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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

Journal:	<i>BMJ Open</i>
Manuscript ID	bmjopen-2019-030502
Article Type:	Protocol
Date Submitted by the Author:	18-Mar-2019
Complete List of Authors:	Newell, Marnie; University of Alberta, Department of Agricultural, Food and Nutritional Science Mackey, John; University of Alberta, Department of Oncology; Alberta Health Services Bigras, Gilbert ; University of Alberta, Department of Laboratory Medicine and Pathology Alvarez-Camacho, Mirey; Alberta Health Services Goruk, Susan; University of Alberta, Department of Agricultural, Food and Nutritional Science Ghosh, Sunita; Alberta Health Services Schmidt, Alison; Alberta Health Services Miede, Deborah; Alberta Health Services Chisotti, Ann; Alberta Health Services Postovit, Lynne; University of Alberta, Department of Oncology Baker, Kristi; University of Alberta, Department of Oncology Mazurak, Vera; University of Alberta, Department of Agricultural, Food and Nutritional Science Courneya, Kerry; University of Alberta, Faculty of Kinesiology, Sport and Recreation Berendt, Richard; University of Alberta, Department of Laboratory Medicine and Pathology Dong, Wei-Feng; University of Alberta, Department of Laboratory Medicine and Pathology Wood, George; University of Alberta, Department of Laboratory Medicine and Pathology Basi, Sanraj; Alberta Health Services Joy, Anil Abraham; Department of Oncology King, Karen; Alberta Health Services Meza-Junco, Judith; Alberta Health Services Zhu, Xiaofu; Alberta Health Services Field, Catherine; University of Alberta, Department of Agricultural, Food and Nutritional Science
Keywords:	ki67, phospholipids, omega-3, apoptosis, proliferation, immune function

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

4 Protocol Number: IIT-0005

5 Version Date: March 17, 2019

6 Marnie Newell¹, John R. Mackey^{2,3}, Gilbert Bigras⁴, Mirey Alvarez-Camacho², Susan
7 Goruk¹, Sunita Ghosh², Alison Schmidt², Deborah Miede², Ann Chisotti², Lynne Postovit³, Kristi
8 Baker³, Vera Mazurak¹, Kerry S. Courneya⁵, Richard Berendt⁴, Wei-Feng Dong⁴, George
9 Wood⁴, Sanraj K. Basi², Anil Abraham Joy², Karen King², Judith Meza-Junco², Xiaofu Zhu² and
10 Catherine J. Field^{1*}

11
12 ¹Department of Agricultural, Food and Nutritional Science, Faculty of Agricultural, Life and
13 Environmental Sciences, University of Alberta

14 ²Alberta Health Services - Cancer Control, Cross Cancer Institute

15 ³Department of Oncology, Faculty of Medicine & Dentistry, University of Alberta

16 ⁴Department of Laboratory Medicine and Pathology, Faculty of Medicine & Dentistry,
17 University of Alberta

18 ⁵Faculty of Kinesiology, Sport, and Recreation, University of Alberta

19 * Corresponding author: Catherine J. Field, PhD, Faculty of Agricultural, Life and Environmental
20 Sciences, University of Alberta, 4-126 Li Ka Shing Centre, Edmonton, Alberta, Canada, T6G
21 2H9. Tel: (780) 492-2597, E-mail: catherine.field@ualberta.ca

22 Word Count: 5055

23 **ABSTRACT**

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

31 *Methods and analysis:* This is a double blind phase II randomized controlled trial of 52 women
32 prescribed neoadjuvant chemotherapy to test if DHA supplementation enhances chemotherapy
33 efficacy. The DHA supplementation group will take 5g/day DHA orally and the placebo group
34 will take equal fat supplement of vegetable oil. The primary outcome will be change in Ki67
35 labelling index from pre-chemotherapy core needle biopsy to definitive surgical specimen.
36
37 Secondary endpoints include assessment of: i) DHA plasma phospholipid content; ii) systemic
38 immune cell types, plasma cytokines and inflammatory markers iii) tumor markers for apoptosis
39 and tumor infiltrating lymphocytes iv) rate of pCR in breast and in axillary nodes v) frequency of
40 grade 3 and 4 chemotherapy associated toxicities and vi) patient perceived quality of life.

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

1
2
3 43 *Ethics and dissemination:* This study has full approval from the Health Research Ethics Board of
4
5 44 Alberta – Cancer Committee (Protocol #: HREBA.CC -18-0381). We expect to present the
6
7 45 findings of this study to the scientific community in peer-reviewed journals and at conferences.
8
9 46 The results of this study will provide evidence for supplementing with DHA during neoadjuvant
10
11 47 chemotherapy treatment for breast cancer.

12
13
14
15 48 Clinical Trial Registration No: NCT03831178

16 17 49 **KEYWORDS**

18
19 50 Ki67, phospholipids, fatty acids, omega-3, apoptosis, proliferation, immune function

20 21 22 51 **STRENGTHS AND LIMITATIONS OF THIS STUDY**

- 23
24
25 52 • This study is the first phase II randomized controlled trial to evaluate DHA
26
27 53 supplementation concomitant with neoadjuvant chemotherapy to treat non-metastatic
28
29 54 breast cancer.
- 30
31
32 55 • The intervention is minimally invasive and side effects from the supplementation are not
33
34 56 expected.
- 35
36
37 57 • This study is powered to examine the key clinical outcome of changes in Ki67 index
38
39 58 from pre-chemotherapy biopsy to surgical excision based on group sample sizes of 23
40
41 59 patients in group one and 23 patients in group two in order to achieve 81% power to
42
43 60 detect a difference between the group proportions of 0.4.
- 44
45
46 61 • This study will measure clinically relevant intermediate outcomes including rate of pCR
47
48 62 in breast and in axillary nodes, rate of grade 3 and 4 chemotherapy associated toxicities
49
50 63 and hospitalizations as well as additional outcomes including plasma phospholipid
51
52 64 content of DHA, markers of immune function (plasma cytokines, chemokines,

1
2
3 65 inflammatory markers and lymphocyte function), tumor markers for apoptosis and tumor
4
5 66 infiltrating lymphocytes and patient perceived quality of life.
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- 7
8 67 • The study will include all subtypes of breast cancer patients undergoing neoadjuvant
9
10 68 chemotherapy but is not powered to assess differences between subtypes.
11

12 13 69 INTRODUCTION

14
15
16 70 Despite improvements in early diagnosis and treatment, breast cancer remains the second leading
17
18 71 cause of cancer related death in women [1]. While neoadjuvant chemotherapy aims to improve
19
20 72 surgical resection outcomes and reduce / eliminate micrometastases [2,3], pathological complete
21
22 73 response (pCR) is not achieved by all patients [3]. Increasing the efficacy of neoadjuvant
23
24 74 treatment without adding additional side-effects would benefit this population.
25
26

27
28 75 DHA is an omega-3 long chain polyunsaturated fatty acid (n-3 LCPUFA). The majority
29
30 76 of n-3 fatty acids are in the form of the 18-carbon fatty acid alpha-linolenic acid (ALA). While
31
32 77 DHA can be synthesized from ALA and other n-3 LCPUFA in the body, endogenous synthesis
33
34 78 is low [4,5]. Consequently, the direct consumption of this fatty acid is the only way to
35
36 79 significantly increase levels of DHA in tissues [6]. Supplementation can increase blood plasma
37
38 80 DHA concentration by 2-fold (500 µM), which can lead to plasma membrane lipid enrichment
39
40 81 [7]. Incorporation of DHA into tumor membrane phospholipids has been shown to reduce
41
42 82 breast cancer cell proliferation [8,9] and increase apoptosis [10-15] in vitro and decreases tumor
43
44 83 growth in animal models [13,15-17]. Additionally, providing/feeding DHA has been shown to
45
46 84 increase the efficacy of different chemotherapeutic drugs in animal models of breast cancer
47
48 85 [11,12,18,19]. While there is limited clinical evidence, it has been shown that increased dietary
49
50 86 intake of n-3 LCPUFA, including DHA, results in increased DHA incorporation in breast
51
52 87 adipose tissue [20] and this correlates with improved response to chemotherapy [21]. In an
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3 88 open label trial with advanced metastatic breast cancer patients, DHA supplementation and
4
5 89 enrichment into plasma phospholipids was associated with improved outcomes [22]. Other
6
7 90 clinical trials have reported that supplementation with n-3 LCPUFA at a wide range of doses
8
9 91 (0.6g-8.6 g/day) increased tolerability of chemotherapeutic drugs in a range of malignancies at
10
11 92 other sites, include lung, pancreatic and colorectal (reviewed in [23]). Consequently, we
12
13 93 hypothesize that the therapeutic index (efficacy: toxicity ratio) of neoadjuvant breast cancer
14
15 94 chemotherapy will be improved with the addition of DHA to the treatment.
16
17
18

19 95 Breast cancer proliferation can be assessed by immunohistochemical (IHC) analysis of
20
21 96 cells staining positive for the nuclear antigen Ki67 [24], as it is expressed in all phases of the
22
23 97 cell cycle, G₁, S, G₂, and M, but not in G₀ [25,26]. The proportion of cells staining for Ki67 is
24
25 98 frequently used as a primary endpoint to measure efficacy of neoadjuvant therapy in clinical
26
27 99 trials. The Ki67 index, defining the change between pre- and post-treatment Ki67, has been
28
29 100 reported to be an independent prognostic factor in luminal A, luminal B, triple-negative, and
30
31 101 HER2+ breast cancer, and has been reported to be a useful surrogate marker of relapse free
32
33 102 survival in luminal B, triple- negative, and HER2+ breast cancer [27,28].
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38 103 **OBJECTIVES**

39
40 104 The objective of this RCT is to assess the efficacy of supplemental DHA combined with
41
42 105 neoadjuvant chemotherapy in treatment naïve women with breast cancer measured by changes in
43
44 106 Ki67 index from biopsy to surgical excision. We hypothesize that DHA supplementation will
45
46 107 increase plasma phospholipid DHA and improve response to neoadjuvant chemotherapy assessed
47
48 108 by decrease in Ki67 index.
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52 109

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

112 Study Design

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

116 METHODS AND ANALYSIS

117 Study Population

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

122 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

123

124 **Intervention**

125 Patients will be prescribed either 5 g/day DHA (in 11- 1g capsules), in the form of DHA
 126 enriched algae-sourced triglyceride oil capsules (life'sDHA™ S40-O400) or 11g placebo
 127 (corn/soy oil blend) per day (capsules from DSM Nutritional Products, Columbia, MD), to be
 128 taken orally throughout the day as tolerated. The intervention will occur for 12-18 weeks (84-
 129 126 days) beginning at the start of the first cycle of chemotherapy and continued through 4-6
 130 cycles of chemotherapy (3 weeks/ cycle). DHA/placebo will be discontinued 21 days after the
 131 last administration of cytotoxic chemotherapy.

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

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

1
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3 141 DHA/placebo. Non-compliance will be assessed as consuming less than 50% of the weekly dose
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5 142 for 2 consecutive cycles. No additional natural health product is permitted beyond a daily multi-
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7
8 143 vitamin.

11 144 **Outcome Measurements**

13 145 Study outcome timelines are summarized in **Table 2**. Briefly, outcomes will be measured
14
15 146 at baseline, within ± 3 days of chemotherapy and/ or post-intervention (surgical excision).
16
17 147 Electronic medical record and or paper chart review of local control, relapse free survival and
18
19
20 148 overall survival will occur at 3, 5, and 10 years to explore possible effects on long-term outcome.

22 149 *Primary Outcome*

24 150 The primary outcome of this study is change in Ki67 from pre-treatment core needle
25
26 151 biopsy to surgical excision. It will be calculated by image analysis and will follow analytical and
27
28
29 152 pre-analytical recommendations of Dowsett et al.[24]. The percent change in Ki67 index at
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31 153 experimental end (surgical excision) from baseline will be determined on a log scale and the
32
33 154 mean percent change in Ki67 level from baseline will be calculated. Ki67 assays will be
34
35 155 performed and reported as part of the routine diagnostic services. A semi-automated computer
36
37 156 algorithm scoring system will be employed as previously described [32] using the platform
38
39
40 157 QuPath [33]. It is expected that 5g DHA/day will result in a clinically relevant decrease in Ki67.

43 158 *Secondary Outcomes*

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46
47 159 1) DHA incorporation into phospholipids: The changes in level of DHA incorporation in plasma
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49 160 phospholipids will be assessed at baseline and at day 20 (± 3 days) of each cycle of
50
51 161 chemotherapy to identify the range of DHA incorporation in this patient population. From our
52
53 162 hypothesis and previously published data [22]. It is expected that supplementing with DHA will

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3 163 result in a significant increase in DHA incorporation. If possible, with the small study size, we
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5 164 will also assess difference in DHA incorporation in patients with different breast cancer
6
7
8 165 subtypes and if subtype or disease stage affects DHA incorporation into plasma, controlling for
9
10 166 the reported dose taken by the patient. The goal is to determine if plasma phospholipid DHA
11
12 167 content can be used to predict treatment outcome.

13
14 168 2) Systemic immune function: Systemic immune function will be assessed on blood samples
15
16 169 obtained at baseline, end of chemotherapy cycle 3 (day 20± 3 days) and at the end of
17
18 170 chemotherapy treatment. Changes in markers of systemic immune cell type and function will be
19
20 171 assessed following supplementation compared to baseline and the change from baseline
21
22 172 compared to patients receiving the placebo. We will also examine the relationship between
23
24 173 changes in activation markers and the level of DHA incorporation, changes in systemic
25
26 174 inflammation (CRP, IL-6, TNF α) and immune function (ability to produce IL-2 after
27
28 175 stimulation in vitro) following DHA supplementation.

29
30 176 3) Identify factors that may affect DHA incorporation into plasma phospholipids: If incorporation
31
32 177 of DHA in plasma phospholipids is variable within the DHA treatment arm, possible factors
33
34 178 that may influence incorporation will be assessed between high and low incorporators. These
35
36 179 parameters will be assessed at end of study from data collected throughout the study.

37
38 180 4) Examine changes in markers for apoptosis: Caspase-3 presence in the excised tumor, as
39
40 181 percent positive cells, will be calculated by image analysis and a comparison of expression
41
42 182 levels at experimental end (surgical excision) to baseline will be determined in patients
43
44 183 receiving DHA supplementation and compared to patients receiving placebo. Proportions of
45
46 184 negative cells, weakly positive cells and strongly positive cells will be scored by two
47
48 185 pathologists and the staining intensity assessed by QuPath [33] will be recorded independently.

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3 186 A 95% confidence interval for the mean percent change in Caspase-3 will be calculated.
4
5 187 Increased apoptosis measured by Caspase-3 is a clinically relevant marker of cell death.
6
7
8 188 5) Examine changes in tumor infiltrating lymphocytes (TILs): CD4+ and CD8+ in the excised
9
10 189 tumor, as number of positive cells for a given area, will be calculated by image analysis and a
11
12 190 comparison of expression levels at experimental end (surgical excision) to baseline will be
13
14 191 determined in patients receiving DHA supplementation and compared to patients receiving
15
16 192 placebo will be made post-treatment. The differences will be compared between treatments and
17
18 193 within the treatment group, related to plasma DHA concentrations. Increased infiltration of
19
20 194 TILs is potential marker that could be used to predict treatment patient outcomes.
21
22
23
24 195 6) Pathological complete response rate (pCR): pCR in resected breast tissue and all sampled
25
26 196 axillary nodes will be assessed as absence of invasive cancer on haematoxylin and eosin
27
28 197 evaluation as per standard of care. Pathologic complete response will be classified as ypT0/is
29
30 198 ypN0 and will be determined at end of study after surgical resection as part of standard of care
31
32 199 assessment.
33
34
35 200 7) Comparison of rate of chemotherapy associated grade 3 and 4 toxicities: Rate of chemotherapy
36
37 201 associated grade 3 and 4 toxicities, and chemotherapy-associated hospitalizations will be
38
39 202 compared between DHA and placebo arms. Any changes will then be examined in regards to
40
41 203 level of supplementation and DHA incorporation. These analyses will be completed at end of
42
43 204 study after surgical resection.
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46
47 205 *Exploratory outcomes*
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- 50 206 1) Quality of life: Assessment in changes in quality of life will be determined by questionnaire
51
52 207 employed at baseline and end of treatment. Comparisons will be assessed from end of treatment
53
54 208 to baseline within and between treatment groups.
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209 2) Exercise behavior: Assessment of exercise behavior will be determined by questionnaire
210 employed at baseline, each cycle of chemotherapy and end of treatment. Comparisons will be
211 assessed from end of treatment to baseline within and between treatment groups.

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

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

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

226 **Participant timeline**

227 Breast cancer patients receiving neoadjuvant chemotherapy account for approximately
228 20% of newly diagnosed breast cancer patients, approximately 10-12/month at the Cross Cancer
229 Institute. Assuming a conservative accrual rate of 30%, accrual is estimated to be completed in
230 14-18 months with 3-4 patients recruited per month. Each patient will be enrolled for the
231 duration of their individual chemotherapy regimen, an estimated 12-18 weeks (84-126 days)

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

236 **Sample Size**

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

249 **Recruitment**

250 Oncologists and clinical trial nurses at the Cross Cancer Institute in Edmonton, Canada
251 will recruit newly diagnosed breast cancer patients. Patients will be screened for eligibility by the
252 clinical trial nurses and eligible, interested patients will receive detailed explanation of the study
253 by the study coordinators and written informed consent will be obtained (**Supplementary File**
254 **1**).

255 **Randomization and Blinding**

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

275 **Data Collection, Management and Analysis**

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

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

282 *Primary Outcome*

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

295 *Secondary Outcomes*

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

1
2
3 300 6 aliquots, and immediately frozen at -70°C for storage. Plasma (concentration and relative
4
5 301 percent) will be extracted by Folch procedure [38,39], phospholipids separated by thin layer
6
7 302 chromatography and fatty acid content measured by gas-liquid chromatography as previously
8
9
10 303 described [40]. The percentage change in DHA from baseline will be compared in each patient
11
12 304 and a 95% t-confidence interval for the mean percent change in the DHA from baseline will be
13
14 305 compared to patients receiving placebo. An internal standard is used to identify and quantify
15
16 306 the fatty acid. This is a standard measure for fatty acid status has coefficient of variation <5%
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18 307 and individual GC peaks are validated against phospholipid standards (GLC-502 and GLC-
19
20
21 308 643) from NuChek (Elysian, MN).

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23
24 309 Phenotyping of immune cell subsets will be measured using whole blood (collected in
25
26 310 EDTA tubes). The various cell types will be identified using specific fluorescently labelled
27
28 311 monoclonal antibodies (mAb) to surface receptors (See Supplementary Table 1 for list of
29
30 312 antibodies). These will be quantified by flow cytometry, as previously described [41]. With
31
32 313 the remaining blood, peripheral mononuclear cells will be isolated and purified on a Ficoll
33
34 314 density gradient of Histopaque 1077 as previously described [41,42]. To measure cytokine
35
36 315 production in isolated lymphocytes, cells will be cultured in media with or without the
37
38 316 mitogens, Phytohemagglutinin (PHA) or Lipopolysaccharide (LPS) for 48 h as previously
39
40 317 described [43]. Supernatant will be collected and stored at -80°C for *ex vivo* measures of
41
42 318 immune function (ability and pattern of cytokines produced after stimulation). IL-1 β , IL-2, IL-
43
44 319 6, IL-10, TNF α , and IFN- γ (pg/ml) cytokines will be measured using electrochemiluminescent
45
46 320 multiplex assays (MesoScale Discovery) or by individual ELISA assays. Cytokines listed
47
48 321 above and inflammatory markers including C-reactive protein (CRP) in plasma will be
49
50 322 measured electrochemiluminescent multiplex assays (MesoScale Discovery) as previously
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3 323 described [44]. Cytokines and inflammatory markers in plasma and cytokines from cultured
4
5 324 lymphocytes will be analyzed when all samples have been collected. Changes in systemic
6
7
8 325 immune function will be assessed in patients compared to baseline and compared between
9
10 326 groups. The data analysis will occur at completion of trial. Cytokines are done in duplicate and
11
12 327 the coefficient of variance is <15%. Phenotypes will be collected as a relative percent of total
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14
15 328 cells and the change compared between treatments.

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18 329 If DHA incorporation in plasma phospholipids and/or tumor tissue is significantly
19
20 330 different within DHA supplementation arm, factors that may influence incorporation will be
21
22 331 compared in low vs high incorporators, to identify possible factors that predict incorporation,
23
24 332 including, including weight (BMI), age, the usual diet and composition of dietary fat of the
25
26
27 333 women (estimated from the FFQ), histology of the tumor (provided from the biopsy) and
28
29 334 amount of DHA consumed (adherence to the supplement).

