Pilot Trial of COGNUTRIN in Breast Cancer Survivors

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Monitoring/Regulatory:	**Moffitt Cancer Center**

CONFIDENTIAL

SCHEMA

Pilot Trial of COGNUTRIN in Breast Cancer Survivors

Screening (In person):

(a) Within 6 months of completing neoadjuvant or adjuvant treatment with anthracyclines and/or taxanes + or – radiation therapy (subjects on concurrent endocrine therapy (TAM, Aromatase inhibitors are also eligible to participate as this is standard of care for this patient population); (b) No evidence of dementia (MMSE >=23) but some evidence of cognitive impairment (each subject will be required to answer in the affirmative: 'do you have problems with memory and attention since having chemotherapy, and do you believe chemotherapy contributed to the problems?'); (c) Collect blood for CBC/CMP/PT/PTT, LDL and antioxidant and biomarkers of inflammation; (d) Conduct Mini-Mental Status Exam

If\yes-

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Baseline/Randomization (In person):

(a) Confirm eligibility based on screening tests; (b) Obtain baseline food records; (c) Complete Demographic and Godin Leisure-Time Exercise Questionnaires; (d) Conduct battery of cognitive tests including Weschler Test of Adult Reading, Hopkins Verbal Learning Test, Revised (Form 1), COWA (T1), Color Trails, Part 1 & 2, Digit Span (Subset of WMS-IV), Symbol Digit Modalities Test, Brief Visuospatial Memory Test-Revised (Form 1), and Rey Complex Figure Copy; (e) Patient's Assessment of Own Functioning Inventory (PAOFI); (f) Structural and resting-state functional magnetic resonance imaging (MRI/fMRI); (g) Complete questionnaires for symptoms of fatigue and depression (PHQ-9 and FSI); (h) Randomize and supply participant with one-month supply of nutritional supplement (COGNUTRIN) or placebo (n=20/arm), multivitamin, and study agent intake and symptom logs

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Intervention:

One of 2 arms, self-administration of nutritional supplement (COGNUTRIN) or placebo for 3 months plus multivitamin

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Weeks 3, 7, & 11:

Remind patient to complete diet records and schedule monthly visits

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Weeks 4 & 8 (+/- 7 days) (In person):

(a) Collect blood for CBC, CMP, PT/PTT; (b) Collect unused nutritional supplement/ placebo, multivitamin, and completed study agent intake and symptom log form; (c) Collect completed diet survey; (d) Supply participant with new one-month supply of nutritional supplement or placebo and study agent intake and symptom logs

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Week 12, 3 months or 90 days (+/- 7 days): End of Intervention (In person):

(a) Collect blood for CBC/CMP/PT/PTT,LDL & biomarkers (inflammation/antioxidant); (b) Collect unused nutritional supplement or placebo, multivitamin, and completed study agent intake and symptom log forms; (c) Collect completed diet survey; (d) Complete Godin Leisure-Time Exercise Questionnaire; (e) Conduct battery of cognitive tests Hopkins Verbal Learning Test, Revised (Form 2), COWA (T2), Color Trails, Part 1 & 2, Digit Span (Subset of WMS-IV), Symbol Digit Modalities Test, Brief Visuospatial Memory Test-Revised (Form 2), and Rey Complex Figure Copy; (f) Patient's Assessment of Own Functioning Inventory (PAOFI); (g) Structural and resting-state functional magnetic resonance imaging (MRI/fMRI); (h) Complete questionnaires for symptoms of fatigue and depression (PHQ-9 and FSI)

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Post-intervention Follow-up: (7 +/- 3 days post-treatment):
Telephone contact to assess signs and symptoms and concomitant medications occurring post-treatment

Endpoints:

Primary:	Changes in cognitive function: Number of impaired neuropsychological tests after 3 months of intervention with COGNUTRIN *vs*. placebo. Safety (Primary): Incidence and severity of adverse events
Secondary:	Changes in self-report measures of cognitive function. Changes in structural and resting-state functional magnetic resonance imaging (fMRI) after 3 months of intervention with COGNUTRIN *vs*. placebo. Changes in symptoms of depression and fatigue with COGNUTRIN *vs*. placebo.
Other:	Changes in inflammatory and oxidative stress biomarkers from serum and plasma cytokine panel including IL1, IL6, IL8, IL10, IL12, GMCSF (Millipore Milleplex system) after 3 months of intervention with COGNUTRIN *vs*. placebo

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1 BACKGROUND

1.1 Disease Background

Cognitive Impairment in Breast Cancer Survivors. Treatment-related neurocognitive impairment related to cancer treatment is most frequently reported in breast cancer patient population who comprise 22% of the 11 million survivors today in the US. [1] It is estimated that most of these women will be diagnosed with stage I to III disease, and a significant proportion will be treated with chemotherapy. Although the benefits of adjuvant chemotherapy are well established, many cancer survivors are at risk for developing physiological and psychological late effects of cancer treatment that might lead to premature mortality and morbidity[2] and compromise their quality of life. One of the most common adverse effects reported by breast cancer patients after treatment is "chemobrain", a phenomenon of cognitive impairment (CI) that is reported in 30-75% of patients treated with chemotherapy for breast and other cancers and lasting anywhere from 6 months to ten years.[3-6] Although most standard chemotherapy regimens at standardized doses appear to not cross the blood-brain barrier, even mild toxicity to the Central Nervous System (CNS) has been shown to produce significant changes to cognitive function (CF).[64] Additionally, although it was first thought that CI that is chemotherapy-induced is acute and reversible suggesting a transient nature of this phenomena, more recent studies have revealed cognitive impairment (CI) to be progressive and lasting anywhere from 6 months to 10 years post treatment in breast cancer survivors.[2-6] Irrespective of baseline CF or type and duration of chemotherapy, there is consistent evidence of CI in patients receiving cytotoxic drugs for cancer treatment.

1.2 Etiology of Cognitive Impairment in Breast Cancer Patients Treated with Chemotherapy. Several studies[4-10] in the past few years have hypothesized this phenomena of "Chemobrain" to be multifactorial and contributed by one or more of the following mechanisms - vascular injury, leukoencephalopathy, oxidative damage, cytokine-induced inflammatory response, direct injury to neuron, autoimmune responses, chemotherapy-induced anemia, abrupt steroid hormone deficit, hypothyroidism and the presence of the apolipoprotein gene.[3, 7-9] We have reported prevalence of chemotherapy-induced loss of thyroid function[8] which has been associated to decrements in working memory, and intentional and executive disturbances similar to those observed in breast cancer survivors. In addition to the biological plausibility, others have proposed psycho-social factors such as stress, anxiety, depression, fatigue, age, social support, education level and intelligence as other influential factors to CI.[8] This study clearly demonstrates the multi-factorial nature of the etiology of this phenomenon and establishes the need for multifaceted approaches to the treatment as we have proposed in this trial.

1.3 Current interventions for the Treatments for Chemotherapy-induced Cognitive Impairment. Studies that hypothesize that CI may be attributed to symptoms of anemia have studied pharmacological interventions to treat anemia with recombinant human erythropoietin.[55] Although these studies have demonstrated an increase in hemoglobin levels, decreased fatigue and improved CF in those cancer patients with anemia, the small sample sizes, use of cohorts with various cancers and treatment regimens and short duration of interventions fail to provide meaningful data as to the efficacy of this intervention. In addition, new trials have provided further evidence against long-term use, cost-effectiveness and side-effects with these agents, particularly thrombic events. Others have evaluated psycho stimulant methylphenidate[53-54] in brain tumor patients and other adults, including breast cancer patients for treatment-related CI and observed significant improvements in CF compared to a placebo group. However, the long-term safety of these agents, including impact on neurochemistry and the amphetamines-like effects continue to be a concern. Most promising, is a single arm pilot study of a brief cognitive-behavioral treatment aimed at managing cognitive dysfunction associated with adjuvant chemotherapy (Memory and Attention Adaptation Training; MAAT) [52], which reported that participants reported high treatment satisfaction and rated MAAT as helpful in improving ability to compensate for memory problems. With the recent understanding of the multi-factorial etiology of CI, similar to those observed in CI related to aging and associated diseases, these structured CT strategies that have already demonstrated efficacy in aging and diseases related to CI, appear to offer promise for the treatment of chemotherapy-induced CI. Over the past 25 years, several studies have demonstrated that among relatively healthy, older adults without dementia, cognitive abilities can be enhanced through a variety of cognitive intervention techniques. [12-16-18, 27] These cognitive training techniques are particularly promising in that transfer of training and have been demonstrated to performance of Timed Instrumental Activities of Daily Living TIADL, [19-20], safer on-road driving performance [21], and maintained health-related quality of life [22-23]. Research has indicated that individuals, who exhibit slowed cognitive speed of processing as measured by UFOV, are most likely to benefit from and experience transfer of speed of processing training to functional outcomes [18].

1.4 Polyphenolic Antioxidants Agents and Cognitive Performance.

Oxidative stress and inflammation contribute to several organ toxicities, including neurotoxicities, after common breast cancer chemotherapy regimen. Doxorubicin and other platinum-based therapies have been documented to cause the generation of free radicals and the induction of oxidative stress, associated with cellular injury.[90] Although the debate continues as to the safety of antioxidant use during chemotherapy to reduce oxidative stress, the utility of these agents to ameliorate treatment-induced consequences post cancer treatment, including CI has not been examined. Much of the work using antioxidant agents in treating CI comes from the aging literature. However, interventions to treat CI using antioxidant therapies with Vitamins A, C and E have been disappointing as a treatment for markers of CI in cancer survivors, similar to those observed in the aging literature.[56-61] Non-vitamin phytochemicals such as polyphenols, which are the most abundant antioxidants in our diets have been shown to possess strong anti-inflammatory properties, as well as stronger antioxidant activity,[27, 36-38,48-51,65-84] as compared to traditional antioxidant vitamins that have never been tested before to improve CI in this cohort.

Figure 1. Protection from oxidative cell induced death by blueberry, green tea, carnosine Vitamin D_3or all 4 combined (NT-020).

Antioxidants, such as polyphenols, not only scavenge reactive oxygen species (ROS)[91-92] but are also involved in modification of protein kinases, apoptosis signaling, and regulating signal transduction via modification of cellular redox reactions.[33,86] Our group has shown that single and combination polyphenolic compounds have demonstrated improvements on cognitive tasks such as the Morris water maze, radial arm maze (water and land), t-maze passive and active avoidance tasks, as well as classical eye blink conditioning tasks. Dr. Bickford[48] established the precedent for cognitive improvement in normal aging rats with dietary supplementation of foods high in ORAC (Oxygen Radical Absorbance Capacity). A combination of plant polyphenols containing a standardized formulation of grape, green tea and rich in blueberry extract (NT-020) (Fig 1) increase actions on the progenitor cell populations and to reduce inflammation and oxidative stress to protect primary neuronal cultures as well as microglial cell cultures from oxidative stress, in addition to neuroprotective and neurogenic effects.[26, 36, 48-51, 93-98] In a recent experiment, we examined the impact of this combination polyphenolic formulation rich in BB anthocyanins (NT-020) on Morris Water Maze performance among young animals (n =10), as well as older animals who received treatment of NT-020 (n = 13) or a control diet (n – 13) for 3 weeks prior to behavioral testing. All animals were tested on the Morris Water Maze test, four trials per day for five days. Figure 2 on the top left shows learning on day 5, based upon the cumulative distance for individual rats on the last day of testing. The animals in the NT-020 arm of study exhibited lower cumulative distance, as compared to the older control animals ($p < .05$). Further, there was much less variability among the treated old animals, as compared to the control animals. We then examined the brains of these rats for neurogenesis and markers of inflammation using unbiased serological measures. As can be seen in Figure 2, there was a significant increase in the numbers of Ki67 and DCX labeled cells in the dentate gyrus of the NT-020 rats that was accompanied by a decrease in OX6 (MHC Class II) positive microglia indicating that there was a reduction in inflammatory mediators.

Plant-derived compounds known as flavonoids may exert particularly powerful actions on mammalian cognition and may reverse age-related declines in memory and learning. In particular, evidence suggests that foods rich in three specific flavonoid sub-groups, the flavanols, anthocyanins [114-116] and/or flavanones, possess the greatest potential to act on the cognitive processes. For example, their specific interactions within the ERK and PI3-kinase/Akt signaling pathways, at the level of receptors or kinases, have been shown to increase the expression of neuroprotective and neuromodulatory proteins and increase the number of, and strength of, connections between neurons. Their effects on the vascular system may also lead to enhancements in cognitive performance through increased brain blood flow and an ability to initiate neurogenesis in the hippocampus. Additional mechanisms have been suggested for the ability of flavonoids to delay the initiation of and/or slow the progression of AD-like pathology and related neurodegenerative disorders, including a potential to inhibit neuronal apoptosis triggered by neurotoxic species (e.g., oxidative stress and neuroinflammation). Berry anthocyanins also improve neuronal and cognitive brain functions, ocular health as well as protect genomic DNA integrity. Recent studies reported by Zafra-Stone S, et al [117] demonstrated that anthocyanins-rich OptiBerry exhibits high antioxidant efficacy as shown by its high oxygen radical absorbance capacity (ORAC) values, significantly inhibited basal MCP-1 and inducible NF-kappa-B transcriptions as well as the inflammatory biomarker IL-8. Together, these processes

act to maintain the number and quality of synaptic connections in key brain regions and thus flavonoids have the potential to prevent the progression of neurodegenerative pathologies and to promote cognitive performance. [116,118]. Preclinical evidence has demonstrated that these polyphenols protect vulnerable neurons and enhance the function of existing neuronal structures, two processes known to underpin neuro-cognitive function [116]. Blueberry anthocyanins can decrease this vulnerability to oxidative stress as assessed in vivo by examining reductions in neuronal signaling and behavioral deficits and in vitro via H2O2-induced decrements in striatal synaptosomal calcium buffering. In preclinical studies, BB anthocyanin supplementations are effective in decreasing indices of inflammation and oxidative stress. To date, the anthocyanins show the most efficacy in penetrating the cell membrane and in providing antioxidant protection. [119] More recent trials have demonstrated that polyphenolic components can be measured in serum and plasma, found to cross the blood brain barrier and are found in measurable levels within the brain.[73] For example NT-020 contains blueberry, anthocyanins in blueberry are considered to be one of the major active fractions and can be found in the brain of several mammalian animal models.[74-76] Our group has considerable experience and demonstrated the safety of polyphenolic[67-72] and n-3 fatty acids[52-53, 84] supplementation in cancer patient cohorts. Anthocyanins from BB thus hold the most promise to prevent or reverse normal or abnormal deteriorations in cognitive performance. With the safety of anthocyanins well established, we predict this treatment regimen with anthocyanins can directly improve neurogenesis that is impacted with cytotoxic agents. [85-86]

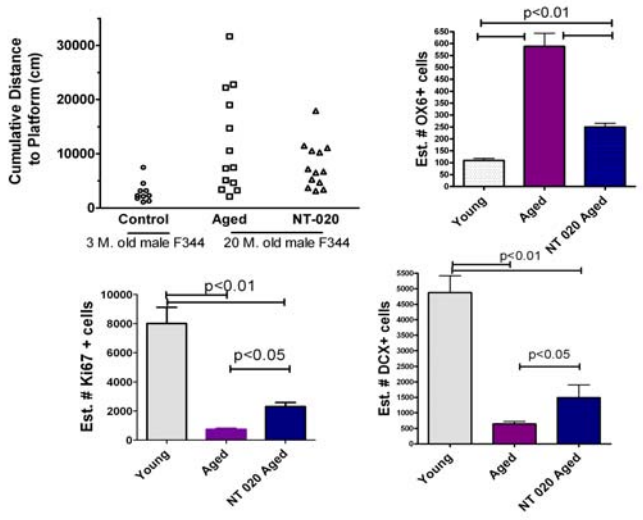


Figure 2. F344 rats of 3 or 20 months of age were treated with Control NIH21 diet or NIH21 diet plus NT-020 10X for 3 weeks prior to testing on a standard Morris water maze, 4 trials per day for 5 days of training. Top left: Cumulative distance scores for each individual rat on day 5 of training. It can be seen that there is a significantly reduced variance for the NT020 rats. When you compare the learning impaired groups (cutoff = 3 SD from young) then the treated learning impaired rats perform at a higher level than the untreated impaired rats. After training brains were prepared for immunostochemistry and sectioned at 40 µm for unbiased stereological measures using the optical fractionator. Top Right: There is a significant increase in activated microglia in the aged dentate gyrus which is reduced in NT-020 treated rats. Bottom left: Numbers of proliferating cells at time of sacrifice (2 weeks post behavior) were estimated using Ki67+ immunolableling were significantly increased in the aged treated rats compared with control. Bottom right: Doublcortin (DCX) positive cells were also increased in the aged NT-020 treated rats suggesting that both proliferation of neural progenitors and cells that proceed to a neuronal lineage are increased by this dietary treatment.

1.5 Anti-inflammatory agents (n-3 fatty Acids) and Cognitive Function: In view of the high omega-3 poly unsaturated fatty acid content of the brain, it is evident that these fats are involved in brain biochemistry, physiology and functioning; and thus in some neuropsychiatric diseases and in the cognitive decline of aging. [52-53, 99-102] DHA (docosahexaenoic acid) is one for the major building structures of membrane phospholipids of brain and is absolutely necessary for neuronal function. Deficiency of DHA alters the course of brain development, perturbs the composition of brain cell membranes, neurones, oligodendrocytes and astrocytes, as well as sub cellular particles such as myelin, nerve endings (synaptosomes) and mitochondria. These alterations induce physicochemical modifications in membranes, lead to biochemical and physiological perturbations, and result in neurosensory and behavioral upset.[99-102] The literature reveals growing mechanistic evidence that cognitive function of the aging brain can be preserved, or loss of function can be diminished with docosahexaenoic acid, a long-chain (n-3) PUFA. In addition, omega-3 polyunsaturated fatty acids (n-3 PUFAs) [52-53, 84] have been shown to modulate levels of proinflammatory cytokines, hepatic acute phase proteins, eicosanoids, and tumor-derived factors in animal models of cancer. Omega-3 fatty acids can be detected in human serum/plasma, shown to cross the blood brain barrier and are the primary component of membranes of the nerve cells in the brain, Deficits in Omega-3 fatty acids are associated with damage to brain biochemistry, structure and cognitive function.[99-100] In contrast, a diet high in n-3 fatty acids reduced beta-amyloid (Abeta) and reduced the amount of plaque in the brain, especially in the hippocampus and parietal cortex, and reduced the amount of APP, the beta-amyloid precursor protein,[101-102] improving CI.

In summary, our team and others have demonstrated that the components of COGNUTRIN®, which includes standard formulations and extracts of blueberry anthocyanins and omega-3 fatty acids, can be detected in human serum/plasma,

shown to cross the blood brain barrier and localize in various brain regions important for learning and memory such as cerebellum, striatum and hippocampus in measurable quantities. Studies have correlated peripheral markers of inflammation, immune function and oxidative stress in serum/plasma with central inflammation and central nervous system functioning. Thus based on the etiologies of cognitive changes, animal and early clinical trial evidence of safety, bioavailability in blood and brain tissue and preliminary evidence of efficacy to improve CF, it is logical to test the effectiveness of a combination standard nutrition supplement that can provide anti-oxidant, anti-inflammatory nutrients critical to the physiochemical composition and functioning of the neurological system to "rescue" tissues from the effects of the oxidative damage and inflammation and reverse chemotherapy-induced cognitive impairment as we propose in this study.

1.6 Structural and functional imaging markers of cognitive function: Self-reported poor concentration or 'chemo-brain' has been validated using neuropsychological tasks and more recently, neuroimaging studies have identified structural [123] and performance differences between patients (i.e. those treated with chemotherapy) and controls [122,124,126]. For example, a recent functional Magnetic Resonance Imaging (fMRI) study has demonstrated that when compared to controls, chemotherapy treated patients show hyporesponsiveness in areas of the brain associated with impaired planning behavior and attentional abilities (i.e. paraphippocampal gyrus, left cuneus, right dorsal striatum, right inferior parietal cortex and left middle temporal gyrus). What is more, functional imaging studies have shown these disruptions last from one month following treatment [126] to 10 years following treatment .[122]

To date, imaging studies have only focused on assessing cognitive disruption induced by chemotherapy. The proposed study aims to replicate these findings and extend them by testing the proposed interventions (e.g. COGNUTRIN®) ability to offset the changes in cognitive performance in breast cancer survivors post chemotherapy treatment.

1.7 Rationale Evidence from epidemiologic studies, as well as our experimental animal literature, suggests that antioxidant and anti-inflammatory agents promote cognitive health among older adults and early stages of Alzheimer's disease.[24-50] To date, interventions with cognitive training alone,[51-52] pharmacological agents to treat chemotherapy-induced anemia,[55] psycho stimulants and antioxidant therapies with Vitamins A, C and E have not demonstrated efficacy or safety in treating CI in cancer survivors.[53-54] In addition, new trials have provided further evidence against long-term use, cost-effectiveness and side-effects with these pharmacological agents, particularly thrombic events, that established the need to identify alternate approaches that can be implemented for a longer durations, with fewer side effects and an established safety profile.[54-56] Both length and quality of survival are important end points.[2] Our group and others have demonstrated the safety of the antioxidant[57-63] and anti-inflammatory agents[61] in high risk and cancer patient cohorts, including breast cancer patients during treatment and the effectiveness of the nutritional supplementation mixture to specifically improve cognitive function in preclinical studies.[24, 45-48] In the current pilot proposal, we propose to examine the safety and influence of an intervention (COGNUTRIN®) using nutritional supplements (n-3 fatty acids and blue berry anthocyanins) on longitudinal changes in cognitive performance in breast cancer survivors post chemotherapy. Our ultimate goal is to provide interventions, both therapeutic and lifestyle that carry the potential to treat or ameliorate these late effects of cancer treatment. Based on the multiple etiology, the varying manifestation and extent of cognitive decline documented in this cohort, we hypothesize that interventions using a combination nutritional supplement with antioxidant and anti-inflammatory agents (COGNUTRIN®) can work synergistically to facilitate reductions in oxidative stress loads and inflammatory cytokines, with significant improvement in cognitive health resulting in improvements in quality of life of breast cancer survivors.

2 OBJECTIVES

2.1 Pilot trial Primary Objectives

1. **Specific Aim 1: Does nutritional supplementation facilitate cognitive function in adult breast cancer survivors, relative to persons who do not receive this supplementation?** We predict that adult breast cancer survivors receiving nutritional supplementation will experience improved cognitive performance as assessed by a battery of standardized tests of cognitive function. The current pilot study will evaluate the feasibility of administration of the nutritional supplement and placebo in this patient population and performing cognitive function testing at baseline and post supplementation.

2. **Specific Aim 2: Is the facilitation of cognitive function in the nutritional supplementation group mediated by changes in markers of oxidative stress and inflammation, relevant to cognitive performance?** We predict that persons receiving nutritional supplementation will exhibit larger changes in antioxidant markers and that these changes will serve to mediate or explain differences in the cognitive performance of the two groups. The current pilot study will evaluate the feasibility of administration of the nutritional supplement and placebo in this patient population and testing antioxidant levels at baseline and after the supplementation.

3. **Specific Aim 3: Does nutritional supplementation facilitate cognitive function in adult breast cancer survivors, relative to persons who do not receive this supplementation as indicated by changes in structural and resting-state functional markers observed utilizing the MRI/fMRI?** We predict that adult breast cancer survivors receiving nutritional supplementation will experience improved cognitive performance as assessed by a battery of structural and functional changes in imaging markers in the brain. The current pilot study will evaluate the feasibility of administration of the nutritional supplement and placebo in this patient population and performe structural and resting-state functional MRI to evaluate changes in these imaging markers at baseline and after the supplementation.

3.0 SUMMARY OF STUDY PLAN

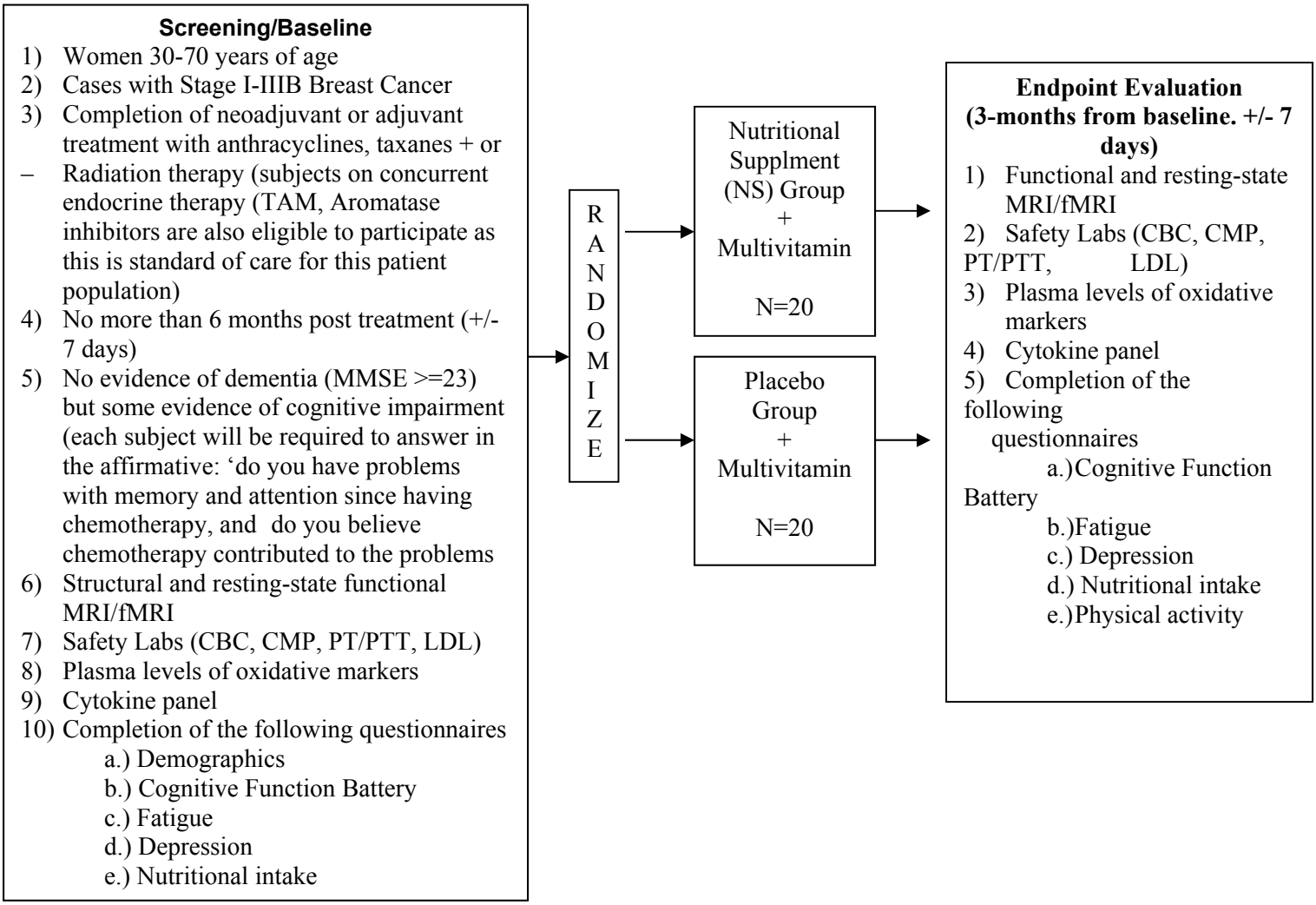
This pilot project is a randomized clinical trial of the feasibility of administering the nutritional supplement and placebo, performing a battery of cognitive function testing, structural and resting-state functional MRIs, and antioxidant levels at baseline and post supplementation in a target group of breast cancer survivors treated with chemotherapy. Prior to study initiation, the Human Subjects Committee will approve all study procedures. This goal of this pilot feasibility study is to provide a comprehensive assessment of a clinical trial's specific challenges as well as strategies to mitigate risks before a well- powered clinical trial can be proposed and implemented.

Research teams that use feasibility studies experience faster clinical trial accrual and significantly fewer delays in enrollment than those that forgo this preliminary assessment. A properly conducted clinical trial feasibility study can provide information regarding:

- Biomarker methodologies/estimations
- Effect size estimations
- Compatibility of the clinical trial treatments and assessments with current patterns of care
- Available patient population
- Likelihood of patients agreeing to participate in the clinical trial
- Potential modifications to clinical trial design and eligibility criteria to enhance enrollment
- Competing clinical trials
- Site capabilities
- Research infrastructure
- Personnel
- Equipment
- Regulatory requirements and timelines
- Local institutional review board/independent ethics committee (IRB/IEC) review timelines
- Investigator grant requirements and site contracts
- Projected patient enrollment rates.

The current feasibility pilot trial is to inform the research team of all these above challenges that occur with clinical trials and can contribute to the proposal and conduct of a well powered clinical trial.

Nutritional Supplementation (COGNUTRIN®) in Breast cancer Patients with Cognitive Impairment: Study Schema



4.0 PARTICIPANT SELECTION

Women who have been diagnosed with stage I-IIIB breast cancer (BCa) and who have completed neoadjuvant or adjuvant treatment with anthracyclines and/or taxanes + or – Radiation therapy within the past 6 months (+/- 7 days) will be invited to be screened for eligibility to participate in this trial (subjects on concurrent endocrine therapy (TAM, Aromatase inhibitors are also eligible to participate as this is standard of care for this patient population). We will, in addition, expand our recruitment at the Moffitt Cancer Center to include the members of the Moffitt Cancer Center Affiliate Network (Total Cancer Care network consisting of 16 community hospital affiliates and 415 community oncologists statewide. With this additional network to recruit this patient population from, we are confident that we will be able to recruit the number of subjects needed for this study from each site. Based on the number of subjects that can be recruited from Moffitt, we conservatively estimate that we will recruit and retain 40 subjects during the 6 months recruitment period. Throughout the recruitment, data collection and retention period of this study we will focus on retaining high participation rates of an ethnically diverse population.

If women are already patients at Moffitt Cancer Center or one of its affiliates, they will be identified by one of the medical oncologists who are co-investigators in this trial based on the diagnosis and treatment received. Women who have been diagnosed with stage I-IIIB breast cancer (BCa) and who have completed neoadjuvant or adjuvant treatment with anthracyclines and/or taxanes + or – Radiation therapy within the past 6 months, (+/- 7 days) will be referred to the clinical research coordinator who will then invite these potentially eligible subjects to be screened for eligibility criteria to participate in this trial. All subjects referred to the research team from affiliate sites will be screened for eligibility. In this pilot clinical trial, all subjects, if identified as eligible based on initial screening in any other site other than the Moffitt Cancer center, will be required to come to the Moffitt cancer center if willing to continue to participate.

Although cognitive complaints are reported frequently after breast cancer treatments, their association with standardized neuropsychological (NP) test performance is not well-established. The lack of association between NP test performance and subjective cognitive complaints in prior studies with breast cancer patients [156-157,160] could reflect the lack of specificity and sensitivity of the self-report tools, or could be due to reliance on global measures rather than domain-specific assessments. There is also a skepticism regarding the validity of cognitive complaints and their relationship to NP test performance in cancer patients. A few more recent studies have suggested that patient self-report is associated with relevant domains of NP function, which is strongest for memory complaints. There is an emerging literature supporting the ability of individuals to subjectively detect changes in cognitive function that precedes statistically significant changes in NP performance or structural imaging abnormalities [158] . There is thus significant support for the value of patient-reported outcomes as a central measurement in evaluation of cancer treatment-related morbidities [159], including domains of cognitive impairment.

Therefore, based on the current body of research, the inclusion criteria in this trial will be based on subjective cognitive complaints in cancer survivors. Inclusion criteria to identify women who met the inclusion criterion for chemotherapy-induced cognitive impairment, we will use the most current approach described by Ferguson et al (2012) [160] where each subject will be required to answer in the affirmative: 'do you have problems with memory and attention since having chemotherapy, and do you believe chemotherapy contributed to the problems?'

4.1 Inclusion Criteria. 1) Women 30-70 years of age 2) Cases with Stage I-IIIB Breast Cancer that have completed neoadjuvant or adjuvant treatment with anthracyclines and/or taxanes + or –Radiation therapy within past 6 months (+/- 7 days) (subjects on concurrent endocrine therapy (TAM, Aromatase inhibitors are also eligible to participate as this is standard of care for this patient population) 3) Able to understand and sign the informed consent. 4) Fluent in reading, comprehension and communication in the English language. (Persons who are unable to meet this requirement are excluded from the current proposal because many of the cognitive tests that are administered are in English and translation of these instruments in other languages is currently unavailable) 5) No evidence of dementia (MMSE >=23) but some evidence of cognitive impairment (each subject will be required to answer in the affirmative: 'do you have problems with memory and attention since having chemotherapy, and do you believe chemotherapy contributed to the problems?'); 6) Each subject must be aware of the nature of his current medical condition and must be willing to give consent after being informed of the experimental nature of therapy, alternatives, potential benefits, side-effects, risks and discomforts. 7) ECOG performance status of 0- 2 (8) Acceptable hemoglobin and hematocrit level based on CBC. (9) All subjects must be willing to be monitored for adequacy of nutritional intake during the intervention, as is the current standard of clinical practice.

4.2 Criteria for exclusion will include: 1) Use of estrogens (oral, dermal or vaginal), progesterone (oral or topical), or androgens during the previous 3 months, 2) Use of over the counter steroid hormonal supplements 3) Patients with advanced or Stage IIIC or IV breast cancer or other cancers. 4) Use of n-3 fatty acids or high dose antioxidant supplements other than what is provided in the trial. 5) History of known allergy to components of the study supplements. 6) Subjects with renal or liver disease (AST/ALT > 5.0 x upper limit of normal as evidenced by impairment of baseline laboratory values); Actual creatinine clearance of <60 utilizing the Cockroft-Gault formula (1976), which employs creatinine measurements and a patient's weight to predict the clearance. (The constant for women is 1.04). 7) Concurrent participation in another chemoprevention trial. 8) Evidence of bleeding diathesis or coagulopathy (PTT and/or either PT or INR > 1.5x upper limit of normal (except for subjects receiving anti-coagulation therapy). Concurrent use of Coumadin or Warfarin will be acceptable and monitoring patients on warfarin or Coumadin will follow the standard of care as dictated by the prescribing physician (PT/PTT). If the prescribing physician is not a Moffitt MD, then the prescribing MD

will be notified by the research staff of the subject participating in the study, and monitors for PT, PTT will be obtained from patient during the 3 month study visit for review. 9) Subjects with metabolic abnormalities (e.g. thyroid disorders, insulin dependent diabetes, rheumatologic disease etc.). Subjects with metabolic disorders (a) who are otherwise eligible, (b) treated for hypothyroidism by their primary MD with Synthroid (levothyroxine) and (c) with the approval of the Moffitt treating oncologist will not be excluded from the study. 10) Patients with known claustrophobia, presence of pacemaker and/or ferromagnetic material in their body that would prohibit MRI imaging (ex. tissue expanders). 11) Medical history of concussions 12) Other acute or chronic medical or psychiatric condition or laboratory abnormality that may increase risk associated with study participation or study drug administration, or may interfere with interpretation of study results, and in the judgment of the investigator would make the subject inappropriate for entry into this study.

Our criteria for inclusion of subjects in this trial is that subjects recruited are able to understand and sign the informed consent. The subjects must also be fluent English-speaking (Persons who are not fluent English speakers are excluded from the current proposal because many of the cognitive tests that are administered are language-based and are heavily influenced by first language). Subjects must also have no evidence of dementia (MMSE >=23) but some evidence of cognitive impairment (each subject will be required to answer in the affirmative: 'do you have problems with memory and attention since having chemotherapy, and do you believe chemotherapy contributed to the problems?');. Each subject must be aware of the nature of his current medical condition and must be willing to give consent after being informed of the experimental nature of therapy, alternatives, potential benefits, side-effects, risks and discomforts. If a subject is determined to be cognitively impaired to the point of not meeting the above criteria at screening by the investigative team, they will not be included in this pilot phase of the clinical trial, making the use of proxy's unnecessary.

4.3 Anticipated problems with subject recruitment and retention: Our group has extensive experience conducting research with breast cancer patients as well as studies collecting neuropsychological data, questionnaires, blood samples, and MRI/fMRI. Thus, we expect difficulties to be minimal. As with all clinical trials, we anticipate that there will a 10% loss of subjects to follow up, although, in our experience, breast cancer survivors tend to be a highly motivated group and the time between assessments is short and subjects will be required to come in monthly for monitoring safety and compliance. Moffitt Cancer center, as the only NCI designated Comprehensive cancer center in the State of Florida has a substantial number of subjects treated for breast cancer.

The recruitment, intervention and retention team (PI, Dr. Kumar, Co-I Dr. Jim and CRC) will initiate, develop and implement procedures to deal with and solve problems with attrition. As we have with our previous and current intervention trials, we will take measures to ensure that loss to follow-up is minimal, implementing several strategies for recruitment and retention as follows:

a. Contact with participants will be maintained by their preferred method or methods: phone numbers, primary and secondary mailing addresses, and e-mail addresses updated at each contact.
b. At each visit, current contact information will be verified and an alternate means of contacting the person will be established with the permission of the participant. For example, friends, family members, and summer addresses will be maintained with the consent of the participant.
c. Reminder calls and/or e-mails the day prior to the appointment.
d. Monetary compensation for travel: Because of the importance of retaining participants for the duration of the study, participants will be provided $50 for each visit to cover travel expenses. Participants traveling more than 100 miles from their home to reach the research site will receive an additional $30 for transportation costs.
e. Empathetic, warm and experienced clinical research coordinators and study staff who can establish rapport with participants.
f. Telephone contact will be encouraged with calls from the research staff to the subject at least once a month between visits.
g. Appointments will be made at the participants' convenience including evenings or early mornings.
h. Monthly and follow up visits will occur in the comfort of the Breast Program clinics to ensure familiarity of staff and environment.
i. Valet parking for all subjects, free of charge.

j. Weekly staff meetings will occur and case studies will be reviewed to observe patterns and etiology of attrition of participants will be discussed.

5.0 PHARMACEUTICAL INFORMATION:

5.1 COGNUTRIN: The choice of the supplement to use in this trial is based on relevant research supporting the various components as well as the use of a commercially available dietary supplement. The supplement to be used will be a combination of 2 standardized formulations (a) antioxidants from blueberry extracts - VitaBlue™ (40% polyphenolics, 12.5% anthocyanins from blueberries (BB) and (b) omega 3-fatty acids - Lovaza®. The standardized agent - Lovaza is manufactured by Glaxo Smith Kline and will be purchased for use in this study. However, the blue berry anthocyanins was purchased from a standard manufacturer and encapsulated at the dose for this specific study using FDA standards in a certified formulary and tested by an independent laboratory for composition (FDA IND 116424). The matching placebos were also manufactured for this study using FDA standards- all of which have been evaluated and approved for use by the FDA. As indicated in the protocol, these are 2 separate products administered separately (pages 8-11). The combination that will be used has met the standards of the FDA and an IND has been approved by the FDA (FDA IND 116424) for use of this agent combination in this study. Our team took into consideration the high patient burden to the subjects due to large size if the agents were combined into one capsule and thus decided to keep them as 2 separate agents.

The supplement COGNUTRIN is a term coined by the PI to denote the combination of 2 standardized formulations (a) VitaBlue™ (40% polyphenolics, 12.5% anthocyanins from blueberries (BB) and (b) n-3 fatty acids- Lovaza®. We have obtained IND (FDA IND 116424) from the FDA for this current indication proposed from the FDA. We have not submitted a patent for this product but hope to explore the potential to license this combination if successful in the future. We are currently working on obtaining a registered trademark with this name that we coined –COGNUTRIN. Our goal is to develop this combination agent scientifically, maintain exclusivity and to not be confused with myriads of supplements in the market place.

5.1.1 VitaBlue™ (BB Anthocyanins): VitaBlue™ is a freeze dried high potency extract from blueberries. VitaBlue™ is created using a unique proprietary process from VDF FutureCeuticals. It comes as a standardized powder containing blueberry anthocyanins that is ready for encapsulation.

VitaBlue™ provides concentrated amounts of healthy blueberry anthocyanins and polyphenols. Made from Wild Blueberry Extract (Lowbush Powder, VitaBlue™, although no major adverse events have been observed with Vitablue, anecdotal evidence suggests that Blueberry can occasionally induce symptoms of food allergy in sensitized individuals; however, no studies have been reported to date. The lack of reported evidence may be due to the low allergenicity of this berry, the small amounts consumed, or the restricted time frame of consumption. Oftentimes a limited or low exposure to certain allergens might be the reason for the limited complaints reported. However, as the promotion of blueberry consumption continues, this situation may change (1). Nonetheless, based on adverse effects reported to other berries, and particularly to members of the same family, it can be said that blueberries may induce symptoms of food allergy in sensitized individuals (2).

For example, a 25-year-old woman reported adverse reactions due to lingonberry (V. vitis-idaea). While eating lingonberry jam, she developed allergic symptoms including itching wheals around her mouth, however, the symptoms dissipated. During a second episode, when she ingested a very small amount of lingonberry jam several days later, she immediately noticed more-intense symptoms, including severe itching on the mouth, tongue and throat, and wheals over the mouth. Symptoms dissipated within an hour, however, it was noted that upon secondary exposure the symptoms were more severe and a skin reactivity test demonstrated a positive allergic response to lingonberry (3). Since there are no reported adverse effects of blueberries, we expect no adverse effects to occur with this agent during this study.

5.1.2: Lovaza® (omega-3-acid ethyl esters): We plan to use a standardized product Lovaza® (omega-3-acid ethyl esters), an FDA-approved prescription omega-3 provides prescription-only potency and purity with each 1-gram Lovaza® (omega-3-acid ethyl esters) gel capsule contains 90% omega-3-acid ethyl esters; 84%

eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) ethyl esters. Lovaza® is provided by GlaxoSmithKline, who has authorized the FDA center for Drug Evaluation and Research, Division of Metabolic and Endocrine Drug Products to reference Lovaza® Capsules IND # 45,998.. Bottles are labeled to comply with study requirements and applicable regulations for investigational drug use. Bottles with study agent will be stored at controlled room temperature (15-30 0 C). The products are both standardized and examined for content, stability and bioavailability and the study will be conducted with an investigator-initiated new drug (IND) approval from the Food and Drug Administration (FDA) as mandated by the National Cancer Institute for Phase I-III clinical trials. Use of n-3 fatty acids may decrease platelet aggregation and subjects with evidence of bleeding diathesis or coagulopathy will not be included in this study and those with concurrent use of Coumadin or warfarin will be observed closely. The follow-up monitoring for PT, PTT for patients on warfarin and/or Coumadin will follow the standard of care as dictated by the prescribing physician. The prescribing MD will be notified by the research staff of the subject participating in the study, and monitors for PT, PTT will be obtained from patients during the monthly clinic visits.

5.2 Rationale for Dose selection and Administration:

5.2.1 Study Agent Lovaza® There is no established Dietary Reference Intake for n-3 fatty acids, yet the Adequate Intake (AI) is set at 1.6 and 1.1 grams/day for men and women respectively. While intake in the United States occurs at much lower than the proposed AI and no signs of deficiency are observed, the AI is proposed to provide optimal health benefits associated with consuming n-3 fatty acids. There is no Upper Limit established for n-3 fatty acids. In early studies using fish oil capsules, we and others have demonstrated the safety of administering 2-6 gms of EPA per day. Anticachectic effects were seen with these doses, with no adverse effects, however, there was no greater response with 6 gms per day relative to 2-4 grams/day. Therefore, the dose selected for this pilot clinical trial, 4 grams per day is comparable to the safe but effective dosage used in our preliminary studies and that of others. Currently prescription doses of 4 grams a day of Lovaza® is used and approved by the FDA to treat hypertriglyceridemia. Furthermore, one, 3 to 4 oz serving of cold water fish such as salmon or sardines contains approximately 1.0-1.5 gms of n-3 fatty acids. The participant will be provided packages of the agent in a capsule form and instructed on compliance, intake, duration of intervention, and monitors to be completed by the research staff. Subjects will be instructed to consume 4 gm Lovaza® in a divided dose (2 gm in the morning and 2 gm at night, to provide a total of 4 gm/day) with meals for a period of 3 months.

5.2.2 Blueberry anthocyanins (VitaBlue): Similarly, there is no established Dietary Reference Intake of the constituents of anthocyanins. The average daily consumption of anthocyanins in a western diet is estimated to be 100 mgs/day. [120]. Anthocyanins are rapidly absorbed and metabolized extensively following a moderate-to-high oral dose in humans. Studies have indicated anywhere from 4-6 hours half-life with complete excretion of parent compound as well as metabolites within 24 hours both in urine and plasma, indicating the need for a dose every 6- 8 hours to have consistent exposure [121]. Similarly administration of anthocyanins at doses ranging from 0.5-2.0 grams daily for 7 days in colorectal cancer patients with dose dependant concentrations detected in plasma and urine with no toxicities. [120]. Studies utilizing anthocyanins at doses of 1.4 grams/day (Mitroslect, Indena S.p.A.) administered as 0.47 grams/capsule TID are currently in progress to provide active cellular antioxidant protection, inhibit inflammatory gene expression and consequently protect against oxidant-induced and inflammatory cell damage and cytotoxicity in obesity-induced inflammation and insulin resistance (NCT01180712) in 40-60 year old males for 21 days. In summary, repeated administration of anthocyanins exerts pharmacodynamic effects and generates concentrations of anthocyanins in human plasma as well as urine with no toxicity. We will thus use a dose of 0.47 grams/capsule dose of anthocyanins TID to total 1.4 grams per day, similar to the dose used in these chemoprevention trials.

5.3 Availability: VitaBlue will be obtained from VDF FutureCeuticals'. The product has earned self-affirmed GRAS (generally recognized as safe) status for use in food and beverages after a comprehensive review by an independent panel of experts. Lovaza® is manufactured and distributed by Glaxo Smith-Kline laboratories and will be obtained from them for use in this study. The capsules have been manufactured by our team utilizing standards established by the FDA in a certified Compounding Center (Carrollwood Compounding Center and Pharmacy) and verified by the Center and by an independent testing group to assure consistent quality of the active agent contained in each capsule. The FDA has verified all these aspects of the agent as well as the placebo manufactured for this study and has provided us an IND approval of

these agents used in this study. Dr. Kumar's team currently holds 3 INDs for agents and placebos developed and approved by the FDA using the same methods for use in her research approved by this IRB.

Placebo's were also manufactured by Carrollwood Compounding Center and Pharmacy.

5.4 Agent Distribution: Agents will only be released by the Investigational pharmacy at MCC after documentation of IRB approval of the protocol and consent is provided and the collection of all essential documents is complete (*e.g.,* signed 1572, current CV, medical license, and lab certifications, *etc*.) and new documents have been submitted to the FDA for the IND.

5.5 Agent Accountability: The PI, or a responsible party designated by the investigator (Investigational pharmacy at MCC), will maintain a careful record of receipt, disposition, and return of all study drugs on the Investigational Agent Accountability Record. All study drug supplies will be kept in a locked, limited access area. The study drug will not be used outside the context of the protocol. Under no circumstances will the investigator or other site personnel supply study drug to other investigators, patients, or clinics, or allow supplies to be used other than directed by this protocol. Study agent will not be transferred from one participant to another.

The investigator will maintain records documenting the receipt, use, loss or other disposition of the investigational product, including batch or code numbers, and account for it's disposition on a subject-by-subject basis, including specific dates and quantities. The source document, documenting the subject's participation in this randomized clinical trial, must be documented in the medical and research records. Destruction will be documented in accordance with institutional practices.

5.6 Packaging and Labels: Bottles are labeled to comply with study requirements and applicable regulations for investigational drug use. Bottle labels include agent name, protocol number, dosing and storage instructions, required warnings for restricted, investigational use, and spaces for recording the subject registration and randomization number. The study agent to be used, Lovaza® (omega-3-acid ethyl esters), an FDA-approved prescription omega-3 provides prescription-only potency and purity with each 1-gram Lovaza® (omega-3-acid ethyl esters) gel capsule contains 90% omega-3-acid ethyl esters; 84% eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) ethyl esters. Lovaza® is provided by GlaxoSmithKline, who has authorized the FDA center for Drug Evaluation and Research, Division of Metabolic and Endocrine Drug Products to reference Lovaza® Capsules IND # 45,998. An investigator-initiated IND will be cross filed by the PI for this clinic trial

5.7 Drug Storage of COGNUTRIN (VITABLUE and n-3 fatty acids): All investigational agents will be received, stored and dispensed by the Investigational pharmacy at MCC. Bottles with study agent will be stored at controlled room temperature (15-30 0 C).The PI, or a responsible party designated by the investigator, will maintain a careful record of receipt, disposition, and return of all study agent on the Investigational Agent Accountability Record. All study drug supplies will be kept in a locked limited access room. The study drug must not be used outside the context of the protocol. Under no circumstances will the investigator or .other site personnel supply study drug to other investigators, patients, or clinics, or allow supplies to be used other than directed by this protocol. Study agent may not be transferred from one patient to another, from one participating center to another participating center or from one protocol to another. All other transfers (i.e. patient or PI moves) must be approved in advance by the study PI. Every attempt will be made to collect all unused study agent supplies to be returned to the Moffitt Research Center PI office. The investigator will maintain records documenting the receipt, use, loss or other disposition of the investigational product, including batch or code numbers, and account for it's disposition on subject-by-subject basis, including specific dates and quantities. The source document, documenting the subject's participation in this clinical trial must be documented in the medical and research records. Destruction will be adequately documented.

5.8 Registration and Randomization: Potential subjects will be screened by the research coordinator using an Eligibility Checklist (Appendix I). The initial screening visit will verify patient eligibility to the study. A minimum of 40 subjects will be randomized to the pilot clinical trial. Within 6 months of completion of treatment with chemotherapy and radiation if applicable, we will screen the patients for cognitive function to determine the eligibility of participants to participate in the randomized trial portion of the study. If found eligible based on the criteria established for cognitive impairment, subjects will be ready for baseline data collection.

The research staff will use the Demographic Questionnaire Form to obtain baseline demographic data, medical and family history, and information regarding other health habits such as alcohol, tobacco and previous nutritional supplement use. Clinical Research Form (CRF): All medical record data (CRF) pertaining to the cancer diagnosis, tumor characteristics, node involvement, stage confirmation, medical history of cancer, menstrual cycle information, other medical history including renal or liver disease, concomitant medications including treatments for cancer and autoimmune diseases will be obtained from all subjects at baseline and changes in these variables monitored. Once clinical eligibility is confirmed, and informed consent is signed, randomization of subjects takes place within the Moffitt SRARS program. SRARS is a web delivered application that records subject registrations and provides randomization assignments. Subjects will be randomized to one of 2 groups. An email is sent to a list of pre-determined recipients (clinical research coordinators) stating the treatment/randomization assigned to the subject.

5.9 Blinding and Unblinding Methods: All requests to unblind treatment assignment will be directed to the Protocol Lead Investigators (Kumar, Moffitt Cancer Center & Research Institute, telephone: 813-745-6885, fax: 813-745-7185, e-mail: Nagi.Kumar@moffitt.org . Dr. Kumar will determine if disclosure of treatment assignment is required after consultation with the Medical Doctor who is the primary MD for the subject. If disclosure of the treatment would affect important medical decisions in a subject experiencing an AE, or could affect decisions regarding safe conduct of the clinical trial, unblinding might be allowed. If disclosure is warranted, the site will be directed to the product label found in the subject's CRF binder or other designated research record. The label has a scratch-off area that will disclose the treatment assignment. Each site will document the unblinding process in the patient chart.

6. AGENT ADMINISTRATION:

6.1 Dose Regimen and Dose Groups: Intervention groups

6.1.1 Placebo Group: All participants in the placebo arm will receive the multivitamin supplement and the cognitive battery tests and structural and resting-state functional imaging at baseline and 3 months. The placebo group used herein will allow us to generate information regarding the reliable change index, so information regarding the naturalistic change in cognitive performance, as well as the potential for practice effects to influence mean-level change data, across the follow-up period is important.

6.1.2 Nutritional Supplement Group (NS).: The choice of the supplement to use in this trial is based on relevant research supporting the various components as well as the use of a commercially available standardized dietary supplement of anthocyanins and n-3 fatty acids. The supplement to be used is a standardized formulation of blueberry extract VitaBlue® (40% polyphenolics, 12.5% anthocyanins from blueberries. The supplement will in addition include Lovaza® (omega-3-acid ethyl esters), an FDA-approved prescription omega-3 provides prescription-only potency and purity with each 1-gram Lovaza® (omega-3-acid ethyl esters) gel capsule contains 90% omega-3-acid ethyl esters; 84% eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) ethyl esters. Lovaza® is provided by GlaxoSmithKline, who has authorized the FDA center for Drug Evaluation and Research, Division of Metabolic and Endocrine Drug Products to reference Lovaza® Capsules IND # 45,998. An investigator-initiated IND has been obtained by PI for use of these agents in this clinical trial (IND # 116424).
Subjects in the supplements treatment arm of the study will be given in the same fashion and time table to the "placebo" patients as the active treatment patients.

6.2 Agent Administration: Intervention will be administered to the patient on an outpatient basis and self-administered by the participants. The daily dose of will be taken in divided doses (Lovaza® 2 tablet BID and Vitablue™ 2 tablet TID). On the day of monthly follow-up visit, capsules should be taken within 4 hours of visit and blood draw for required lab work.

Two bottles containing Lovaza and 1 bottle of anthocyanins capsules will be dispensed to all participants upon randomization with adequate number of capsules, to ensure that the subject has sufficient study drug to last until the next planned clinic visit. At baseline and at each monthly visit up to the 3-month final visit, participants will receive these supplements in person to take home with them. At that point, participants will receive instructions regarding how many supplement pills they are to take a day.

Schedule of dose administration of study agents

Arm of Study	Breakfast	Lunch	Dinner
Treatment arm	2 tablets Lovaza 2 tablets Vitablue	2 tablets Vitablue	2 tablets Lovaza 2 tablets Vitablue 1 tablet multivitamin/mineral
Placebo arm	4 tablets matching placebo tablets	2 tablets matching placebo	4 tablets matching placebo tablets 1 tablet multivitamin/mineral

6.3 Contraindications: COGNUTRIN ingredients - n-3 fatty acids may be contraindicated in subjects with bleeding disorders and anthocyanins may be contraindicated in people who are sensitive or allergic to berries.

During study participation, subjects will be instructed to limit their consumption of the following berries to 1 cup per day: blackberry, blueberry, chokeberry, black currant, elderberry, and black raspberry. They will be asked to limit their consumption of the following omega 3's to 2-3 servings (4 oz per serving) a week: walnuts, flaxseed oil, canola oil, fish oils, and fatty fish such as salmon, trout, mackerel, herring, sardines, pilchards, kipper, eel, whitebait, fresh tuna, anchovies, swordfish, bloater, cacha, carp, hilsa, jack fish, katla, orange roughy, and pangas.

6.4 Concomitant Medications: During study participation, subjects will be instructed to suspend consumption of additional supplements containing anthocyanins or omega 3's. Subjects are considered enrolled in the trial from the time that the informed consent document is signed until the time that the subject is off study. All medications (prescription and over-the-counter), taken by the participant while on study will be documented on the concomitant medication CRF and will include: start and stop date, dose and route of administration, and indication.

6.5 Dose and Toxicity Management: We will ensure that our protocol and informed consent comply with FDA recommendations. In addition, we have planned monthly visits to monitor daily symptoms monitor and will perform an interview for adverse events, unusual symptoms, change in medical condition and concomitant medications use. Any abnormality observed will be communicated by the research coordinator to the study MD for evaluation and for continuation in the study. Subjects in the study will be dropped from study until which time the symptom is determined to be unrelated to the study agent as determined by the study MD. We will provide each subject with a 24- hour contact phone number and specific instruction on contacting the research team including the MD in this study. In addition, evaluation of safety in this subject population has been added as a specific aim of the trial with a formal comparison of safety between active and placebo arms, further illustrating our commitment to ensuring subject safety in this and future clinical trials.

A patient's treatment will be discontinued in any of the following circumstances: (a) Allergic reaction to product; (b) Dramatic change in stool frequency; (c) Start of other treatments that may cause malabsorption; (d) Request of patient of MD or patient; (e) Unusual symptoms reported during follow-up or other unusual symptoms or blood work results observed during the study period; and, (f) Persistent nausea not controlled with antiemetics. There are no reductions in the Cognutrin® dose. If adverse events occur that require holding Cognutrin ® (until etiology is determined), the dose will remain the same once treatment resumes. Any toxicities associated or possibly associated with Cognutrin ® treatment will be managed according to standard medical practice. Subjects will be assessed clinically for toxicity prior to, during intervention on a monthly basis and at end of intervention. If >grade III toxicity occurs because of Cognutrin ® at any time during the study, treatment with Cognutrin ® will be discontinued. We will also inquire about hypersensitivity to fish/and or shellfish and symptoms of atrial fibrillation at each visit. All subjects will be followed by their medical oncologists for their cancer treatment, using the routine surveillance guidelines as established by the Cancer Center during the study.

There are no alternative agents that are currently available to treat or ameliorate cognitive impairment observed in this target population. To date, interventions with cognitive training alone, pharmacological agents to treat chemotherapy-induced anemia, psycho stimulants and antioxidant therapies with Vitamins A, C and E have not demonstrated efficacy nor safety in treating CI. We and others have demonstrated in preclinical and clinical trials that plant-based substances and n-3 fatty acids with potent antioxidant and anti-inflammatory properties can be detected in plasma and serum, cross the blood-brain barrier, localize in various brain regions important for learning and memory such as cerebellum, striatum

and hippocampus and increase neurogenesis. However, these interventions have not been evaluated to ameliorate CI in cancer patients treated with chemotherapy. The results of this study may inform the design of a well-powered clinical trial to test the effectiveness of this intervention to ameliorate these late effects of cancer treatment and ultimately improve quality of survival in this patient population. However, no guarantee of personal benefit based on participation will be made to subjects recruited in this study. Instead, participants will be told that results are expected to contribute to increased knowledge about possible interventions to remediate chemotherapy-induced changes in cognitive performance. Among persons in the nutritional supplementation intervention conditions, based on existing evidence from the ageing literature, some improvements to cognitive performance are anticipated.

On the other hand, the risks to participants of this study can be divided into three groups a) risk due to collection of blood, b) potential risk due to study agent intake; c) Magnetic resonance Imaging (MRI). We have taken careful steps to implement p**rocedures for minimizing and protecting against potential risks:**

(a) Reduce risks of specimen collection: Participants undergoing venipuncture typically experience brief pain during needle insertion; occasionally experience bruising or the need for additional needle sticks to access "good vein", and rarely experience an infection at the site of needle insertion. Sterile equipment, accepted clinical practices, and trained; experienced staff will be used to reduce the risks of specimen collection. In addition the site will be cleaned before drawing blood, pressure will be applied until the bleeding stops and a sterile dressing will be used to cover the puncture site.

(b) Reduce risk due to study agent: Based upon our team's considerable experience administering anthocyanins and n-3 fatty acid supplementation in preclinical and clinical trials, we have proposed periodic monitoring of organ function (CBC, CMP) including liver function tests throughout the study. Moreover, all of the substances in the nutritional supplements are found in everyday foods and are not at levels that greatly exceed a typical dietary intake. We have obtained detailed information and potential side effects of each of the agents used in the study and paid attention to the criteria for inclusion and exclusion and dose to reduce side effects as follows: **Study Agent** Lovaza® There is no established Dietary Reference Intake for n-3 fatty acids, yet the Adequate Intake (AI) is set at 1.6 and 1.1 grams/day for men and women respectively. While intake in the United States occurs at much lower than the proposed AI and no signs of deficiency are observed, the AI is proposed to provide optimal health benefits associated with consuming n-3 fatty acids. There is no Upper Limit established for n-3 fatty acids. In early studies using fish oil capsules, we and others have demonstrated the safety of administering 2-6 gms of EPA per day. Anticachectic effects were seen with these doses, with no adverse effects, however, there was no greater response with 6 gms per day relative to 2-4 grams/day. Therefore, the dose selected for this pilot clinical trial, 4 grams per day is comparable to the safe but effective dosage used in our preliminary studies and that of others. Currently prescription doses of 4 grams a day of Lovaza® is used and approved by the FDA to treat hypertriglyceridemia. Furthermore, one, 3 to 4 oz serving of cold water fish such as salmon or sardines contains approximately 1.0-1.5 gms of n-3 fatty acids. The participant will be provided packages of the agent in a capsule form and instructed on compliance, intake, duration of intervention, and monitors to be completed by the research staff. Subjects will be instructed to consume 4 gm Lovaza® in a divided dose (2 gm in the morning, 2 gm at night, to provide a total of 4 gm/day) with meals for a period of 3 months. **Blueberry anthocyanins (VitaBlue):** Similarly, there is no established Dietary Reference Intake of the constituents of anthocyanins. The average daily consumption of anthocyanins in a western diet is estimated to be 100 mgs/day. Anthocyanins are rapidly absorbed and metabolized extensively following a moderate-to-high oral dose in humans. Studies have indicated anywhere from 4-6 hours half-life with complete excretion of parent compound as well as metabolites within 24 hours both in urine and plasma, indicating the need for a dose every 6- 8 hours to have consistent exposure [121]. Similarly administration of anthocyanins at doses ranging from 0.5-2.0 grams daily for 7 days in colorectal cancer patients with dose dependant concentrations detected in plasma and urine with no toxicities. Studies utilizing anthocyanins at doses of 1.4 grams/day .(Mitroslect, Indena S.p.A.) administered as 0.47 grams/capsule TID are currently in progress to provide active cellular antioxidant protection, inhibit inflammatory gene expression and consequently protect against oxidant-induced and inflammatory cell damage and cytotoxicity in obesity-induced inflammation and insulin resistance in 40-60 year old males for 21 days. In summary, repeated administration of anthocyanins exerts pharmacodynamic effects and generates concentrations of anthocyanins in human plasma as well as urine with no toxicity. We will thus use a dose of 0.47 grams/capsule dose of anthocyanins TID to total 1.4 grams per day, similar to the dose used in these chemoprevention trials.

Nevertheless, we will record adverse events and will develop a data safety and monitoring plan to ensure that study participants are not negatively affected by study compounds. We will in addition obtain symptoms and concomitant medications that may be potentially related to study agent intake using daily monitors. All of the substances in the nutritional supplements are found in everyday foods and are not at levels that greatly exceed a typical dietary intake. Nevertheless, we will observe and report adverse events and will develop a data safety and monitoring plan to ensure that study participants are not negatively affected by study compounds. Our group is currently involved in several active clinical trials where these agents are being used and under IND approval from the FDA. We will keep abreast of the literature in the area of potential drug-drug, drug-nutrient interactions and other contraindications in the evolving research and will amend the study appropriately to ensure subject safety.

(c) Risks due to MRI: For most people, there is no danger associated with having an MRI scan. However, there may be some risk of psychological distress for the individuals posed by the confining environment of the scanner. We will therefore verify that participants are not claustrophobic, thereby reducing the probability of claustrophobia. The scanning environment poses potential risk from the magnet; specifically that metallic objects brought into the scanning room are dangerous to the individual. However the well-established Moffitt Cancer Center and University Diagnostic Institute protocol for treatment of patients in this environment minimizes risk.

Other: The research team has considerable experience with administering cognitive tests to young and older adults and in all cases, participants will be encouraged to do "their best", which seems to minimize test taking anxiety. Experienced personnel will administer all self-report and cognitive performance measures. The research study staff will carefully monitor participants' reactions and provide information upon request. All participants will be provided with Dr. Kumar's name and telephone number.

Based on the unavailability of effective and safe treatments to ameliorate cognitive impairment resulting from chemotherapy and the potential benefit a study such as this can provide, combined with the careful attention paid to minimize and eliminate risks due to participation, the benefits outweigh the risks.

Adverse events requiring delays or permanent discontinuation of Cognutrin® are listed in the table below:

Event	**Action**
Grade 1 & 2 constitutional, dermatological, gastrointestinal (GI), metabolic and pain symptoms Examples: Rash Allergic rhinitis, cough, bronchitis. Abdominal Pain Belching/Gas Nausea/Vomiting Diarrhea	No Action Required Inform MD Document event in research and medical record
Grade 3	Discontinue Cognutrin ®/Notify Study PI
Grade 4	Discontinue Cognutrin ®/Notify Study PI
Grade 2, 3, or 4 abnormal liver function tests[1,2] as determined by NCI CTC version 4.0	Discontinue Cognutrin ®/Notify Study PI and Monitor until it returns to normal

1. Tests used to monitor liver function include: albumin, total and direct bilirubin, alkaline phosphatase, AST, ALT, total protein, PT/PTT,)
2. In the event that PT/PTT is abnormal due to anticoagulant therapy, treatment and/or drug discontinuation will be at the discretion of the site investigator/MD.

Regardless of the reason for holding study drug treatment, the maximum allowable length of treatment interruption is 1 week. Interruption of intake of >1 week will result in discontinuance of subject from the study. Patients who have an ongoing Cognutrin ®-related Grade 3-4 or serious adverse event at the time of discontinuation from study treatment will continue to be followed until resolution of the event or until the event is considered irreversible by the study MDs.

In addition, a standard multivitamin/ mineral preparation will be provided free of charge during the study duration, to assure a consistent baseline intake of essential vitamins and minerals among all study participants during the study period and to encourage subjects from not using other supplements that may contain components of the study agents. Subjects in the treatment and placebo arms will be provided these multivitamin/mineral supplements and instructed to consume 1 tablet daily with the dinner meal.

In our experience with cancer survivors both clinically and in research studies, over 75-85% of breast cancer survivors use a multivitamin/mineral supplement. Several of these supplements have added ingredients including botanicals and biologics from unknown as well as known sources. The quality, content, standardization techniques, safety as well as stability of the content of these supplements have been a challenge to our team. In order to provide a standard dose to all subjects in the study .ensure safety and prevent any overdosing on other supplements, similar to the content of COGNUTRIN, we have provided a standard multivitamin/mineral to the subjects in our clinical trials with botanicals and biologics.

Our group is currently involved in several active clinical trials where these agents are being used and under IND approval from the FDA. We will keep abreast of the literature in the area of potential drug-drug, drug-nutrient interactions and other contraindications in the evolving research and will amend the study appropriately to ensure subject safety.

The research team has considerable experience with administering cognitive tests to young and older adults and in all cases, participants will be encouraged to do "their best", which seems to minimize test taking anxiety. Experienced personnel will administer all self-report and cognitive performance measures. The research study staff will carefully monitor participants' reactions and provide information upon request. All participants will be provided with Dr. Kumar's name and telephone number to clarify or answer any questions.

6.6 Adherence/Compliance: During study participation, subjects will be asked to: self-administer the study medication; complete diet recall forms at specified time periods (baseline, 1, 2 and 3 months); avoid consumption of additional nutritional supplements and multivitamins containing the recommended daily allowance (RDA) of Vitamin D. Subjects are expected to: maintain ≥85% compliance with study agent intake; comply with dietary, medication and supplement restrictions; and complete the diet recall forms to the best of their ability. Subjects will be interviewed at the monthly scheduled time points, at which time pill counts will be performed to verify compliance with study requirements. Subjects discovered not to be complying with study requirements will have the requirements re-explained to them.

6.7 Agent Destruction/Disposal

At the completion of the study, there will be a final reconciliation of drug shipped, drug consumed, and drug remaining. This reconciliation will be logged on the drug reconciliation form, signed and dated. Any discrepancies noted will be investigated, resolved, and documented prior to destruction of unused study drug. Drug destroyed on site will be documented in the study files.

7 CLINICAL EVALUATIONS AND PROCEDURES

7.1 Table 1: Schedule and Sequence of Data Collection

Assessment Schedule

	Screening	Baseline/ Randomization		M1 +/- 7 days		M2 +/- 7 days		End of Treatment/M3 +/- 7 days	7 Day Follow Up
		W1	W3	W4	W7	W8	W11	W12	
Informed Consent	X								
Concomitant Medications	X	X		X		X		X	X
Relevant Medical History/Current Medical Condition	X								
Mini-Mental Status Exam, 2nd Edition (MMSE)	X								
Self-Reported Cognitive	X								

Impairment Since Chemotherapy									
Wechsler Test of Adult Reading (WTAR)		X							
Hopkins Verbal Learning Test, Revised (HVLT-R): Form 1		X							
Hopkins Verbal Learning Test, Revised (HVLT-R): Form 2								X	
Controlled Oral Word Association Test (COWA): T1		X							
Controlled Oral Word Association Test (COWA): T2								X	
Color Trails – Part 1 (Form A)		X						X	
Color Trails – Part 2 (Form A)		X						X	
Brief Visuospatial Memory Test – