	X														
Medical history or current medical conditions	X														
Height	X														
Weight	X	X												X	
Vital Signs	X	X		X		X		X		X		X		X	
Physical Exam	X	X		X		X		X		X		X		X	
You will be asked about your ability to carry out daily activities	X	X												X	
Questionnaires about your symptoms and well-being (ESAS questionnaire)	X	X		X		X		X		X		X		X	
Quality of life questionnaire	X													X	
Exercise questionnaire	X	X		X		X		X		X		X		X	
Food frequency questionnaire		X (anytime within the first cycle)													
A sample of your tumour will be analyzed for	X														X

disease-related biomarkers (signs related to your disease)														

Assessments (Part 2 of 2)	**Screening (within 21 days before chemotherapy)**	**Chemotherapy Cycle 1**		**Chemotherapy Cycle 2**		**Chemotherapy Cycle 3**		**Chemotherapy Cycle 4**		**Chemotherapy Cycle 5**		**Chemotherapy Cycle 6**		**End of Treatment**	**Surgery**
		Day 1[2]	**Day 20 (+/- 3 days)**	**Day 1**	**Day 20 (+/- 3 days)**	**Day 1**	**Day 20 (+/- 3 days)**	**Day 1**	**Day 20 (+/- 3 days)**	**Day 1**	**Day 20 (+/-3 days**	**Day 1**	**Day 20 (+/-3 days**	**Within 28 days after last dose**	
Blood sample for routine tests to monitor your health	X							X					X		
Blood will be collected to measure signs of immune function	X							X					X		
Blood will be collected to measure the level of study treatment in your blood lipids	X			X		X		X		X		X	X		
Treatment: DHA/Placebo		Days 1-21		Days 1-21		Days 1-21		Days 1-21		Days 1-21		Days 1-21			
Diary completion with your capsule intake		Days 1 -21		Days 1 -21		Days 1 -21		Days 1 -21		Days 1 -21		Days 1 -21			
Confirmation of previous or current medications	X	X		X		X		X		X		X		X	
You will be asked about any side effects which may or not be related to the study	X	X		X		X		X		X		X		X	

treatment														
We will collect results from your surgery report														X

OPTIONAL RESEARCH

The researchers doing this study are interested in doing additional optional research. You will be given a separate optional study consent form(s) to read and sign if you wish to give permission to this. You may decide not to participate in the "optional" study and still participate in this main study.

WHAT ARE THE POTENTIAL SIDE EFFECTS FROM PARTICIPATING IN THIS STUDY?

You may experience side effects from participating in this study. Some side effects are known and are listed below, but there may be side effects that are not expected. You should discuss these with the study doctor.

There are no known side effects of this omega 3 (DHA) supplement. A non-medicinal ingredient in this nutritional supplement that may cause an allergic reaction includes gelatin.

The risks and side-effects of the standard or usual treatment will be explained to you as part of your standard care. These risks are not included in this consent form.

A Data and Safety Monitoring Board (DSMB), an independent group of experts, will be reviewing the data throughout the conduct of the study to ensure continuing participant safety as well as scientific validity and quality of the research.

WHAT ARE THE REPRODUCTIVE RISKS?

There appears to be no effect of the nutritional product on the human reproductive system.

WHAT ARE THE BENEFITS OF PARTICIPATING IN THIS STUDY?

Participation in this study may or may not be of personal benefit to you. However, based on the results of this study, it is hoped that in the long-term, patient care can be improved.

WHAT ARE MY RESPONSIBILITIES AS A STUDY PARTICIPANT?

If you choose to participate in this study, you will be expected to:

- Tell the study doctor about your current medical conditions;
- Tell the study doctor about all prescription and non-prescription medications and supplements, including vitamins and herbals, that you may be taking and check with the study doctor before starting, stopping or changing any of these. This is for your safety as these may interact with the intervention you receive on this study;
- Tell the study doctor if you are thinking about participating on another research study;

- Attend all scheduled study visits and undergo all of the procedures described above;
- Return any unused DHA / placebo products;
- Return any *diaries and food frequency questionnaires* taken home to complete;
- Tell the study doctor if you become pregnant while participating on this study;
- Avoid taking fish oil supplements, or any supplements containing DHA.
- Stop taking other *supplements of vitamin C, vitamin E, or β-carotene exceeding the DRI (daily recommended intake), or other anti-oxidant supplements. A multivitamin with vitamin C, E, and β-carotene below the DRI are permitted (75 mg/day vitamin C, 15 mg/day vitamin E, and 700 µg/day β-carotene). A member of the research staff will go through the details of multivitamin intake to ensure it is within the guidelines.*
- *DHA supplement/ placebo capsules are* meant for you alone, and must not be shared with others. If someone accidently takes the capsules, the *intake should be recorded in medication diary, and the study staff should be informed.*

HOW LONG WILL I BE PARTICIPATING IN THIS STUDY?

The study intervention will last as long as it takes for you to receive your chemotherapy (about *12-18 weeks)*.
You may be seen more often if the study doctor determines that this is necessary or if your cancer *gets worse*.

WILL THERE BE ANY LONG-TERM FOLLOW-UP INVOLVED WITH THIS STUDY?

No matter which group you are randomized to, and even if you stop receiving the study intervention early, we would like to keep track of your health for *10 years to look at the long-term effects of your participation on the study*. We would do this *by accessing electronic or paper medical chart review at 3, 5 and 10 years after treatment.*

In the event it is necessary to further evaluate the safety or efficacy of the *DHA supplement*, it may be necessary to have access to additional information about your health status. The study team may attempt to obtain study-related information about your health from you or from other private sources, including your care physician and *electronic or paper medical chart review*. This may include contacting you again by phone or letter, but only if you have not withdrawn your consent for future contact. However, contacting you, your care physician or using other private sources of information, is optional, please indicate your decision using the check boxes below.

You give permission to the study doctor or member of the study team to attempt to obtain study-related information about your health status to further evaluate the safety or efficacy of *DHA supplementation*. This may include contacting your care physician, or by contacting you by phone or letter (i.e., future contact).

☐ Yes ☐ No Participant's Initials: _____

Name/phone number of care physician: _____

In addition, the study team may also attempt to obtain study-relevant information about your health information from public sources such as national patient registries (e.g., cancer registries)

If the study doctor needs to follow up with you but cannot locate you, either because you have moved and not updated your contact information or if, for some reason, your contact information is no longer accurate, the study doctor would like to obtain your new contact information (e.g., address, telephone number) by calling or writing to the persons you've named as your secondary contacts. This is optional, please indicate your decision using the check boxes below.

You give permission to the study doctor or member of the study team to contact your secondary contacts if the study doctor or study team no longer have accurate contact information for you.

☐ Yes ☐ No Participant's Initials: _____

Name/phone number of secondary contacts: _____

If the study doctor cannot obtain information through your secondary contacts, he/she would like to ask for assistance of a third party that specializes in locating persons. The study doctor may only share limited information about you (name and last known address) with a third party locator. None of your personal health or study-related information will be shared with the third party locator. The third party locator will consult public sources and databases to obtain your current contact information but will not contact you. The third party locator will only share this information with the study doctor or study team to help complete the follow-up stage of the study. Only the study doctor or a member of the study team will attempt to contact you directly. This is optional, please indicate your decision using the check boxes below.

If the study doctor is not able to obtain your contact information from your secondary contacts, you give permission for the study doctor to provide your name and last location to a third party that specializes in locating persons.

☐ Yes ☐ No Participant's Initials: _____

CAN I CHOOSE TO LEAVE THIS STUDY EARLY?

You can choose to end your participation in this research (called early withdrawal) at any time without having to provide a reason. If you choose to withdraw early from the study without finishing the intervention, procedure or follow-up, you are encouraged to contact the study doctor or study staff.

You may be asked questions about your experience with the study intervention, and to have laboratory tests and physical examinations considered necessary to safely stop your study involvement.

You may withdraw your permission to use information that was collected about you for this study at any time by letting the study doctor know. However, this would also mean that you withdraw from the study.

Information that was recorded before you withdrew will be used by the researchers for the purposes of the study, but no additional information will be collected or sent to the sponsor after you withdraw your permission.

CAN MY PARTICIPATION IN THIS STUDY END EARLY?

The study doctor may stop your participation in the study early, and without your consent, for reasons such as:

- The intervention does not work for you;
- You are unable to tolerate the study intervention;
- You are unable to complete all required study procedures;
- New information shows that the study intervention is no longer in your best interest;
- The study doctor no longer feels this is the best treatment for you;
- A regulatory authority (for example, Health Canada) or the research ethics board withdraws permission for the study to continue;
- Your treatment assignment becomes known to others (the study doctor or study staff);

If you are removed from the study, the study doctor will discuss the reasons with you and plans will be made for your continued care outside of the study.

HOW WILL MY PERSONAL INFORMATION BE KEPT CONFIDENTIAL?

If you decide to participate in this study, the study doctor and study staff will only collect the information they need for this study.

Records identifying you, including information collect from your medical files/records, such as your Electronic Medical Records (EMR), Netcare, charts, etc., will be kept confidential to the extent permitted by the applicable laws, will not be disclosed or made publicly available, except as described in this consent document.

Authorized representatives of the following organizations may look at your identifiable medical/clinical study records at the site where these records are held for quality assurance purposes and/or to verify that the information collected for the study is correct and follows proper laws and guidelines:

- Members of the Regulatory/Audit team at *Cross Cancer Institute,* for quality assurance purposes;
- The Health Research Ethics Board of Alberta – Cancer Committee, which oversees the ethical conduct of this study;
- Health Canada, which oversees the use of natural health products/drugs/devices in Canada and the conduct of clinical trials;

Authorized representatives of the above organizations and of the University of Alberta may **receive** information related to the study from your medical/clinical study records that will be kept confidential in a secure location and may be used in current or future relevant health research. Your name or other information that may identify you will not be provided (i.e., the information will be de-identified). The records received by these organizations will be coded with a number. The key that indicates what number you have been assigned will be kept secure by the researchers directly involved with your study and will not be released.

Any disclosure of your identifiable health information will be done in accordance with federal and provincial laws including the Alberta Health Information Act (HIA). The organizations listed above are required to have organizational policies and procedures to protect the information they see or receive about you, except where disclosure may be required by law. The study doctor will ensure that any personal health information collected for this study is kept in a secure and confidential location at the *Cross Cancer Institute, Edmonton Alberta* as also required by law.

If the results of this study are published, your identity will remain confidential. It is expected that the information collected during the study will be *used in analyses and will be published/presented to the scientific community at meetings and in journals*. This information may also be used as part of a submission to regulatory authorities around the world to support the approval of this intervention.

Even though the likelihood that someone may identify you from the study data is very small, it can never be completely eliminated. Every effort will be made to keep your identifiable information confidential, and to follow the ethical and legal rules about collecting, using and disclosing this information.

WILL MY HEALTHCARE PROVIDER(S) BE INFORMED OF MY PARTICIPATION IN THIS STUDY?

Your family doctor/health care provider will not be informed by the study team that you are taking part in the study. You can choose to let your family doctor/health care provider know, if you like. If you are undecided, the study doctor can discuss this with you.

WILL THERE BE ANY COSTS INVOLVED WITH PARTICIPATING IN THIS STUDY?

The DHA supplement/ placebo will be given to you free of charge while you take part in this study.

Taking part in this study may result in added costs to you. For example:

- There may be costs associated with hospital visits. For instance, parking, transportation, or snacks/meals during the study.

Possible Costs After the Study is Complete

You may not be able to receive the study intervention after your participation in the study is completed. There are several possible reasons for this, some of which are:

- The intervention may not turn out to be effective or safe;
- The intervention may not be approved for use in Canada;
- Your caregivers may not feel it is the best option for you;
- You may decide it is too expensive and insurance coverage may not be available;
- The intervention, even if approved in Canada, may not be available free of charge.

The study doctor will discuss these options with you.

WILL I BE COMPENSATED FOR PARTICIPATING IN THIS STUDY?

You will not be paid for taking part in this study.

It is possible that the research conducted using your samples and/or study data may eventually lead to the development of new diagnostic tests, new drugs or devices, or other commercial products. There are no plans to provide payment to you if this happens.

In the case of research-related side effects or injury, as a direct result of participating in this research, you will receive all medical treatments or services recommended by your doctors.

Although no funds have been set aside to compensate you in the event of injury or illness related to the study treatment or procedures, you do not give up any of your legal rights for compensation by signing this form.

WHAT ARE MY RIGHTS AS A PARTICIPANT IN THIS STUDY?

You will be told, in a timely manner, about new information that may be relevant to your willingness to stay in this study.

You have the right to be informed of the results of this study once the entire study is complete. If you would like to be informed of these results, please contact the study doctor.

The results of this study will be available on a clinical registry; refer to the section titled “Where can I find online information about this study?”.

Your rights to privacy are legally protected by federal and provincial laws that require safeguards to ensure that your privacy is respected.

By signing this form you do not give up any of your legal rights against the hospital, investigators, sponsor, involved institutions for compensation or their agents, nor does this form relieve these parties from their legal and professional responsibilities.

IS THERE CONFLICT OF INTEREST RELATED TO THIS STUDY?

There are no conflicts of interest declared between the study doctor and sponsor of this study.

WHAT IF RESEARCHERS DISCOVER SOMETHING ABOUT ME AS A RESEARCH PARTICIPANT?

During the study, the researchers may learn something about you that they didn’t expect. For example, the researchers may *find out that you have another medical condition*.

If any clinically important information about your health is obtained as a result of your participation in this study, you will be given the opportunity at that time to decide whether you wish to be made aware of that information.

WHERE CAN I FIND ONLINE INFORMATION ABOUT THIS STUDY?

A description of this clinical trial will be available on *http://www.ClinicalTrials.gov*.

The study registration number to use this website is: NCT03831178

This website will not include information that can identify you. You can search for this website at any time.

WHO DO I CONTACT FOR QUESTIONS?

If you have questions about taking part in this study, or if you suffer a research-related injury, you should talk to the study doctor, co-investigator or study nurse. These person(s) are:

Dr. John Mackey	780-432-8221
Name	Telephone
Name	Telephone
Name	Telephone

He can also be paged through the Cross Cancer Institute switchboard at (780) 432-8771.

If you have questions about your rights as a participant or about ethical issues related to this study and you would like to talk to someone who is not involved in the conduct of the study, please contact the Office of the Health Research Ethics Board of Alberta – Cancer Committee at:

Telephone: 780-423-5727 Toll Free: 1-877-423-5727

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SIGNATURES

Part 1 - to be completed by the potential participant.

	Yes	No
Do you understand that you have been asked to take part in a research study?	☐	☐
Do you understand why this study is being done?	☐	☐
Do you understand the potential benefits of taking part in this study?	☐	☐
Do you understand the risks of taking part in this study and the risks of becoming pregnant or fathering a child during this study?	☐	☐
Do you understand what you will be asked to do should you decide to take part in this study?	☐	☐
Do you understand the alternatives to participating in this study?	☐	☐
Do you understand that you are free to leave the study at any time, without out having to give reason and without affecting your future health care?	☐	☐
Do you understand who will see your records, including health information that identifies you?	☐	☐
Do you understand that by signing this consent form you are giving us permission to access your health information and specimens if applicable?	☐	☐
Do you understand that by signing this consent form that you do not give up any of your legal rights?	☐	☐
Have you had enough opportunity to ask questions and discuss this study?	☐	☐

By signing this form I agree, to participate in this study.

Signature of Participant	PRINTED NAME	Date

Part 2 - to be completed by the study doctor or designee who conducted the informed consent discussion. Only compete this section if the potential participant has **agreed** to participate.

I believe that the person signing this form understands what is involved in the study and has freely decided to participate.

Signature of Person Conducting the Consent Discussion	PRINTED NAME	Date

Part 3 - to be completed only if the participant is unable to read or requires assistance of an oral translator/interpreter.

- The informed consent form was accurately explained to, and apparently understood by the participant.
- Informed consent was freely given by *or on behalf of* the participant.

Signature of Impartial Witness/Interpreter	PRINTED NAME	Date

You will be given a copy of this signed and dated consent form prior to participating in this study.

Informed Consent Form for Participation in Optional Research

DHA for Women with Breast Cancer in the Neoadjuvant Setting (DHA WIN)

DHA to improve effectiveness of Chemotherapy in Breast Cancer

Protocol ID: IIT-0005

Researcher: Dr. John Mackey
Department of Medical Oncology
Cross Cancer Institute
780-432-8221

Funder(s)/Sponsor: Alberta Health Services- Cross Cancer Institute

INTRODUCTION

In addition to the main study, you also are being invited to take part in optional research. Although it is optional, the study of human samples and data focusing on the prevention, diagnosis and treatment of cancer and other diseases is an important part of research. Taking part in this optional research is voluntary. You still can take part in the main study, and will continue to receive treatment and care even if you say “no” to any or all of this optional research now or later. This form and your discussion with the researcher/study staff will give you the information you need to make your decision.

WHY IS THIS OPTIONAL RESEARCH BEING DONE?

The researchers conducting this research are interested in doing the following:

- Biomarker research for the main study using tumour tissue / blood already collected
- Bio-banking for use in future research using tumour tissue / blood already collected

As part of this optional research, the researchers would like to examine your tumour tissue/blood samples to look for any **biomarkers** (small “signature” molecules or indicators) in your cancer cells or circulating in your blood. These biomarkers might help predict which patients are most likely to be affected by the study drug. This is called biomarker research.

Bio-banking is the collection, storage, and use of human body samples and related health information for future research. It provides an important resource for health research

locally, across Canada, and around the world. The researchers doing the main study are also interested in storing your tissue/blood samples for future research. The research that may be done on your samples in the future is unknown at this time. It may be related to your condition or it may be used to address research questions that are unrelated.

Some of this research may be about genes. Genes carry information about features, such as hair or eye colour. This research may include looking at changes in genes found in you and in people who are related to you. These changes may be inherited (passed on in families). This is called hereditary genetic testing. Researchers also may be interested in the way that genes affect health and disease, or how your body responds to treatment.

WHAT WILL HAPPEN DURING THIS OPTIONAL RESEARCH?

You may take part in all or some of the optional research described here, it is your choice. If you agree to take part:

- the samples used for this optional research have already been collected as part of your standard of care. No further biopsies or surgeries are needed for this purpose.
- the blood samples used for this optional research will be those left over or remaining from your participation in the main study. No further biopsies or surgeries are needed for this purpose.

HOW WILL MY SAMPLES BE HANDLED?

Your sample(s) and some related health information already collected from your participation in the main study will be sent to the Nutritional Immunology laboratory at the University of Alberta, Edmonton, AB, for analysis. The samples and data will be kept indefinitely or until they are used up, destroyed or returned to the hospital where you had your surgery or biopsy.

Qualified researchers can submit a request to use the materials stored at the University of Alberta. Your samples and related health information will be used only by researchers whose requests have been accepted by the sponsor and who have met regulatory requirements and secured ethics approval for their research. The samples and data may be sent to other countries. Your name or any other information that could directly identify you will not be given to these researchers.

The results of research done on your samples will not be added to your personal health records and you or the researcher will not know the results.

WHAT ARE THE RISKS OF PARTICIPATING IN THIS OPTIONAL RESEARCH?

Risks related to sample collection:

- Since the tissue sample(s) already have been collected for the main study or as part of your standard of care, no additional physical risks are expected.

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Risks related to the disclosure of personal health information:

- There is a risk that someone could get access to the personal information in your personal health records or other information researchers have stored about you.
- There is a risk that someone could trace the information in a central or public database back to you. Even without your name or other identifiers, your genetic information is unique to you. The researchers believe the chance that someone will identify you is very small, but the risk may change in the future as people come up with new ways of tracing information.
- New health information about inherited traits that might affect you or your blood relatives could be found during a study. The researchers believe the chance these things will happen is very small, but cannot promise that they will not occur.
- Due to the rapid pace of technological advances, the potential future use of genetic information is unknown and therefore the potential future risks also are unknown.
- There may be risks to eligibility for employment or insurance if the results of genetic testing were inadvertently disclosed to certain parties.
- Genetic information cannot be protected from court-ordered disclosure.

WHAT ARE THE POTENTIAL BENEFITS OF PARTICIPATING IN THIS OPTIONAL RESEARCH?

You will not benefit directly from taking part in this optional research. However, research done with your donated samples or health information may benefit other patients with your condition or other similar or related condition(s).

HOW WILL MY PERSONAL INFORMATION BE KEPT PRIVATE?

Your privacy is very important to the researchers and they will make every effort to protect it. Here are the steps they will take:

- When your sample(s) are sent to the laboratory, no information identifying you (such as your name, date of birth, health insurance number) will be provided or shared. Samples may be identified by your study code.
- The samples that are provided to researchers by the Cross Cancer Institute are identified only by that biobank code; researchers will not know who you are.
- The list that links the samples to your personal identifiers (i.e., name) will be kept separate from your sample(s) and health information in a secure and confidential location at the main study site. If you change your mind about participating in this optional research, this list will be used to locate and return or destroy your samples. Decoding can only be done by the researcher or an individual authorized by the researcher.
- Study records will be kept for 25 years.
- A record of your participation in this optional study will be kept with your main study records and may be monitored for quality assurance.

Information that identifies you, will be kept confidential and, to the extent permitted by the applicable laws, will not be disclosed or made publicly available except as described in this document. If research results are published, your name and other personal information will not be used.

Qualified representatives of the sponsor will make sure the study has been done properly by checking your records at the researcher's site. Regulatory authorities, such as Health Canada and the applicable Research Ethics Board also may wish to check that the study has been done properly, and may also have direct access to your personal health information. Except as expressly stated in this section, all of the information provided in the main study consent form about confidentiality and direct access to your personal health information applies to this optional research consent form.

WHAT IF RESEARCHERS DISCOVER SOMETHING ABOUT ME DURING THE STUDY?

During the study, the researchers may learn something about you that they didn't expect. For example, the researchers may find out that you have another medical condition.

If any new clinically important information about your health is obtained as a result of your participation in this optional research, you will be given the opportunity to decide whether you wish to be made aware of that information.

WILL THERE BE ANY COSTS OR COMPENSATION INVOLVED WITH THS RESEARCH?

There are no costs to you. You will not be paid for taking part. No samples or information/data will be sold.

It is possible that the research conducted using your samples and/or my data may eventually lead to the development of new diagnostic tests, new drugs or other commercial products. There are no plans to provide payment to you if this happens.

WHAT ARE MY RIGHTS AS A PARTICIPANT IN THIS OPTIONAL RESEARCH?

You will be told, in a timely manner, about new information that may be relevant to your willingness to stay in this study.

If you decide you no longer want your samples or related health information to be used, you should tell the researcher. Any sample(s) that remain(s) in the laboratory will be destroyed (if blood) or returned to the hospital where you had your original biopsy or surgery (if tumour block). If tests have already been done on your sample and included in an analysis or publication, it will not be possible to withdraw these results.

You will be given a copy of this signed and dated consent form prior to participating in this

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study.

IS THERE ANY CONFLICT OF INTEREST RELATED TO THIS OPTIONAL RESEARCH?
There are no current or potential conflicts of interest concerning the optional research study.

WHO DO I CONTACT FOR QUESTIONS RELATED TO THIS OPTIONAL RESEARCH?

If you have questions about the use of your samples/data for optional research, or if you suffer a research-related injury, contact the researcher of this optional study:

Catherine J Field	780-492-5297
Name	Telephone Number

If you have questions about your rights as a participant or about ethical issues related to this optional research and you would like to speak to someone not involved in its conduct, please contact the Office of the Health Research Ethics Board of Alberta – Cancer Committee at: 780-423-5727 or toll-free 1-877-423-5727.

UNDERSTANDING AND SIGNATURES PAGE

Please circle your answer to show whether or not you would like to take part in the optional research:

I agree that samples which were already collected and related health information may be used for the optional research described above.

YES NO

I agree that my samples and related health information may be kept in a biobank for use in future health research related to my condition or may be used to address research questions that are unrelated.

YES NO

I agree that the researcher, or their representative, may contact me or my physician to see if I wish to learn about results from this research.

YES NO

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SIGNATURES

PARTICIPANT ACKNOWLEDGEMENT

- I understand the information within this optional consent form.
- All of my questions have been answered to my satisfaction.
- I am aware of the risks and potential benefits to me of participating in this optional research.
- I allow access to my personal health information and samples as explained in this form.
- I understand that I do not give up any of my legal rights by signing this consent form.
- I agree to take part in this optional research as described and where "YES" above has been circled.

_____	_____	_____
Signature of Participant	Printed Name	Date

STUDY TEAM ACKNOWLEDGEMENT

I believe that the person signing this form understands what is involved in this optional research and has freely decided to participate.

_____	_____	_____
Signature of Person Conducting the Consent Discussion	Printed Name	Date

PARTICIPANT ASSISTANCE (IMPARTIAL WITNESS)

This section is to be completed only if the participant is unable to read the consent document. The individual assisting the participant must be impartial.

- The informed consent form was accurately explained to, and apparently understood by the research participant.
- Informed consent was freely given by the participant.

_____	_____	_____
Signature of Impartial Witness	Printed Name	Date

TRANSLATOR/INTERPRETER ACKNOWLEDGEMENT

This section is to be completed only if the participant requires the assistance of a qualified oral translator/interpreter. The interpreter must be impartial.

- The informed consent discussion was accurately explained to, and apparently

understood by the research participant.

- A sight translation of the consent document was provided by the interpreter as directed by the research staff conducting the consent process.

Signature of Interpreter	Printed Name	Date

You will be given a copy of this signed and dated consent form prior to participating in this optional research.

SPIRIT

STANDARD PROTOCOL ITEMS: RECOMMENDATIONS FOR INTERVENTIONAL TRIALS

SPIRIT 2013 Checklist: Recommended items to address in a clinical trial protocol and related documents*

Section/item	Item No	Description	Reported on Page No
Administrative information			
Title	1	Descriptive title identifying the study design, population, interventions, and, if applicable, trial acronym	1
Trial registration	2a	Trial identifier and registry name. If not yet registered, name of intended registry	a
	2b	All items from the World Health Organization Trial Registration Data Set	Supp. files
Protocol version	3	Date and version identifier	1
Funding	4	Sources and types of financial, material, and other support	22
Roles and responsibilities	5a	Names, affiliations, and roles of protocol contributors	1
	5b	Name and contact information for the trial sponsor	1
	5c	Role of study sponsor and funders, if any, in study design; collection, management, analysis, and interpretation of data; writing of the report; and the decision to submit the report for publication, including whether they will have ultimate authority over any of these activities	22
	5d	Composition, roles, and responsibilities of the coordinating centre, steering committee, endpoint adjudication committee, data management team, and other individuals or groups overseeing the trial, if applicable (see Item 21a for data monitoring committee)	20-21
Introduction			
Background and rationale	6a	Description of research question and justification for undertaking the trial, including summary of relevant studies (published and unpublished) examining benefits and harms for each intervention	4-5

	6b	Explanation for choice of comparators	7
Objectives	7	Specific objectives or hypotheses	5
Trial design	8	Description of trial design including type of trial (eg, parallel group, crossover, factorial, single group), allocation ratio, and framework (eg, superiority, equivalence, noninferiority, exploratory)	6, Fig 1, Fig 2
Methods: Participants, interventions, and outcomes			
Study setting	9	Description of study settings (eg, community clinic, academic hospital) and list of countries where data will be collected. Reference to where list of study sites can be obtained	6
Eligibility criteria	10	Inclusion and exclusion criteria for participants. If applicable, eligibility criteria for study centres and individuals who will perform the interventions (eg, surgeons, psychotherapists)	Table 1 (page 1,2)
Interventions	11a	Interventions for each group with sufficient detail to allow replication, including how and when they will be administered	7
	11b	Criteria for discontinuing or modifying allocated interventions for a given trial participant (eg, drug dose change in response to harms, participant request, or improving/worsening disease)	8
	11c	Strategies to improve adherence to intervention protocols, and any procedures for monitoring adherence (eg, drug tablet return, laboratory tests)	8
	11d	Relevant concomitant care and interventions that are permitted or prohibited during the trial	-
Outcomes	12	Primary, secondary, and other outcomes, including the specific measurement variable (eg, systolic blood pressure), analysis metric (eg, change from baseline, final value, time to event), method of aggregation (eg, median, proportion), and time point for each outcome. Explanation of the clinical relevance of chosen efficacy and harm outcomes is strongly recommended	8-11 Table 3
Participant timeline	13	Time schedule of enrolment, interventions (including any run-ins and washouts), assessments, and visits for participants. A schematic diagram is highly recommended (see Figure)	12, Table 2

Sample size	14	Estimated number of participants needed to achieve study objectives and how it was determined, including clinical and statistical assumptions supporting any sample size calculations	12
Recruitment	15	Strategies for achieving adequate participant enrolment to reach target sample size	13
Methods: Assignment of interventions (for controlled trials)			
Allocation:			
Sequence generation	16a	Method of generating the allocation sequence (eg, computer-generated random numbers), and list of any factors for stratification. To reduce predictability of a random sequence, details of any planned restriction (eg, blocking) should be provided in a separate document that is unavailable to those who enrol participants or assign interventions	13
Allocation concealment mechanism	16b	Mechanism of implementing the allocation sequence (eg, central telephone; sequentially numbered, opaque, sealed envelopes), describing any steps to conceal the sequence until interventions are assigned	13
Implementation	16c	Who will generate the allocation sequence, who will enrol participants, and who will assign participants to interventions	13
Blinding (masking)	17a	Who will be blinded after assignment to interventions (eg, trial participants, care providers, outcome assessors, data analysts), and how	13
	17b	If blinded, circumstances under which unblinding is permissible, and procedure for revealing a participant's allocated intervention during the trial	13
Methods: Data collection, management, and analysis			
Data collection methods	18a	Plans for assessment and collection of outcome, baseline, and other trial data, including any related processes to promote data quality (eg, duplicate measurements, training of assessors) and a description of study instruments (eg, questionnaires, laboratory tests) along with their reliability and validity, if known. Reference to where data collection forms can be found, if not in the protocol	14-18 Table 3

	18b	Plans to promote participant retention and complete follow-up, including list of any outcome data to be collected for participants who discontinue or deviate from intervention protocols	-
Data management	19	Plans for data entry, coding, security, and storage, including any related processes to promote data quality (eg, double data entry; range checks for data values). Reference to where details of data management procedures can be found, if not in the protocol	18
Statistical methods	20a	Statistical methods for analysing primary and secondary outcomes. Reference to where other details of the statistical analysis plan can be found, if not in the protocol	19-20 Table 3
	20b	Methods for any additional analyses (eg, subgroup and adjusted analyses)	19-20
	20c	Definition of analysis population relating to protocol non-adherence (eg, as randomised analysis), and any statistical methods to handle missing data (eg, multiple imputation)	-
Methods: Monitoring			
Data monitoring	21a	Composition of data monitoring committee (DMC); summary of its role and reporting structure; statement of whether it is independent from the sponsor and competing interests; and reference to where further details about its charter can be found, if not in the protocol. Alternatively, an explanation of why a DMC is not needed	20
	21b	Description of any interim analyses and stopping guidelines, including who will have access to these interim results and make the final decision to terminate the trial	21
Harms	22	Plans for collecting, assessing, reporting, and managing solicited and spontaneously reported adverse events and other unintended effects of trial interventions or trial conduct	20
Auditing	23	Frequency and procedures for auditing trial conduct, if any, and whether the process will be independent from investigators and the sponsor	21
Ethics and dissemination			

Research ethics approval	24	Plans for seeking research ethics committee/institutional review board (REC/IRB) approval	21
Protocol amendments	25	Plans for communicating important protocol modifications (eg, changes to eligibility criteria, outcomes, analyses) to relevant parties (eg, investigators, REC/IRBs, trial participants, trial registries, journals, regulators)	21
Consent or assent	26a	Who will obtain informed consent or assent from potential trial participants or authorised surrogates, and how (see Item 32)	21 Supp file
	26b	Additional consent provisions for collection and use of participant data and biological specimens in ancillary studies, if applicable	21 Supp file
Confidentiality	27	How personal information about potential and enrolled participants will be collected, shared, and maintained in order to protect confidentiality before, during, and after the trial	22
Declaration of interests	28	Financial and other competing interests for principal investigators for the overall trial and each study site	22
Access to data	29	Statement of who will have access to the final trial dataset, and disclosure of contractual agreements that limit such access for investigators	22
Ancillary and post-trial care	30	Provisions, if any, for ancillary and post-trial care, and for compensation to those who suffer harm from trial participation	22
Dissemination policy	31a	Plans for investigators and sponsor to communicate trial results to participants, healthcare professionals, the public, and other relevant groups (eg, via publication, reporting in results databases, or other data sharing arrangements), including any publication restrictions	22
	31b	Authorship eligibility guidelines and any intended use of professional writers	22
	31c	Plans, if any, for granting public access to the full protocol, participant-level dataset, and statistical code	-
Appendices			
Informed consent materials	32	Model consent form and other related documentation given to participants and authorised surrogates	Supp file

Biological specimens	33	Plans for collection, laboratory evaluation, and storage of biological specimens for genetic or molecular analysis in the current trial and for future use in ancillary studies, if applicable	Supp file

*It is strongly recommended that this checklist be read in conjunction with the SPIRIT 2013 Explanation & Elaboration for important clarification on the items. Amendments to the protocol should be tracked and dated. The SPIRIT checklist is copyrighted by the SPIRIT Group under the Creative Commons "Attribution-NonCommercial-NoDerivs 3.0 Unported" license.

Supplemental Table 2: World Health Organization Trial Registration Data Set DHA WIN Summary

Data Category	**Information**
Primary registry and trial identifying number	ClinicalTrials.gov: NCT03831178
Date of registration in primary registry	February 5, 2019
Secondary identifying numbers	IIT-0005
Sources of monetary or material support	Canadian Institutes of Health Research (CIHR), AHS Cancer Control Alberta, Butler Family Foundation
Primary sponsor	AHS Cancer Control Alberta
Secondary sponsors	University of Alberta
Contact for public queries	Deborah Miede: Deborah.Miede@albertahealthservices.ca
Contact for scientific queries	Catherine Field: Catherine.field@ualberta.ca
Public title	DHA WIN
Scientific title	Docosahexaenoic acid (DHA) for Women with breast cancer in the neoadjuvant setting
Country of recruitment	Canada
Health condition or problems studied	Breast cancer
Interventions	DHA supplementation (5 g/ day) or equal amount of vegetable oil placebo for the duration of the participants chemotherapy treatment
Key inclusion and exclusion criteria	Inclusion: ECOG Performance status of 0 or 1; Hematology and biochemistry assessments within normal range; ability to take oral medication; adequate tissue specimen for diagnosis, biomarkers and endpoint Ki67 assays Exclusion: Patients undergoing surgery prior to chemotherapy; Current or previous (within 2 months) daily use (>1 day/week) use of omega-3, fish oil, or other supplements or foods containing DHA (at daily doses > 200 mg); Known allergy to soy or corn; Continued intake of supplements containing Vitamin C, Vitamin E or β-carotene exceeding the DRI, or other anti-oxidant supplements; History of deep venous thrombosis, active thrombophlebitis, pulmonary embolism, stroke, acute myocardial infarction, congestive cardiac failure, untreated

	hypertension, known inherited hypercoagulable disorder; Diagnosis of any other malignancy within the previous year except for adequately treated basal cell or squamous cell skin cancer
Study type	Randomized controlled trial
Date of first enrolment	Expected April 2019
Target sample size	52
Recruitment status	Not yet recruiting
Primary outcomes	Percent change in Ki67 index from baseline to surgical excision
Key secondary outcomes	Percent of DHA in plasma phospholipids, systemic immune function; Identify factors that may affect DHA incorporation into plasma phospholipids; Examine changes in markers for apoptosis and tumor infiltrating lymphocytes; pathological complete response; Comparison of rate of chemotherapy associated grade 3 and 4 toxicities

Supplemental Table 3: Fatty Acid Composition of DHA supplement and Placebo

Fatty Acid	DHA capsule	Placebo
16:0	16.9	10.9
18:0	0.1	2.7
18:1n-9	4.8	23.2
18:2n6	0.5	53.5
18:3n-3	<0.1	4.7
20:5n-3	1.0	<0.1
22:5n-3	0.5	<0.1
22:5n-6	18.1	<0.1
22:6n-3	43.4	<0.1

Supplemental Table 4: List of Antibodies used for immune cell phenotyping

CD1a	FITC	300104
CD1c	BV421	331526
CD3	FITC	300306
CD4	APC	357408
CD8	PerCP/Cy5.5	344710
CD11b	PE	301306
CD11c	APC	301614
CD14	APC	367118
CD16	PE	302008
CD20	FITC	302304
CD25	PE	302606
CD27	PECy7	356412
CD28	APC	302912
CD45RA	PE	304108
CD45RO	FITC	304204
CD56	APC	362504
CD86	PCP	374210
CD95	BV421	305624
CD103	PECy7	350212
CD107	PE	328608
CD141	PECy7	344110
CD152	PE	369604
CD183	PerCP/Cy5.5	353720
CD196	PE	353410
CD279	APC	329908
FOXP3	FITC	320106
HLADR	PerCP/Cy5.5	307630

BMJ Open

Protocol of a double blind, phase II randomized controlled trial to compare Docosahexaenoic acid (DHA) concomitant with neoadjuvant chemotherapy versus neoadjuvant chemotherapy alone in the treatment of breast cancer: DHA WIN

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Primary Subject Heading:	Oncology
Secondary Subject Heading:	Nutrition and metabolism, Immunology (including allergy), Patient-

	centred medicine
Keywords:	ki67, phospholipids, omega-3, apoptosis, proliferation, immune function

Protocol of a double blind, phase II randomized controlled trial to compare Docosahexaenoic acid (DHA) concomitant with neoadjuvant chemotherapy versus neoadjuvant chemotherapy alone in the treatment of breast cancer: DHA WIN

Protocol Number: IIT-0005

Version Date: August 8, 2019

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Word Count: 5751

ABSTRACT

Introduction: Neoadjuvant chemotherapy for breast cancer treatment is prescribed to facilitate surgery, provide confirmation of drug sensitive disease and the achievement of pathological complete response (pCR) predicts improved long-term outcomes. Docosahexaenoic acid (DHA) has been shown to reduce tumor growth in preclinical models when combined with chemotherapy and is known to beneficially modulate systemic immune function. The purpose of this trial is to investigate the benefit of DHA supplementation in combination with neoadjuvant chemotherapy in patients with breast cancer.

Methods and analysis: This is a double blind phase II randomized controlled trial of 52 women prescribed neoadjuvant chemotherapy to test if DHA supplementation enhances chemotherapy efficacy. The DHA supplementation group will take 5g/day DHA orally and the placebo group will take an equal fat supplement of vegetable oil. The primary outcome will be change in Ki67 labelling index from pre-chemotherapy core needle biopsy to definitive surgical specimen.

Secondary endpoints include assessment of: i) DHA plasma phospholipid content; ii) systemic immune cell types, plasma cytokines and inflammatory markers; iii) tumor markers for apoptosis and tumor infiltrating lymphocytes; iv) rate of pCR in breast and in axillary nodes; v) frequency of grade 3 and 4 chemotherapy associated toxicities and vi) patient perceived quality of life.

The trial has 81% power to detect a significant between-group difference in Ki67 index with a two-sided t-test of less than 0.0497, and accounts for 10% drop-out rate.

Ethics and dissemination: This study has full approval from the Health Research Ethics Board of Alberta – Cancer Committee (Protocol #: HREBA.CC -18-0381). We expect to present the findings of this study to the scientific community in peer-reviewed journals and at conferences. The results of this study will provide evidence for supplementing with DHA during neoadjuvant chemotherapy treatment for breast cancer.

Clinical Trial Registration No: NCT03831178

KEYWORDS

Ki67, phospholipids, fatty acids, omega-3, apoptosis, proliferation, immune function

STRENGTHS AND LIMITATIONS OF THIS STUDY

- This study is the first phase II randomized controlled trial to evaluate DHA supplementation concomitant with neoadjuvant chemotherapy to treat non-metastatic breast cancer.
- The intervention is minimally invasive and side effects from the supplementation are not expected.
- This study is powered to examine the key clinical outcome of changes in Ki67 index from pre-chemotherapy biopsy to surgical excision based on group sample sizes of 23 patients in group one and 23 patients in group two in order to achieve 81% power to detect a difference between the group proportions of 0.4.
- This study will measure clinically relevant intermediate outcomes including rate of pCR in breast and in axillary nodes, rate of grade 3 and 4 chemotherapy associated toxicities and hospitalizations as well as additional outcomes including plasma phospholipid content of DHA, markers of immune function (plasma cytokines, inflammatory markers

and lymphocyte function), tumor markers for apoptosis and tumor infiltrating lymphocytes and patient perceived quality of life.

- The study will include all subtypes of breast cancer patients undergoing neoadjuvant chemotherapy but is not powered to assess differences between subtypes.

INTRODUCTION

Despite improvements in early diagnosis and treatment, breast cancer remains the second leading cause of cancer related death in women [1]. While neoadjuvant chemotherapy aims to improve surgical resection outcomes and reduce/eliminate micrometastases [2,3], pathological complete response (pCR) is not achieved by all patients [3]. Increasing the efficacy of neoadjuvant treatment without adding additional side-effects would benefit this population.

DHA is an omega-3 long chain polyunsaturated fatty acid (n-3 LCPUFA). The majority of n-3 fatty acids are in the form of the 18-carbon fatty acid alpha-linolenic acid (ALA). While DHA can be synthesized from ALA and other n-3 LCPUFA in the body, endogenous synthesis is low [4,5]. Consequently, the direct consumption of this fatty acid is the only way to significantly increase levels of DHA in tissues [6]. Supplementation can increase blood plasma DHA concentration by 2-fold (500 μM), which can lead to plasma membrane lipid enrichment [7]. Incorporation of DHA into tumor membrane phospholipids has been shown to reduce breast cancer cell proliferation [8,9] and increase apoptosis [10-15] in vitro and decreases tumor growth in animal models [13,15-17]. Additionally, providing/feeding DHA has been shown to increase the efficacy of different chemotherapeutic drugs in animal models of breast cancer [11,12,18,19]. While there is limited clinical evidence, it has been shown that increased dietary intake of n-3 LCPUFA, including DHA, results in increased DHA incorporation in breast adipose tissue [20] and this correlates with improved response to chemotherapy [21]. In an

open label trial with advanced metastatic breast cancer patients, DHA supplementation and enrichment into plasma phospholipids was associated with improved outcomes [22]. Other clinical trials have reported that supplementation with n-3 LCPUFA at a wide range of doses (0.6 g-8.6 g/day) increased tolerability of chemotherapeutic drugs in a range of malignancies at other sites, include lung, pancreatic and colorectal (reviewed in [23]). Consequently, we hypothesize that the therapeutic index (efficacy: toxicity ratio) of neoadjuvant breast cancer chemotherapy will be improved with the addition of DHA to the treatment.

Breast cancer proliferation can be assessed by immunohistochemical (IHC) analysis of cells staining positive for the nuclear antigen Ki67 [24], as it is expressed in all phases of the cell cycle, G_1, S, G_2, and M, but not in G_0 [25,26]. The proportion of cells staining for Ki67 is frequently used as a primary endpoint to measure efficacy of neoadjuvant therapy in clinical trials. The Ki67 index, defining the change between pre- and post-treatment Ki67, has been reported to be an independent prognostic factor in luminal A, luminal B, triple-negative, and HER2+ breast cancer, and has been reported to be a useful surrogate marker of relapse free survival in luminal B, triple-negative, and HER2+ breast cancer [27,28].

OBJECTIVES

The objective of this RCT is to assess the efficacy of supplemental DHA combined with neoadjuvant chemotherapy in treatment naïve women with breast cancer measured by changes in Ki67 index from biopsy to surgical excision. We hypothesize that DHA supplementation will increase plasma phospholipid DHA and improve response to neoadjuvant chemotherapy assessed by a decrease in the Ki67 index.

This protocol follows the Standard Protocol Items for Randomized Trials (SPIRIT) guideline (Spirit Checklist: Supplemental Table 1, WHO Checklist: Supplemental Table 2) [29,30].

Study Design

The DHA-WIN trial will be a two-arm, double blind phase II randomized controlled trial comparing DHA supplementation and placebo (vegetable oil). The proposed study design with outcomes depicted is shown in **Figure 1** and SPIRIT participant flow chart is shown in **Figure 2**.

METHODS AND ANALYSIS

Study Population

Eligible women with invasive breast cancer (clinical stage I, II or III) for whom systemic chemotherapy [31] is recommended prior to surgery. The study will occur at the Cross Cancer Institute, with central laboratory and clinical analyses occurring the University of Alberta, both in Edmonton, Alberta, Canada. Inclusion and exclusion criteria are listed in **Table 1**.

Table 1: Inclusion and Exclusion Criteria for DHAWIN

Inclusion Criteria
1) ECOG Performance status of 0 or 1
2) Hematology and biochemistry assessments [CBC and differential, partial thromboplastin time (PTT), prothrombin time/ international normalized ratio (PT/INR), aspartate aminotransferase **(**AST**),** alkaline phosphatase (ALP), bilirubin, and creatinine] within normal range unless determined not clinically significant by the qualified investigator
3) Ability to take oral medications
4) Adequate tissue specimen for diagnosis, biomarkers, and endpoint Ki67 assays
Exclusion Criteria
1. Patients undergoing surgery prior to chemotherapy
2. Current or previous (within 2 months) daily use (>1 day/week) use of omega-3, fish oil, or other supplements or foods containing DHA (at daily doses > 200 mg)
3. Known allergy to soy or corn
4. Continued intake of supplements containing Vitamin C, Vitamin E or β-carotene exceeding the DRI, or other anti-oxidant supplements
5. Symptomatic but untreated cholelithiasis

6. History of deep venous thrombosis, active thrombophlebitis, pulmonary embolism, stroke, acute myocardial infarction, congestive cardiac failure, untreated hypertension, known inherited hypercoagulable disorder
7. Diagnosis of any other malignancy within the previous year except for adequately treated basal cell or squamous cell skin cancer
8. Medically documented history of a psychiatric disorder that would preclude consent
9. Partial or complete loss of vision or diplopia, from ophthalmic vascular disease
10. Hypersensitivity to any component of the container

Intervention

All women in this trial will receive standard of care chemotherapy throughout the duration of the trial. Breast cancer chemotherapy is developed in a guideline-coordinated system by a single team residing at the Cross Cancer Institute. Consequently, there are only two chemotherapy regimens that are used for neoadjuvant chemotherapy in this population. Each of the two regimens are six cycles in length and given at three-week intervals with a resultant chemotherapy regimen duration of 18 weeks. Both regimens are docetaxel based. For HER2 negative disease, patients universally receive the FEC-D (fluorouracil, epirubicin, cyclophosphamide; docetaxel) [32] regimen as neoadjuvant therapy, while HER2 positive patients receive the DCH regimen (docetaxel, carboplatinum, trastuzumab) [33].

Patients will be prescribed either 5 g/day DHA (in 11- 1g capsules), in the form of DHA enriched algae-sourced triglyceride oil capsules (life'sDHA™ S40-O400) or 11g placebo (corn/soy oil blend) per day (capsules from DSM Nutritional Products, Columbia, MD, Supplemental Table 3 for the main fatty acid content of DHA and the placebo). The placebo is balanced for PUFA content with linoleic acid to match the DHA treatment. The amount of additional linoleic acid in the diet of this group is not expected to increase inflammation [34] and has not been shown to elicit a tumoral response [35]. The capsules are to be taken orally

throughout the day as tolerated (at any time, with or without food). Capsules are identical in appearance and composition (other than the oils) to maintain blinding of participants and study staff. As the DHA source is an algae-synthesized triglyceride, there are no differences in texture or taste.

All patients will begin a cytotoxic chemotherapy regimen intended to require 18 weeks for delivery. The intervention (DHA or placebo) will commence at the start of the first cycle of chemotherapy and continue through 4-6 cycles of chemotherapy (3 weeks/cycle). Should a patient not be able to complete the full six cycles of therapy, the timing of surgery remains 3-5 weeks after the last cycle of chemotherapy is delivered. As local guidelines mandate surgery between 3 and 5 weeks from the last round of chemotherapy, DHA/placebo will be continued until this time (21-35 days after the last administration of cytotoxic chemotherapy).

All patients will be dispensed an additional bottle of DHA/placebo capsules at the beginning of the study to account for circumstances where their treatment is delayed due to treatment associated toxicities (including but not limited to vomiting, diarrhea, abnormalities in blood work, fatigue or severe mouth sores). The patients will be requested to continue taking the DHA or placebo as tolerated and will be dispensed additional capsules as necessary. The extra capsules will remain with the patient until the end of the study.

Patients will be encouraged to take the supplements as tolerated (throughout the day at any time, with or without food). Treatment adherence will be monitored by a review of the patient dosing diary and recording the number of any remaining capsules returned at the end of study visit following the last dose of DHA/placebo. Non-compliance will be assessed as consuming less than 50% of the weekly dose for 2 consecutive cycles. No additional natural health product is permitted beyond a daily multi-vitamin.

Outcome Measurements

Study outcome timelines are summarized in **Table 2**. Briefly, outcomes will be measured at baseline, within ± 3 days of chemotherapy and/or post-intervention (surgical excision). Electronic medical record and/or paper chart review of local control, relapse free survival and overall survival will occur at 3, 5, and 10 years to explore possible effects on long-term outcome.

Primary Outcome

The primary outcome of this study is the change in Ki67 from pre-treatment core needle biopsy to surgical excision. It will be calculated by image analysis and will follow analytical and pre-analytical recommendations of Dowsett et al.[24]. The percent change in Ki67 index at experimental end (surgical excision) from baseline will be determined on a log scale and the mean percent change in Ki67 level from baseline will be calculated. Ki67 assays will be performed and reported as part of the routine diagnostic services. A semi-automated computer algorithm scoring system will be employed as previously described [36] using the platform QuPath [37]. It is expected that 5g DHA/day will result in a clinically relevant decrease in Ki67.

Secondary Outcomes

1) DHA incorporation into phospholipids: The changes in level of DHA incorporation in plasma phospholipids will be assessed at baseline and at day 1 (±3 days) of each cycle of chemotherapy (2-6) and end of cycle 6 to identify the range of DHA incorporation in this patient population. The use of plasma rather than red blood cells or whole blood for this study is supported by the recent recommendations for best practices for fatty acids described by Brenna et al [38]. Analysis of the plasma phospholipid rather than plasma total lipids avoids the postprandial fluctuation of the triacylglycerol pool and is believed to adequately represent the cell membrane composition [38]. From our hypothesis and previously published data [22], it is expected that

supplementing with DHA will result in a significant increase in DHA incorporation. If possible, with the small study size, we will also assess differences in DHA incorporation in patients with different breast cancer subtypes and if subtype or disease stage affects DHA incorporation into plasma, controlling for the reported dose taken by the patient. The goal is to determine if plasma phospholipid DHA content can be used to predict treatment outcomes. We will also assess incorporation of other essential fatty acids (linoleic, linolenic, arachidonic, eicosapentaenoic, docosapentaenoic) to determine if there are differences between or within treatment groups.

2) Systemic immune function: Systemic immune function will be assessed on blood samples obtained at baseline, beginning of chemotherapy cycle 4 (day 1± 3 days) and at the end of chemotherapy treatment. Changes in markers of systemic immune cell type and function will be assessed following supplementation compared to baseline and the change from baseline compared to patients receiving the placebo. We will also examine the relationship between changes in activation markers and the level of DHA incorporation, changes in systemic inflammation (CRP, IL-6, TNFα) and immune function (ability to produce IL-2 after stimulation in vitro) following DHA supplementation.

3) Identify factors that may affect DHA incorporation into plasma phospholipids: if incorporation of DHA into plasma phospholipids is variable within the DHA treatment arm, possible factors that may influence incorporation will be assessed between high and low incorporators. These parameters will be assessed at the end of the study from data collected throughout the study.

4) Examine changes in markers for apoptosis: Caspase-3 presence in the excised tumor, as percent positive cells, will be calculated by image analysis and a comparison of expression levels at experimental end (surgical excision) to baseline will be determined in patients

receiving DHA supplementation and compared to patients receiving placebo. Proportions of negative cells, weakly positive cells and strongly positive cells will be scored by two pathologists and the staining intensity, assessed by QuPath, [37] will be recorded independently. Increased apoptosis measured by Caspase-3 is a clinically relevant marker of cell death.

5) Examine changes in tumor infiltrating lymphocytes (TILs): CD4+ and CD8+ in the excised tumor, as a number of positive cells for a given area, will be calculated by image analysis. A comparison post-treatment of expression levels at experimental end (surgical excision) to baseline will be determined in patients receiving DHA supplementation and compared to patients receiving placebo. The differences will be compared between treatments and within the treatment group, related to plasma DHA concentrations. Increased infiltration of TILs is potential marker that could be used to predict treatment patient outcomes.

6) Pathological complete response (pCR) rate: pCR in resected breast tissue and all sampled axillary nodes will be assessed as absence of invasive cancer by haematoxylin and eosin evaluation as per standard of care. Pathologic complete response will be classified as ypT0/is ypN0 and will be determined at the end of study after surgical resection as part of standard of care assessment.

7) Comparison of rate of chemotherapy associated grade 3 and 4 toxicities: Rate of chemotherapy associated grade 3 and 4 toxicities, and chemotherapy-associated hospitalizations will be compared between DHA and placebo arms. Any changes will then be examined in regards to level of supplementation and DHA incorporation. These analyses will be completed at the end of study after surgical resection.

Exploratory outcomes

1) Food frequency questionnaire (FFQ): Assessment of the FFQ to compare the estimated (pre-diagnosis) usual intake of macronutrients on an energy basis (including fat content and composition) between the two groups at baseline. In the future, the overall medians/means of the subjects in this study will be compared to age-matched women in the Alberta Tomorrow Project.

2) Quality of life: Assessment in changes in quality of life will be determined by questionnaire employed at baseline and end of treatment. Comparisons will be assessed from end of treatment to baseline within and between treatment groups.

3) Exercise behavior: Assessment of exercise behavior will be determined by questionnaire employed at baseline, each cycle of chemotherapy and end of treatment. Comparisons will be assessed from end of treatment to baseline within and between treatment groups.

4) Breast conservation: The rate of breast conservation, specifically the rate of lumpectomy and mastectomy, will be determined by review of surgical and pathological reports at the end of study after surgical resection.

5) Volume of surgical blood loss: High intakes of n-3 LCPUFA (that contain some DHA) have been studied to determine if they increase bleeding time [39,40]. We will review surgical report estimates of blood loss to see if there is a qualitative or quantitative difference between placebo and treatment arms, once adjusted for the magnitude of surgery (lumpectomy vs. mastectomy vs. mastectomy + immediate reconstruction; sentinel node dissection vs. full axillary dissection). It is not expected that we will see a difference as it is eicosapentaenoic acid (EPA, the precursor to DHA) that has antithrombotic and antiplatelet properties [41].

6) Local control, relapse free survival and overall survival: Local control, relapse free survival and overall survival will be analyzed by review of electronic medical records, registry reports,

and/or paper medical charts at 3, 5, and 10 years to explore possible effects on long-term outcome.

Participant timeline

Breast cancer patients receiving neoadjuvant chemotherapy account for approximately 20% of newly diagnosed breast cancer patients, approximately 10-12/month at the Cross Cancer Institute. Assuming a conservative accrual rate of 30%, accrual is estimated to be completed in 14-18 months with 3-4 patients recruited per month. Each patient will be enrolled for the duration of their individual chemotherapy regimen, an estimated 12-18 weeks (84-126 days) beginning at the start of the first cycle of chemotherapy and continued through 4-6 cycles of chemotherapy (3 weeks/cycle). The intervention will be discontinued 21-35 days after the last administration of cytotoxic chemotherapy when surgery to remove the tumor occurs. See Figure 1 for a schematic of the participant timeline.

Sample Size

Fifty-two women prescribed neoadjuvant breast cancer chemotherapy will be enrolled in a 2-arm trial with 26 participants/arm. The sample size calculation is based on the primary objective, which is to determine the efficacy of supplemental DHA provided with standard neoadjuvant as measured by change in the Ki67 index from biopsy to surgical excision. Group sample sizes of 23 patients in each group are required to achieve 81% power to detect a difference between the group proportions of 0.4. The proportion in group one is assumed to be 0.3 under the null hypothesis and 0.7 under the alternate hypothesis. The proportion in group two which is the control group is 0.3. The test statistic used is the two-sided t-test. The significance level of the test was targeted at 0.05 and the significance level actually achieved by this design is about 0.0497. Assuming a dropout rate estimated at approximately 10% for this patient population

which is approximately 5 patients, a total of 52 patients (26 patients in the DHA supplementation group, and 26 in the placebo group) is required for the study.

Recruitment

Oncologists and clinical trial nurses at the Cross Cancer Institute in Edmonton, Canada will recruit newly diagnosed breast cancer patients. Patients will be screened for eligibility by the clinical trial nurses and eligible, interested patients will receive a detailed explanation of the study by the study coordinators and written informed consent will be obtained (**Supplemental File 1**).

Randomization and Blinding

A biostatistician will generate a patient randomization list and randomized bottle numbers by covariate-adaptive randomization (block randomization). The randomized bottle numbers will be provided to DSM for labeling for both the DHA and placebo groups and the randomized bottle list will also be provided to the unblinded Clinical Trials Coordinator (CTC, Clinical Trials Unit) and the unblinded pharmacist. Patients will be stratified by histological subtype and then randomized. The allocation of the study arm (as the study is blinded, hence, the study arm A and B will be used as this will not identify the placebo or intervention arm) and a unique study identifier will be conducted using the REDCap database. The key to the study arm A and B will only be provided to the unblinded CTC, statistician and the pharmacist. The study coordinator will enter the new patient information in REDCap and assign the unique ID and arm. This information will be shared with the unblinded CTC and the unblinded pharmacy staff. The pharmacy staff will assign the correct bottle numbers based on the study arm at day 1 of each chemotherapy cycle. Following the allocation of the bottle numbers, this information will then be shared with the study coordinator and the unblinded CTC. The bottle ID will be entered in the

REDCap database by the study co-ordinator. All future bottle allocations with the unique bottle ID will be entered into the REDCap database. The key to the study arm will be kept in password protected computers and will only be shared in an urgent need for breaking of the blind. When a blinding code is broken, the date and reason for unblinding must be fully documented in source documents and entered on the case report form. Every effort should be made by site staff to ensure that the treatment arm in which the unblinded patient is assigned is communicated only to those site staff that require the information for treatment purposes. To assist in maintaining the blind of the patients, supplements and placebo are identical in size, shape, color and texture, in addition to identical bottles for dispensing. Patients, pathologists, physicians, and researchers will be blinded to patient enrolment in the study and throughout trial. Blinding will only be dropped after analysis of fatty acids, systemic immune function and Ki67 is complete.

Data Collection, Management and Analysis

Study methods are summarized in **Table 3**. Briefly, data will be collected and measured at baseline, within ± 3 days of chemotherapy and/or post-intervention (surgical excision). Electronic medical record and/or paper chart review of local control, relapse free survival and overall survival will occur at 3, 5, and 10 years. All data will be entered and maintained in the REDCap trial database. Baseline measurements will be analyzed once all participants have been enrolled and all other analyses will occur at completion of trial.

Primary Outcome

Ki67 will be tested by immunohistochemistry (IHC) by the diagnostic biomarker laboratory at the Cross Cancer Institute using the MIB1 antibody on 4 μm sections from formalin fixed paraffin embedded (FFPE) needle core biopsy surgical specimens. At final

analyses, Ki67 staining will be repeated as a single IHC stain and interpreted by image analysis. At the time of Ki67 interpretation, slides will be de-identified and coded to ensure the pathologist is blinded to the experimental group. In addition, the original single stained slides will be interpreted visually by research staff. All Ki67 values (routine and image analysis) will be recorded as absolute percentage and H-score in the REDCap trial database and the participants' case report form. The Ki67 index is validated and used in clinic as a marker of proliferation. The Ki67 index (absolute % and H-score [42] of biopsy and surgical resection (after chemotherapy) will be compared on each participant and between participants receiving DHA compared to placebo.

Secondary Outcomes

DHA incorporation into plasma phospholipids will be measured in venous blood from patients at baseline (time of enrolment in trial), and at day 1 (± 3 days) of each chemotherapy cycle by a technician blinded to the treatment group. Venous blood will be collected in coated EDTA tubes and centrifuged at 750x g for 10 min to obtain plasma. Red blood cells will be immediately frozen and banked at -80°C for storage for future secondary analysis. Plasma will be separated into 6 aliquots and immediately frozen at -80°C for storage. Plasma will be extracted by the Folch procedure [43,44], phospholipids separated by thin layer chromatography and fatty acid content (concentration and relative percent) measured by gas-liquid chromatography as previously described [45]. The percentage change in DHA from baseline will be compared in each patient and a 95% t-confidence interval for the mean percent change in DHA from baseline will be compared to patients receiving placebo. An internal standard is used to identify and quantify the fatty acids. This standard measure for fatty acid

status has coefficient of variation <5% and individual GC peaks are validated against phospholipid standards (GLC-502 and GLC-643) from NuChek (Elysian, MN).

Phenotyping of immune cell subsets will be measured using whole blood (collected in EDTA tubes). The various cell types will be identified using specific fluorescently labelled monoclonal antibodies (mAb) to surface receptors (See Supplementary Table 4 for list of antibodies). These will be quantified by flow cytometry, as previously described [46]. With the remaining blood, peripheral mononuclear cells will be isolated and purified on a Ficoll density gradient of Histopaque 1077 as previously described [46,47]. To measure cytokine production in isolated lymphocytes, cells will be cultured in media with or without the mitogens, Phytohemagglutinin (PHA) or Lipopolysaccharide (LPS), for 48 h as previously described [48]. Supernatant will be collected and stored at -80°C for *ex vivo* measures of immune function (ability and pattern of cytokines produced after stimulation). IL-1 β, IL-2, IL-6, IL-10, TNF α, and IFN-γ (pg/ml) cytokines will be measured using electrochemiluminescent multiplex assays (MesoScale Discovery) or by individual ELISA assays. Cytokines listed above and inflammatory markers including C-reactive protein (CRP) in plasma will be measured by electrochemiluminescent multiplex assays (MesoScale Discovery) as previously described [49]. Cytokines and inflammatory markers in plasma and cytokines from cultured lymphocytes will be analyzed when all samples have been collected. Changes in systemic immune function will be assessed in patients compared to baseline and compared between groups. The data analysis will occur at completion of trial. Cytokines are done in duplicate and the coefficient of variance is <15%. Phenotypes will be collected as a relative percent of total cells and the change compared between treatments. Additionally, white blood cells that are not used for the immune assays will be assessed for fatty acid composition.

If DHA incorporation into plasma phospholipids is significantly different within the DHA supplementation arm, factors that may influence incorporation will be compared in low vs. high incorporators, to identify possible factors that predict incorporation including BMI, age, the estimated macronutrient intake and composition of dietary fat of the women (estimated from the FFQ), histology of the tumor (provided from the biopsy), the amount of DHA consumed (adherence to the supplement) and length of time DHA consumed (if treatment is ended early) . We will also assess incorporation of other fatty acids (palmitic, oleic, linoleic, linolenic, arachidonic, eicosapentaenoic, docosapentaenoic) to determine if there are differences between or within treatment groups.

Caspase-3 changes and changes in CD4 and CD8 will be tested by IHC by the diagnostic biomarker laboratory at the Cross Cancer Institute on 4 µm sections from FFPE surgical specimens. At final analyses, IHC staining will be interpreted by image analysis. At time of interpretation, slides will be de-identified and coded to maintain the blind. All values (routine and image analysis) will be recorded as absolute percentage. Caspase-3 is a validated marker of apoptosis and CD4 and CD8 are validated markers for lymphocytes. The calculated indices (absolute % and H-score) of biopsy and surgical resection will be compared on each participant and between participants receiving DHA compared to placebo.

Pathological complete response in resected breast tissue and axillary nodes will be assessed in hematoxylin and eosin stained tissue for evidence of invasive disease according to standard of care and recorded in patients' case report form. The rate of pathological complete

response in breast tissue and axillary nodes after surgical resection will be compared between participants receiving DHA supplementation compared to placebo.

Grade 3 and 4 toxicities will be assessed and recorded by the clinical trial nurse. Toxicities will be assessed on day 1 (± 3 days) of each chemotherapy cycle. Dates of hospitalization will be recorded in patients' case report form. Rates of chemotherapy-associated grade 3/4 toxicities, all grade neuropathy and hospitalizations will be compared between DHA supplementation and placebo arms as scored by a medical oncologist in a standardized toxicity/ neuropathy form for each cycle of chemotherapy.

Exploratory outcomes:

The FFQ is a validated questionnaire for macronutrient intake [50-52]. The quality of life questionnaire is a validated questionnaire from European Organization for Research and Treatment of Cancer- Quality of Life Questionnaire-C30 (EORTC-QLQ-C30) [53]. Exercise behavior will be assessed using the modified Godin Leisure-Time Exercise Questionnaire (GLTEQ) [54,55]. Assessment of changes in quality of life and exercise behavior will be compared from timepoints collected to baseline within and between treatment groups. We do not expect the supplement/placebo to influence this variable but since exercise alters immune function, quality of life and tumor growth we have included it herein to determine if it changes during therapy.

The rate of breast conservation, specifically the rate of lumpectomy and modified radical mastectomy, will be determined by surgical and pathological reports at time of surgical resection. Volume estimates of blood loss will be assessed by review of surgical reports to see if there is a qualitative or quantitative difference between placebo and treatment arms, once adjusted for the type of surgery (lumpectomy vs. mastectomy vs. mastectomy + immediate

reconstruction; sentinel node dissection vs. full axillary dissection). Local control, relapse free survival and overall survival will be analyzed by electronic medical record and/or paper medical chart review at 3, 5, and 10 years. Data will be validated by a medical oncologist.

Data Management

All data will be entered and maintained in the REDCap trial database. Direct access to clinical and laboratory information on enrolled trial patients will be limited to the principal investigator, co-investigators, trainees/staff who have had the appropriate training and approval and study nurses and study coordinators who will have access to the source documents through the electronic medical record and laboratory information system at the Cross Cancer Institute. All patients will have biopsy and tumor samples for analysis and we do not expect any missing data for the primary endpoint (Ki67). If supplement compliance is below 50% for more than two consecutive cycles, or if participants do not complete chemotherapy (to a minimum of 4 cycles), they will be excluded from final analysis of the primary endpoint. If patients do not have sufficient blood samples for the secondary analyses (DHA incorporation, systemic immune function), analysis will be performed using data from the remaining patients.

Statistical Methods

Primary Outcome:

The percent change in Ki67 will be determined as an absolute percentage and H-score. The number of patients showing a decrease and the 95% confidence interval for the mean percent change in the Ki67 level from baseline in patients receiving DHA supplementation will be compared to patients receiving placebo. The mean change will be measured using an independent t-test between the two groups.

Secondary Outcomes:

Paired t-tests will be used to compare the mean percent change in the plasma DHA level of the patients after each cycle of chemotherapy with their baseline values. If the data is not normally distributed, the Wilcoxon signed rank test will be used to compare the plasma DHA level after each cycle of chemotherapy with baseline. The difference in plasma phospholipid DHA from baseline and between DHA supplementation and placebo arms will be calculated, and the 95% confidence interval for the mean percent change in DHA level from baseline and groups will be assessed.

If systemic immune function data is not normally distributed, it will be log transformed prior to analysis and the normality assumptions will be tested again. Repeated measures ANOVA with post hoc analysis will be used to determine if there is an effect of treatment on immune function.

Factors affecting DHA incorporation will be examined by independent t-tests to compare the mean values between the DHA and placebo groups. Chi-square tests will be conducted to determine correlation between two categorical variables for the outcome measures listed.

The within subject and between subject variability between the two groups for the mean percent change in apoptosis and tumor infiltrating lymphocyte markers will be tested using the generalized estimating equation (GEE) method.

The 95% confidence interval using independent t-tests will be conducted for the mean percent change in pathological complete response and rates of grade 3 and 4 chemotherapy associated toxicities and hospitalization in patients receiving DHA supplementation compared to patients receiving placebo.

Exploratory outcomes:

Independent t-tests for macronutrient and fat content obtained from the food frequency questionnaire will be examined between groups. Paired t-tests for continuous variables and McNemar's test for categorical variables will be assessed for mean percent change in events between treatment arms for the quality of life and exercise questionnaires. Chi-square tests will be used to compare the degree of breast conservation and the volume of surgical blood loss will employ an independent t-test between the two study arms. Rate of local control will be compared between treatment arms using t-test of proportions. Recurrence-free survival and survival will be analyzed using the log rank test on Kaplan-Meier survival curves.

SAS software, version 9.4 (SAS Institute Inc., Cary, NC), will be used for statistical analysis. A p-value <0.05 level will be used for all statistical significance. Two-sided tests will be used for all statistical tests.

Data Monitoring

The trial activities performed at the Cross Cancer Institute will be monitored by the Cross Cancer Institute, Investigator Initiated Trials Data Safety Monitoring Board (DSMB). The DSMB is independent of the investigator and is composed of representatives from both medical and radiation oncology.

The investigator will assess the relationship between protocol treatment and the occurrence of adverse events (AEs) and this assessment will be recorded in the database for adverse events. This study will use the International Common Terminology Criteria for Adverse Events (CTCAE), version 5.0, for adverse event reporting. The reporting period for adverse events will start at the time the patient takes the first dose of DHA/placebo through and including 28 calendar days after last administration of study agent. If serious adverse reaction to treatment occurs, the Natural and Non-prescription Health Products Directorate (NNHPD),

Clinical Trial Unit, Health Ethics Research Board of Alberta, Cancer Committee (HREBA.CC) and DSM will be notified as per guidelines. After 25 evaluable patients, all data and results will be submitted to the DSMB for review.

Auditing

As per the SPIRIT guidelines, the investigators, Cross Cancer Institute and the University of Alberta will permit trial-related monitoring, audits, REB, DSMB review, and regulatory inspection(s), providing direct access to paper and/or electronic documentation pertaining to the clinical study (e.g. CRFs, source documents such as hospital patient charts and investigator study files). All site facilities related to the study conduct could be visited during an audit (e.g. pharmacy, laboratory, outpatient department) and are agreed to co-operate and provide assistance at reasonable times and places with respect to any auditing activity.

Patient and Public Involvement

Patients were not involved in the protocol development or study design. However, oncologists and clinical trial nurses who work in the breast tumor group are involved in patient screening to assess eligibility for the study. The HREBA-CC approved informed consent will be obtained from patients prior to their involvement in the study and it informs patients of their right to withdraw at any time. At the end of the trial, results will be disseminated to the public through seminars, public talks and in peer-reviewed journals.

Ethics and dissemination

DHA WIN has received Health Canada approval (#HC6-24-c220167), full ethical approval from the Health Research Ethics Board of Alberta – Cancer Committee (Protocol #: HREBA.CC- 18-0381) and is registered at clinicaltrials.gov (Identifier: NCT03831178).

Protocol amendments will be submitted to HREBA.CC, Health Canada and the clinical trial registry prior to study implementation according to regulatory requirements. The formal consent of a participant, using the HREBA-CC-approved consent form (Supplemental File 1), will be obtained by a clinical trial nurse before the participant is enrolled in the study and will be signed by the patient, and the principle investigator. A voluntary optional consent form for use of participant data and biological specimens (Supplemental File 2), will be offered at time of enrollment. Patient confidentiality and anonymity will be maintained and identities protected from unauthorized parties.

Access to data will be restricted to the primary investigators and statistician. They will grant access to other team members as governed and approved by ethics. Ancillary care post-trial will occur as routine standard of care for all participants. Our objective is to determine the efficacy of using DHA supplementation concomitant with chemotherapy and as such our results will be disseminated to clinicians for implementation in future treatment paradigms. The results will be submitted to peer-reviewed journals and presented at national and international conferences.

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Competing Interests Statement

There are no financial or competing interests or conflicts to declare.

Author Contributions

MN and CJF wrote the manuscript. JRM, GB, MAC, SGo, SGh, AS, DM, AC, LP, KB, VM, KSC, RB, WFD, GW, SKB, AAJ, KK, JMJ, and XZ contributed to the study design and reviewed the manuscript drafts. JRM, SKB, AAJ, KK, JMJ, and XZ provided oncological expertise. GB, RB, WFD, and GW provided pathology and immunohistochemistry expertise. SGo and KB contributed to the design of the immunologic component of the study; SGh designed the statistical models for all components of the study. AS, DM, AC, and MAC obtained all regulatory and operational approvals (Health Canada, HREBA, Clinical Trials registration and site approvals). CJF, LP, VM, KB, JRM were co-applicants on the successful CIHR grant that designed the immune component of the trial. KSC contributed expertise for the QoL and exercise component of the study. All authors reviewed drafts of the manuscript. Authors of the data manuscripts will include at least the principal investigator, medical director (J. Mackey) and any co-investigators who have i) included eligible patients in the trial (by order of inclusion) and/or ii) contributed significantly to the design, conduct and data interpretation regarding companion basic science studies.

Appendices:

Supplemental File 1: Informed consent

Supplemental File 2: Optional consent

Supplemental Table 1: Spirit Checklist

Supplemental Table 2: WHO Checklist

Supplemental Table 3: Main fatty acid content of DHA supplement and placebo

Supplemental Table 4: List of Antibodies for Immune cell subset identification

FIGURE LEGENDS

Figure 1 Flowchart of Trial Design with Endpoints and Proposed Experimental Analyses

Figure 2 SPIRIT patient flow diagram of the DHA WIN trial

Table 2: DHA WIN assessment schedule based on the Standard Protocol Items: Recommendations for Interventional Trials (SPIRIT)

	STUDY PERIOD												
		Chemotherapy											
	Enrolment	**Cycle 1**		**Cycle 2**		**Cycle 3**		**Cycle 4**		**Cycle 5**		**Cycle 6**	**Surgery**
TIMEPOINT**	$-t_1$	***Day 1*** [1]	***Day 20***	***Day 1***	***Day 20***	***Day 1***	***Day 20***	***Day 1***	***Day 20***	***Day 1***	***Day 20***	***Day 1***	t_x
ENROLMENT:													
Eligibility screen	X												
Informed consent	X												
Randomization	X												
INTERVENTIONS: [2]													
DHA 5 grams/day (11 - 1g capsules)		←	—	—	—	—	—	—	—	—	—	→	
Vegetable oil placebo (11 -1g capsules/day)		←	—	—	—	—	—	—	—	—	—	→	
Medication Diary		←	—	—	—	—	—	—	—	—	—	→	
Assessment of Compliance				X		X		X		X		X	X
ASSESSMENTS:													
BASELINE / ONGOING													

Demographic data collection	X												
Tumor analysis for Grade/ER/PR/HER2[3]	X												
Physical Exam / anthropometric measurements	X	X		X		X		X		X		X	X
Relevant medical history /current medical conditions	X			X		X		X		X		X	X
ESAS questionnaire	X	X		X		X		X		X		X	X
Blood Chemistry	X											X [4]	
CBC and differential	X							X				X [4]	
Adverse Events		X		X		X		X		X		X	X
Assessment of Relevant Toxicities		X		X		X		X		X		X	
Primary Outcome													
Tumor analysis of Ki67	X												X
Secondary Outcome													
Assessment of immune function:	X							X				X[4]	
Assessment of DHA incorporation	X			X		X		X		X		X	

Tumor analysis of apoptosis and TILs	X												X
Exploratory Outcomes													
Grade 1, 2 neuropathy assessment		X		X		X		X		X		X	X	
Pathological complete response														X
Breast conservation														X
Assessment of surgical blood loss														X
Study Associated Questionnaires														
Food frequency questionnaire[5]	X													
Quality of life questionnaire	X												X	
Godin Exercise Questionnaire	X			X		X		X		X		X	X	

ESAS: Edmonton Symptom Assessment System

(1) Day 1 is the day 1 of chemotherapy cycle.

(2) If patients' chemotherapy is delayed due to associated toxicities, they will be encouraged to continue taking the DHA/placebo capsules as tolerated.

(3) From previously collected biopsy.

(4) Tests required at the end of the last round of chemotherapy (i.e., end of cycle 4, 5 or 6 as per patients' individual treatment plan).

(5) Food frequency questionnaire can be completed anytime within the first cycle (21 days) of chemotherapy.

Table 3: Variables, Measures and methods of analysis

VARIABLE / OUTCOME	OUTCOME MEASURE	METHOD	STATISTICAL ANALYSIS
Primary:			
Efficacy of supplemental DHA provided with standard neoadjuvant chemotherapy as measured by change in Ki67	Ki67 labelling index	Immunohistochemistry	95% t-confidence interval for mean percent change in Ki67. Independent t-test to compare change between the study groups [36]
Secondary:			
1. DHA incorporation into plasma phospholipids	Fatty acid composition of plasma phospholipids	Gas chromatography	Paired t-test will be used to compare the mean percent change in the DHA level of patients after each cycle with their baseline values. If the data is not normally distributed, the Wilcoxon signed rank test will be employed for this comparison. A 95% t-confidence interval for the mean percent change in the DHA from baseline will be compared to patients receiving placebo
2. Systemic immune function	a) Immune cell subset identification b) Plasma cytokines	a) Flow cytometry b and c) ELISA and MesoScale	Repeated Measures ANOVA with post-hoc analysis

	c) Ex vivo stimulated immune cell response		
3. Identify factors that may affect DHA incorporation into tumor tissue and plasma phospholipids.	Factors assessed after calculating high and low DHA incorporators: a) Weight (BMI) b) Age c) The usual diet estimated from the FFQ d) Composition of dietary fat estimated from the FFQ e) Histology of the tumor (provided from the biopsy) f) Amount of DHA consumed (adherence to the supplement) g) % incorporation of other fatty acids		Independent t-test will be conducted to compare the mean values between the two study groups. Chi-square test will be conducted to determine correlation between two categorical variables for outcome measures listed
4. Examine changes in markers for apoptosis	Caspase-3	Immunohistochemistry	Within subject and between subject variability between the two groups will be tested using generalized estimating equation (GEE) method
5. Examine changes in markers for tumor	CD4+/CD8+	Immunohistochemistry	Within subject and between subject variability between the two groups will be

infiltrating lymphocytes			tested using generalized estimating equations (GEE) method
6. Describe the rate of pathological complete response in breast and in axillary nodes	Absence of invasive cancer on haematoxylin and eosin evaluation	Immunohistochemistry	pCR= ypT0/is ypN0 95% t-confidence interval using independent t-test for mean percent change between treatment groups
7. Describe the rate of grade 3 and 4 chemotherapy associated toxicities.	Rate of grade 3 /4 toxicities and chemotherapy associated hospitalizations	Chart review	95% t-confidence interval using independent t-test for mean percent change in events between treatment groups
Exploratory Outcomes			
1. Food Frequency Questionnaire	DHQ II questionnaire	Questionnaire	Independent t-test of macronutrient and fat content / composition between groups
2. Quality of Life	Baseline and Endpoint questionnaires	Questionnaire	Paired t-test for continuous variables and McNemar's for categorical variables for mean percent change in events between treatment groups
3. Exercise	Godin Exercise questionnaire	Questionnaire	Paired t-test for continuous variables and McNemar's for categorical variables for mean percent change in events between treatment groups
4. Assess the rate of breast conservation	Rate of lumpectomy and mastectomy.	Chart review	Chi-square tests

5. Assess the volume of surgical blood loss.	Review surgical reports for quantitative / qualitative loss of blood	Chart review	Independent t-test
6. Analyze local control, relapse free survival and overall survival	Electronic medical record and / or paper medical chart review at.3, 5, and 10 years to explore possible effects on long-term outcome	Chart review	Kaplan-Meier estimates along with the survival curves, log-rank test will be used for statistical comparison between groups

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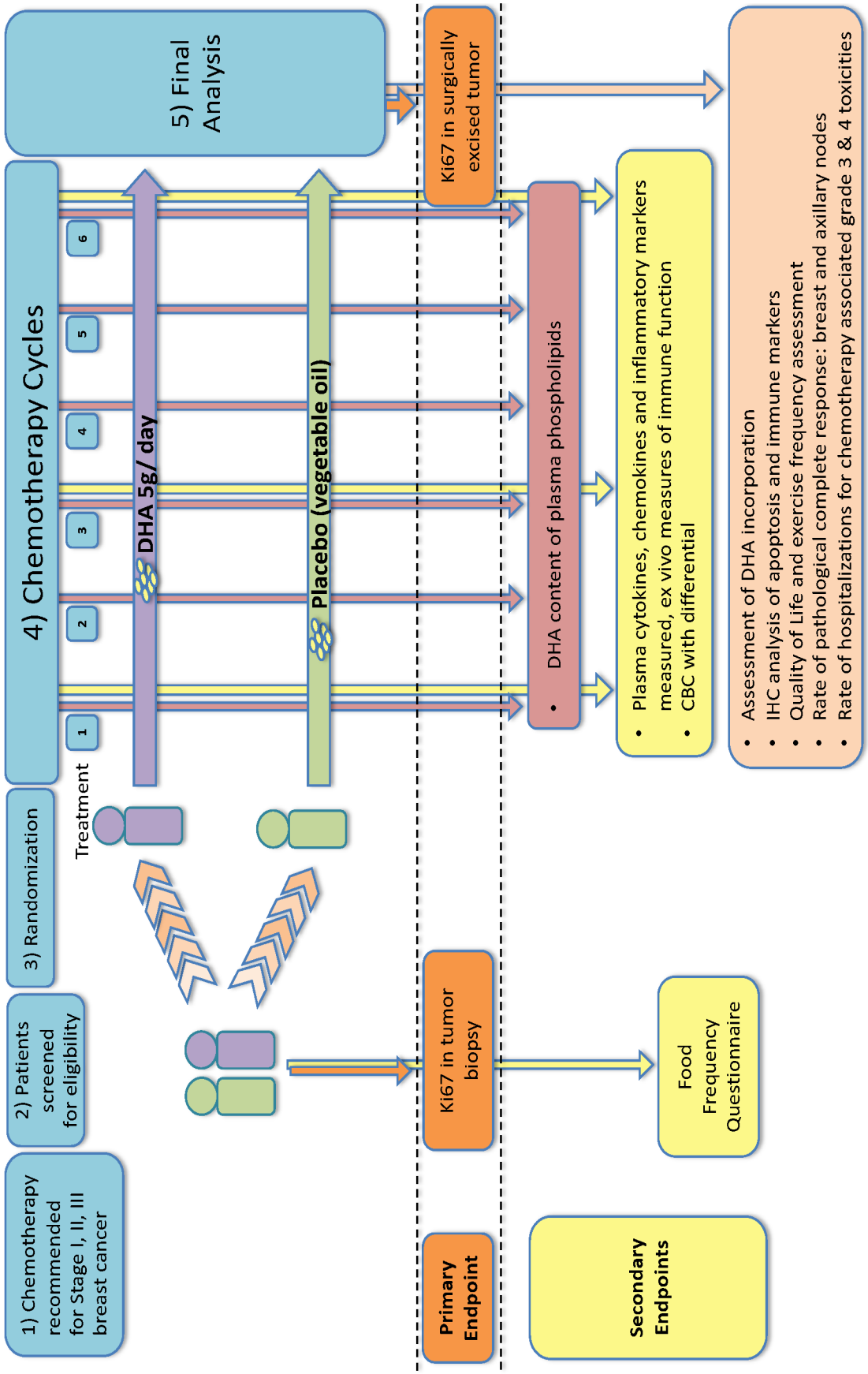
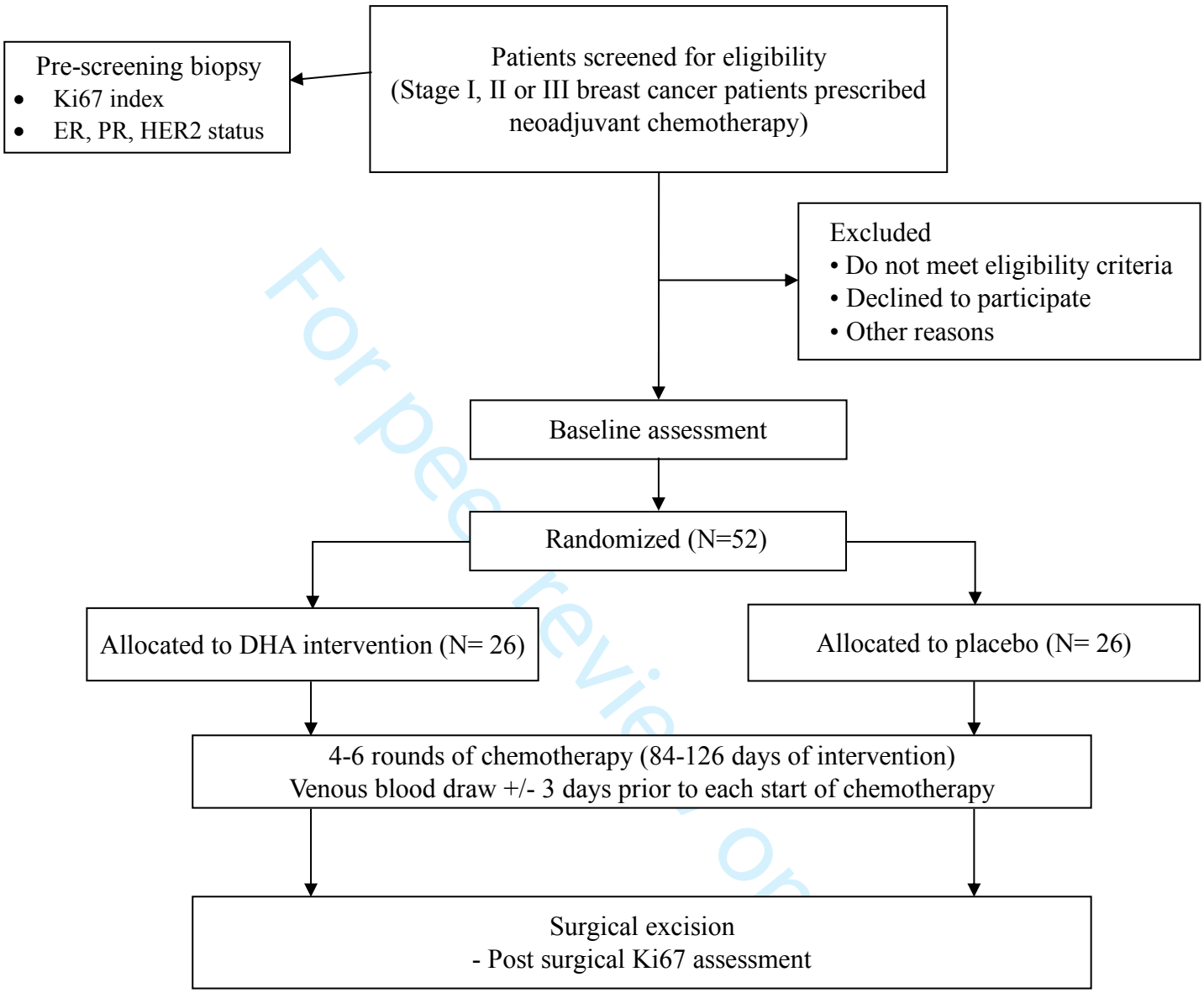


Figure 1

Figure 2



Alberta Health Services

Informed Consent Form for Participation in a Research Study

DHA for Women with Breast Cancer in the Neoadjuvant Setting

DHA to improve effectiveness of Chemotherapy in Breast Cancer

Protocol ID: *IIT-0005*

Study Doctor: *Dr. John Mackey*
Department of Medical Oncology
Cross Cancer Institute
780-432-8221

Sponsor/Funder(s): *Alberta Health Services- Cross Cancer Institute*

Emergency Contact Number (24 hours / 7 days a week): *780-965-8824*

Non-Emergency contact numbers are noted at the end of this document under the section heading "WHO DO I CONTACT FOR QUESTIONS?".

For assistance with terminology within this consent form, please refer to the Canadian Cancer Society Glossary of Terms at http://info.cancer.ca/e/glossary/glossary.html.

You are being invited to participate in a research study because you have stage I, II or III breast cancer which has not spread to distant parts of the body and will be receiving chemotherapy prior to surgery. This consent form provides detailed information about the study to assist you with making an informed decision. Please read this document carefully and ask any questions you may have. All questions should be answered to your satisfaction before you decide whether to participate.

The study staff will tell you about timelines for making your decision. You may find it helpful to discuss the study with family and friends so that you can make the best possible decision within the given timelines.

Taking part in this study is voluntary. You may choose not to take part or, if you choose to participate, you may leave the study at any time without giving a reason. Deciding not to take part or deciding to leave the study will not result in any penalty or any loss of medical or health-related benefits to which you are entitled.

The study doctor, who is one of the researchers, will discuss this study with you and will answer any questions you may have. If you do consent to participate in this study, you will need to sign and date this consent form. You will receive a copy of the signed form.

WHAT IS THE BACKGROUND INFORMATION FOR THIS STUDY?

Docosahexaenoic acid (DHA) is an omega-3 fatty acid commonly found in fish and fish oil. In the body, DHA is found in the membranes of cells. DHA is important for brain development, and in the immune system. DHA is also beneficial in heart disease. A diet high in DHA can reduce the incidence of breast cancer.

Incubating breast cancer cells with DHA in cell culture (cells in a dish in a laboratory) decreases the growth of the breast cancer cells, and increases the death of these cells. This is specific to cancer cells, since DHA has no effect on normal breast cells. When breast cancer cells are treated with chemotherapy drugs and DHA, DHA increases the effectiveness of chemotherapy resulting in increased death of the cancer cells.

When mice with breast tumors are fed DHA and treated with chemotherapy their tumors are much smaller than mice who are not fed DHA. In a previous clinical trial, women with metastatic breast cancer were given DHA supplements and treated with chemotherapy. DHA supplements appeared to improve the response to chemotherapy for some women.

Taking DHA may also reduce some side effects of chemotherapy in women with breast cancer. In these previous trials, no side-effects of taking DHA supplements were found.

Health Canada, the regulatory body that oversees the use of natural health products, drugs and devices in Canada, has not approved the sale or use of this DHA supplement to treat this kind of cancer, although they have allowed its use in this study.

The Health Research Ethics Board of Alberta – Cancer Committee (HREBA-CC), which oversees the ethical acceptability of research involving humans, has reviewed and granted ethics approval for this study.

WHY IS THIS STUDY BEING DONE?

This study will test if taking a DHA supplement during chemotherapy for breast cancer increases the effectiveness of the chemotherapy. The purpose of this study is to find out what effects a new agent, DHA supplementation, has on you and your breast cancer.

The investigators of this study are also interested in exploring the factors that may affect DHA incorporation in your blood, such as your weight and height, usual food intake (including amount and type of fat eaten), tumor type and the amount of DHA supplement consumed in the study.

WHAT ARE OTHER OPTIONS IF I DECIDE NOT TO PARTICIPATE IN THIS STUDY?

You do not have to take part in this study, in order to receive continued medical care. Other alternatives in addition to standard care may include:

- Other experimental studies may be available if you decide not take part in this study.
- Continuing regular observation and routine follow-up care e.g., symptom management

Please talk to the study doctor or your care doctor about the known benefits and risks of these other options before you decide to take part in this study. Your study or care doctor can also discuss with you what will happen if you decide not to undertake any treatment at this time.

HOW MANY PEOPLE WILL TAKE PART IN THIS STUDY?

Up to 52 people will take part in this study.

WHAT WILL HAPPEN DURING THIS STUDY?

ASSIGNMENT TO A GROUP

If you decide to participate then you will be "randomized" into one of the groups described below. Randomization means that you are put into a group by chance (like flipping a coin). There is no way to predict which group you will be assigned to. You will have an equal chance of being placed in either DHA supplementation or placebo group. Neither you, the study staff, nor the study doctor can choose what group you will be in.

This is a double-blinded study, which means that neither you nor the study doctor or study staff will know which group you are in. This is done so that you and the study doctor will not be influenced by expectations of the effects of the study agent. Your treatment will be identified if medically necessary by a process referred to as unblinding. Requests to reveal your assignment for your information or participation in other research studies will not be considered until the study has been completed and the results are known.

STUDY INTERVENTION

Group 1 (Experimental intervention): standard intervention of neoadjuvant chemotherapy plus experimental intervention of DHA supplementation.
If you are randomized into this group, you will take DHA capsules by mouth every day during chemotherapy treatment (4-6 cycles of chemotherapy, which would last approximately12-18 weeks)

Group 2 (Non-experimental intervention): standard intervention of neoadjuvant chemotherapy

If you are randomized into this group you will take placebo capsules containing corn/soy oil by mouth every day during chemotherapy treatment (4-6 cycles of chemotherapy, which would last approximately *12-18 weeks).*

Other important information on study intervention:
If you have side effects while you are on this study, the study doctor may make changes to the intervention.

STUDY PROCEDURES

Established Procedures

The following established procedures will be done as part of this study. Some of these procedures may be done as part of your standard care, in which case the results may be used. Some may be done more frequently than if you were not taking part in this study. Some of these procedures may be done solely for the purpose of the study. If the results show that you are not able to continue participating in the study, the study doctor will let you know.

Screening:

- Signed Informed Consent
- Review of inclusion / exclusion criteria
- Confirmation of no known allergies to soybean or corn oil (participants with allergies to soy or corn will be excluded from the study).
- Demographic data
- Physical examination
- You will be asked about your ability to carry out daily activities
- Body height and weight
- Vital signs
- Documentation of the diagnosis and disease stage
- Confirmation of no previous or concomitant treatment
- Complete medical / oncological history and consultation
- Questionnaire about your symptoms and well-being (ESAS questionnaire)
- Quality of Life questionnaire
- Exercise questionnaire
- Food frequency questionnaire (to be completed before the end of the first cycle of chemotherapy)
- Blood sample
- Your biopsy sample will be analyzed for standard tumor analysis: Grade; ER/PR/HER2; Ki67 to be requested if not already performed and other disease-related biomarkers.
- Adverse events before start of treatment

Chemotherapy Cycles (will take place prior to each chemotherapy administration):

- Physical exam
- You will be asked about your ability to carry out daily activities (cycle 1 and upon completion of your chemotherapy).
- Weight
- Vital signs
- Adverse events
- Blood sample
- You will take the DHA/placebo capsules by mouth every day during chemotherapy treatment (4-6 cycles of chemotherapy, which would last approximately 12-18 weeks)
- Quality of Life questionnaire (only at end of 6th cycle)
- Exercise questionnaire

Upon completion of chemotherapy:

If you undergo a surgical procedure to remove the tumor after chemotherapy, we will collect information from your records regarding the extent of the surgical procedure and amount of blood loss. In addition, your tumor sample will be reassessed for Ki67 and other disease-related biomarkers.

Alberta Health Services

Questionnaires

You will be provided with a questionnaire about food intake by research staff during cycle 1 of this study. The purpose of the questionnaire is to determine the amount of DHA in your diet, and other foods that can affect DHA in the body. The questionnaire will take about 1 hr. to complete and it can be done online or on paper. If you decide to complete it online, you will receive a link and a password to complete the questionnaire at home. If you don't have access to a computer or prefer a paper version, a printed questionnaire will be offered to you.

You will also be asked to complete questionnaires about your symptoms and well-being (ESAS questionnaire and exercise questionnaire) at the beginning of each chemotherapy cycle. It may take you 15-20 minutes to complete both questionnaires.

The information you provide is for research purposes only and will remain strictly confidential. Some of the questions are personal; you may choose not to answer them.

Participant Diaries

You will be asked to keep a diary to record *your study supplement capsules intake.* Please record *the times and number of capsules when you take the capsules each day*. You will be asked to return the diary to *the Cross Cancer Institute at the end of each cycle*.

MANDATORY SAMPLE COLLECTION

The researchers doing this study need to do tests on samples as described below. *The biopsy sample will be examined to make sure you have the type of cancer that is being studied in the research study. The surgical resection will be examined and compared to the biopsy sample to see how the cancer cells respond to DHA supplementation. Blood samples will be examined to see how DHA supplementation affects the amount of DHA in these samples, and if DHA alters immune cells.*

The collection of these samples is a necessary part of this study and will be used only for this purpose. The samples will not be sold.

Once these tests have been completed, any leftover samples will be returned to the facility from which they were obtained if needed or destroyed, unless you wish to give permission for other future research purposes, in which case you will be given a separate optional consent form to sign.

Hereditary genetic testing (to look at whether cancer runs in your family) will not be done on these samples.

Reports about research tests done with your samples will be given to the study doctor(s). If you would like to learn the results of this research, please let them know.

Tissue Collection (Mandatory)

A small sample of your tissue that has already been removed by a previous surgery or biopsy will be obtained by the researchers doing this study. No further surgeries or biopsies are required of you for this purpose.

As part of your standard of care and necessary for this study, you will have had a tissue biopsy. Upon completion of your chemotherapy treatment and as part of your standard of care, you may undergo a surgical procedure to remove the tumor from your breast. The amount of tissue to be removed will depend on the size and location of the tumor. Your doctor will give you more details regarding this procedure.

A sample of the tissues obtained from the initial biopsy and from the subsequent breast surgery will be sent to a laboratory at the *Cross Cancer Institute, and at the University of Alberta in Edmonton, Alberta, Canada,* where they will be examined to confirm your diagnosis *and examine how DHA alters tumour growth, and the amount of DHA in tumour cells*.

Blood Collection (Mandatory)

Blood samples will be taken by inserting a needle into a vein in your arm. These will be taken at the same time as your study related tests whenever possible upon *entry to the study, at the beginning of every cycle of chemotherapy (every three weeks), on day 20 of cycle 3 and before surgery. One tablespoon of blood will be collected for this study at those times*. These blood samples will be sent to a laboratory at the *Cross Cancer Institute and the University of Alberta in Edmonton, Alberta, Canada* where they will be examined to *measure the different cells in your blood, and the amount of DHA in these cells.*

Identification of Samples

To protect your identity, the information that will be on your samples will be limited to the *pathology identification number, and an identification number for the study.*
Despite protections being in place, there is a risk of unintentional release of information that could lead to loss of privacy. Due to technological advances in genetics, there is also a risk of unintentional release of genetic information from the samples. This information can be linked back to you and can lead to possible future discrimination in employment or insurance, against you or your biological relatives.

Withdrawal of Samples

If you no longer want your samples to be used in this research, you should tell the study doctor. The study doctor will ensure the samples are returned to the hospital from which they were obtained, if needed, or destroyed.

You can request withdrawal of your sample(s) until *you have received your blinded capsules* when the samples will be made anonymous. It won't be possible to return samples after this because the researchers will not know which samples are yours.

You will not be able to continue to participate in this study if required samples are withdrawn.

Assessments (Part 1 of 2)	**Screening** (within 21 days before chemotherapy)	**Chemotherapy Cycle 1**		**Chemotherapy Cycle 2**		**Chemotherapy Cycle 3**		**Chemotherapy Cycle 4**		**Chemotherapy Cycle 5**		**Chemotherapy Cycle 6**		**End of Treatment**	**Surgery**
		Day 1[2]	**Day 20 (+/- 3 days)**	**Day 1**	**Day 20 (+/- 3 days)**	**Day 1**	**Day 20 (+/- 3 days)**	**Day 1**	**Day 20 (+/- 3 days)**	**Day 1**	**Day 20 (+/-3 days**	**Day 1**	**Day 20 (+/-3 days**	**Within 28 days after last dose**	
Informed Consent	X														
Demographic data collection	X														
Medical history or current medical conditions	X														
Height	X														
Weight	X	X												X	
Vital Signs	X	X		X		X		X		X		X		X	
Physical Exam	X	X		X		X		X		X		X		X	
You will be asked about your ability to carry out daily activities	X	X												X	
Questionnaires about your symptoms and well-being (ESAS questionnaire)	X	X		X		X		X		X		X		X	
Quality of life questionnaire	X													X	
Exercise questionnaire	X	X		X		X		X		X		X		X	
Food frequency questionnaire		X (anytime within the first cycle)													
A sample of your tumour will be analyzed for	X														X

disease-related biomarkers (signs related to your disease)														

Assessments (Part 2 of 2)	**Screening (within 21 days before chemotherapy)**	**Chemotherapy Cycle 1**		**Chemotherapy Cycle 2**		**Chemotherapy Cycle 3**		**Chemotherapy Cycle 4**		**Chemotherapy Cycle 5**		**Chemotherapy Cycle 6**		**End of Treatment**	**Surgery**
		Day 1[2]	**Day 20 (+/- 3 days)**	**Day 1**	**Day 20 (+/- 3 days)**	**Day 1**	**Day 20 (+/- 3 days)**	**Day 1**	**Day 20 (+/- 3 days)**	**Day 1**	**Day 20 (+/-3 days**	**Day 1**	**Day 20 (+/-3 days**	**Within 28 days after last dose**	
Blood sample for routine tests to monitor your health	X							X					X		
Blood will be collected to measure signs of immune function	X							X					X		
Blood will be collected to measure the level of study treatment in your blood lipids	X			X		X		X		X		X	X		
Treatment: DHA/Placebo		Days 1-21		Days 1-21		Days 1-21		Days 1-21		Days 1-21		Days 1-21			
Diary completion with your capsule intake		Days 1 -21		Days 1 -21		Days 1 -21		Days 1 -21		Days 1 -21		Days 1 -21			
Confirmation of previous or current medications	X	X		X		X		X		X		X		X	
You will be asked about any side effects which may or not be related to the study	X	X		X		X		X		X		X		X	

treatment														
We will collect results from your surgery report														X

OPTIONAL RESEARCH

The researchers doing this study are interested in doing additional optional research. You will be given a separate optional study consent form(s) to read and sign if you wish to give permission to this. You may decide not to participate in the "optional" study and still participate in this main study.

WHAT ARE THE POTENTIAL SIDE EFFECTS FROM PARTICIPATING IN THIS STUDY?

You may experience side effects from participating in this study. Some side effects are known and are listed below, but there may be side effects that are not expected. You should discuss these with the study doctor.

There are no known side effects of this omega 3 (DHA) supplement. A non-medicinal ingredient in this nutritional supplement that may cause an allergic reaction includes gelatin.

The risks and side-effects of the standard or usual treatment will be explained to you as part of your standard care. These risks are not included in this consent form.

A Data and Safety Monitoring Board (DSMB), an independent group of experts, will be reviewing the data throughout the conduct of the study to ensure continuing participant safety as well as scientific validity and quality of the research.

WHAT ARE THE REPRODUCTIVE RISKS?

There appears to be no effect of the nutritional product on the human reproductive system.

WHAT ARE THE BENEFITS OF PARTICIPATING IN THIS STUDY?

Participation in this study may or may not be of personal benefit to you. However, based on the results of this study, it is hoped that in the long-term, patient care can be improved.

WHAT ARE MY RESPONSIBILITIES AS A STUDY PARTICIPANT?

If you choose to participate in this study, you will be expected to:

- Tell the study doctor about your current medical conditions;
- Tell the study doctor about all prescription and non-prescription medications and supplements, including vitamins and herbals, that you may be taking and check with the study doctor before starting, stopping or changing any of these. This is for your safety as these may interact with the intervention you receive on this study;
- Tell the study doctor if you are thinking about participating on another research study;

- Attend all scheduled study visits and undergo all of the procedures described above;
- Return any unused DHA / placebo products;
- Return any *diaries and food frequency questionnaires* taken home to complete;
- Tell the study doctor if you become pregnant while participating on this study;
- Avoid taking fish oil supplements, or any supplements containing DHA.
- Stop taking other *supplements of vitamin C, vitamin E, or β-carotene exceeding the DRI (daily recommended intake), or other anti-oxidant supplements. A multivitamin with vitamin C, E, and β-carotene below the DRI are permitted (75 mg/day vitamin C, 15 mg/day vitamin E, and 700 µg/day β-carotene). A member of the research staff will go through the details of multivitamin intake to ensure it is within the guidelines.*
- *DHA supplement/ placebo capsules are* meant for you alone, and must not be shared with others. If someone accidently takes the capsules, the *intake should be recorded in medication diary, and the study staff should be informed.*

HOW LONG WILL I BE PARTICIPATING IN THIS STUDY?

The study intervention will last as long as it takes for you to receive your chemotherapy (about *12-18 weeks)*.
You may be seen more often if the study doctor determines that this is necessary or if your cancer *gets worse*.

WILL THERE BE ANY LONG-TERM FOLLOW-UP INVOLVED WITH THIS STUDY?

No matter which group you are randomized to, and even if you stop receiving the study intervention early, we would like to keep track of your health for *10 years to look at the long-term effects of your participation on the study*. We would do this *by accessing electronic or paper medical chart review at 3, 5 and 10 years after treatment.*

In the event it is necessary to further evaluate the safety or efficacy of the *DHA supplement*, it may be necessary to have access to additional information about your health status. The study team may attempt to obtain study-related information about your health from you or from other private sources, including your care physician and *electronic or paper medical chart review*. This may include contacting you again by phone or letter, but only if you have not withdrawn your consent for future contact. However, contacting you, your care physician or using other private sources of information, is optional, please indicate your decision using the check boxes below.

You give permission to the study doctor or member of the study team to attempt to obtain study-related information about your health status to further evaluate the safety or efficacy of *DHA supplementation*. This may include contacting your care physician, or by contacting you by phone or letter (i.e., future contact).

☐ Yes ☐ No Participant's Initials: _____

Name/phone number of care physician: _____

In addition, the study team may also attempt to obtain study-relevant information about your health information from public sources such as national patient registries (e.g., cancer registries)

If the study doctor needs to follow up with you but cannot locate you, either because you have moved and not updated your contact information or if, for some reason, your contact information is no longer accurate, the study doctor would like to obtain your new contact information (e.g., address, telephone number) by calling or writing to the persons you've named as your secondary contacts. This is optional, please indicate your decision using the check boxes below.

You give permission to the study doctor or member of the study team to contact your secondary contacts if the study doctor or study team no longer have accurate contact information for you.

☐ Yes ☐ No Participant's Initials: _____

Name/phone number of secondary contacts: _____

If the study doctor cannot obtain information through your secondary contacts, he/she would like to ask for assistance of a third party that specializes in locating persons. The study doctor may only share limited information about you (name and last known address) with a third party locator. None of your personal health or study-related information will be shared with the third party locator. The third party locator will consult public sources and databases to obtain your current contact information but will not contact you. The third party locator will only share this information with the study doctor or study team to help complete the follow-up stage of the study. Only the study doctor or a member of the study team will attempt to contact you directly. This is optional, please indicate your decision using the check boxes below.

If the study doctor is not able to obtain your contact information from your secondary contacts, you give permission for the study doctor to provide your name and last location to a third party that specializes in locating persons.

☐ Yes ☐ No Participant's Initials: _____

CAN I CHOOSE TO LEAVE THIS STUDY EARLY?

You can choose to end your participation in this research (called early withdrawal) at any time without having to provide a reason. If you choose to withdraw early from the study without finishing the intervention, procedure or follow-up, you are encouraged to contact the study doctor or study staff.

You may be asked questions about your experience with the study intervention, and to have laboratory tests and physical examinations considered necessary to safely stop your study involvement.

You may withdraw your permission to use information that was collected about you for this study at any time by letting the study doctor know. However, this would also mean that you withdraw from the study.

Information that was recorded before you withdrew will be used by the researchers for the purposes of the study, but no additional information will be collected or sent to the sponsor after you withdraw your permission.

CAN MY PARTICIPATION IN THIS STUDY END EARLY?

The study doctor may stop your participation in the study early, and without your consent, for reasons such as:

- The intervention does not work for you;
- You are unable to tolerate the study intervention;
- You are unable to complete all required study procedures;
- New information shows that the study intervention is no longer in your best interest;
- The study doctor no longer feels this is the best treatment for you;
- A regulatory authority (for example, Health Canada) or the research ethics board withdraws permission for the study to continue;
- Your treatment assignment becomes known to others (the study doctor or study staff);

If you are removed from the study, the study doctor will discuss the reasons with you and plans will be made for your continued care outside of the study.

HOW WILL MY PERSONAL INFORMATION BE KEPT CONFIDENTIAL?

If you decide to participate in this study, the study doctor and study staff will only collect the information they need for this study.

Records identifying you, including information collect from your medical files/records, such as your Electronic Medical Records (EMR), Netcare, charts, etc., will be kept confidential to the extent permitted by the applicable laws, will not be disclosed or made publicly available, except as described in this consent document.

Authorized representatives of the following organizations may look at your identifiable medical/clinical study records at the site where these records are held for quality assurance purposes and/or to verify that the information collected for the study is correct and follows proper laws and guidelines:

- Members of the Regulatory/Audit team at *Cross Cancer Institute,* for quality assurance purposes;
- The Health Research Ethics Board of Alberta – Cancer Committee, which oversees the ethical conduct of this study;
- Health Canada, which oversees the use of natural health products/drugs/devices in Canada and the conduct of clinical trials;

Authorized representatives of the above organizations and of the University of Alberta may **receive** information related to the study from your medical/clinical study records that will be kept confidential in a secure location and may be used in current or future relevant health research. Your name or other information that may identify you will not be provided (i.e., the information will be de-identified). The records received by these organizations will be coded with a number. The key that indicates what number you have been assigned will be kept secure by the researchers directly involved with your study and will not be released.

Any disclosure of your identifiable health information will be done in accordance with federal and provincial laws including the Alberta Health Information Act (HIA). The organizations listed above are required to have organizational policies and procedures to protect the information they see or receive about you, except where disclosure may be required by law. The study doctor will ensure that any personal health information collected for this study is kept in a secure and confidential location at the *Cross Cancer Institute, Edmonton Alberta* as also required by law.

If the results of this study are published, your identity will remain confidential. It is expected that the information collected during the study will be *used in analyses and will be published/presented to the scientific community at meetings and in journals*. This information may also be used as part of a submission to regulatory authorities around the world to support the approval of this intervention.

Even though the likelihood that someone may identify you from the study data is very small, it can never be completely eliminated. Every effort will be made to keep your identifiable information confidential, and to follow the ethical and legal rules about collecting, using and disclosing this information.

WILL MY HEALTHCARE PROVIDER(S) BE INFORMED OF MY PARTICIPATION IN THIS STUDY?

Your family doctor/health care provider will not be informed by the study team that you are taking part in the study. You can choose to let your family doctor/health care provider know, if you like. If you are undecided, the study doctor can discuss this with you.

WILL THERE BE ANY COSTS INVOLVED WITH PARTICIPATING IN THIS STUDY?

The DHA supplement/ placebo will be given to you free of charge while you take part in this study.

Taking part in this study may result in added costs to you. For example:

- There may be costs associated with hospital visits. For instance, parking, transportation, or snacks/meals during the study.

Possible Costs After the Study is Complete

You may not be able to receive the study intervention after your participation in the study is completed. There are several possible reasons for this, some of which are:

- The intervention may not turn out to be effective or safe;
- The intervention may not be approved for use in Canada;
- Your caregivers may not feel it is the best option for you;
- You may decide it is too expensive and insurance coverage may not be available;
- The intervention, even if approved in Canada, may not be available free of charge.

The study doctor will discuss these options with you.

WILL I BE COMPENSATED FOR PARTICIPATING IN THIS STUDY?

You will not be paid for taking part in this study.

It is possible that the research conducted using your samples and/or study data may eventually lead to the development of new diagnostic tests, new drugs or devices, or other commercial products. There are no plans to provide payment to you if this happens.

In the case of research-related side effects or injury, as a direct result of participating in this research, you will receive all medical treatments or services recommended by your doctors.

Although no funds have been set aside to compensate you in the event of injury or illness related to the study treatment or procedures, you do not give up any of your legal rights for compensation by signing this form.

WHAT ARE MY RIGHTS AS A PARTICIPANT IN THIS STUDY?

You will be told, in a timely manner, about new information that may be relevant to your willingness to stay in this study.

You have the right to be informed of the results of this study once the entire study is complete. If you would like to be informed of these results, please contact the study doctor.

The results of this study will be available on a clinical registry; refer to the section titled “Where can I find online information about this study?”.

Your rights to privacy are legally protected by federal and provincial laws that require safeguards to ensure that your privacy is respected.

By signing this form you do not give up any of your legal rights against the hospital, investigators, sponsor, involved institutions for compensation or their agents, nor does this form relieve these parties from their legal and professional responsibilities.

IS THERE CONFLICT OF INTEREST RELATED TO THIS STUDY?

There are no conflicts of interest declared between the study doctor and sponsor of this study.

WHAT IF RESEARCHERS DISCOVER SOMETHING ABOUT ME AS A RESEARCH PARTICIPANT?

During the study, the researchers may learn something about you that they didn’t expect. For example, the researchers may *find out that you have another medical condition*.

If any clinically important information about your health is obtained as a result of your participation in this study, you will be given the opportunity at that time to decide whether you wish to be made aware of that information.

WHERE CAN I FIND ONLINE INFORMATION ABOUT THIS STUDY?

A description of this clinical trial will be available on *http://www.ClinicalTrials.gov*.

The study registration number to use this website is: NCT03831178

This website will not include information that can identify you. You can search for this website at any time.

WHO DO I CONTACT FOR QUESTIONS?

If you have questions about taking part in this study, or if you suffer a research-related injury, you should talk to the study doctor, co-investigator or study nurse. These person(s) are:

Dr. John Mackey	780-432-8221
Name	Telephone
Name	Telephone
Name	Telephone

He can also be paged through the Cross Cancer Institute switchboard at (780) 432-8771.

If you have questions about your rights as a participant or about ethical issues related to this study and you would like to talk to someone who is not involved in the conduct of the study, please contact the Office of the Health Research Ethics Board of Alberta – Cancer Committee at:

Telephone: 780-423-5727 Toll Free: 1-877-423-5727

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SIGNATURES

Part 1 - to be completed by the potential participant.

	Yes	No
Do you understand that you have been asked to take part in a research study?	☐	☐
Do you understand why this study is being done?	☐	☐
Do you understand the potential benefits of taking part in this study?	☐	☐
Do you understand the risks of taking part in this study and the risks of becoming pregnant or fathering a child during this study?	☐	☐
Do you understand what you will be asked to do should you decide to take part in this study?	☐	☐
Do you understand the alternatives to participating in this study?	☐	☐
Do you understand that you are free to leave the study at any time, without out having to give reason and without affecting your future health care?	☐	☐
Do you understand who will see your records, including health information that identifies you?	☐	☐
Do you understand that by signing this consent form you are giving us permission to access your health information and specimens if applicable?	☐	☐
Do you understand that by signing this consent form that you do not give up any of your legal rights?	☐	☐
Have you had enough opportunity to ask questions and discuss this study?	☐	☐

By signing this form I agree, to participate in this study.

Signature of Participant	PRINTED NAME	Date

Part 2 - to be completed by the study doctor or designee who conducted the informed consent discussion. Only compete this section if the potential participant has **agreed** to participate.

I believe that the person signing this form understands what is involved in the study and has freely decided to participate.

Signature of Person Conducting the Consent Discussion	PRINTED NAME	Date

Part 3 - to be completed only if the participant is unable to read or requires assistance of an oral translator/interpreter.

- The informed consent form was accurately explained to, and apparently understood by the participant.
- Informed consent was freely given by *or on behalf of* the participant.

Signature of Impartial Witness/Interpreter	PRINTED NAME	Date

You will be given a copy of this signed and dated consent form prior to participating in this study.

Informed Consent Form for Participation in Optional Research

DHA for Women with Breast Cancer in the Neoadjuvant Setting (DHA WIN)

DHA to improve effectiveness of Chemotherapy in Breast Cancer

Protocol ID: IIT-0005

Researcher: Dr. John Mackey
Department of Medical Oncology
Cross Cancer Institute
780-432-8221

Funder(s)/Sponsor: Alberta Health Services- Cross Cancer Institute

INTRODUCTION

In addition to the main study, you also are being invited to take part in optional research. Although it is optional, the study of human samples and data focusing on the prevention, diagnosis and treatment of cancer and other diseases is an important part of research. Taking part in this optional research is voluntary. You still can take part in the main study, and will continue to receive treatment and care even if you say “no” to any or all of this optional research now or later. This form and your discussion with the researcher/study staff will give you the information you need to make your decision.

WHY IS THIS OPTIONAL RESEARCH BEING DONE?

The researchers conducting this research are interested in doing the following:

- Biomarker research for the main study using tumour tissue / blood already collected
- Bio-banking for use in future research using tumour tissue / blood already collected

As part of this optional research, the researchers would like to examine your tumour tissue/blood samples to look for any **biomarkers** (small “signature” molecules or indicators) in your cancer cells or circulating in your blood. These biomarkers might help predict which patients are most likely to be affected by the study drug. This is called biomarker research.

Bio-banking is the collection, storage, and use of human body samples and related health information for future research. It provides an important resource for health research

locally, across Canada, and around the world. The researchers doing the main study are also interested in storing your tissue/blood samples for future research. The research that may be done on your samples in the future is unknown at this time. It may be related to your condition or it may be used to address research questions that are unrelated.

Some of this research may be about genes. Genes carry information about features, such as hair or eye colour. This research may include looking at changes in genes found in you and in people who are related to you. These changes may be inherited (passed on in families). This is called hereditary genetic testing. Researchers also may be interested in the way that genes affect health and disease, or how your body responds to treatment.

WHAT WILL HAPPEN DURING THIS OPTIONAL RESEARCH?

You may take part in all or some of the optional research described here, it is your choice. If you agree to take part:

- the samples used for this optional research have already been collected as part of your standard of care. No further biopsies or surgeries are needed for this purpose.
- the blood samples used for this optional research will be those left over or remaining from your participation in the main study. No further biopsies or surgeries are needed for this purpose.

HOW WILL MY SAMPLES BE HANDLED?

Your sample(s) and some related health information already collected from your participation in the main study will be sent to the Nutritional Immunology laboratory at the University of Alberta, Edmonton, AB, for analysis. The samples and data will be kept indefinitely or until they are used up, destroyed or returned to the hospital where you had your surgery or biopsy.

Qualified researchers can submit a request to use the materials stored at the University of Alberta. Your samples and related health information will be used only by researchers whose requests have been accepted by the sponsor and who have met regulatory requirements and secured ethics approval for their research. The samples and data may be sent to other countries. Your name or any other information that could directly identify you will not be given to these researchers.

The results of research done on your samples will not be added to your personal health records and you or the researcher will not know the results.

WHAT ARE THE RISKS OF PARTICIPATING IN THIS OPTIONAL RESEARCH?

Risks related to sample collection:

- Since the tissue sample(s) already have been collected for the main study or as part of your standard of care, no additional physical risks are expected.

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Risks related to the disclosure of personal health information:

- There is a risk that someone could get access to the personal information in your personal health records or other information researchers have stored about you.
- There is a risk that someone could trace the information in a central or public database back to you. Even without your name or other identifiers, your genetic information is unique to you. The researchers believe the chance that someone will identify you is very small, but the risk may change in the future as people come up with new ways of tracing information.
- New health information about inherited traits that might affect you or your blood relatives could be found during a study. The researchers believe the chance these things will happen is very small, but cannot promise that they will not occur.
- Due to the rapid pace of technological advances, the potential future use of genetic information is unknown and therefore the potential future risks also are unknown.
- There may be risks to eligibility for employment or insurance if the results of genetic testing were inadvertently disclosed to certain parties.
- Genetic information cannot be protected from court-ordered disclosure.

WHAT ARE THE POTENTIAL BENEFITS OF PARTICIPATING IN THIS OPTIONAL RESEARCH?

You will not benefit directly from taking part in this optional research. However, research done with your donated samples or health information may benefit other patients with your condition or other similar or related condition(s).

HOW WILL MY PERSONAL INFORMATION BE KEPT PRIVATE?

Your privacy is very important to the researchers and they will make every effort to protect it. Here are the steps they will take:

- When your sample(s) are sent to the laboratory, no information identifying you (such as your name, date of birth, health insurance number) will be provided or shared. Samples may be identified by your study code.
- The samples that are provided to researchers by the Cross Cancer Institute are identified only by that biobank code; researchers will not know who you are.
- The list that links the samples to your personal identifiers (i.e., name) will be kept separate from your sample(s) and health information in a secure and confidential location at the main study site. If you change your mind about participating in this optional research, this list will be used to locate and return or destroy your samples. Decoding can only be done by the researcher or an individual authorized by the researcher.
- Study records will be kept for 25 years.
- A record of your participation in this optional study will be kept with your main study records and may be monitored for quality assurance.

Information that identifies you, will be kept confidential and, to the extent permitted by the applicable laws, will not be disclosed or made publicly available except as described in this document. If research results are published, your name and other personal information will not be used.

Qualified representatives of the sponsor will make sure the study has been done properly by checking your records at the researcher's site. Regulatory authorities, such as Health Canada and the applicable Research Ethics Board also may wish to check that the study has been done properly, and may also have direct access to your personal health information. Except as expressly stated in this section, all of the information provided in the main study consent form about confidentiality and direct access to your personal health information applies to this optional research consent form.

WHAT IF RESEARCHERS DISCOVER SOMETHING ABOUT ME DURING THE STUDY?

During the study, the researchers may learn something about you that they didn't expect. For example, the researchers may find out that you have another medical condition.

If any new clinically important information about your health is obtained as a result of your participation in this optional research, you will be given the opportunity to decide whether you wish to be made aware of that information.

WILL THERE BE ANY COSTS OR COMPENSATION INVOLVED WITH THS RESEARCH?

There are no costs to you. You will not be paid for taking part. No samples or information/data will be sold.

It is possible that the research conducted using your samples and/or my data may eventually lead to the development of new diagnostic tests, new drugs or other commercial products. There are no plans to provide payment to you if this happens.

WHAT ARE MY RIGHTS AS A PARTICIPANT IN THIS OPTIONAL RESEARCH?

You will be told, in a timely manner, about new information that may be relevant to your willingness to stay in this study.

If you decide you no longer want your samples or related health information to be used, you should tell the researcher. Any sample(s) that remain(s) in the laboratory will be destroyed (if blood) or returned to the hospital where you had your original biopsy or surgery (if tumour block). If tests have already been done on your sample and included in an analysis or publication, it will not be possible to withdraw these results.

You will be given a copy of this signed and dated consent form prior to participating in this

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study.

IS THERE ANY CONFLICT OF INTEREST RELATED TO THIS OPTIONAL RESEARCH?
There are no current or potential conflicts of interest concerning the optional research study.

WHO DO I CONTACT FOR QUESTIONS RELATED TO THIS OPTIONAL RESEARCH?

If you have questions about the use of your samples/data for optional research, or if you suffer a research-related injury, contact the researcher of this optional study:

Catherine J Field	780-492-5297
Name	Telephone Number

If you have questions about your rights as a participant or about ethical issues related to this optional research and you would like to speak to someone not involved in its conduct, please contact the Office of the Health Research Ethics Board of Alberta – Cancer Committee at: 780-423-5727 or toll-free 1-877-423-5727.

UNDERSTANDING AND SIGNATURES PAGE

Please circle your answer to show whether or not you would like to take part in the optional research:

I agree that samples which were already collected and related health information may be used for the optional research described above.

YES NO

I agree that my samples and related health information may be kept in a biobank for use in future health research related to my condition or may be used to address research questions that are unrelated.

YES NO

I agree that the researcher, or their representative, may contact me or my physician to see if I wish to learn about results from this research.

YES NO

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SIGNATURES

PARTICIPANT ACKNOWLEDGEMENT

- I understand the information within this optional consent form.
- All of my questions have been answered to my satisfaction.
- I am aware of the risks and potential benefits to me of participating in this optional research.
- I allow access to my personal health information and samples as explained in this form.
- I understand that I do not give up any of my legal rights by signing this consent form.
- I agree to take part in this optional research as described and where "YES" above has been circled.

_____	_____	_____
Signature of Participant	Printed Name	Date

STUDY TEAM ACKNOWLEDGEMENT

I believe that the person signing this form understands what is involved in this optional research and has freely decided to participate.

_____	_____	_____
Signature of Person Conducting the Consent Discussion	Printed Name	Date

PARTICIPANT ASSISTANCE (IMPARTIAL WITNESS)

This section is to be completed only if the participant is unable to read the consent document. The individual assisting the participant must be impartial.

- The informed consent form was accurately explained to, and apparently understood by the research participant.
- Informed consent was freely given by the participant.

_____	_____	_____
Signature of Impartial Witness	Printed Name	Date

TRANSLATOR/INTERPRETER ACKNOWLEDGEMENT

This section is to be completed only if the participant requires the assistance of a qualified oral translator/interpreter. The interpreter must be impartial.

- The informed consent discussion was accurately explained to, and apparently

understood by the research participant.

- A sight translation of the consent document was provided by the interpreter as directed by the research staff conducting the consent process.

Signature of Interpreter	Printed Name	Date

You will be given a copy of this signed and dated consent form prior to participating in this optional research.

SPIRIT

STANDARD PROTOCOL ITEMS: RECOMMENDATIONS FOR INTERVENTIONAL TRIALS

SPIRIT 2013 Checklist: Recommended items to address in a clinical trial protocol and related documents*

Section/item	Item No	Description	Reported on Page No
Administrative information			
Title	1	Descriptive title identifying the study design, population, interventions, and, if applicable, trial acronym	1
Trial registration	2a	Trial identifier and registry name. If not yet registered, name of intended registry	a
	2b	All items from the World Health Organization Trial Registration Data Set	Supp. files
Protocol version	3	Date and version identifier	1
Funding	4	Sources and types of financial, material, and other support	22
Roles and responsibilities	5a	Names, affiliations, and roles of protocol contributors	1
	5b	Name and contact information for the trial sponsor	1
	5c	Role of study sponsor and funders, if any, in study design; collection, management, analysis, and interpretation of data; writing of the report; and the decision to submit the report for publication, including whether they will have ultimate authority over any of these activities	22
	5d	Composition, roles, and responsibilities of the coordinating centre, steering committee, endpoint adjudication committee, data management team, and other individuals or groups overseeing the trial, if applicable (see Item 21a for data monitoring committee)	20-21
Introduction			
Background and rationale	6a	Description of research question and justification for undertaking the trial, including summary of relevant studies (published and unpublished) examining benefits and harms for each intervention	4-5

	6b	Explanation for choice of comparators	7
Objectives	7	Specific objectives or hypotheses	5
Trial design	8	Description of trial design including type of trial (eg, parallel group, crossover, factorial, single group), allocation ratio, and framework (eg, superiority, equivalence, noninferiority, exploratory)	6, Fig 1, Fig 2
Methods: Participants, interventions, and outcomes			
Study setting	9	Description of study settings (eg, community clinic, academic hospital) and list of countries where data will be collected. Reference to where list of study sites can be obtained	6
Eligibility criteria	10	Inclusion and exclusion criteria for participants. If applicable, eligibility criteria for study centres and individuals who will perform the interventions (eg, surgeons, psychotherapists)	Table 1 (page 1,2)
Interventions	11a	Interventions for each group with sufficient detail to allow replication, including how and when they will be administered	7
	11b	Criteria for discontinuing or modifying allocated interventions for a given trial participant (eg, drug dose change in response to harms, participant request, or improving/worsening disease)	8
	11c	Strategies to improve adherence to intervention protocols, and any procedures for monitoring adherence (eg, drug tablet return, laboratory tests)	8
	11d	Relevant concomitant care and interventions that are permitted or prohibited during the trial	-
Outcomes	12	Primary, secondary, and other outcomes, including the specific measurement variable (eg, systolic blood pressure), analysis metric (eg, change from baseline, final value, time to event), method of aggregation (eg, median, proportion), and time point for each outcome. Explanation of the clinical relevance of chosen efficacy and harm outcomes is strongly recommended	8-11 Table 3
Participant timeline	13	Time schedule of enrolment, interventions (including any run-ins and washouts), assessments, and visits for participants. A schematic diagram is highly recommended (see Figure)	12, Table 2

Sample size	14	Estimated number of participants needed to achieve study objectives and how it was determined, including clinical and statistical assumptions supporting any sample size calculations	12
Recruitment	15	Strategies for achieving adequate participant enrolment to reach target sample size	13
Methods: Assignment of interventions (for controlled trials)			
Allocation:			
Sequence generation	16a	Method of generating the allocation sequence (eg, computer-generated random numbers), and list of any factors for stratification. To reduce predictability of a random sequence, details of any planned restriction (eg, blocking) should be provided in a separate document that is unavailable to those who enrol participants or assign interventions	13
Allocation concealment mechanism	16b	Mechanism of implementing the allocation sequence (eg, central telephone; sequentially numbered, opaque, sealed envelopes), describing any steps to conceal the sequence until interventions are assigned	13
Implementation	16c	Who will generate the allocation sequence, who will enrol participants, and who will assign participants to interventions	13
Blinding (masking)	17a	Who will be blinded after assignment to interventions (eg, trial participants, care providers, outcome assessors, data analysts), and how	13
	17b	If blinded, circumstances under which unblinding is permissible, and procedure for revealing a participant's allocated intervention during the trial	13
Methods: Data collection, management, and analysis			
Data collection methods	18a	Plans for assessment and collection of outcome, baseline, and other trial data, including any related processes to promote data quality (eg, duplicate measurements, training of assessors) and a description of study instruments (eg, questionnaires, laboratory tests) along with their reliability and validity, if known. Reference to where data collection forms can be found, if not in the protocol	14-18 Table 3

	18b	Plans to promote participant retention and complete follow-up, including list of any outcome data to be collected for participants who discontinue or deviate from intervention protocols	-
Data management	19	Plans for data entry, coding, security, and storage, including any related processes to promote data quality (eg, double data entry; range checks for data values). Reference to where details of data management procedures can be found, if not in the protocol	18
Statistical methods	20a	Statistical methods for analysing primary and secondary outcomes. Reference to where other details of the statistical analysis plan can be found, if not in the protocol	19-20 Table 3
	20b	Methods for any additional analyses (eg, subgroup and adjusted analyses)	19-20
	20c	Definition of analysis population relating to protocol non-adherence (eg, as randomised analysis), and any statistical methods to handle missing data (eg, multiple imputation)	-
Methods: Monitoring			
Data monitoring	21a	Composition of data monitoring committee (DMC); summary of its role and reporting structure; statement of whether it is independent from the sponsor and competing interests; and reference to where further details about its charter can be found, if not in the protocol. Alternatively, an explanation of why a DMC is not needed	20
	21b	Description of any interim analyses and stopping guidelines, including who will have access to these interim results and make the final decision to terminate the trial	21
Harms	22	Plans for collecting, assessing, reporting, and managing solicited and spontaneously reported adverse events and other unintended effects of trial interventions or trial conduct	20
Auditing	23	Frequency and procedures for auditing trial conduct, if any, and whether the process will be independent from investigators and the sponsor	21
Ethics and dissemination			

Research ethics approval	24	Plans for seeking research ethics committee/institutional review board (REC/IRB) approval	21
Protocol amendments	25	Plans for communicating important protocol modifications (eg, changes to eligibility criteria, outcomes, analyses) to relevant parties (eg, investigators, REC/IRBs, trial participants, trial registries, journals, regulators)	21
Consent or assent	26a	Who will obtain informed consent or assent from potential trial participants or authorised surrogates, and how (see Item 32)	21 Supp file
	26b	Additional consent provisions for collection and use of participant data and biological specimens in ancillary studies, if applicable	21 Supp file
Confidentiality	27	How personal information about potential and enrolled participants will be collected, shared, and maintained in order to protect confidentiality before, during, and after the trial	22
Declaration of interests	28	Financial and other competing interests for principal investigators for the overall trial and each study site	22
Access to data	29	Statement of who will have access to the final trial dataset, and disclosure of contractual agreements that limit such access for investigators	22
Ancillary and post-trial care	30	Provisions, if any, for ancillary and post-trial care, and for compensation to those who suffer harm from trial participation	22
Dissemination policy	31a	Plans for investigators and sponsor to communicate trial results to participants, healthcare professionals, the public, and other relevant groups (eg, via publication, reporting in results databases, or other data sharing arrangements), including any publication restrictions	22
	31b	Authorship eligibility guidelines and any intended use of professional writers	22
	31c	Plans, if any, for granting public access to the full protocol, participant-level dataset, and statistical code	-
Appendices			
Informed consent materials	32	Model consent form and other related documentation given to participants and authorised surrogates	Supp file

Biological specimens	33	Plans for collection, laboratory evaluation, and storage of biological specimens for genetic or molecular analysis in the current trial and for future use in ancillary studies, if applicable	Supp file

*It is strongly recommended that this checklist be read in conjunction with the SPIRIT 2013 Explanation & Elaboration for important clarification on the items. Amendments to the protocol should be tracked and dated. The SPIRIT checklist is copyrighted by the SPIRIT Group under the Creative Commons "Attribution-NonCommercial-NoDerivs 3.0 Unported" license.

Supplemental Table 2: World Health Organization Trial Registration Data Set DHA WIN Summary

Data Category	**Information**
Primary registry and trial identifying number	ClinicalTrials.gov: NCT03831178
Date of registration in primary registry	February 5, 2019
Secondary identifying numbers	IIT-0005
Sources of monetary or material support	Canadian Institutes of Health Research (CIHR), AHS Cancer Control Alberta, Butler Family Foundation
Primary sponsor	AHS Cancer Control Alberta
Secondary sponsors	University of Alberta
Contact for public queries	Deborah Miede: Deborah.Miede@albertahealthservices.ca
Contact for scientific queries	Catherine Field: Catherine.field@ualberta.ca
Public title	DHA WIN
Scientific title	Docosahexaenoic acid (DHA) for Women with breast cancer in the neoadjuvant setting
Country of recruitment	Canada
Health condition or problems studied	Breast cancer
Interventions	DHA supplementation (5 g/ day) or equal amount of vegetable oil placebo for the duration of the participants chemotherapy treatment
Key inclusion and exclusion criteria	Inclusion: ECOG Performance status of 0 or 1; Hematology and biochemistry assessments within normal range; ability to take oral medication; adequate tissue specimen for diagnosis, biomarkers and endpoint Ki67 assays Exclusion: Patients undergoing surgery prior to chemotherapy; Current or previous (within 2 months) daily use (>1 day/week) use of omega-3, fish oil, or other supplements or foods containing DHA (at daily doses > 200 mg); Known allergy to soy or corn; Continued intake of supplements containing Vitamin C, Vitamin E or β-carotene exceeding the DRI, or other anti-oxidant supplements; History of deep venous thrombosis, active thrombophlebitis, pulmonary embolism, stroke, acute myocardial infarction, congestive cardiac failure, untreated

	hypertension, known inherited hypercoagulable disorder; Diagnosis of any other malignancy within the previous year except for adequately treated basal cell or squamous cell skin cancer
Study type	Randomized controlled trial
Date of first enrolment	Expected August 2019
Target sample size	52
Recruitment status	Not yet recruiting
Primary outcomes	Percent change in Ki67 index from baseline to surgical excision
Key secondary outcomes	Percent of DHA in plasma phospholipids; systemic immune function; Identify factors that may affect DHA incorporation into plasma phospholipids; Examine changes in markers for apoptosis and tumor infiltrating lymphocytes; pathological complete response; Comparison of rate of chemotherapy associated grade 3 and 4 toxicities

Supplemental Table 3: Fatty Acid Composition of DHA supplement and Placebo

Fatty Acid	DHA capsule	Placebo
16:0	16.9	10.9
18:0	0.1	2.7
18:1n-9	4.8	23.2
18:2n6	0.5	53.5
18:3n-3	<0.1	4.7
20:5n-3	1.0	<0.1
22:5n-3	0.5	<0.1
22:5n-6	18.1	<0.1
22:6n-3	43.4	<0.1

Supplemental Table 4: List of Antibodies used for immune cell phenotyping

CD1a	FITC	300104
CD1c	BV421	331526
CD3	FITC	300306
CD4	APC	357408
CD8	PerCP/Cy5.5	344710
CD11b	PE	301306
CD11c	APC	301614
CD14	APC	367118
CD16	PE	302008
CD20	FITC	302304
CD25	PE	302606
CD27	PECy7	356412
CD28	APC	302912
CD45RA	PE	304108
CD45RO	FITC	304204
CD56	APC	362504
CD86	PCP	374210
CD95	BV421	305624
CD103	PECy7	350212
CD107	PE	328608
CD141	PECy7	344110
CD152	PE	369604
CD183	PerCP/Cy5.5	353720
CD196	PE	353410
CD279	APC	329908
FOXP3	FITC	320106
HLADR	PerCP/Cy5.5	307630