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31
32 335 Caspase-3 changes and changes in CD4 and CD8 will be tested by immunohistochemistry
33
34 336 (IHC) by the diagnostic biomarker laboratory at the Cross Cancer Institute on 4 µm sections
35
36
37 337 from formalin fixed paraffin embedded (FFPE) surgical specimens. At final analyses, IHC
38
39 338 staining will be interpreted by image analysis. At time of interpretation, slides will be de-
40
41 339 identified and coded to maintain the blind. All values (routine and image analysis) will be
42
43
44 340 recorded as absolute percentage and as log-transformation. Caspase-3 is a validated marker of
45
46 341 apoptosis and CD4 and CD8 are validated marker for lymphocytes. The calculated indices
47
48 342 (absolute %, log transformed and H-score) of biopsy and surgical resection will be compared on
49
50 343 each participant and between participants receiving DHA compared to placebo.

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3 344 Pathological complete response in resected breast tissue and axillary nodes will be
4
5 345 assessed in hematoxylin and eosin stained tissue for evidence of invasive disease according to
6
7 346 standard of care. Pathological complete response will be assessed following breast surgery as
8
9
10 347 per standard of care and recorded in patient's case report form. The rate of pathological
11
12 348 complete response in breast tissue and axillary nodes after surgical resection will be compared
13
14 349 between participants receiving DHA supplementation compared to placebo.

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16
17 350 Grade 3 and 4 toxicities will be assessed and recorded by the clinical trial nurse.
18
19 351 Toxicities will be assessed on day 20 (\pm 3 days) of each chemotherapy cycle. Dates of
20
21 352 hospitalization will be recorded in patient's case report form. Rates of chemotherapy-associated
22
23 353 grade 3/4 toxicities, all grade neuropathy and hospitalizations will compared between DHA
24
25 354 supplementation and placebo arms as scored by a medical oncologist in a standardized toxicity/
26
27 355 neuropathy form for each cycle of chemotherapy.

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31 356 *Exploratory outcomes:*

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33 357 The quality of life questionnaire is a validated questionnaire from European Organization
34
35 358 for Research and Treatment of Cancer- Quality of Life Questionnaire-C30 (EORTC-QLQ-C30)
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37 359 [45]. Exercise behavior will be assessed using the modified Godin Leisure-Time Exercise
38
39 360 Questionnaire (GLTEQ) [46]. Assessment in changes in quality of life and exercise behavior will
40
41 361 be assessed from timepoints collected to baseline within and between treatment groups.

42
43 362 The rate of breast conservation, specifically the rate of lumpectomy and modified radical
44
45 363 mastectomy, will be determined by surgical and pathologic reports at time of surgical resection.
46
47 364 Volume estimates of blood loss will be assessed by review of surgical report estimates of blood
48
49 365 loss to see if there is a qualitative or quantitative difference between placebo and treatment arms,
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51 366 once adjusted for the type of surgery (lumpectomy vs. mastectomy vs. mastectomy + immediate
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3 367 reconstruction; sentinel node dissection vs. full axillary dissection). Local control, relapse free
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5 368 survival and overall survival will be analyzed by electronic medical record and / or paper
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7 369 medical chart review at 3, 5, and 10 years. Data will be validated by a medical oncologist.
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9

10 370 **Data Management**

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14 371 All data will be entered and maintained in REDCap trial database. Direct access to
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16 372 clinical and laboratory information on the enrolled trial patients will be limited to the principal
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18 373 investigator, co-investigators, trainees/staff who have had the appropriate training and approval
19
20 374 and study nurses and study coordinators who will have access to the source documents through
21
22 375 the electronic medical record and laboratory information system at the Cross Cancer Institute.
23
24 376 All patients will have biopsy and tumor samples for analysis and we do not expect any missing
25
26 377 data for the primary endpoint (Ki67). If supplement compliance is below 50% for more than
27
28 378 two consecutive cycles, or participants do not complete chemotherapy (to a minimum of 4
29
30 379 cycles), they will be excluded from final analysis of the primary end point. If patients do not
31
32 380 have sufficient blood samples for the secondary analyses (DHA incorporation, systemic
33
34 381 immune function), analysis will be performed using data from the remaining patients.
35
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40 382 **Statistical Methods**

41 383 *Primary Outcome:*

42
43 384 The percent change in Ki67 will be determined as an absolute percentage and H-score. The
44
45 385 number of patients showing a decrease and the 95% confidence interval for the mean percent
46
47 386 change in the Ki67 level from baseline in patients receiving DHA supplementation will be
48
49 387 compared to patients receiving placebo. Test of proportions will be used to compare the
50
51 388 proportions between the two groups.
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389 *Secondary Outcomes:*

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

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

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

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

407 *Exploratory outcomes:*

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

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3 412 free survival and survival will be analyzed using the log rank test on Kaplan-Meier survival
4
5 413 curves.

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8 414 SAS software, version 9.4 (SAS Institute Inc., Cary, NC), will be used for statistical analysis.

9
10 415 A p-value <0.05 level will be used for all statistical significance. Two-sided tests will be used
11
12 416 for all statistical tests.

13 14 15 417 **Data Monitoring**

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18 418 The trial activities performed at the Cross Cancer Institute will be monitored by the
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20 419 Cross Cancer Institute, Investigator Initiated Trials Data Safety Monitoring Board (DSMB).
21
22 420 The DSMB is independent of the investigator and is composed of representatives from both
23
24 421 medical and radiation oncology.

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27
28 422 The investigator will assess the relationship between protocol treatment and the
29
30 423 occurrence of adverse events (AEs) and this assessment will be recorded in the database for
31
32 424 adverse events. This study will use the International Common Terminology Criteria for
33
34 425 Adverse Events (CTCAE), version 5.0, for adverse event reporting. The reporting period for
35
36 426 adverse events will start at the time the patient takes the first dose of DHA/placebo through and
37
38 427 including 28 calendar days after last administration of study agent. If serious adverse reaction
39
40 428 to treatment occurs, the Natural and Non-prescription Health Products Directorate (NNHPD),
41
42 429 Clinical Trial Unit, Health Ethics Research Board of Alberta, Cancer Committee (HREBA.CC)
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44 430 and DSM will be notified as per guidelines. After 25 evaluable patients, all data and results will
45
46 431 be submitted to the DSMB for review.

47 48 49 50 51 432 **Auditing**

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3 433 As per the SPIRIT guidelines, the investigators, Cross Cancer Institute and the
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5 434 University of Alberta will permit trial-related monitoring, audits, REB, DSMB review, and
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8 435 regulatory inspection(s), providing direct access to paper and/or electronic documentation
9
10 436 pertaining to the clinical study (e.g. CRFs, source documents such as hospital patient charts and
11
12 437 investigator study files). All site facilities related to the study conduct could be visited during
13
14
15 438 an audit (e.g. pharmacy, laboratory, outpatient department) and are agreed to co-operate and
16
17 439 provide assistance at reasonable times and places with respect to any auditing activity.
18
19

20 440 **Patient and Public Involvement**

21
22 441 Patients were not involved in the protocol development or study design. However,
23
24 442 oncologists and clinical trial nurses who work in the breast tumor group are involved in patient
25
26
27 443 screening to assess eligibility for the study. The HREBA-CC approved informed consent will be
28
29 444 obtained from patients prior to their involvement in the study and it informs patients of their right
30
31 445 to withdraw at anytime. At the end of the trial, results will be disseminated to the public through
32
33
34 446 seminars, public talks and in peer-reviewed journals.
35

36 447 **Ethics and dissemination**

37
38 448 DHAWIN has received Health Canada approval (#HC6-24-c220167), full ethical approval
39
40 449 from the Health Research Ethics Board of Alberta – Cancer Committee (Protocol #:
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42
43 450 HREBA.CC- 18-0381) and is registered at clinicaltrials.gov (Identifier: NCT03831178).
44
45 451 Protocol amendments will be submitted to HREBA.CC, Health Canada and the clinical trial
46
47 452 registry prior to study implementation according to regulatory requirements. The formal consent
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49 453 of a participant, using the HREBA-CC-approved consent form (Supplementary File 1), will be
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51 454 obtained by a clinical trial nurse before the participant is enrolled in the study and will be signed
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53
54 455 by the patient, and the principle investigator. A voluntary optional consent form for use of
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3 456 participant data and biological specimens (Supplementary File 2), will be offered at time of
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5 457 enrollment. Patient confidentiality and anonymity will be maintained and identities protected
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7
8 458 from unauthorized parties.

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10 459 Access to data will be restricted to the primary investigators and statistician. They will
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12 460 grant access to other team members as governed and approved by ethics. Ancillary care post-trial
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14 461 will occur as routine standard of care for all participants. Our objective is to determine the
15
16 462 efficacy of using DHA supplementation concomitant with chemotherapy and as such our results
17
18 463 will be disseminated to clinicians for implementation in future treatment paradigms. The results
19
20 464 will be submitted to peer-reviewed journals and presented at national and international
21
22 465 conferences.

23 24 25 26 466 **Funding Statement**

27
28
29 467 This study is supported by the Canadian Institutes of Health Research [Grant Number:
30
31 468 RES0037745], Cross Cancer Institute Investigator Initiated Trials [Grant Number: IIT-0005]
32
33 469 and a gift from the Butler Family Foundation, Edmonton Alberta.

34 35 36 37 470 **Competing Interests Statement**

38
39
40
41 471 There are no financial or competing interests or conflicts to declare.

42 43 44 45 472 **Author Contributions**

46
47
48 473 MN and CJF designed and wrote the manuscript. All authors contributed to the study
49
50 474 design and reviewed the manuscript drafts. MAC obtained all regulatory approvals
51
52 475 (Health Canada, HREBA and Clinical Trials registration). Authors of the data
53
54 476 manuscripts will include at least the Principal Investigator, medical director (J.

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2
3 477 Mackey) and any co-investigators who have i) included eligible patients in the trial (by
4
5 478 order of inclusion) and/or ii) contributed significantly to the design, conduct and data
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7
8 479 interpretation regarding companion basic science studies.
9

10 480 Appendices:

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12 481 Supplemental File 1: Informed consent
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14 482 Supplemental File 2: Optional consent
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17 483 Supplemental Table 1: List of Antibodies for Immune cell subset identification
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19 484 **FIGURE LEGENDS**

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22 485 **Figure 1** Flowchart of Trial Design with Endpoints and Proposed Experimental Analyses
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24 486 **Figure 2** SPIRIT patient flow diagram of the DHA WIN trial
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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	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	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												

488 ESAS: Edmonton Symptom Assessment System

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

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

491 (3) From previously collected biopsy.

492 (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).

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

494

495 **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 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.	<ul style="list-style-type: none"> 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

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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	Chisquare tests
4. Assess the volume of surgical blood loss.	Review surgical reports for quantitative / qualitative loss of blood	Chart review	Chisquare 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

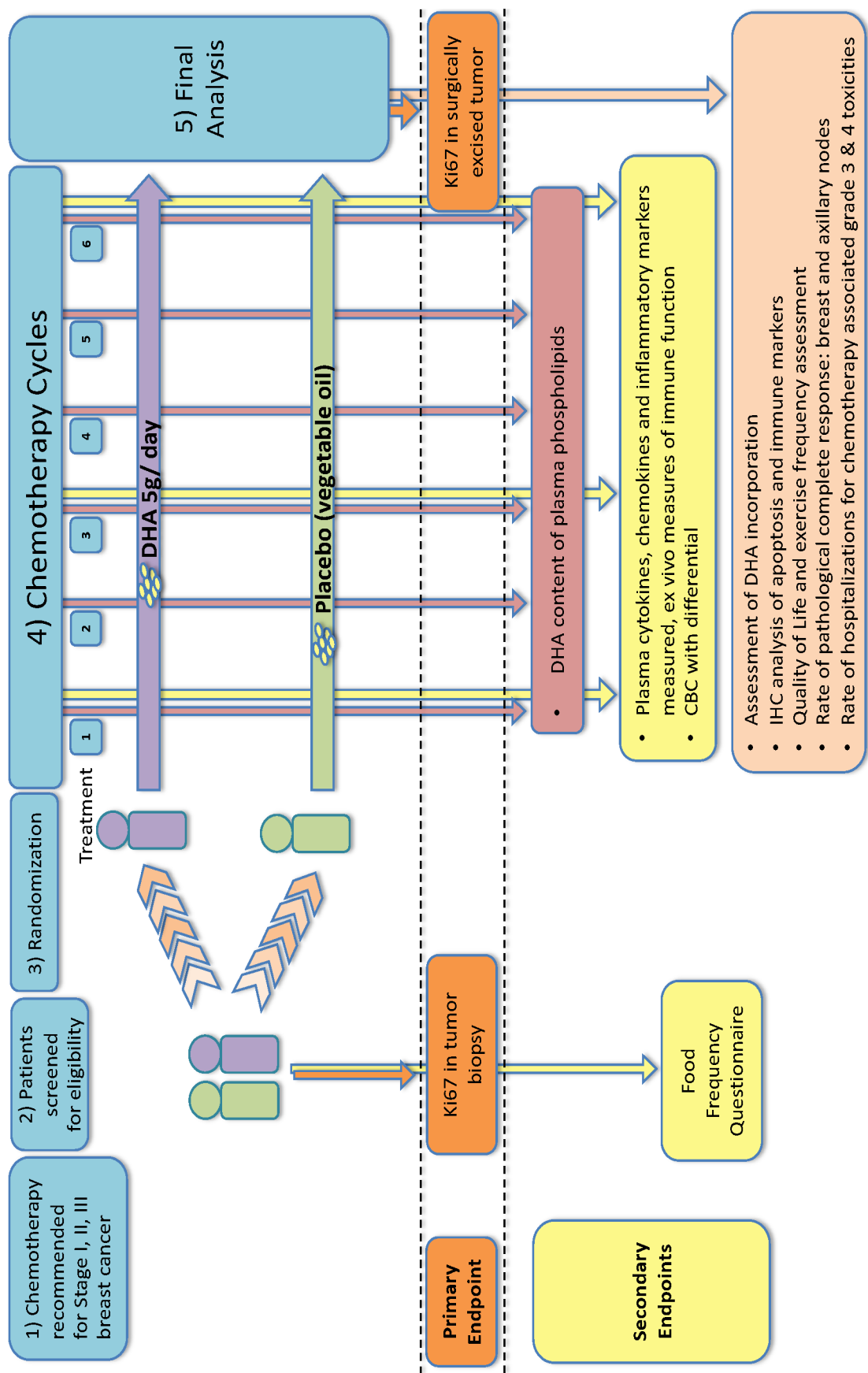
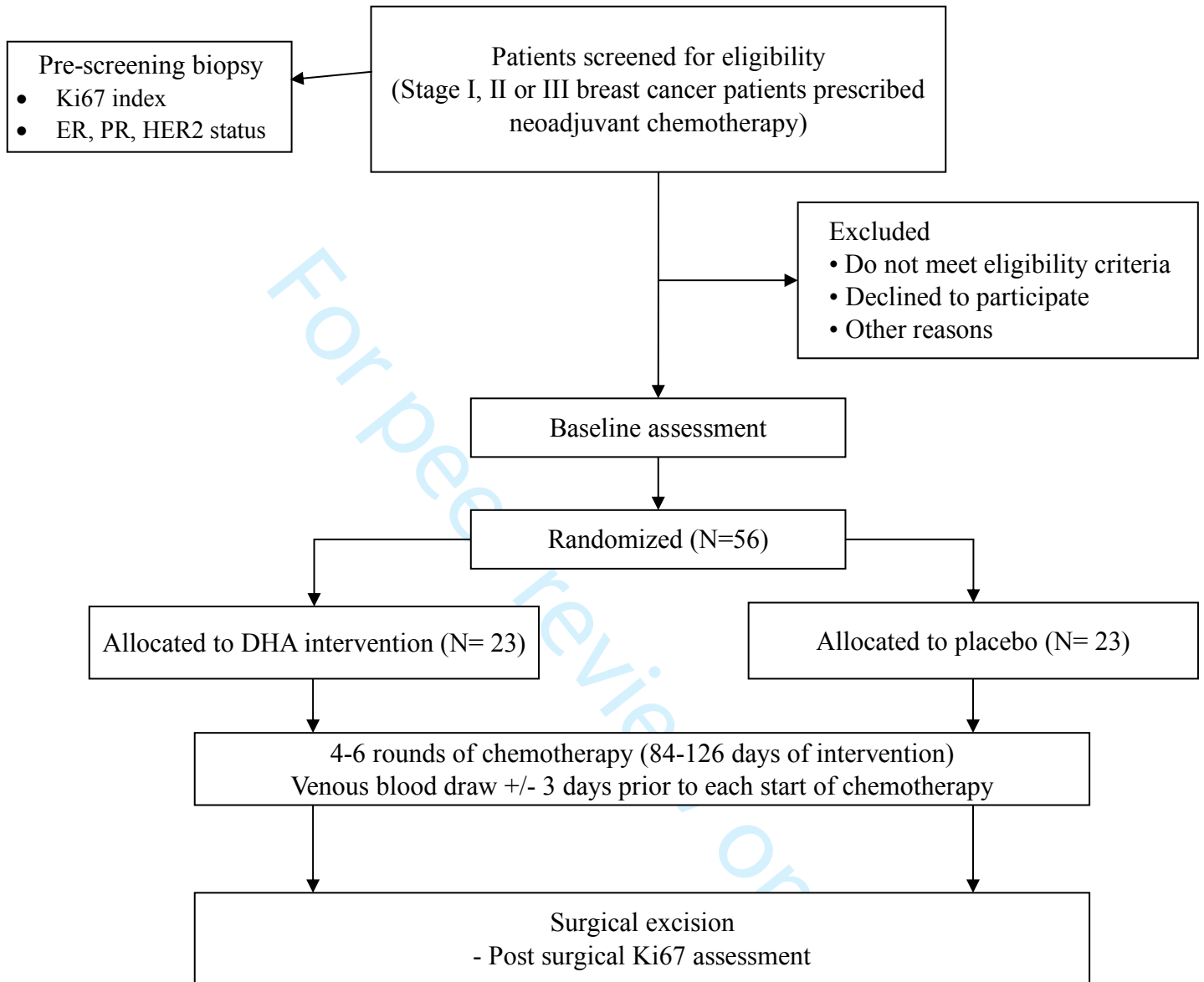


Figure 2



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

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	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

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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.

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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.

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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 approximately 12-18 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.

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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

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3 undergo a surgical procedure to remove the tumor from your breast. The amount of tissue to be
4 removed will depend on the size and location of the tumor. Your doctor will give you more
5 details regarding this procedure.
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8 A sample of the tissues obtained from the initial biopsy and from the subsequent breast surgery
9 will be sent to a laboratory at the *Cross Cancer Institute, and at the University of Alberta in*
10 *Edmonton, Alberta, Canada, where they will be examined to confirm your diagnosis and*
11 *examine how DHA alters tumour growth, and the amount of DHA in tumour cells.*
12

13 Blood Collection (Mandatory)

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15 Blood samples will be taken by inserting a needle into a vein in your arm. These will be taken at
16 the same time as your study related tests whenever possible upon *entry to the study, at the*
17 *beginning of every cycle of chemotherapy (every three weeks), on day 20 of cycle 3 and before*
18 *surgery. One tablespoon of blood will be collected for this study at those times.* These blood
19 samples will be sent to a laboratory at the *Cross Cancer Institute and the University of Alberta in*
20 *Edmonton, Alberta, Canada where they will be examined to measure the different cells in your*
21 *blood, and the amount of DHA in these cells.*
22

23 Identification of Samples

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

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37 If you no longer want your samples to be used in this research, you should tell the study doctor.
38 The study doctor will ensure the samples are returned to the hospital from which they were
39 obtained, if needed, or destroyed.
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42 You can request withdrawal of your sample(s) until *you have received your blinded capsules*
43 *when the samples will be made anonymous.* It won't be possible to return samples after this
44 because the researchers will not know which samples are yours.
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47 *You will not be able to continue to participate in this study if required samples are withdrawn.*
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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 Within 30 days after last dose	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)		
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	

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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	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	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	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	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	X	X	X	X	X	
We will collect results from your surgery report														X

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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;

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- 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 μ g/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 accidentally 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: _____

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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.

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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.

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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.

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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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Dr. John Mackey, Cross Cancer Institute, 11560 University Ave, Edmonton, AB, www.albertahealthservices.ca

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SIGNATURES

Part 1 - to be completed by the potential participant.

	<u>Yes</u>	<u>No</u>
Do you understand that you have been asked to take part in a research study?	<input type="checkbox"/>	<input type="checkbox"/>
Do you understand why this study is being done?	<input type="checkbox"/>	<input type="checkbox"/>
Do you understand the potential benefits of taking part in this study?	<input type="checkbox"/>	<input type="checkbox"/>
Do you understand the risks of taking part in this study and the risks of becoming pregnant or fathering a child during this study?	<input type="checkbox"/>	<input type="checkbox"/>
Do you understand what you will be asked to do should you decide to take part in this study?	<input type="checkbox"/>	<input type="checkbox"/>
Do you understand the alternatives to participating in this study?	<input type="checkbox"/>	<input type="checkbox"/>
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?	<input type="checkbox"/>	<input type="checkbox"/>
Do you understand who will see your records, including health information that identifies you?	<input type="checkbox"/>	<input type="checkbox"/>
Do you understand that by signing this consent form you are giving us permission to access your health information and specimens if applicable?	<input type="checkbox"/>	<input type="checkbox"/>
Do you understand that by signing this consent form that you do not give up any of your legal rights?	<input type="checkbox"/>	<input type="checkbox"/>
Have you had enough opportunity to ask questions and discuss this study?	<input type="checkbox"/>	<input type="checkbox"/>

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

Signature of Participant	PRINTED NAME	Date
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Part 2 - to be completed by the study doctor or designee who conducted the informed consent discussion. Only complete 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
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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
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****You will be given a copy of this signed and dated consent form prior to participating in this study.****

For peer review only

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
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3 locally, across Canada, and around the world. The researchers doing the main study are
4 also interested in storing your tissue/blood samples for future research. The research that
5 may be done on your samples in the future is unknown at this time. It may be related to
6 your condition or it may be used to address research questions that are unrelated.
7

8 Some of this research may be about genes. Genes carry information about features, such
9 as hair or eye colour. This research may include looking at changes in genes found in you
10 and in people who are related to you. These changes may be inherited (passed on in
11 families). This is called hereditary genetic testing. Researchers also may be interested in
12 the way that genes affect health and disease, or how your body responds to treatment.
13
14

15 **WHAT WILL HAPPEN DURING THIS OPTIONAL RESEARCH?**

16
17 You may take part in all or some of the optional research described here, it is your choice.
18 If you agree to take part:
19

- 20 • the samples used for this optional research have already been collected as part of
21 your standard of care. No further biopsies or surgeries are needed for this purpose.
- 22 • the blood samples used for this optional research will be those left over or remaining
23 from your participation in the main study. No further biopsies or surgeries are
24 needed for this purpose.
25
26

27 **HOW WILL MY SAMPLES BE HANDLED?**

28
29 Your sample(s) and some related health information already collected from your
30 participation in the main study will be sent to the Nutritional Immunology laboratory at the
31 University of Alberta, Edmonton, AB, for analysis. The samples and data will be kept
32 indefinitely or until they are used up, destroyed or returned to the hospital where you had
33 your surgery or biopsy.
34
35

36
37 Qualified researchers can submit a request to use the materials stored at the University of
38 Alberta. Your samples and related health information will be used only by researchers
39 whose requests have been accepted by the sponsor and who have met regulatory
40 requirements and secured ethics approval for their research. The samples and data may be
41 sent to other countries. Your name or any other information that could directly identify you
42 will not be given to these researchers.
43
44

45
46 The results of research done on your samples will not be added to your personal health
47 records and you or the researcher will not know the results.
48

49 **WHAT ARE THE RISKS OF PARTICIPATING IN THIS OPTIONAL RESEARCH?**

50
51 Risks related to sample collection:

- 52 • Since the tissue sample(s) already have been collected for the main study or as part of
53 your standard of care, no additional physical risks are expected.
54
55

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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.

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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 THIS 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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2
3 study.

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5
6 **IS THERE ANY CONFLICT OF INTEREST RELATED TO THIS OPTIONAL RESEARCH?**

7 There are no current or potential conflicts of interest concerning the optional research
8 study.
9

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11
12
13 **WHO DO I CONTACT FOR QUESTIONS RELATED TO THIS OPTIONAL RESEARCH?**

14
15 If you have questions about the use of your samples/data for optional research, or if you
16 suffer a research-related injury, contact the researcher of this optional study:
17

18
19
20 Catherine J Field
21 Name

22 780-492-5297
23 Telephone Number

24
25
26
27 If you have questions about your rights as a participant or about ethical issues related to
28 this optional research and you would like to speak to someone not involved in its conduct,
29 please contact the Office of the Health Research Ethics Board of Alberta – Cancer
30 Committee at: 780-423-5727 or toll-free 1-877-423-5727.
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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 Printed Name Date
the Consent Discussion

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 Printed Name Date
Witness

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.

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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 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

1			
2		6b	Explanation for choice of comparators
3			7
4	Objectives	7	Specific objectives or hypotheses
5			5
6	Trial design	8	Description of trial design including type of trial (eg,
7			parallel group, crossover, factorial, single group),
8			allocation ratio, and framework (eg, superiority,
9			equivalence, noninferiority, exploratory)
10			6, Fig 1,
11			Fig 2
12	Methods: Participants, interventions, and outcomes		
13			
14	Study setting	9	Description of study settings (eg, community clinic,
15			academic hospital) and list of countries where data
16			will be collected. Reference to where list of study
17			sites can be obtained
18			6
19	Eligibility criteria	10	Inclusion and exclusion criteria for participants. If
20			applicable, eligibility criteria for study centres and
21			individuals who will perform the interventions (eg,
22			surgeons, psychotherapists)
23			Table 1
24			(page 1,2)
25	Interventions	11a	Interventions for each group with sufficient detail to
26			allow replication, including how and when they will
27			be administered
28			7
29		11b	Criteria for discontinuing or modifying allocated
30			interventions for a given trial participant (eg, drug
31			dose change in response to harms, participant
32			request, or improving/worsening disease)
33			8
34		11c	Strategies to improve adherence to intervention
35			protocols, and any procedures for monitoring
36			adherence (eg, drug tablet return, laboratory tests)
37			8
38		11d	Relevant concomitant care and interventions that are
39			permitted or prohibited during the trial
40			-
41	Outcomes	12	Primary, secondary, and other outcomes, including
42			the specific measurement variable (eg, systolic blood
43			pressure), analysis metric (eg, change from baseline,
44			final value, time to event), method of aggregation
45			(eg, median, proportion), and time point for each
46			outcome. Explanation of the clinical relevance of
47			chosen efficacy and harm outcomes is strongly
48			recommended
49			8-11
50			Table 3
51	Participant timeline	13	Time schedule of enrolment, interventions (including
52			any run-ins and washouts), assessments, and visits
53			for participants. A schematic diagram is highly
54			recommended (see Figure)
55			12, Table
56			2
57			
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1				
2	Sample size	14	Estimated number of participants needed to achieve	12
3			study objectives and how it was determined,	
4			including clinical and statistical assumptions	
5			supporting any sample size calculations	
6				
7	Recruitment	15	Strategies for achieving adequate participant	13
8			enrolment to reach target sample size	
9				

Methods: Assignment of interventions (for controlled trials)

Allocation:

14	Sequence	16a	Method of generating the allocation sequence (eg,	13
15	generation		computer-generated random numbers), and list of	
16			any factors for stratification. To reduce predictability	
17			of a random sequence, details of any planned	
18			restriction (eg, blocking) should be provided in a	
19			separate document that is unavailable to those who	
20			enrol participants or assign interventions	
21				
22				
23				
24	Allocation	16b	Mechanism of implementing the allocation sequence	13
25	concealment		(eg, central telephone; sequentially numbered,	
26	mechanism		opaque, sealed envelopes), describing any steps to	
27			conceal the sequence until interventions are	
28			assigned	
29				
30				
31	Implementation	16c	Who will generate the allocation sequence, who will	13
32			enrol participants, and who will assign participants to	
33			interventions	
34				
35	Blinding (masking)	17a	Who will be blinded after assignment to interventions	13
36			(eg, trial participants, care providers, outcome	
37			assessors, data analysts), and how	
38				
39				
40		17b	If blinded, circumstances under which unblinding is	13
41			permissible, and procedure for revealing a	
42			participant's allocated intervention during the trial	
43				

Methods: Data collection, management, and analysis

46	Data collection	18a	Plans for assessment and collection of outcome,	14-18
47	methods		baseline, and other trial data, including any related	Table 3
48			processes to promote data quality (eg, duplicate	
49			measurements, training of assessors) and a	
50			description of study instruments (eg, questionnaires,	
51			laboratory tests) along with their reliability and	
52			validity, if known. Reference to where data collection	
53			forms can be found, if not in the protocol	
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2		18b	Plans to promote participant retention and complete
3			follow-up, including list of any outcome data to be
4			collected for participants who discontinue or deviate
5			from intervention protocols
6			
7	Data management	19	Plans for data entry, coding, security, and storage,
8			including any related processes to promote data
9			quality (eg, double data entry; range checks for data
10			values). Reference to where details of data
11			management procedures can be found, if not in the
12			protocol
13			
14			
15	Statistical methods	20a	Statistical methods for analysing primary and
16			secondary outcomes. Reference to where other
17			details of the statistical analysis plan can be found, if
18			not in the protocol
19			
20			
21			
22		20b	Methods for any additional analyses (eg, subgroup
23			and adjusted analyses)
24			
25		20c	Definition of analysis population relating to protocol
26			non-adherence (eg, as randomised analysis), and
27			any statistical methods to handle missing data (eg,
28			multiple imputation)
29			
30			
31	Methods: Monitoring		
32	Data monitoring	21a	Composition of data monitoring committee (DMC);
33			summary of its role and reporting structure;
34			statement of whether it is independent from the
35			sponsor and competing interests; and reference to
36			where further details about its charter can be found,
37			if not in the protocol. Alternatively, an explanation of
38			why a DMC is not needed
39			
40			
41			
42		21b	Description of any interim analyses and stopping
43			guidelines, including who will have access to these
44			interim results and make the final decision to
45			terminate the trial
46			
47	Harms	22	Plans for collecting, assessing, reporting, and
48			managing solicited and spontaneously reported
49			adverse events and other unintended effects of trial
50			interventions or trial conduct
51			
52			
53	Auditing	23	Frequency and procedures for auditing trial conduct,
54			if any, and whether the process will be independent
55			from investigators and the sponsor
56			
57			
58	Ethics and dissemination		
59			
60			

1				
2	Research ethics	24	Plans for seeking research ethics	21
3	approval		committee/institutional review board (REC/IRB)	
4			approval	
5				
6	Protocol	25	Plans for communicating important protocol	21
7	amendments		modifications (eg, changes to eligibility criteria,	
8			outcomes, analyses) to relevant parties (eg,	
9			investigators, REC/IRBs, trial participants, trial	
10			registries, journals, regulators)	
11				
12				
13	Consent or assent	26a	Who will obtain informed consent or assent from	21
14			potential trial participants or authorised surrogates,	Supp file
15			and how (see Item 32)	
16				
17		26b	Additional consent provisions for collection and use	21
18			of participant data and biological specimens in	Supp file
19			ancillary studies, if applicable	
20				
21				
22	Confidentiality	27	How personal information about potential and	22
23			enrolled participants will be collected, shared, and	
24			maintained in order to protect confidentiality before,	
25			during, and after the trial	
26				
27				
28	Declaration of	28	Financial and other competing interests for principal	22
29	interests		investigators for the overall trial and each study site	
30				
31	Access to data	29	Statement of who will have access to the final trial	22
32			dataset, and disclosure of contractual agreements	
33			that limit such access for investigators	
34				
35	Ancillary and post-	30	Provisions, if any, for ancillary and post-trial care,	22
36	trial care		and for compensation to those who suffer harm from	
37			trial participation	
38				
39				
40	Dissemination	31a	Plans for investigators and sponsor to communicate	22
41	policy		trial results to participants, healthcare professionals,	
42			the public, and other relevant groups (eg, via	
43			publication, reporting in results databases, or other	
44			data sharing arrangements), including any	
45			publication restrictions	
46				
47				
48		31b	Authorship eligibility guidelines and any intended use	22
49			of professional writers	
50				
51		31c	Plans, if any, for granting public access to the full	-
52			protocol, participant-level dataset, and statistical	
53			code	
54				
55				
56	Appendices			
57				
58	Informed consent	32	Model consent form and other related documentation	Supp file
59	materials		given to participants and authorised surrogates	
60				

1				
2	Biological	33	Plans for collection, laboratory evaluation, and	Supp file
3	specimens		storage of biological specimens for genetic or	
4			molecular analysis in the current trial and for future	
5			use in ancillary studies, if applicable	
6				

7 *It is strongly recommended that this checklist be read in conjunction with the SPIRIT 2013
8 Explanation & Elaboration for important clarification on the items. Amendments to the
9 protocol should be tracked and dated. The SPIRIT checklist is copyrighted by the SPIRIT
10 Group under the Creative Commons "[Attribution-NonCommercial-NoDerivs 3.0 Unported](#)"
11 license.
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For peer review only

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

Journal:	<i>BMJ Open</i>
Manuscript ID	bmjopen-2019-030502.R1
Article Type:	Protocol
Date Submitted by the Author:	21-May-2019
Complete List of Authors:	Newell, Marnie; University of Alberta, Department of Agricultural, Food and Nutritional Science Mackey, John; University of Alberta, Department of Oncology; Alberta Health Services Bigras, Gilbert ; University of Alberta, Department of Laboratory Medicine and Pathology Alvarez-Camacho, Mirey; Alberta Health Services Goruk, Susan; University of Alberta, Department of Agricultural, Food and Nutritional Science Ghosh, Sunita; Alberta Health Services Schmidt, Alison; Alberta Health Services Miede, Deborah; Alberta Health Services Chisotti, Ann; Alberta Health Services Postovit, Lynne; University of Alberta, Department of Oncology Baker, Kristi; University of Alberta, Department of Oncology Mazurak, Vera; University of Alberta, Department of Agricultural, Food and Nutritional Science Courneya, Kerry; University of Alberta, Faculty of Kinesiology, Sport and Recreation Berendt, Richard; University of Alberta, Department of Laboratory Medicine and Pathology Dong, Wei-Feng; University of Alberta, Department of Laboratory Medicine and Pathology Wood, George; University of Alberta, Department of Laboratory Medicine and Pathology Basi, Sanraj; Alberta Health Services Joy, Anil Abraham; Department of Oncology King, Karen; Alberta Health Services Meza-Junco, Judith; Alberta Health Services Zhu, Xiaofu; Alberta Health Services Field, Catherine; University of Alberta, Department of Agricultural, Food and Nutritional Science
Primary Subject Heading:	Oncology
Secondary Subject Heading:	Nutrition and metabolism, Immunology (including allergy)

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

SCHOLARONE™
Manuscripts

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

4 Protocol Number: IIT-0005

5 Version Date: March 17, 2019

6 Marnie Newell¹, John R. Mackey^{2,3}, Gilbert Bigras⁴, Mirey Alvarez-Camacho², Susan
7 Goruk¹, Sunita Ghosh², Alison Schmidt², Deborah Miede², Ann Chisotti², Lynne Postovit³, Kristi
8 Baker³, Vera Mazurak¹, Kerry S. Courneya⁵, Richard Berendt⁴, Wei-Feng Dong⁴, George
9 Wood⁴, Sanraj K. Basi², Anil Abraham Joy², Karen King², Judith Meza-Junco², Xiaofu Zhu² and
10 Catherine J. Field^{1*}

11
12 ¹Department of Agricultural, Food and Nutritional Science, Faculty of Agricultural, Life and
13 Environmental Sciences, University of Alberta

14 ²Alberta Health Services - Cancer Control, Cross Cancer Institute

15 ³Department of Oncology, Faculty of Medicine & Dentistry, University of Alberta

16 ⁴Department of Laboratory Medicine and Pathology, Faculty of Medicine & Dentistry,
17 University of Alberta

18 ⁵Faculty of Kinesiology, Sport, and Recreation, University of Alberta

19 * Corresponding author: Catherine J. Field, PhD, Faculty of Agricultural, Life and Environmental
20 Sciences, University of Alberta, 4-126 Li Ka Shing Centre, Edmonton, Alberta, Canada, T6G
21 2H9. Tel: (780) 492-2597, E-mail: catherine.field@ualberta.ca

22 Word Count: 5804

23 **ABSTRACT**

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

31 *Methods and analysis:* This is a double blind phase II randomized controlled trial of 52 women
32 prescribed neoadjuvant chemotherapy to test if DHA supplementation enhances chemotherapy
33 efficacy. The DHA supplementation group will take 5g/day DHA orally and the placebo group
34 will take equal fat supplement of vegetable oil. The primary outcome will be change in Ki67
35 labelling index from pre-chemotherapy core needle biopsy to definitive surgical specimen.
36
37 Secondary endpoints include assessment of: i) DHA plasma phospholipid content; ii) systemic
38 immune cell types, plasma cytokines and inflammatory markers iii) tumor markers for apoptosis
39 and tumor infiltrating lymphocytes iv) rate of pCR in breast and in axillary nodes v) frequency of
40 grade 3 and 4 chemotherapy associated toxicities and vi) patient perceived quality of life.

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

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2
3 43 *Ethics and dissemination:* This study has full approval from the Health Research Ethics Board of
4
5 44 Alberta – Cancer Committee (Protocol #: HREBA.CC -18-0381). We expect to present the
6
7 45 findings of this study to the scientific community in peer-reviewed journals and at conferences.
8
9 46 The results of this study will provide evidence for supplementing with DHA during neoadjuvant
10
11 47 chemotherapy treatment for breast cancer.

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15 48 Clinical Trial Registration No: NCT03831178

16 17 49 **KEYWORDS**

18
19 50 Ki67, phospholipids, fatty acids, omega-3, apoptosis, proliferation, immune function

20 21 22 51 **STRENGTHS AND LIMITATIONS OF THIS STUDY**

- 23
24
25 52 • This study is the first phase II randomized controlled trial to evaluate DHA
26
27 53 supplementation concomitant with neoadjuvant chemotherapy to treat non-metastatic
28
29 54 breast cancer.
- 30
31
32 55 • The intervention is minimally invasive and side effects from the supplementation are not
33
34 56 expected.
- 35
36
37 57 • This study is powered to examine the key clinical outcome of changes in Ki67 index
38
39 58 from pre-chemotherapy biopsy to surgical excision based on group sample sizes of 23
40
41 59 patients in group one and 23 patients in group two in order to achieve 81% power to
42
43 60 detect a difference between the group proportions of 0.4.
- 44
45
46 61 • This study will measure clinically relevant intermediate outcomes including rate of pCR
47
48 62 in breast and in axillary nodes, rate of grade 3 and 4 chemotherapy associated toxicities
49
50 63 and hospitalizations as well as additional outcomes including plasma phospholipid
51
52 64 content of DHA, markers of immune function (plasma cytokines, inflammatory markers

- 1
2
3 65 and lymphocyte function), tumor markers for apoptosis and tumor infiltrating
4
5 66 lymphocytes and patient perceived quality of life.
6
7
8 67 • The study will include all subtypes of breast cancer patients undergoing neoadjuvant
9
10 68 chemotherapy but is not powered to assess differences between subtypes.
11
12

13 69 INTRODUCTION

14
15
16 70 Despite improvements in early diagnosis and treatment, breast cancer remains the second leading
17
18 71 cause of cancer related death in women [1]. While neoadjuvant chemotherapy aims to improve
19
20 72 surgical resection outcomes and reduce / eliminate micrometastases [2,3], pathological complete
21
22 73 response (pCR) is not achieved by all patients [3]. Increasing the efficacy of neoadjuvant
23
24 74 treatment without adding additional side-effects would benefit this population.
25
26
27

28 75 DHA is an omega-3 long chain polyunsaturated fatty acid (n-3 LCPUFA). The majority
29
30 76 of n-3 fatty acids are in the form of the 18-carbon fatty acid alpha-linolenic acid (ALA). While
31
32 77 DHA can be synthesized from ALA and other n-3 LCPUFA in the body, endogenous synthesis
33
34 78 is low [4,5]. Consequently, the direct consumption of this fatty acid is the only way to
35
36 79 significantly increase levels of DHA in tissues [6]. Supplementation can increase blood plasma
37
38 80 DHA concentration by 2-fold (500 μ M), which can lead to plasma membrane lipid enrichment
39
40 81 [7]. Incorporation of DHA into tumor membrane phospholipids has been shown to reduce
41
42 82 breast cancer cell proliferation [8,9] and increase apoptosis [10-15] in vitro and decreases tumor
43
44 83 growth in animal models [13,15-17]. Additionally, providing/feeding DHA has been shown to
45
46 84 increase the efficacy of different chemotherapeutic drugs in animal models of breast cancer
47
48 85 [11,12,18,19]. While there is limited clinical evidence, it has been shown that increased dietary
49
50 86 intake of n-3 LCPUFA, including DHA, results in increased DHA incorporation in breast
51
52 87 adipose tissue [20] and this correlates with improved response to chemotherapy [21]. In an
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3 88 open label trial with advanced metastatic breast cancer patients, DHA supplementation and
4
5 89 enrichment into plasma phospholipids was associated with improved outcomes [22]. Other
6
7 90 clinical trials have reported that supplementation with n-3 LCPUFA at a wide range of doses
8
9 91 (0.6g-8.6 g/day) increased tolerability of chemotherapeutic drugs in a range of malignancies at
10
11 92 other sites, include lung, pancreatic and colorectal (reviewed in [23]). Consequently, we
12
13 93 hypothesize that the therapeutic index (efficacy: toxicity ratio) of neoadjuvant breast cancer
14
15 94 chemotherapy will be improved with the addition of DHA to the treatment.
16
17
18

19 95 Breast cancer proliferation can be assessed by immunohistochemical (IHC) analysis of
20
21 96 cells staining positive for the nuclear antigen Ki67 [24], as it is expressed in all phases of the
22
23 97 cell cycle, G₁, S, G₂, and M, but not in G₀ [25,26]. The proportion of cells staining for Ki67 is
24
25 98 frequently used as a primary endpoint to measure efficacy of neoadjuvant therapy in clinical
26
27 99 trials. The Ki67 index, defining the change between pre- and post-treatment Ki67, has been
28
29 100 reported to be an independent prognostic factor in luminal A, luminal B, triple-negative, and
30
31 101 HER2+ breast cancer, and has been reported to be a useful surrogate marker of relapse free
32
33 102 survival in luminal B, triple- negative, and HER2+ breast cancer [27,28].
34
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38 103 **OBJECTIVES**

39
40 104 The objective of this RCT is to assess the efficacy of supplemental DHA combined with
41
42 105 neoadjuvant chemotherapy in treatment naïve women with breast cancer measured by changes in
43
44 106 Ki67 index from biopsy to surgical excision. We hypothesize that DHA supplementation will
45
46 107 increase plasma phospholipid DHA and improve response to neoadjuvant chemotherapy assessed
47
48 108 by decrease in Ki67 index.
49
50
51

52 109

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

112 Study Design

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

116 METHODS AND ANALYSIS

117 Study Population

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

122 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

123

124 **Intervention**

125 All women in this trial will receive standard of care chemotherapy throughout the duration of the
 126 trial. Breast cancer chemotherapy is developed in a guideline-coordinated system by a single
 127 team residing at the Cross Cancer Institute. Consequently, there are only two chemotherapy
 128 regimens that are used for neoadjuvant chemotherapy in this population. Each of the two
 129 regimens are six cycles in length and given at three-week intervals with a resultant chemotherapy
 130 regimen duration of 18 weeks. Both regimens are docetaxel based. For HER2 negative disease,
 131 patients universally receive the FEC-D (fluorouracil, epirubicin, cyclophosphamide; docetaxel)
 132 [32] regimen as neoadjuvant therapy, while HER2 positive patients receive the DCH regimen
 133 (docetaxel, carboplatinum, trastuzumab) [33].

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

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3 141 throughout the day as tolerated (at anytime, with or without food). Capsules are identical in
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5 142 appearance and composition (other than the oils) to maintain blinding of participants and study
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8 143 staff. As the DHA source is an algae-synthesized triglyceride, there are no differences in texture
9
10 144 or taste.

11
12
13 145 All patients will begin a cytotoxic chemotherapy regimen intended to require 18 weeks
14
15 146 for delivery. The intervention (DHA or placebo) will commence at the start of the first cycle of
16
17 147 chemotherapy and continue through 4-6 cycles of chemotherapy (3 weeks/ cycle). Should a
18
19 148 patient not be able to complete the full six cycles of therapy, the timing of surgery remains 3-5
20
21 149 weeks after completion of the last cycle of chemotherapy delivered. As local guidelines mandate
22
23 150 surgery between 3 and 5 weeks from the last round of chemotherapy, DHA/placebo will be
24
25 151 continued until this time (21-35 days after the last administration of cytotoxic chemotherapy).

26
27 152 All patients will be dispensed an additional bottle of DHA/placebo capsules at the
28
29 153 beginning of the study to account for circumstances where their treatment is delayed due to
30
31 154 treatment associated toxicities (including but not limited to vomiting, diarrhea, abnormalities in
32
33 155 blood work, fatigue or severe mouth sores). The patients will be requested to continue taking the
34
35 156 DHA or placebo as tolerated and will be dispensed additional capsules as necessary. The extra
36
37 157 capsules will remain with the patient until the end of the study.

38
39 158 Patients will be encouraged to take the supplements as tolerated (throughout the day at
40
41 159 anytime, with or without food). Treatment adherence will be monitored by review of patient
42
43 160 dosing diary and recording the number of any remaining capsules returned at the end of study
44
45 161 visit following the last dose of DHA/placebo. Non-compliance will be assessed as consuming
46
47 162 less than 50% of the weekly dose for 2 consecutive cycles. No additional natural health product
48
49 163 is permitted beyond a daily multi-vitamin.

164 Outcome Measurements

165 Study outcome timelines are summarized in **Table 2**. Briefly, outcomes will be measured
166 at baseline, within ± 3 days of chemotherapy and/ or post-intervention (surgical excision).

167 Electronic medical record and or paper chart review of local control, relapse free survival and
168 overall survival will occur at 3, 5, and 10 years to explore possible effects on long-term outcome.

169 *Primary Outcome*

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

178 *Secondary Outcomes*

179 1) DHA incorporation into phospholipids: The changes in level of DHA incorporation in plasma
180 phospholipids will be assessed at baseline and at day 1 (± 3 days) of each cycle of chemotherapy
181 (2-6) and end of cycle 6 to identify the range of DHA incorporation in this patient population.

182 The use plasma rather than red blood cells or whole blood for this study is supported by the
183 recent recommendations for best practices for fatty acids described by Brenna et al [38].

184 Analysis of the plasma phospholipid rather than plasma total lipids avoids the postprandial
185 fluctuation of the triacylglycerol pool and is believed to adequately represent the cell membrane
186 composition [38]. From our hypothesis and previously published data [22], it is expected that

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

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

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

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

1
2
3 210 receiving DHA supplementation and compared to patients receiving placebo. Proportions of
4
5 211 negative cells, weakly positive cells and strongly positive cells will be scored by two
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7 212 pathologists and the staining intensity assessed by QuPath [37] will be recorded independently.
8
9
10 213 Increased apoptosis measured by Caspase-3 is a clinically relevant marker of cell death.
11
12 214 5) Examine changes in tumor infiltrating lymphocytes (TILs): CD4+ and CD8+ in the excised
13
14 215 tumor, as number of positive cells for a given area, will be calculated by image analysis and a
15
16 216 comparison of expression levels at experimental end (surgical excision) to baseline will be
17
18 217 determined in patients receiving DHA supplementation and compared to patients receiving
19
20 218 placebo will be made post-treatment. The differences will be compared between treatments and
21
22 219 within the treatment group, related to plasma DHA concentrations. Increased infiltration of
23
24 220 TILs is potential marker that could be used to predict treatment patient outcomes.
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27
28 221 6) Pathological complete response rate (pCR): pCR in resected breast tissue and all sampled
29
30 222 axillary nodes will be assessed as absence of invasive cancer on haematoxylin and eosin
31
32 223 evaluation as per standard of care. Pathologic complete response will be classified as ypT0/is
33
34 224 ypN0 and will be determined at end of study after surgical resection as part of standard of care
35
36 225 assessment.
37
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39
40 226 7) Comparison of rate of chemotherapy associated grade 3 and 4 toxicities: Rate of chemotherapy
41
42 227 associated grade 3 and 4 toxicities, and chemotherapy-associated hospitalizations will be
43
44 228 compared between DHA and placebo arms. Any changes will then be examined in regards to
45
46 229 level of supplementation and DHA incorporation. These analyses will be completed at end of
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48 230 study after surgical resection.
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52 231 *Exploratory outcomes*
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3 232 1) Food frequency questionnaire (FFQ): Assessment of the FFQ to compare the estimated (pre-
4
5 233 diagnosis) usual intake of macronutrients on an energy basis (including fat content and
6
7 234 composition) between our two groups at baseline. In the future, the overall medians/ means of
8
9 235 the subjects in this study will be compared to age-matched women in the Alberta Tomorrow
10
11 236 Project.
- 12
13
14 237 2) Quality of life: Assessment in changes in quality of life will be determined by questionnaire
15
16 238 employed at baseline and end of treatment. Comparisons will be assessed from end of treatment
17
18 239 to baseline within and between treatment groups.
- 19
20
21 240 3) Exercise behavior: Assessment of exercise behavior will be determined by questionnaire
22
23 241 employed at baseline, each cycle of chemotherapy and end of treatment. Comparisons will be
24
25 242 assessed from end of treatment to baseline within and between treatment groups.
- 26
27
28 243 4) Breast conservation: The rate of breast conservation, specifically the rate of lumpectomy and
29
30 244 mastectomy, will be determined by review of surgical and pathologic reports at end of study
31
32 245 after surgical resection.
- 33
34
35 246 5) Volume of surgical blood loss: High intakes of n-3 LCPUFA (that contain some DHA) have
36
37 247 been studied to determine if they increase bleeding time [39,40]. We will review surgical
38
39 248 report estimates of blood loss to see if there is a qualitative or quantitative difference between
40
41 249 placebo and treatment arms, once adjusted for the magnitude of surgery (lumpectomy vs.
42
43 250 mastectomy vs. mastectomy + immediate reconstruction; sentinel node dissection vs. full
44
45 251 axillary dissection). It is not expected that we will see a difference as it is eicosapentaenoic
46
47 252 acid (EPA, the precursor to DHA) that has antithrombotic and antiplatelet properties [41].
- 48
49
50 253 6) Local control, relapse free survival and overall survival: Local control, relapse free survival
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52 254 and overall survival will be analyzed by review of electronic medical records, registry reports,
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255 and / or paper medical charts at 3, 5, and 10 years to explore possible effects on long-term
256 outcome.

257 **Participant timeline**

258 Breast cancer patients receiving neoadjuvant chemotherapy account for approximately
259 20% of newly diagnosed breast cancer patients, approximately 10-12/month at the Cross Cancer
260 Institute. Assuming a conservative accrual rate of 30%, accrual is estimated to be completed in
261 14-18 months with 3-4 patients recruited per month. Each patient will be enrolled for the
262 duration of their individual chemotherapy regimen, an estimated 12-18 weeks (84-126 days)
263 beginning at the start of the first cycle of chemotherapy and continued through 4-6 cycles of
264 chemotherapy (3 weeks/ cycle). The intervention will be discontinued 21-35 days after the last
265 administration of cytotoxic chemotherapy when surgery to remove the tumor occurs. See Figure
266 1 for schematic of the participant timeline.

267 **Sample Size**

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

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

280 **Recruitment**

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

285 **Randomization and Blinding**

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

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3 301 with the unique bottle ID will be entered into the REDCap database. The key to the study arm
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5 302 will be kept in password protected computers and will only be shared in an urgent need for
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7 303 breaking of the blind. When a blinding code is broken, the date and reason for unblinding must
8
9 304 be fully documented in source documents and entered on case report form. Every effort should
10
11 305 be made by site staff to ensure that the treatment arm in which the unblinded patient is assigned
12
13 306 is communicated only to those site staff that require the information for treatment purposes. To
14
15 307 assist in maintaining the blind of the patients, supplements and placebo are identical in size,
16
17 308 shape, color and texture, in addition to identical bottles for dispensing. Patients, pathologists,
18
19 309 physicians, and researchers will be blinded to patient enrolment in the study and throughout trial.
20
21 310 Blinding will only be dropped after analysis of fatty acids, systemic immune function and Ki67
22
23 311 is complete.

312 **Data Collection, Management and Analysis**

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

319 *Primary Outcome*

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

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3 323 analyses, Ki67 staining will be repeated as single IHC stain and interpreted by image analysis.
4
5 324 At time of Ki67 interpretation, slides will be de-identified and coded to ensure the pathologist is
6
7
8 325 blinded to the experimental group. In addition, the original single stained slides will be
9
10 326 interpreted visually by research staff. All Ki67 values (routine and image analysis) will be
11
12 327 recorded as absolute percentage and as log-transformation in REDCap trial database and
13
14 328 participant's case report form. The Ki67 index is validated and used in clinic as marker of
15
16 329 proliferation. The Ki67 index (absolute %, log transformed and H-score [42] of biopsy and
17
18 330 surgical resection (after chemotherapy) will be compared on each participant and between
19
20 331 participants receiving DHA compared to placebo.
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25 332 *Secondary Outcomes*

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28 333 DHA incorporation into plasma phospholipids will be measured in venous blood from
29
30 334 patients at baseline (time of enrolment in trial), and at day 20 (\pm 3 days) of each chemotherapy
31
32 335 cycle by a technician blinded to the treatment group. Venous blood will be collected in coated
33
34 336 EDTA tubes and centrifuged at 750x g for 10 min to obtain plasma. Red blood cells will be
35
36 337 immediately frozen and banked at -70°C for storage for future secondary analysis. Plasma will
37
38 338 be separated in 6 aliquots, and immediately frozen at -70°C for storage. Plasma (concentration
39
40 339 and relative percent) will be extracted by Folch procedure [43,44], phospholipids separated by
41
42 340 thin layer chromatography and fatty acid content measured by gas-liquid chromatography as
43
44 341 previously described [45]. The percentage change in DHA from baseline will be compared in
45
46 342 each patient and a 95% t-confidence interval for the mean percent change in the DHA from
47
48 343 baseline will be compared to patients receiving placebo. An internal standard is used to
49
50 344 identify and quantify the fatty acid. This is a standard measure for fatty acid status has
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3 345 coefficient of variation <5% and individual GC peaks are validated against phospholipid
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5 346 standards (GLC-502 and GLC-643) from NuChek (Elysian, MN).
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8 347 Phenotyping of immune cell subsets will be measured using whole blood (collected in
9
10 348 EDTA tubes). The various cell types will be identified using specific fluorescently labelled
11
12 349 monoclonal antibodies (mAb) to surface receptors (See Supplementary Table 4 for list of
13
14 350 antibodies). These will be quantified by flow cytometry, as previously described [46]. With
15
16 351 the remaining blood, peripheral mononuclear cells will be isolated and purified on a Ficoll
17
18 352 density gradient of Histopaque 1077 as previously described [46,47]. To measure cytokine
19
20 353 production in isolated lymphocytes, cells will be cultured in media with or without the
21
22 354 mitogens, Phytohemagglutinin (PHA) or Lipopolysaccharide (LPS) for 48 h as previously
23
24 355 described [48]. Supernatant will be collected and stored at -80°C for *ex vivo* measures of
25
26 356 immune function (ability and pattern of cytokines produced after stimulation). IL-1 β , IL-2, IL-
27
28 357 6, IL-10, TNF α , and IFN- γ (pg/ml) cytokines will be measured using electrochemiluminescent
29
30 358 multiplex assays (MesoScale Discovery) or by individual ELISA assays. Cytokines listed
31
32 359 above and inflammatory markers including C-reactive protein (CRP) in plasma will be
33
34 360 measured electrochemiluminescent multiplex assays (MesoScale Discovery) as previously
35
36 361 described [49]. Cytokines and inflammatory markers in plasma and cytokines from cultured
37
38 362 lymphocytes will be analyzed when all samples have been collected. Changes in systemic
39
40 363 immune function will be assessed in patients compared to baseline and compared between
41
42 364 groups. The data analysis will occur at completion of trial. Cytokines are done in duplicate and
43
44 365 the coefficient of variance is <15%. Phenotypes will be collected as a relative percent of total
45
46 366 cells and the change compared between treatments. Additionally, white blood cells that are not
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48 367 used for the immune assays will be assessed for fatty acid composition.
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6 369 If DHA incorporation in plasma phospholipids is significantly different within the DHA
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8 370 supplementation arm, factors that may influence incorporation will be compared in low vs high
9
10 371 incorporators, to identify possible factors that predict incorporation, including, including BMI,
11
12 372 age, the estimated macronutrient intake and composition of dietary fat of the women
13
14
15 373 (estimated from the FFQ), histology of the tumor (provided from the biopsy), the amount of
16
17 374 DHA consumed (adherence to the supplement) and length of time DHA consumed (if treatment
18
19 375 is ended early) . We will also assess incorporation of other fatty acids (palmitic, oleic, linoleic,
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21 376 linolenic, eicosapentaenoic, docosapentaenoic) to determine if there are differences between or
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23
24 377 within treatment groups
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27 378 Caspase-3 changes and changes in CD4 and CD8 will be tested by
28
29 379 immunohistochemistry (IHC) by the diagnostic biomarker laboratory at the Cross Cancer
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31 380 Institute on 4 µm sections from formalin fixed paraffin embedded (FFPE) surgical specimens.
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34 381 At final analyses, IHC staining will be interpreted by image analysis. At time of interpretation,
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36 382 slides will be de-identified and coded to maintain the blind. All values (routine and image
37
38 383 analysis) will be recorded as absolute percentage and as log-transformation. Caspase-3 is a
39
40 384 validated marker of apoptosis and CD4 and CD8 are validated marker for lymphocytes. The
41
42 385 calculated indices (absolute %, log transformed and H-score) of biopsy and surgical resection
43
44 386 will be compared on each participant and between participants receiving DHA compared to
45
46 387 placebo.
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51 388 Pathological complete response in resected breast tissue and axillary nodes will be
52
53 389 assessed in hematoxylin and eosin stained tissue for evidence of invasive disease according to
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3 390 standard of care. Pathological complete response will be assessed following breast surgery as
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5 391 per standard of care and recorded in patient's case report form. The rate of pathological
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8 392 complete response in breast tissue and axillary nodes after surgical resection will be compared
9
10 393 between participants receiving DHA supplementation compared to placebo.

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12 394 Grade 3 and 4 toxicities will be assessed and recorded by the clinical trial nurse.

13
14 395 Toxicities will be assessed on day 1 (\pm 3 days) of each chemotherapy cycle. Dates of
15
16
17 396 hospitalization will be recorded in patient's case report form. Rates of chemotherapy-associated
18
19 397 grade 3/4 toxicities, all grade neuropathy and hospitalizations will be compared between DHA
20
21 398 supplementation and placebo arms as scored by a medical oncologist in a standardized toxicity/
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24 399 neuropathy form for each cycle of chemotherapy.

25
26 400 *Exploratory outcomes:*

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28 401 The FFQ is a validated questionnaire for macronutrient intake [50-52]. The quality of life
29
30 402 questionnaire is a validated questionnaire from European Organization for Research and
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33 403 Treatment of Cancer- Quality of Life Questionnaire-C30 (EORTC-QLQ-C30) [53]. Exercise
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35 404 behavior will be assessed using the modified Godin Leisure-Time Exercise Questionnaire
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37 405 (GLTEQ) [54,55]. Assessment in changes in quality of life and exercise behavior will be
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40 406 assessed from timepoints collected to baseline within and between treatment groups. We do not
41
42 407 expect the supplement/ placebo to influence this variable but since exercise alters immune
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44 408 function, quality of life and tumor growth we have included it herein to determine if it changes
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47 409 during therapy.

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49 410 The rate of breast conservation, specifically the rate of lumpectomy and modified radical
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51 411 mastectomy, will be determined by surgical and pathologic reports at time of surgical resection.
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54 412 Volume estimates of blood loss will be assessed by review of surgical report estimates of blood
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3 413 loss to see if there is a qualitative or quantitative difference between placebo and treatment arms,
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5 414 once adjusted for the type of surgery (lumpectomy vs. mastectomy vs. mastectomy + immediate
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7 415 reconstruction; sentinel node dissection vs. full axillary dissection). Local control, relapse free
8
9 416 survival and overall survival will be analyzed by electronic medical record and / or paper
10
11 417 medical chart review at 3, 5, and 10 years. Data will be validated by a medical oncologist.
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15 418 **Data Management**

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19 419 All data will be entered and maintained in REDCap trial database. Direct access to
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21 420 clinical and laboratory information on the enrolled trial patients will be limited to the principal
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23 421 investigator, co-investigators, trainees/staff who have had the appropriate training and approval
24
25 422 and study nurses and study coordinators who will have access to the source documents through
26
27 423 the electronic medical record and laboratory information system at the Cross Cancer Institute.
28
29 424 All patients will have biopsy and tumor samples for analysis and we do not expect any missing
30
31 425 data for the primary endpoint (Ki67). If supplement compliance is below 50% for more than
32
33 426 two consecutive cycles, or participants do not complete chemotherapy (to a minimum of 4
34
35 427 cycles), they will be excluded from final analysis of the primary end point. If patients do not
36
37 428 have sufficient blood samples for the secondary analyses (DHA incorporation, systemic
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39 429 immune function), analysis will be performed using data from the remaining patients.
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45 430 **Statistical Methods**

46 431 *Primary Outcome:*

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49 432 The percent change in Ki67 will be determined as an absolute percentage and H-score. The
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51 433 number of patients showing a decrease and the 95% confidence interval for the mean percent
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53 434 change in the Ki67 level from baseline in patients receiving DHA supplementation will be
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3 435 compared to patients receiving placebo. The mean change will be measured using independent
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5 436 t-test between the two groups.
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8 437 *Secondary Outcomes:*
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10 438 Paired t-test will be used to compare the mean percent change in the plasma DHA level of the
11
12 439 patients after each cycle of chemotherapy with their baseline values. If the data is not normally
13
14 440 distributed, the Wilcoxon signed rank test will be used to compare the plasma DHA level after
15
16 441 each cycle of chemotherapy with baseline. The difference in plasma phospholipid DHA from
17
18 442 baseline and between DHA supplementation and placebo arms will be calculated, and the 95%
19
20 443 confidence interval for the mean percent change in DHA level from baseline and groups will be
21
22 444 assessed.
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27 445 If systemic immune function data is not normally distributed, it will be log transformed prior
28
29 446 to analysis and the normality assumptions will be tested again. Repeated measures ANOVA with
30
31 447 post hoc analysis will be used to determine if there is an effect of treatment on immune function.
32
33

34 448 Factors affecting DHA incorporation will be examined by independent t-test to compare the
35
36 449 mean values between the DHA and placebo groups. Chi-square tests will be conducted to
37
38 450 determine correlation between two categorical variables for the outcome measures listed.
39
40 451 The within subject and between subject variability between the two groups for the mean percent
41
42 452 change in apoptosis, tumor infiltrating lymphocyte markers will be tested using generalized
43
44 453 estimating equation (GEE) method.
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48 454 The 95% confidence interval using independent t-test will be conducted for the mean percent
49
50 455 change in pathological complete response and rates of grade 3 and 4 chemotherapy associated
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52 456 toxicities and hospitalization in patients receiving DHA supplementation compared to patients
53
54 457 receiving placebo.
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3 458 *Exploratory outcomes:*
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5 459 Independent t-test for macronutrient and fat content obtained from the food frequency
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8 460 questionnaire will be examined between groups. Paired t-test for continuous variables and
9
10 461 McNemar's test for categorical variables will be assessed for mean percent change in events
11
12 462 between treatment arms for the quality of life and exercise questionnaires. Chi-square tests will
13
14 463 be used to compare the degree of breast conservation and the volume of surgical blood loss will
15
16 464 employ an independent t-test between the two study arms. Rate of local control will be compared
17
18 465 between treatment arms using t-test of proportions. Recurrence-free survival and survival will be
19
20 466 analyzed using the log rank test on Kaplan-Meier survival curves.
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24 467 SAS software, version 9.4 (SAS Institute Inc., Cary, NC), will be used for statistical
25
26 468 analysis. A p-value <0.05 level will be used for all statistical significance. Two-sided tests will
27
28 469 be used for all statistical tests.
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31 470 **Data Monitoring**
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34 471 The trial activities performed at the Cross Cancer Institute will be monitored by the
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36 472 Cross Cancer Institute, Investigator Initiated Trials Data Safety Monitoring Board (DSMB).
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38 473 The DSMB is independent of the investigator and is composed of representatives from both
39
40 474 medical and radiation oncology.
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44 475 The investigator will assess the relationship between protocol treatment and the
45
46 476 occurrence of adverse events (AEs) and this assessment will be recorded in the database for
47
48 477 adverse events. This study will use the International Common Terminology Criteria for
49
50 478 Adverse Events (CTCAE), version 5.0, for adverse event reporting. The reporting period for
51
52 479 adverse events will start at the time the patient takes the first dose of DHA/placebo through and
53
54 480 including 28 calendar days after last administration of study agent. If serious adverse reaction
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3 481 to treatment occurs, the Natural and Non-prescription Health Products Directorate (NNHPD),
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5 482 Clinical Trial Unit, Health Ethics Research Board of Alberta, Cancer Committee (HREBA.CC)
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7
8 483 and DSM will be notified as per guidelines. After 25 evaluable patients, all data and results will
9
10 484 be submitted to the DSMB for review.

13 485 **Auditing**

16 486 As per the SPIRIT guidelines, the investigators, Cross Cancer Institute and the
17
18 487 University of Alberta will permit trial-related monitoring, audits, REB, DSMB review, and
19
20 488 regulatory inspection(s), providing direct access to paper and/or electronic documentation
21
22 489 pertaining to the clinical study (e.g. CRFs, source documents such as hospital patient charts and
23
24 490 investigator study files). All site facilities related to the study conduct could be visited during
25
26 491 an audit (e.g. pharmacy, laboratory, outpatient department) and are agreed to co-operate and
27
28 492 provide assistance at reasonable times and places with respect to any auditing activity.
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33 493 **Patient and Public Involvement**

36 494 Patients were not involved in the protocol development or study design. However,
37
38 495 oncologists and clinical trial nurses who work in the breast tumor group are involved in patient
39
40 496 screening to assess eligibility for the study. The HREBA-CC approved informed consent will be
41
42 497 obtained from patients prior to their involvement in the study and it informs patients of their right
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44 498 to withdraw at anytime. At the end of the trial, results will be disseminated to the public through
45
46 499 seminars, public talks and in peer-reviewed journals.
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50 500 **Ethics and dissemination**

51
52 501 DHAWIN has received Health Canada approval (#HC6-24-c220167), full ethical approval
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54 502 from the Health Research Ethics Board of Alberta – Cancer Committee (Protocol #:
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3 503 HREBA.CC- 18-0381) and is registered at clinicaltrials.gov (Identifier: NCT03831178).
4
5 504 Protocol amendments will be submitted to HREBA.CC, Health Canada and the clinical trial
6
7 505 registry prior to study implementation according to regulatory requirements. The formal consent
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9 506 of a participant, using the HREBA-CC-approved consent form (Supplemental File 1), will be
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11 507 obtained by a clinical trial nurse before the participant is enrolled in the study and will be signed
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13 508 by the patient, and the principle investigator. A voluntary optional consent form for use of
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15 509 participant data and biological specimens (Supplemental File 2), will be offered at time of
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17 510 enrollment. Patient confidentiality and anonymity will be maintained and identities protected
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19 511 from unauthorized parties.
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24 512 Access to data will be restricted to the primary investigators and statistician. They will
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26 513 grant access to other team members as governed and approved by ethics. Ancillary care post-trial
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28 514 will occur as routine standard of care for all participants. Our objective is to determine the
29
30 515 efficacy of using DHA supplementation concomitant with chemotherapy and as such our results
31
32 516 will be disseminated to clinicians for implementation in future treatment paradigms. The results
33
34 517 will be submitted to peer-reviewed journals and presented at national and international
35
36 518 conferences.
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40 519 **Funding Statement**

41
42
43 520 This study is supported by the Canadian Institutes of Health Research [Grant Number:
44
45 521 RES0037745], Cross Cancer Institute Investigator Initiated Trials [Grant Number: IIT-0005]
46
47 522 and a gift from the Butler Family Foundation, Edmonton Alberta.
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51 523 **Competing Interests Statement**

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55 524 There are no financial or competing interests or conflicts to declare.
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525 **Author Contributions**

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

542 **Appendices:**

543 Supplemental File 1: Informed consent

544 Supplemental File 2: Optional consent

545 Supplemental Table 1: Spirit Checklist

546 Supplemental Table 2: WHO Checklist

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

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3 548 Supplemental Table 4: List of Antibodies for Immune cell subset identification
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5 549 **FIGURE LEGENDS**
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8 550 **Figure 1** Flowchart of Trial Design with Endpoints and Proposed Experimental Analyses
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10 551 **Figure 2** SPIRIT patient flow diagram of the DHA WIN trial
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For peer review only

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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	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	X

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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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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 cytokine	a) Flow cytometry b and c) ELISA and MesoScale	Repeated Measures ANOVA with post hoc analysis

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

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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
	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
3. Exercise			
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

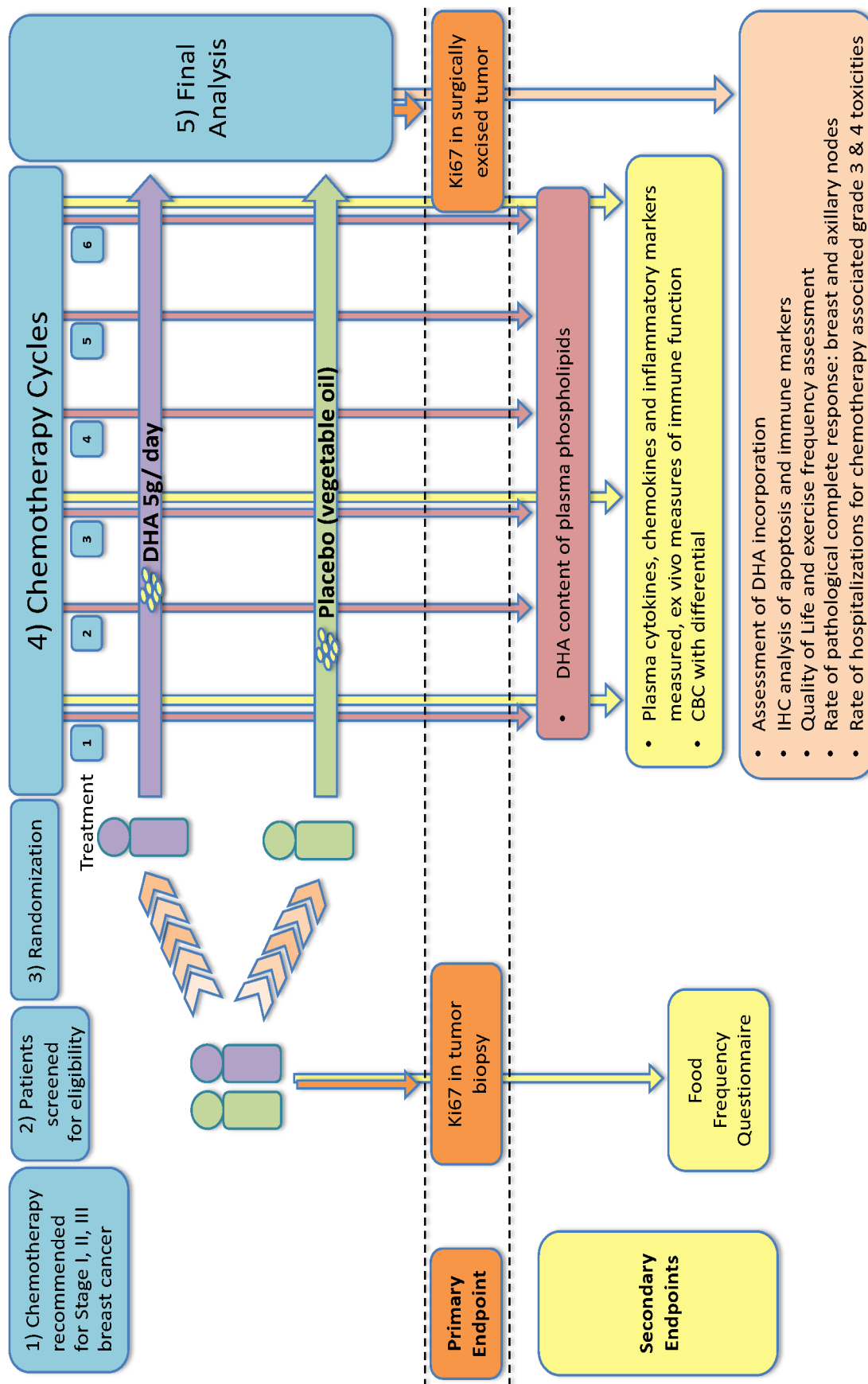
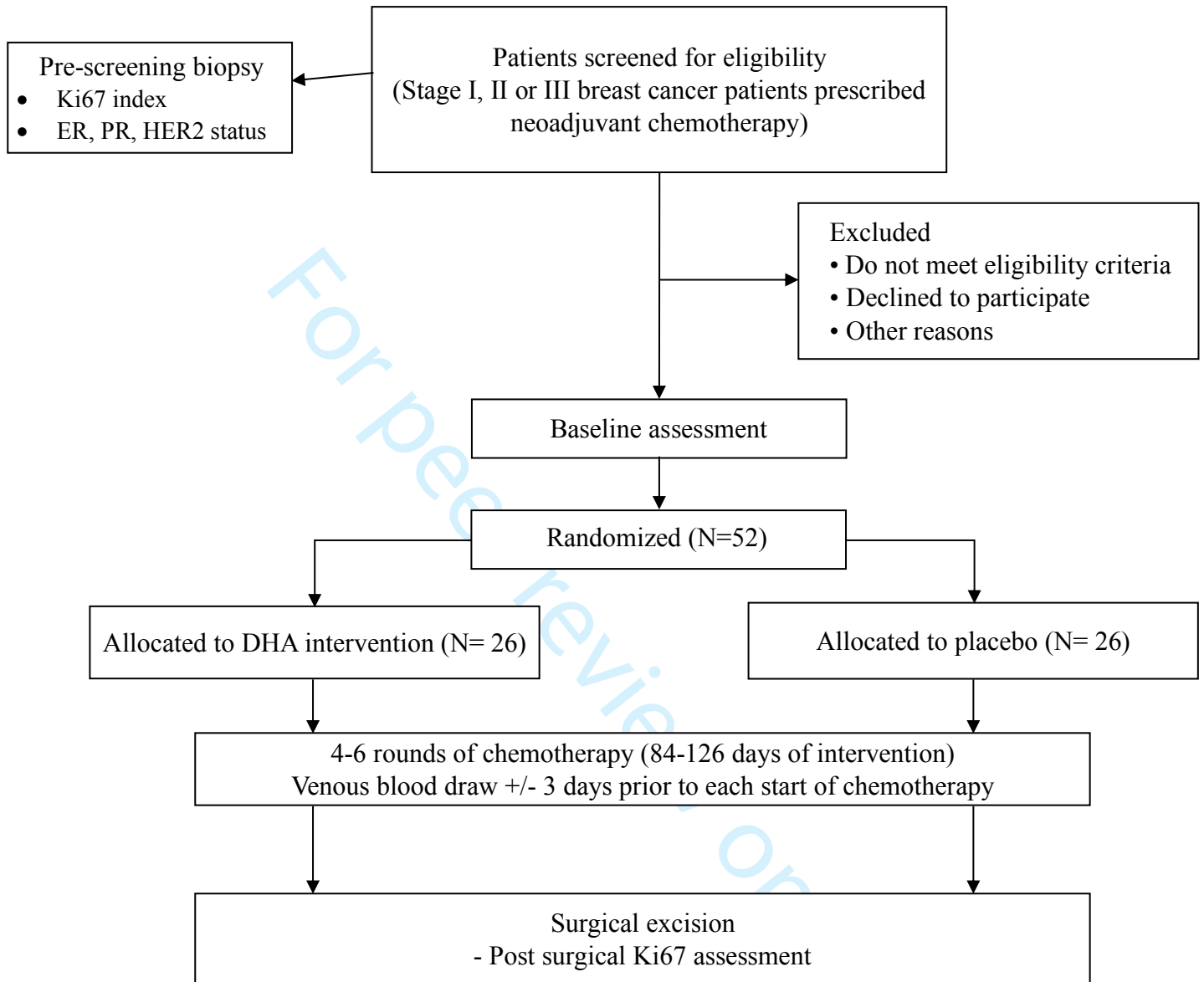


Figure 2



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.

Dr. John Mackey, Cross Cancer Institute, 11560 University Ave, Edmonton, AB, www.albertahealthservices.ca

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.

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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 approximately 12-18 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.

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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 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.

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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 Within 28 days after last dose	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)		
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

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disease-related biomarkers (signs related to your disease)														
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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 (Within 28 days after last dose)	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)		
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	

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HREBA-CC ICF DHA WIN

treatment																	
We will collect results from your surgery report																	X

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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;

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- 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 μ g/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 accidentally 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: _____

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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.

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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.

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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.

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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.



SIGNATURES

Part 1 - to be completed by the potential participant.

	<u>Yes</u>	<u>No</u>
Do you understand that you have been asked to take part in a research study?	<input type="checkbox"/>	<input type="checkbox"/>
Do you understand why this study is being done?	<input type="checkbox"/>	<input type="checkbox"/>
Do you understand the potential benefits of taking part in this study?	<input type="checkbox"/>	<input type="checkbox"/>
Do you understand the risks of taking part in this study and the risks of becoming pregnant or fathering a child during this study?	<input type="checkbox"/>	<input type="checkbox"/>
Do you understand what you will be asked to do should you decide to take part in this study?	<input type="checkbox"/>	<input type="checkbox"/>
Do you understand the alternatives to participating in this study?	<input type="checkbox"/>	<input type="checkbox"/>
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?	<input type="checkbox"/>	<input type="checkbox"/>
Do you understand who will see your records, including health information that identifies you?	<input type="checkbox"/>	<input type="checkbox"/>
Do you understand that by signing this consent form you are giving us permission to access your health information and specimens if applicable?	<input type="checkbox"/>	<input type="checkbox"/>
Do you understand that by signing this consent form that you do not give up any of your legal rights?	<input type="checkbox"/>	<input type="checkbox"/>
Have you had enough opportunity to ask questions and discuss this study?	<input type="checkbox"/>	<input type="checkbox"/>

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

For peer review only

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
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Version date: August 17, 2018

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Ethics ID: HREBA.CC-18-0381

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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.

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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 THIS 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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2
3 study.

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6 **IS THERE ANY CONFLICT OF INTEREST RELATED TO THIS OPTIONAL RESEARCH?**

7 There are no current or potential conflicts of interest concerning the optional research
8 study.
9

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

14
15 If you have questions about the use of your samples/data for optional research, or if you
16 suffer a research-related injury, contact the researcher of this optional study:
17

18
19
20 Catherine J Field
21 Name

22 780-492-5297
23 Telephone Number

24
25
26
27 If you have questions about your rights as a participant or about ethical issues related to
28 this optional research and you would like to speak to someone not involved in its conduct,
29 please contact the Office of the Health Research Ethics Board of Alberta – Cancer
30 Committee at: 780-423-5727 or toll-free 1-877-423-5727.
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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.

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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

1			
2		6b	Explanation for choice of comparators 7
3			
4	Objectives	7	Specific objectives or hypotheses 5
5			
6	Trial design	8	Description of trial design including type of trial (eg, 6, Fig 1, parallel group, crossover, factorial, single group), allocation ratio, and framework (eg, superiority, equivalence, noninferiority, exploratory) Fig 2
7			
8			
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12	Methods: Participants, interventions, and outcomes		
13			
14	Study setting	9	Description of study settings (eg, community clinic, 6 academic hospital) and list of countries where data will be collected. Reference to where list of study sites can be obtained
15			
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19	Eligibility criteria	10	Inclusion and exclusion criteria for participants. If 7 applicable, eligibility criteria for study centres and individuals who will perform the interventions (eg, surgeons, psychotherapists) Table 1 (page 1,2)
20			
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25	Interventions	11a	Interventions for each group with sufficient detail to 7 allow replication, including how and when they will be administered
26			
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30		11b	Criteria for discontinuing or modifying allocated 8 interventions for a given trial participant (eg, drug dose change in response to harms, participant request, or improving/worsening disease)
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35		11c	Strategies to improve adherence to intervention 8 protocols, and any procedures for monitoring adherence (eg, drug tablet return, laboratory tests)
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40		11d	Relevant concomitant care and interventions that are 7 permitted or prohibited during the trial -
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42			
43	Outcomes	12	Primary, secondary, and other outcomes, including 8-11 the specific measurement variable (eg, systolic blood pressure), analysis metric (eg, change from baseline, final value, time to event), method of aggregation Table 3 (eg, median, proportion), and time point for each outcome. Explanation of the clinical relevance of chosen efficacy and harm outcomes is strongly recommended
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53	Participant timeline	13	Time schedule of enrolment, interventions (including 12, Table any run-ins and washouts), assessments, and visits 2 for participants. A schematic diagram is highly recommended (see Figure)
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2	Sample size	14	Estimated number of participants needed to achieve	12
3			study objectives and how it was determined,	
4			including clinical and statistical assumptions	
5			supporting any sample size calculations	
6				
7	Recruitment	15	Strategies for achieving adequate participant	13
8			enrolment to reach target sample size	
9				

Methods: Assignment of interventions (for controlled trials)

Allocation:

14				
15	Sequence	16a	Method of generating the allocation sequence (eg,	13
16	generation		computer-generated random numbers), and list of	
17			any factors for stratification. To reduce predictability	
18			of a random sequence, details of any planned	
19			restriction (eg, blocking) should be provided in a	
20			separate document that is unavailable to those who	
21			enrol participants or assign interventions	
22				
23				
24	Allocation	16b	Mechanism of implementing the allocation sequence	13
25	concealment		(eg, central telephone; sequentially numbered,	
26	mechanism		opaque, sealed envelopes), describing any steps to	
27			conceal the sequence until interventions are	
28			assigned	
29				
30				
31	Implementation	16c	Who will generate the allocation sequence, who will	13
32			enrol participants, and who will assign participants to	
33			interventions	
34				
35	Blinding (masking)	17a	Who will be blinded after assignment to interventions	13
36			(eg, trial participants, care providers, outcome	
37			assessors, data analysts), and how	
38				
39				
40		17b	If blinded, circumstances under which unblinding is	13
41			permissible, and procedure for revealing a	
42			participant's allocated intervention during the trial	
43				

Methods: Data collection, management, and analysis

46	Data collection	18a	Plans for assessment and collection of outcome,	14-18
47	methods		baseline, and other trial data, including any related	Table 3
48			processes to promote data quality (eg, duplicate	
49			measurements, training of assessors) and a	
50			description of study instruments (eg, questionnaires,	
51			laboratory tests) along with their reliability and	
52			validity, if known. Reference to where data collection	
53			forms can be found, if not in the protocol	
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2		18b	Plans to promote participant retention and complete
3			follow-up, including list of any outcome data to be
4			collected for participants who discontinue or deviate
5			from intervention protocols
6			
7	Data management	19	Plans for data entry, coding, security, and storage,
8			including any related processes to promote data
9			quality (eg, double data entry; range checks for data
10			values). Reference to where details of data
11			management procedures can be found, if not in the
12			protocol
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15	Statistical methods	20a	Statistical methods for analysing primary and
16			secondary outcomes. Reference to where other
17			details of the statistical analysis plan can be found, if
18			not in the protocol
19			
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21			
22		20b	Methods for any additional analyses (eg, subgroup
23			and adjusted analyses)
24			
25		20c	Definition of analysis population relating to protocol
26			non-adherence (eg, as randomised analysis), and
27			any statistical methods to handle missing data (eg,
28			multiple imputation)
29			
30			
31	Methods: Monitoring		
32	Data monitoring	21a	Composition of data monitoring committee (DMC);
33			summary of its role and reporting structure;
34			statement of whether it is independent from the
35			sponsor and competing interests; and reference to
36			where further details about its charter can be found,
37			if not in the protocol. Alternatively, an explanation of
38			why a DMC is not needed
39			
40			
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42		21b	Description of any interim analyses and stopping
43			guidelines, including who will have access to these
44			interim results and make the final decision to
45			terminate the trial
46			
47	Harms	22	Plans for collecting, assessing, reporting, and
48			managing solicited and spontaneously reported
49			adverse events and other unintended effects of trial
50			interventions or trial conduct
51			
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53	Auditing	23	Frequency and procedures for auditing trial conduct,
54			if any, and whether the process will be independent
55			from investigators and the sponsor
56			
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58	Ethics and dissemination		
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2	Research ethics	24	Plans for seeking research ethics	21
3	approval		committee/institutional review board (REC/IRB)	
4			approval	
5				
6	Protocol	25	Plans for communicating important protocol	21
7	amendments		modifications (eg, changes to eligibility criteria,	
8			outcomes, analyses) to relevant parties (eg,	
9			investigators, REC/IRBs, trial participants, trial	
10			registries, journals, regulators)	
11				
12				
13	Consent or assent	26a	Who will obtain informed consent or assent from	21
14			potential trial participants or authorised surrogates,	Supp file
15			and how (see Item 32)	
16				
17		26b	Additional consent provisions for collection and use	21
18			of participant data and biological specimens in	Supp file
19			ancillary studies, if applicable	
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22	Confidentiality	27	How personal information about potential and	22
23			enrolled participants will be collected, shared, and	
24			maintained in order to protect confidentiality before,	
25			during, and after the trial	
26				
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28	Declaration of	28	Financial and other competing interests for principal	22
29	interests		investigators for the overall trial and each study site	
30				
31	Access to data	29	Statement of who will have access to the final trial	22
32			dataset, and disclosure of contractual agreements	
33			that limit such access for investigators	
34				
35	Ancillary and post-	30	Provisions, if any, for ancillary and post-trial care,	22
36	trial care		and for compensation to those who suffer harm from	
37			trial participation	
38				
39				
40	Dissemination	31a	Plans for investigators and sponsor to communicate	22
41	policy		trial results to participants, healthcare professionals,	
42			the public, and other relevant groups (eg, via	
43			publication, reporting in results databases, or other	
44			data sharing arrangements), including any	
45			publication restrictions	
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48		31b	Authorship eligibility guidelines and any intended use	22
49			of professional writers	
50				
51		31c	Plans, if any, for granting public access to the full	-
52			protocol, participant-level dataset, and statistical	
53			code	
54				
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56	Appendices			
57				
58	Informed consent	32	Model consent form and other related documentation	Supp file
59	materials		given to participants and authorised surrogates	
60				

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2	Biological	33	Plans for collection, laboratory evaluation, and	Supp file
3	specimens		storage of biological specimens for genetic or	
4			molecular analysis in the current trial and for future	
5			use in ancillary studies, if applicable	
6				

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

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	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

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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

Journal:	<i>BMJ Open</i>
Manuscript ID	bmjopen-2019-030502.R2
Article Type:	Protocol
Date Submitted by the Author:	09-Aug-2019
Complete List of Authors:	Newell, Marnie; University of Alberta, Department of Agricultural, Food and Nutritional Science Mackey, John; University of Alberta, Department of Oncology; Alberta Health Services Bigras, Gilbert ; University of Alberta, Department of Laboratory Medicine and Pathology Alvarez-Camacho, Mirey; Alberta Health Services Goruk, Susan; University of Alberta, Department of Agricultural, Food and Nutritional Science Ghosh, Sunita; Alberta Health Services Schmidt, Alison; Alberta Health Services Miede, Deborah; Alberta Health Services Chisotti, Ann; Alberta Health Services Postovit, Lynne; University of Alberta, Department of Oncology Baker, Kristi; University of Alberta, Department of Oncology Mazurak, Vera; University of Alberta, Department of Agricultural, Food and Nutritional Science Courneya, Kerry; University of Alberta, Faculty of Kinesiology, Sport and Recreation Berendt, Richard; University of Alberta, Department of Laboratory Medicine and Pathology Dong, Wei-Feng; University of Alberta, Department of Laboratory Medicine and Pathology Wood, George; University of Alberta, Department of Laboratory Medicine and Pathology Basi, Sanraj; Alberta Health Services Joy, Anil Abraham; Department of Oncology King, Karen; Alberta Health Services Meza-Junco, Judith; Alberta Health Services Zhu, Xiaofu; Alberta Health Services Field, Catherine; University of Alberta, Department of Agricultural, Food and Nutritional Science
Primary Subject Heading:	Oncology
Secondary Subject Heading:	Nutrition and metabolism, Immunology (including allergy), Patient-

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



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

4 Protocol Number: IIT-0005

5 Version Date: August 8, 2019

6 Marnie Newell¹, John R. Mackey^{2,3}, Gilbert Bigras⁴, Mirey Alvarez-Camacho², Susan
7 Goruk¹, Sunita Ghosh², Alison Schmidt², Deborah Miede², Ann Chisotti², Lynne Postovit³, Kristi
8 Baker³, Vera Mazurak¹, Kerry S. Courneya⁵, Richard Berendt⁴, Wei-Feng Dong⁴, George
9 Wood⁴, Sanraj K. Basi², Anil Abraham Joy², Karen King², Judith Meza-Junco², Xiaofu Zhu² and
10 Catherine J. Field^{1*}

11
12 ¹Department of Agricultural, Food and Nutritional Science, Faculty of Agricultural, Life and
13 Environmental Sciences, University of Alberta

14 ²Alberta Health Services - Cancer Control, Cross Cancer Institute

15 ³Department of Oncology, Faculty of Medicine & Dentistry, University of Alberta

16 ⁴Department of Laboratory Medicine and Pathology, Faculty of Medicine & Dentistry,
17 University of Alberta

18 ⁵Faculty of Kinesiology, Sport, and Recreation, University of Alberta

19 * Corresponding author: Catherine J. Field, PhD, Faculty of Agricultural, Life and Environmental
20 Sciences, University of Alberta, 4-126 Li Ka Shing Centre, Edmonton, Alberta, Canada, T6G
21 2H9. Tel: (780) 492-2597, E-mail: catherine.field@ualberta.ca

22 Word Count: 5751

23 **ABSTRACT**

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

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

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

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

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3 43 *Ethics and dissemination:* This study has full approval from the Health Research Ethics Board of
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5 44 Alberta – Cancer Committee (Protocol #: HREBA.CC -18-0381). We expect to present the
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7 45 findings of this study to the scientific community in peer-reviewed journals and at conferences.
8
9 46 The results of this study will provide evidence for supplementing with DHA during neoadjuvant
10
11 47 chemotherapy treatment for breast cancer.

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15 48 Clinical Trial Registration No: NCT03831178

16 17 49 **KEYWORDS**

18
19 50 Ki67, phospholipids, fatty acids, omega-3, apoptosis, proliferation, immune function

20 21 22 51 **STRENGTHS AND LIMITATIONS OF THIS STUDY**

- 23
24
25 52 • This study is the first phase II randomized controlled trial to evaluate DHA
26
27 53 supplementation concomitant with neoadjuvant chemotherapy to treat non-metastatic
28
29 54 breast cancer.
- 30
31
32 55 • The intervention is minimally invasive and side effects from the supplementation are not
33
34 56 expected.
- 35
36
37 57 • This study is powered to examine the key clinical outcome of changes in Ki67 index
38
39 58 from pre-chemotherapy biopsy to surgical excision based on group sample sizes of 23
40
41 59 patients in group one and 23 patients in group two in order to achieve 81% power to
42
43 60 detect a difference between the group proportions of 0.4.
- 44
45
46 61 • This study will measure clinically relevant intermediate outcomes including rate of pCR
47
48 62 in breast and in axillary nodes, rate of grade 3 and 4 chemotherapy associated toxicities
49
50 63 and hospitalizations as well as additional outcomes including plasma phospholipid
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52 64 content of DHA, markers of immune function (plasma cytokines, inflammatory markers

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3 65 and lymphocyte function), tumor markers for apoptosis and tumor infiltrating
4
5 66 lymphocytes and patient perceived quality of life.
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8 67 • The study will include all subtypes of breast cancer patients undergoing neoadjuvant
9
10 68 chemotherapy but is not powered to assess differences between subtypes.
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13 69 INTRODUCTION

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16 70 Despite improvements in early diagnosis and treatment, breast cancer remains the second leading
17
18 71 cause of cancer related death in women [1]. While neoadjuvant chemotherapy aims to improve
19
20 72 surgical resection outcomes and reduce/eliminate micrometastases [2,3], pathological complete
21
22 73 response (pCR) is not achieved by all patients [3]. Increasing the efficacy of neoadjuvant
23
24 74 treatment without adding additional side-effects would benefit this population.
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27

28 75 DHA is an omega-3 long chain polyunsaturated fatty acid (n-3 LCPUFA). The majority
29
30 76 of n-3 fatty acids are in the form of the 18-carbon fatty acid alpha-linolenic acid (ALA). While
31
32 77 DHA can be synthesized from ALA and other n-3 LCPUFA in the body, endogenous synthesis
33
34 78 is low [4,5]. Consequently, the direct consumption of this fatty acid is the only way to
35
36 79 significantly increase levels of DHA in tissues [6]. Supplementation can increase blood plasma
37
38 80 DHA concentration by 2-fold (500 μ M), which can lead to plasma membrane lipid enrichment
39
40 81 [7]. Incorporation of DHA into tumor membrane phospholipids has been shown to reduce
41
42 82 breast cancer cell proliferation [8,9] and increase apoptosis [10-15] in vitro and decreases tumor
43
44 83 growth in animal models [13,15-17]. Additionally, providing/feeding DHA has been shown to
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46 84 increase the efficacy of different chemotherapeutic drugs in animal models of breast cancer
47
48 85 [11,12,18,19]. While there is limited clinical evidence, it has been shown that increased dietary
49
50 86 intake of n-3 LCPUFA, including DHA, results in increased DHA incorporation in breast
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52 87 adipose tissue [20] and this correlates with improved response to chemotherapy [21]. In an
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3 88 open label trial with advanced metastatic breast cancer patients, DHA supplementation and
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5 89 enrichment into plasma phospholipids was associated with improved outcomes [22]. Other
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7 90 clinical trials have reported that supplementation with n-3 LCPUFA at a wide range of doses
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9 91 (0.6 g-8.6 g/day) increased tolerability of chemotherapeutic drugs in a range of malignancies at
10
11 92 other sites, include lung, pancreatic and colorectal (reviewed in [23]). Consequently, we
12
13 93 hypothesize that the therapeutic index (efficacy: toxicity ratio) of neoadjuvant breast cancer
14
15 94 chemotherapy will be improved with the addition of DHA to the treatment.
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18

19 95 Breast cancer proliferation can be assessed by immunohistochemical (IHC) analysis of
20
21 96 cells staining positive for the nuclear antigen Ki67 [24], as it is expressed in all phases of the
22
23 97 cell cycle, G₁, S, G₂, and M, but not in G₀ [25,26]. The proportion of cells staining for Ki67 is
24
25 98 frequently used as a primary endpoint to measure efficacy of neoadjuvant therapy in clinical
26
27 99 trials. The Ki67 index, defining the change between pre- and post-treatment Ki67, has been
28
29 100 reported to be an independent prognostic factor in luminal A, luminal B, triple-negative, and
30
31 101 HER2+ breast cancer, and has been reported to be a useful surrogate marker of relapse free
32
33 102 survival in luminal B, triple-negative, and HER2+ breast cancer [27,28].
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38 103 **OBJECTIVES**

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40 104 The objective of this RCT is to assess the efficacy of supplemental DHA combined with
41
42 105 neoadjuvant chemotherapy in treatment naïve women with breast cancer measured by changes in
43
44 106 Ki67 index from biopsy to surgical excision. We hypothesize that DHA supplementation will
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46 107 increase plasma phospholipid DHA and improve response to neoadjuvant chemotherapy assessed
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48 108 by a decrease in the Ki67 index.
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110 This protocol follows the Standard Protocol Items for Randomized Trials (SPIRIT) guideline
 111 (Spirit Checklist: Supplemental Table 1, WHO Checklist: Supplemental Table 2) [29,30].

112 Study Design

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

116 METHODS AND ANALYSIS

117 Study Population

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

122 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

123

124 **Intervention**

125 All women in this trial will receive standard of care chemotherapy throughout the duration of the
 126 trial. Breast cancer chemotherapy is developed in a guideline-coordinated system by a single
 127 team residing at the Cross Cancer Institute. Consequently, there are only two chemotherapy
 128 regimens that are used for neoadjuvant chemotherapy in this population. Each of the two
 129 regimens are six cycles in length and given at three-week intervals with a resultant chemotherapy
 130 regimen duration of 18 weeks. Both regimens are docetaxel based. For HER2 negative disease,
 131 patients universally receive the FEC-D (fluorouracil, epirubicin, cyclophosphamide; docetaxel)
 132 [32] regimen as neoadjuvant therapy, while HER2 positive patients receive the DCH regimen
 133 (docetaxel, carboplatinum, trastuzumab) [33].

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

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3 141 throughout the day as tolerated (at any time, with or without food). Capsules are identical in
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5 142 appearance and composition (other than the oils) to maintain blinding of participants and study
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8 143 staff. As the DHA source is an algae-synthesized triglyceride, there are no differences in texture
9
10 144 or taste.

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12
13 145 All patients will begin a cytotoxic chemotherapy regimen intended to require 18 weeks
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15 146 for delivery. The intervention (DHA or placebo) will commence at the start of the first cycle of
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17 147 chemotherapy and continue through 4-6 cycles of chemotherapy (3 weeks/cycle). Should a
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19 148 patient not be able to complete the full six cycles of therapy, the timing of surgery remains 3-5
20
21 149 weeks after the last cycle of chemotherapy is delivered. As local guidelines mandate surgery
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23 150 between 3 and 5 weeks from the last round of chemotherapy, DHA/placebo will be continued
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25 151 until this time (21-35 days after the last administration of cytotoxic chemotherapy).

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28 152 All patients will be dispensed an additional bottle of DHA/placebo capsules at the
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30 153 beginning of the study to account for circumstances where their treatment is delayed due to
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32 154 treatment associated toxicities (including but not limited to vomiting, diarrhea, abnormalities in
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34 155 blood work, fatigue or severe mouth sores). The patients will be requested to continue taking the
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36 156 DHA or placebo as tolerated and will be dispensed additional capsules as necessary. The extra
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38 157 capsules will remain with the patient until the end of the study.

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41 158 Patients will be encouraged to take the supplements as tolerated (throughout the day at
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43 159 any time, with or without food). Treatment adherence will be monitored by a review of the
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45 160 patient dosing diary and recording the number of any remaining capsules returned at the end of
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47 161 study visit following the last dose of DHA/placebo. Non-compliance will be assessed as
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49 162 consuming less than 50% of the weekly dose for 2 consecutive cycles. No additional natural
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51 163 health product is permitted beyond a daily multi-vitamin.

164 Outcome Measurements

165 Study outcome timelines are summarized in **Table 2**. Briefly, outcomes will be measured
166 at baseline, within ± 3 days of chemotherapy and/or post-intervention (surgical excision).

167 Electronic medical record and/or paper chart review of local control, relapse free survival and
168 overall survival will occur at 3, 5, and 10 years to explore possible effects on long-term outcome.

169 *Primary Outcome*

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

178 *Secondary Outcomes*

179 1) DHA incorporation into phospholipids: The changes in level of DHA incorporation in plasma
180 phospholipids will be assessed at baseline and at day 1 (± 3 days) of each cycle of chemotherapy
181 (2-6) and end of cycle 6 to identify the range of DHA incorporation in this patient population.
182 The use of plasma rather than red blood cells or whole blood for this study is supported by the
183 recent recommendations for best practices for fatty acids described by Brenna et al [38].
184 Analysis of the plasma phospholipid rather than plasma total lipids avoids the postprandial
185 fluctuation of the triacylglycerol pool and is believed to adequately represent the cell membrane
186 composition [38]. From our hypothesis and previously published data [22], it is expected that

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

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

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

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

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3 210 receiving DHA supplementation and compared to patients receiving placebo. Proportions of
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5 211 negative cells, weakly positive cells and strongly positive cells will be scored by two
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7 212 pathologists and the staining intensity, assessed by QuPath, [37] will be recorded
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10 213 independently. Increased apoptosis measured by Caspase-3 is a clinically relevant marker of
11
12 214 cell death.

13
14 215 5) Examine changes in tumor infiltrating lymphocytes (TILs): CD4+ and CD8+ in the excised
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16 216 tumor, as a number of positive cells for a given area, will be calculated by image analysis. A
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18 217 comparison post-treatment of expression levels at experimental end (surgical excision) to
19
20 218 baseline will be determined in patients receiving DHA supplementation and compared to
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22 219 patients receiving placebo. The differences will be compared between treatments and within
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24 220 the treatment group, related to plasma DHA concentrations. Increased infiltration of TILs is
25
26 221 potential marker that could be used to predict treatment patient outcomes.

27
28 222 6) Pathological complete response (pCR) rate: pCR in resected breast tissue and all sampled
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30 223 axillary nodes will be assessed as absence of invasive cancer by haematoxylin and eosin
31
32 224 evaluation as per standard of care. Pathologic complete response will be classified as ypT0/is
33
34 225 ypN0 and will be determined at the end of study after surgical resection as part of standard of
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36 226 care assessment.

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38 227 7) Comparison of rate of chemotherapy associated grade 3 and 4 toxicities: Rate of chemotherapy
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40 228 associated grade 3 and 4 toxicities, and chemotherapy-associated hospitalizations will be
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42 229 compared between DHA and placebo arms. Any changes will then be examined in regards to
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44 230 level of supplementation and DHA incorporation. These analyses will be completed at the end
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46 231 of study after surgical resection.

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49 232 *Exploratory outcomes*

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3 233 1) Food frequency questionnaire (FFQ): Assessment of the FFQ to compare the estimated (pre-
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5 234 diagnosis) usual intake of macronutrients on an energy basis (including fat content and
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7 235 composition) between the two groups at baseline. In the future, the overall medians/means of
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9 236 the subjects in this study will be compared to age-matched women in the Alberta Tomorrow
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11 237 Project.
- 12
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14 238 2) Quality of life: Assessment in changes in quality of life will be determined by questionnaire
15
16 239 employed at baseline and end of treatment. Comparisons will be assessed from end of treatment
17
18 240 to baseline within and between treatment groups.
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20
21 241 3) Exercise behavior: Assessment of exercise behavior will be determined by questionnaire
22
23 242 employed at baseline, each cycle of chemotherapy and end of treatment. Comparisons will be
24
25 243 assessed from end of treatment to baseline within and between treatment groups.
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27
28 244 4) Breast conservation: The rate of breast conservation, specifically the rate of lumpectomy and
29
30 245 mastectomy, will be determined by review of surgical and pathological reports at the end of
31
32 246 study after surgical resection.
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35 247 5) Volume of surgical blood loss: High intakes of n-3 LCPUFA (that contain some DHA) have
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37 248 been studied to determine if they increase bleeding time [39,40]. We will review surgical
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39 249 report estimates of blood loss to see if there is a qualitative or quantitative difference between
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41 250 placebo and treatment arms, once adjusted for the magnitude of surgery (lumpectomy vs.
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43 251 mastectomy vs. mastectomy + immediate reconstruction; sentinel node dissection vs. full
44
45 252 axillary dissection). It is not expected that we will see a difference as it is eicosapentaenoic
46
47 253 acid (EPA, the precursor to DHA) that has antithrombotic and antiplatelet properties [41].
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50 254 6) Local control, relapse free survival and overall survival: Local control, relapse free survival
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52 255 and overall survival will be analyzed by review of electronic medical records, registry reports,
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3 256 and/or paper medical charts at 3, 5, and 10 years to explore possible effects on long-term
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5 257 outcome.

8 258 **Participant timeline**

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10 259 Breast cancer patients receiving neoadjuvant chemotherapy account for approximately
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12 260 20% of newly diagnosed breast cancer patients, approximately 10-12/month at the Cross Cancer
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14 261 Institute. Assuming a conservative accrual rate of 30%, accrual is estimated to be completed in
15
16 262 14-18 months with 3-4 patients recruited per month. Each patient will be enrolled for the
17
18 263 duration of their individual chemotherapy regimen, an estimated 12-18 weeks (84-126 days)
19
20 264 beginning at the start of the first cycle of chemotherapy and continued through 4-6 cycles of
21
22 265 chemotherapy (3 weeks/cycle). The intervention will be discontinued 21-35 days after the last
23
24 266 administration of cytotoxic chemotherapy when surgery to remove the tumor occurs. See Figure
25
26 267 1 for a schematic of the participant timeline.

31 268 **Sample Size**

32
33
34 269 Fifty-two women prescribed neoadjuvant breast cancer chemotherapy will be enrolled in a 2-arm
35
36 270 trial with 26 participants/arm. The sample size calculation is based on the primary objective,
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38 271 which is to determine the efficacy of supplemental DHA provided with standard neoadjuvant as
39
40 272 measured by change in the Ki67 index from biopsy to surgical excision. Group sample sizes of
41
42 273 23 patients in each group are required to achieve 81% power to detect a difference between the
43
44 274 group proportions of 0.4. The proportion in group one is assumed to be 0.3 under the null
45
46 275 hypothesis and 0.7 under the alternate hypothesis. The proportion in group two which is the
47
48 276 control group is 0.3. The test statistic used is the two-sided t-test. The significance level of the
49
50 277 test was targeted at 0.05 and the significance level actually achieved by this design is about
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52 278 0.0497. Assuming a dropout rate estimated at approximately 10% for this patient population
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279 which is approximately 5 patients, a total of 52 patients (26 patients in the DHA supplementation
280 group, and 26 in the placebo group) is required for the study.

281 **Recruitment**

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

287 **Randomization and Blinding**

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

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3 302 REDCap database by the study co-ordinator. All future bottle allocations with the unique bottle
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5 303 ID will be entered into the REDCap database. The key to the study arm will be kept in password
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8 304 protected computers and will only be shared in an urgent need for breaking of the blind. When a
9
10 305 blinding code is broken, the date and reason for unblinding must be fully documented in source
11
12 306 documents and entered on the case report form. Every effort should be made by site staff to
13
14 307 ensure that the treatment arm in which the unblinded patient is assigned is communicated only to
15
16 308 those site staff that require the information for treatment purposes. To assist in maintaining the
17
18 309 blind of the patients, supplements and placebo are identical in size, shape, color and texture, in
19
20 310 addition to identical bottles for dispensing. Patients, pathologists, physicians, and researchers
21
22 311 will be blinded to patient enrolment in the study and throughout trial. Blinding will only be
23
24 312 dropped after analysis of fatty acids, systemic immune function and Ki67 is complete.
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313 **Data Collection, Management and Analysis**

314 Study methods are summarized in **Table 3**. Briefly, data will be collected and measured
315 at baseline, within ± 3 days of chemotherapy and/or post-intervention (surgical excision).
316 Electronic medical record and/or paper chart review of local control, relapse free survival and
317 overall survival will occur at 3, 5, and 10 years. All data will be entered and maintained in the
318 REDCap trial database. Baseline measurements will be analyzed once all participants have been
319 enrolled and all other analyses will occur at completion of trial.
320

320 *Primary Outcome*

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

1
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3 324 analyses, Ki67 staining will be repeated as a single IHC stain and interpreted by image analysis.
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5 325 At the time of Ki67 interpretation, slides will be de-identified and coded to ensure the
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8 326 pathologist is blinded to the experimental group. In addition, the original single stained slides
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10 327 will be interpreted visually by research staff. All Ki67 values (routine and image analysis) will
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12 328 be recorded as absolute percentage and H-score in the REDCap trial database and the
13
14 329 participants' case report form. The Ki67 index is validated and used in clinic as a marker of
15
16 330 proliferation. The Ki67 index (absolute % and H-score [42] of biopsy and surgical resection
17
18 331 (after chemotherapy) will be compared on each participant and between participants receiving
19
20 332 DHA compared to placebo.
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25 333 *Secondary Outcomes*

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28 334 DHA incorporation into plasma phospholipids will be measured in venous blood from
29
30 335 patients at baseline (time of enrolment in trial), and at day 1 (\pm 3 days) of each chemotherapy
31
32 336 cycle by a technician blinded to the treatment group. Venous blood will be collected in coated
33
34 337 EDTA tubes and centrifuged at 750x g for 10 min to obtain plasma. Red blood cells will be
35
36 338 immediately frozen and banked at -80°C for storage for future secondary analysis. Plasma will
37
38 339 be separated into 6 aliquots and immediately frozen at -80°C for storage. Plasma will be
39
40 340 extracted by the Folch procedure [43,44], phospholipids separated by thin layer
41
42 341 chromatography and fatty acid content (concentration and relative percent) measured by gas-
43
44 342 liquid chromatography as previously described [45]. The percentage change in DHA from
45
46 343 baseline will be compared in each patient and a 95% t-confidence interval for the mean percent
47
48 344 change in DHA from baseline will be compared to patients receiving placebo. An internal
49
50 345 standard is used to identify and quantify the fatty acids. This standard measure for fatty acid
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3 346 status has coefficient of variation <5% and individual GC peaks are validated against
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5 347 phospholipid standards (GLC-502 and GLC-643) from NuChek (Elysian, MN).
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7

8 348 Phenotyping of immune cell subsets will be measured using whole blood (collected in
9
10 349 EDTA tubes). The various cell types will be identified using specific fluorescently labelled
11
12 350 monoclonal antibodies (mAb) to surface receptors (See Supplementary Table 4 for list of
13
14 351 antibodies). These will be quantified by flow cytometry, as previously described [46]. With
15
16 352 the remaining blood, peripheral mononuclear cells will be isolated and purified on a Ficoll
17
18 353 density gradient of Histopaque 1077 as previously described [46,47]. To measure cytokine
19
20 354 production in isolated lymphocytes, cells will be cultured in media with or without the
21
22 355 mitogens, Phytohemagglutinin (PHA) or Lipopolysaccharide (LPS), for 48 h as previously
23
24 356 described [48]. Supernatant will be collected and stored at -80°C for *ex vivo* measures of
25
26 357 immune function (ability and pattern of cytokines produced after stimulation). IL-1 β , IL-2, IL-
27
28 358 6, IL-10, TNF α , and IFN- γ (pg/ml) cytokines will be measured using electrochemiluminescent
29
30 359 multiplex assays (MesoScale Discovery) or by individual ELISA assays. Cytokines listed
31
32 360 above and inflammatory markers including C-reactive protein (CRP) in plasma will be
33
34 361 measured by electrochemiluminescent multiplex assays (MesoScale Discovery) as previously
35
36 362 described [49]. Cytokines and inflammatory markers in plasma and cytokines from cultured
37
38 363 lymphocytes will be analyzed when all samples have been collected. Changes in systemic
39
40 364 immune function will be assessed in patients compared to baseline and compared between
41
42 365 groups. The data analysis will occur at completion of trial. Cytokines are done in duplicate and
43
44 366 the coefficient of variance is <15%. Phenotypes will be collected as a relative percent of total
45
46 367 cells and the change compared between treatments. Additionally, white blood cells that are not
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48 368 used for the immune assays will be assessed for fatty acid composition.
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6 370 If DHA incorporation into plasma phospholipids is significantly different within the
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8 371 DHA supplementation arm, factors that may influence incorporation will be compared in low
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10 372 vs. high incorporators, to identify possible factors that predict incorporation including BMI,
11
12 373 age, the estimated macronutrient intake and composition of dietary fat of the women
13
14 374 (estimated from the FFQ), histology of the tumor (provided from the biopsy), the amount of
15
16 375 DHA consumed (adherence to the supplement) and length of time DHA consumed (if treatment
17
18 376 is ended early) . We will also assess incorporation of other fatty acids (palmitic, oleic, linoleic,
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20 377 linolenic, arachidonic, eicosapentaenoic, docosapentaenoic) to determine if there are
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22 378 differences between or within treatment groups.
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27 379 Caspase-3 changes and changes in CD4 and CD8 will be tested by IHC by the diagnostic
28
29 380 biomarker laboratory at the Cross Cancer Institute on 4 μ m sections from FFPE surgical
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31 381 specimens. At final analyses, IHC staining will be interpreted by image analysis. At time of
32
33 382 interpretation, slides will be de-identified and coded to maintain the blind. All values (routine
34
35 383 and image analysis) will be recorded as absolute percentage. Caspase-3 is a validated marker of
36
37 384 apoptosis and CD4 and CD8 are validated markers for lymphocytes. The calculated indices
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39 385 (absolute % and H-score) of biopsy and surgical resection will be compared on each participant
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41 386 and between participants receiving DHA compared to placebo.
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47 387 Pathological complete response in resected breast tissue and axillary nodes will be
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49 388 assessed in hematoxylin and eosin stained tissue for evidence of invasive disease according to
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51 389 standard of care and recorded in patients' case report form. The rate of pathological complete
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3 390 response in breast tissue and axillary nodes after surgical resection will be compared between
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5 391 participants receiving DHA supplementation compared to placebo.
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8 392 Grade 3 and 4 toxicities will be assessed and recorded by the clinical trial nurse.
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10 393 Toxicities will be assessed on day 1 (\pm 3 days) of each chemotherapy cycle. Dates of
11
12 394 hospitalization will be recorded in patients' case report form. Rates of chemotherapy-associated
13
14 395 grade 3/4 toxicities, all grade neuropathy and hospitalizations will be compared between DHA
15
16 396 supplementation and placebo arms as scored by a medical oncologist in a standardized toxicity/
17
18 397 neuropathy form for each cycle of chemotherapy.
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21 398 *Exploratory outcomes:*
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24 399 The FFQ is a validated questionnaire for macronutrient intake [50-52]. The quality of life
25
26 400 questionnaire is a validated questionnaire from European Organization for Research and
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28 401 Treatment of Cancer- Quality of Life Questionnaire-C30 (EORTC-QLQ-C30) [53]. Exercise
29
30 402 behavior will be assessed using the modified Godin Leisure-Time Exercise Questionnaire
31
32 403 (GLTEQ) [54,55]. Assessment of changes in quality of life and exercise behavior will be
33
34 404 compared from timepoints collected to baseline within and between treatment groups. We do not
35
36 405 expect the supplement/placebo to influence this variable but since exercise alters immune
37
38 406 function, quality of life and tumor growth we have included it herein to determine if it changes
39
40 407 during therapy.
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44 408 The rate of breast conservation, specifically the rate of lumpectomy and modified radical
45
46 409 mastectomy, will be determined by surgical and pathological reports at time of surgical
47
48 410 resection. Volume estimates of blood loss will be assessed by review of surgical reports to see if
49
50 411 there is a qualitative or quantitative difference between placebo and treatment arms, once
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52 412 adjusted for the type of surgery (lumpectomy vs. mastectomy vs. mastectomy + immediate
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3 413 reconstruction; sentinel node dissection vs. full axillary dissection). Local control, relapse free
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5 414 survival and overall survival will be analyzed by electronic medical record and/or paper medical
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7 415 chart review at 3, 5, and 10 years. Data will be validated by a medical oncologist.
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10 416 **Data Management**

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14 417 All data will be entered and maintained in the REDCap trial database. Direct access to
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16 418 clinical and laboratory information on enrolled trial patients will be limited to the principal
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18 419 investigator, co-investigators, trainees/staff who have had the appropriate training and approval
19
20 420 and study nurses and study coordinators who will have access to the source documents through
21
22 421 the electronic medical record and laboratory information system at the Cross Cancer Institute.
23
24 422 All patients will have biopsy and tumor samples for analysis and we do not expect any missing
25
26 423 data for the primary endpoint (Ki67). If supplement compliance is below 50% for more than
27
28 424 two consecutive cycles, or if participants do not complete chemotherapy (to a minimum of 4
29
30 425 cycles), they will be excluded from final analysis of the primary endpoint. If patients do not
31
32 426 have sufficient blood samples for the secondary analyses (DHA incorporation, systemic
33
34 427 immune function), analysis will be performed using data from the remaining patients.
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40 428 **Statistical Methods**

41 429 *Primary Outcome:*

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43 430 The percent change in Ki67 will be determined as an absolute percentage and H-score. The
44
45 431 number of patients showing a decrease and the 95% confidence interval for the mean percent
46
47 432 change in the Ki67 level from baseline in patients receiving DHA supplementation will be
48
49 433 compared to patients receiving placebo. The mean change will be measured using an
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51 434 independent t-test between the two groups.
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3 435 *Secondary Outcomes:*
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5 436 Paired t-tests will be used to compare the mean percent change in the plasma DHA level of
6
7 437 the patients after each cycle of chemotherapy with their baseline values. If the data is not normally
8
9 438 distributed, the Wilcoxon signed rank test will be used to compare the plasma DHA level after
10
11 439 each cycle of chemotherapy with baseline. The difference in plasma phospholipid DHA from
12
13 440 baseline and between DHA supplementation and placebo arms will be calculated, and the 95%
14
15 441 confidence interval for the mean percent change in DHA level from baseline and groups will be
16
17 442 assessed.
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20
21 443 If systemic immune function data is not normally distributed, it will be log transformed prior
22
23 444 to analysis and the normality assumptions will be tested again. Repeated measures ANOVA with
24
25 445 post hoc analysis will be used to determine if there is an effect of treatment on immune function.
26
27

28 446 Factors affecting DHA incorporation will be examined by independent t-tests to compare the
29
30 447 mean values between the DHA and placebo groups. Chi-square tests will be conducted to
31
32 448 determine correlation between two categorical variables for the outcome measures listed.
33
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35 449 The within subject and between subject variability between the two groups for the mean percent
36
37 450 change in apoptosis and tumor infiltrating lymphocyte markers will be tested using the generalized
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39 451 estimating equation (GEE) method.
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43 452 The 95% confidence interval using independent t-tests will be conducted for the mean percent
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45 453 change in pathological complete response and rates of grade 3 and 4 chemotherapy associated
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47 454 toxicities and hospitalization in patients receiving DHA supplementation compared to patients
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49 455 receiving placebo.
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52 456 *Exploratory outcomes:*
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3 457 Independent t-tests for macronutrient and fat content obtained from the food frequency
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5 458 questionnaire will be examined between groups. Paired t-tests for continuous variables and
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7
8 459 McNemar's test for categorical variables will be assessed for mean percent change in events
9
10 460 between treatment arms for the quality of life and exercise questionnaires. Chi-square tests will
11
12 461 be used to compare the degree of breast conservation and the volume of surgical blood loss will
13
14 462 employ an independent t-test between the two study arms. Rate of local control will be compared
15
16 463 between treatment arms using t-test of proportions. Recurrence-free survival and survival will be
17
18 464 analyzed using the log rank test on Kaplan-Meier survival curves.

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21 465 SAS software, version 9.4 (SAS Institute Inc., Cary, NC), will be used for statistical
22
23 466 analysis. A *p*-value <0.05 level will be used for all statistical significance. Two-sided tests will
24
25 467 be used for all statistical tests.

26 468 **Data Monitoring**

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29 469 The trial activities performed at the Cross Cancer Institute will be monitored by the
30
31 470 Cross Cancer Institute, Investigator Initiated Trials Data Safety Monitoring Board (DSMB).
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33 471 The DSMB is independent of the investigator and is composed of representatives from both
34
35 472 medical and radiation oncology.

36
37 473 The investigator will assess the relationship between protocol treatment and the
38
39 474 occurrence of adverse events (AEs) and this assessment will be recorded in the database for
40
41 475 adverse events. This study will use the International Common Terminology Criteria for
42
43 476 Adverse Events (CTCAE), version 5.0, for adverse event reporting. The reporting period for
44
45 477 adverse events will start at the time the patient takes the first dose of DHA/placebo through and
46
47 478 including 28 calendar days after last administration of study agent. If serious adverse reaction
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49 479 to treatment occurs, the Natural and Non-prescription Health Products Directorate (NNHPD),
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3 480 Clinical Trial Unit, Health Ethics Research Board of Alberta, Cancer Committee (HREBA.CC)
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5 481 and DSM will be notified as per guidelines. After 25 evaluable patients, all data and results will
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8 482 be submitted to the DSMB for review.
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10 11 483 **Auditing**

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14 484 As per the SPIRIT guidelines, the investigators, Cross Cancer Institute and the
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16 485 University of Alberta will permit trial-related monitoring, audits, REB, DSMB review, and
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18 486 regulatory inspection(s), providing direct access to paper and/or electronic documentation
19
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21 487 pertaining to the clinical study (e.g. CRFs, source documents such as hospital patient charts and
22
23 488 investigator study files). All site facilities related to the study conduct could be visited during
24
25 489 an audit (e.g. pharmacy, laboratory, outpatient department) and are agreed to co-operate and
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27
28 490 provide assistance at reasonable times and places with respect to any auditing activity.
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31 491 **Patient and Public Involvement**

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33 492 Patients were not involved in the protocol development or study design. However,
34
35 493 oncologists and clinical trial nurses who work in the breast tumor group are involved in patient
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37 494 screening to assess eligibility for the study. The HREBA-CC approved informed consent will be
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39 495 obtained from patients prior to their involvement in the study and it informs patients of their right
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41 496 to withdraw at any time. At the end of the trial, results will be disseminated to the public through
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43 497 seminars, public talks and in peer-reviewed journals.
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45 498 **Ethics and dissemination**

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48 499 DHA WIN has received Health Canada approval (#HC6-24-c220167), full ethical
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50 500 approval from the Health Research Ethics Board of Alberta – Cancer Committee (Protocol #:
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52 501 HREBA.CC- 18-0381) and is registered at clinicaltrials.gov (Identifier: NCT03831178).
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3 502 Protocol amendments will be submitted to HREBA.CC, Health Canada and the clinical trial
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5 503 registry prior to study implementation according to regulatory requirements. The formal consent
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7 504 of a participant, using the HREBA-CC-approved consent form (Supplemental File 1), will be
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9 505 obtained by a clinical trial nurse before the participant is enrolled in the study and will be signed
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11 506 by the patient, and the principle investigator. A voluntary optional consent form for use of
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13 507 participant data and biological specimens (Supplemental File 2), will be offered at time of
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15 508 enrollment. Patient confidentiality and anonymity will be maintained and identities protected
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17 509 from unauthorized parties.
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22 510 Access to data will be restricted to the primary investigators and statistician. They will
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24 511 grant access to other team members as governed and approved by ethics. Ancillary care post-trial
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26 512 will occur as routine standard of care for all participants. Our objective is to determine the
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28 513 efficacy of using DHA supplementation concomitant with chemotherapy and as such our results
29
30 514 will be disseminated to clinicians for implementation in future treatment paradigms. The results
31
32 515 will be submitted to peer-reviewed journals and presented at national and international
33
34 516 conferences.
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37 517 **Funding Statement**

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41 518 This study is supported by the Canadian Institutes of Health Research [Grant Number:
42
43 519 RES0037745], Cross Cancer Institute Investigator Initiated Trials [Grant Number: IIT-0005]
44
45 520 and a gift from the Butler Family Foundation, Edmonton Alberta.
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49 521 **Competing Interests Statement**

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51
52 522 There are no financial or competing interests or conflicts to declare.
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523 **Author Contributions**

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

540 **Appendices:**

541 Supplemental File 1: Informed consent

542 Supplemental File 2: Optional consent

543 Supplemental Table 1: Spirit Checklist

544 Supplemental Table 2: WHO Checklist

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

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3 546 Supplemental Table 4: List of Antibodies for Immune cell subset identification
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5 547 **FIGURE LEGENDS**
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8 548 **Figure 1** Flowchart of Trial Design with Endpoints and Proposed Experimental Analyses
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10 549 **Figure 2** SPIRIT patient flow diagram of the DHA WIN trial
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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	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	X

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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/placibo 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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558 **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 cytokines	a) Flow cytometry b and c) ELISA and MesoScale	Repeated Measures ANOVA with post-hoc analysis

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

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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

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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

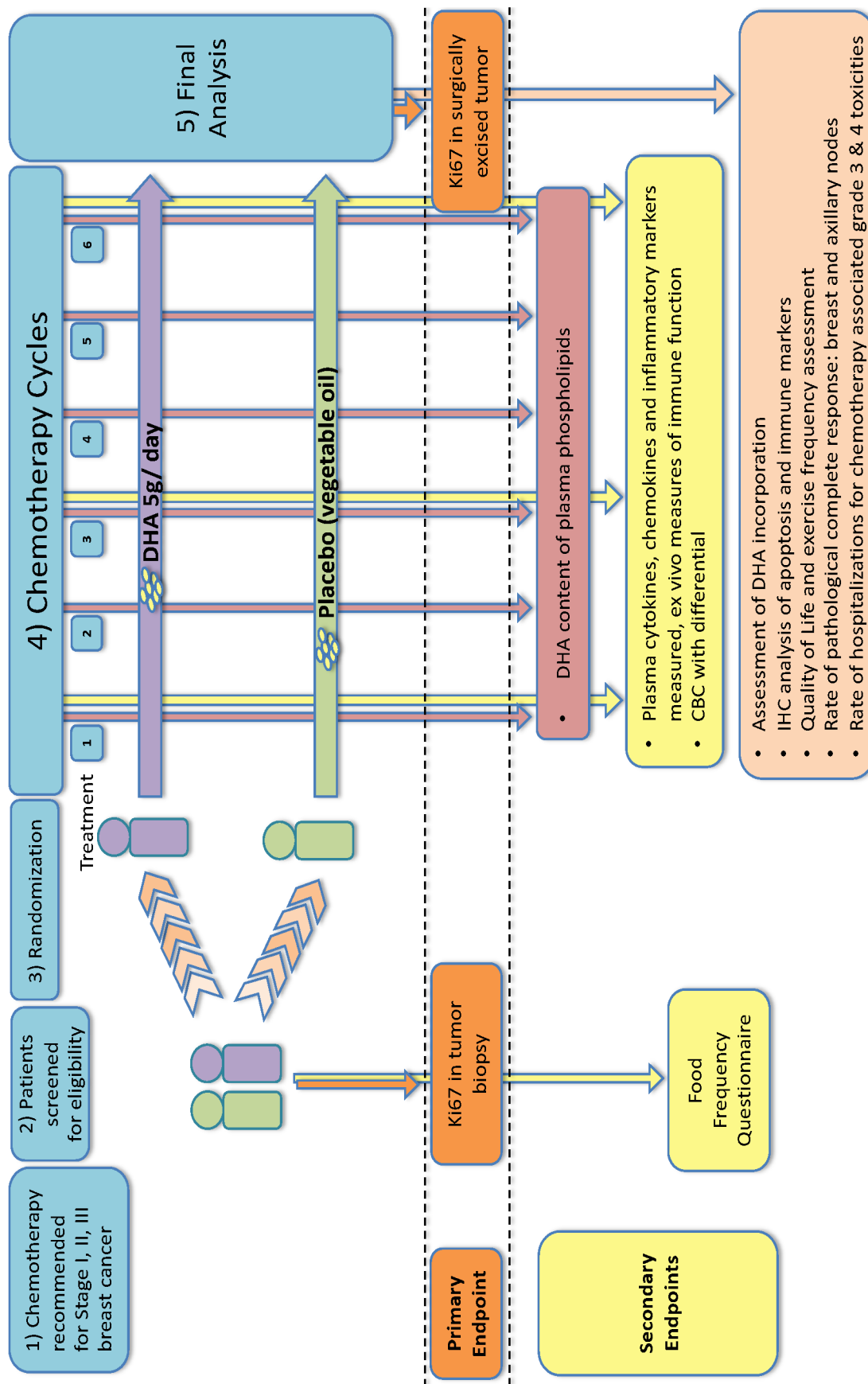
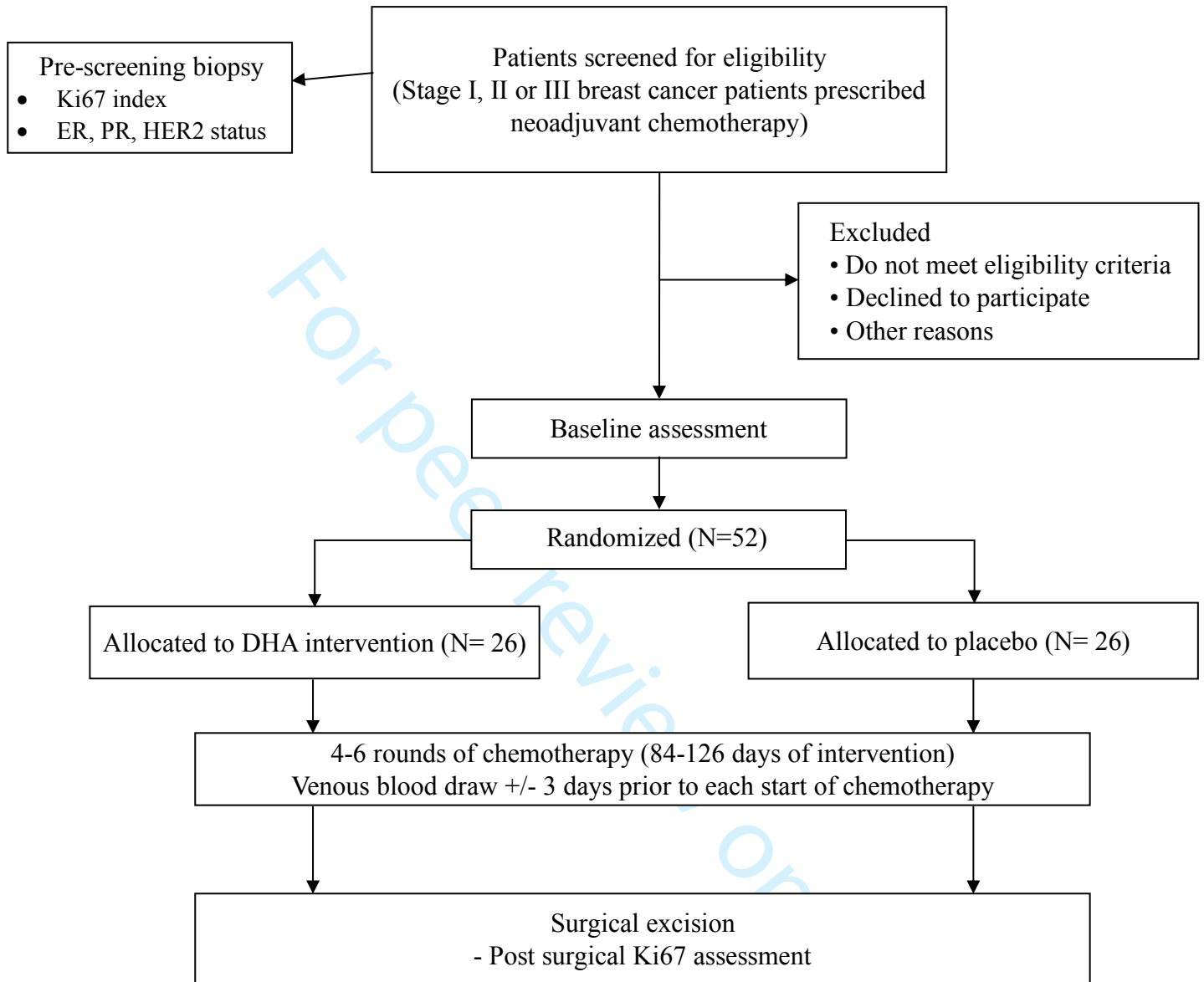


Figure 2



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.

Dr. John Mackey, Cross Cancer Institute, 11560 University Ave, Edmonton, AB, www.albertahealthservices.ca

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.

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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 approximately 12-18 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.

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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 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.

Dr. John Mackey, Cross Cancer Institute, 11560 University Ave, Edmonton, AB, www.albertahealthservices.ca

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 Within 28 days after last dose	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)		
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

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disease-related biomarkers (signs related to your disease)														
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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 (Within 28 days after last dose)	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)		
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	

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HREBA-CC ICF DHA WIN

treatment																	
We will collect results from your surgery report																	X

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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;

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- 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 μ g/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 accidentally 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: _____

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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.

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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.

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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.

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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.



SIGNATURES

Part 1 - to be completed by the potential participant.

	<u>Yes</u>	<u>No</u>
Do you understand that you have been asked to take part in a research study?	<input type="checkbox"/>	<input type="checkbox"/>
Do you understand why this study is being done?	<input type="checkbox"/>	<input type="checkbox"/>
Do you understand the potential benefits of taking part in this study?	<input type="checkbox"/>	<input type="checkbox"/>
Do you understand the risks of taking part in this study and the risks of becoming pregnant or fathering a child during this study?	<input type="checkbox"/>	<input type="checkbox"/>
Do you understand what you will be asked to do should you decide to take part in this study?	<input type="checkbox"/>	<input type="checkbox"/>
Do you understand the alternatives to participating in this study?	<input type="checkbox"/>	<input type="checkbox"/>
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?	<input type="checkbox"/>	<input type="checkbox"/>
Do you understand who will see your records, including health information that identifies you?	<input type="checkbox"/>	<input type="checkbox"/>
Do you understand that by signing this consent form you are giving us permission to access your health information and specimens if applicable?	<input type="checkbox"/>	<input type="checkbox"/>
Do you understand that by signing this consent form that you do not give up any of your legal rights?	<input type="checkbox"/>	<input type="checkbox"/>
Have you had enough opportunity to ask questions and discuss this study?	<input type="checkbox"/>	<input type="checkbox"/>

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

Signature of Participant	PRINTED NAME	Date
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Part 2 - to be completed by the study doctor or designee who conducted the informed consent discussion. Only complete 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
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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
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****You will be given a copy of this signed and dated consent form prior to participating in this study.****

For peer review only

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
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3 locally, across Canada, and around the world. The researchers doing the main study are
4 also interested in storing your tissue/blood samples for future research. The research that
5 may be done on your samples in the future is unknown at this time. It may be related to
6 your condition or it may be used to address research questions that are unrelated.
7

8 Some of this research may be about genes. Genes carry information about features, such
9 as hair or eye colour. This research may include looking at changes in genes found in you
10 and in people who are related to you. These changes may be inherited (passed on in
11 families). This is called hereditary genetic testing. Researchers also may be interested in
12 the way that genes affect health and disease, or how your body responds to treatment.
13
14

15 **WHAT WILL HAPPEN DURING THIS OPTIONAL RESEARCH?**

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

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

23 **HOW WILL MY SAMPLES BE HANDLED?**

24 Your sample(s) and some related health information already collected from your
25 participation in the main study will be sent to the Nutritional Immunology laboratory at the
26 University of Alberta, Edmonton, AB, for analysis. The samples and data will be kept
27 indefinitely or until they are used up, destroyed or returned to the hospital where you had
28 your surgery or biopsy.
29

30 Qualified researchers can submit a request to use the materials stored at the University of
31 Alberta. Your samples and related health information will be used only by researchers
32 whose requests have been accepted by the sponsor and who have met regulatory
33 requirements and secured ethics approval for their research. The samples and data may be
34 sent to other countries. Your name or any other information that could directly identify you
35 will not be given to these researchers.
36

37 The results of research done on your samples will not be added to your personal health
38 records and you or the researcher will not know the results.
39

40 **WHAT ARE THE RISKS OF PARTICIPATING IN THIS OPTIONAL RESEARCH?**

41 Risks related to sample collection:

- 42 • Since the tissue sample(s) already have been collected for the main study or as part of
43 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.

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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 THIS 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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3 study.

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6 **IS THERE ANY CONFLICT OF INTEREST RELATED TO THIS OPTIONAL RESEARCH?**

7 There are no current or potential conflicts of interest concerning the optional research
8 study.
9

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

14
15 If you have questions about the use of your samples/data for optional research, or if you
16 suffer a research-related injury, contact the researcher of this optional study:
17

18
19
20 Catherine J Field
21 Name

22 780-492-5297
23 Telephone Number

24
25
26
27 If you have questions about your rights as a participant or about ethical issues related to
28 this optional research and you would like to speak to someone not involved in its conduct,
29 please contact the Office of the Health Research Ethics Board of Alberta – Cancer
30 Committee at: 780-423-5727 or toll-free 1-877-423-5727.
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56 Dr. John Mackey, Cross Cancer Institute, 11560 University Ave, Edmonton AB, www.albertahealthservices.ca

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58 Version date: August 17, 2018

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Ethics ID: HREBA.CC-18-0381

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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 Printed Name Date
the Consent Discussion

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 Printed Name Date
Witness

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.

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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

1			
2		6b	Explanation for choice of comparators 7
3			
4	Objectives	7	Specific objectives or hypotheses 5
5			
6	Trial design	8	Description of trial design including type of trial (eg, 6, Fig 1, parallel group, crossover, factorial, single group), allocation ratio, and framework (eg, superiority, equivalence, noninferiority, exploratory) Fig 2
7			
8			
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12	Methods: Participants, interventions, and outcomes		
13			
14	Study setting	9	Description of study settings (eg, community clinic, 6 academic hospital) and list of countries where data will be collected. Reference to where list of study sites can be obtained
15			
16			
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19	Eligibility criteria	10	Inclusion and exclusion criteria for participants. If 7 applicable, eligibility criteria for study centres and individuals who will perform the interventions (eg, surgeons, psychotherapists) Table 1 (page 1,2)
20			
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24			
25	Interventions	11a	Interventions for each group with sufficient detail to 7 allow replication, including how and when they will be administered
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30		11b	Criteria for discontinuing or modifying allocated 8 interventions for a given trial participant (eg, drug dose change in response to harms, participant request, or improving/worsening disease)
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35		11c	Strategies to improve adherence to intervention 8 protocols, and any procedures for monitoring adherence (eg, drug tablet return, laboratory tests)
36			
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40		11d	Relevant concomitant care and interventions that are 7 permitted or prohibited during the trial
41			
42			
43	Outcomes	12	Primary, secondary, and other outcomes, including 8-11 the specific measurement variable (eg, systolic blood pressure), analysis metric (eg, change from baseline, final value, time to event), method of aggregation Table 3 (eg, median, proportion), and time point for each outcome. Explanation of the clinical relevance of chosen efficacy and harm outcomes is strongly recommended
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53	Participant timeline	13	Time schedule of enrolment, interventions (including 12, Table any run-ins and washouts), assessments, and visits 2 for participants. A schematic diagram is highly recommended (see Figure)
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2	Sample size	14	Estimated number of participants needed to achieve	12
3			study objectives and how it was determined,	
4			including clinical and statistical assumptions	
5			supporting any sample size calculations	
6				
7	Recruitment	15	Strategies for achieving adequate participant	13
8			enrolment to reach target sample size	
9				

Methods: Assignment of interventions (for controlled trials)

Allocation:

14	Sequence	16a	Method of generating the allocation sequence (eg,	13
15	generation		computer-generated random numbers), and list of	
16			any factors for stratification. To reduce predictability	
17			of a random sequence, details of any planned	
18			restriction (eg, blocking) should be provided in a	
19			separate document that is unavailable to those who	
20			enrol participants or assign interventions	
21				
22				
23				
24	Allocation	16b	Mechanism of implementing the allocation sequence	13
25	concealment		(eg, central telephone; sequentially numbered,	
26	mechanism		opaque, sealed envelopes), describing any steps to	
27			conceal the sequence until interventions are	
28			assigned	
29				
30				
31	Implementation	16c	Who will generate the allocation sequence, who will	13
32			enrol participants, and who will assign participants to	
33			interventions	
34				
35	Blinding (masking)	17a	Who will be blinded after assignment to interventions	13
36			(eg, trial participants, care providers, outcome	
37			assessors, data analysts), and how	
38				
39				
40		17b	If blinded, circumstances under which unblinding is	13
41			permissible, and procedure for revealing a	
42			participant's allocated intervention during the trial	
43				

Methods: Data collection, management, and analysis

46	Data collection	18a	Plans for assessment and collection of outcome,	14-18
47	methods		baseline, and other trial data, including any related	Table 3
48			processes to promote data quality (eg, duplicate	
49			measurements, training of assessors) and a	
50			description of study instruments (eg, questionnaires,	
51			laboratory tests) along with their reliability and	
52			validity, if known. Reference to where data collection	
53			forms can be found, if not in the protocol	
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2		18b	Plans to promote participant retention and complete
3			follow-up, including list of any outcome data to be
4			collected for participants who discontinue or deviate
5			from intervention protocols
6			
7	Data management	19	Plans for data entry, coding, security, and storage,
8			including any related processes to promote data
9			quality (eg, double data entry; range checks for data
10			values). Reference to where details of data
11			management procedures can be found, if not in the
12			protocol
13			
14			
15	Statistical methods	20a	Statistical methods for analysing primary and
16			secondary outcomes. Reference to where other
17			details of the statistical analysis plan can be found, if
18			not in the protocol
19			
20			
21		20b	Methods for any additional analyses (eg, subgroup
22			and adjusted analyses)
23			
24		20c	Definition of analysis population relating to protocol
25			non-adherence (eg, as randomised analysis), and
26			any statistical methods to handle missing data (eg,
27			multiple imputation)
28			
29			
30			
31	Methods: Monitoring		
32	Data monitoring	21a	Composition of data monitoring committee (DMC);
33			summary of its role and reporting structure;
34			statement of whether it is independent from the
35			sponsor and competing interests; and reference to
36			where further details about its charter can be found,
37			if not in the protocol. Alternatively, an explanation of
38			why a DMC is not needed
39			
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41		21b	Description of any interim analyses and stopping
42			guidelines, including who will have access to these
43			interim results and make the final decision to
44			terminate the trial
45			
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47	Harms	22	Plans for collecting, assessing, reporting, and
48			managing solicited and spontaneously reported
49			adverse events and other unintended effects of trial
50			interventions or trial conduct
51			
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53	Auditing	23	Frequency and procedures for auditing trial conduct,
54			if any, and whether the process will be independent
55			from investigators and the sponsor
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58	Ethics and dissemination		
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2	Research ethics	24	Plans for seeking research ethics	21
3	approval		committee/institutional review board (REC/IRB)	
4			approval	
5				
6	Protocol	25	Plans for communicating important protocol	21
7	amendments		modifications (eg, changes to eligibility criteria,	
8			outcomes, analyses) to relevant parties (eg,	
9			investigators, REC/IRBs, trial participants, trial	
10			registries, journals, regulators)	
11				
12				
13	Consent or assent	26a	Who will obtain informed consent or assent from	21
14			potential trial participants or authorised surrogates,	Supp file
15			and how (see Item 32)	
16				
17		26b	Additional consent provisions for collection and use	21
18			of participant data and biological specimens in	Supp file
19			ancillary studies, if applicable	
20				
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22	Confidentiality	27	How personal information about potential and	22
23			enrolled participants will be collected, shared, and	
24			maintained in order to protect confidentiality before,	
25			during, and after the trial	
26				
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28	Declaration of	28	Financial and other competing interests for principal	22
29	interests		investigators for the overall trial and each study site	
30				
31	Access to data	29	Statement of who will have access to the final trial	22
32			dataset, and disclosure of contractual agreements	
33			that limit such access for investigators	
34				
35	Ancillary and post-	30	Provisions, if any, for ancillary and post-trial care,	22
36	trial care		and for compensation to those who suffer harm from	
37			trial participation	
38				
39				
40	Dissemination	31a	Plans for investigators and sponsor to communicate	22
41	policy		trial results to participants, healthcare professionals,	
42			the public, and other relevant groups (eg, via	
43			publication, reporting in results databases, or other	
44			data sharing arrangements), including any	
45			publication restrictions	
46				
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48		31b	Authorship eligibility guidelines and any intended use	22
49			of professional writers	
50				
51		31c	Plans, if any, for granting public access to the full	-
52			protocol, participant-level dataset, and statistical	
53			code	
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56	Appendices			
57				
58	Informed consent	32	Model consent form and other related documentation	Supp file
59	materials		given to participants and authorised surrogates	
60				

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2	Biological	33	Plans for collection, laboratory evaluation, and	Supp file
3	specimens		storage of biological specimens for genetic or	
4			molecular analysis in the current trial and for future	
5			use in ancillary studies, if applicable	
6				

7 *It is strongly recommended that this checklist be read in conjunction with the SPIRIT 2013
8 Explanation & Elaboration for important clarification on the items. Amendments to the
9 protocol should be tracked and dated. The SPIRIT checklist is copyrighted by the SPIRIT
10 Group under the Creative Commons "[Attribution-NonCommercial-NoDerivs 3.0 Unported](#)"
11 license.
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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	<p>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</p> <p>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</p>

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	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

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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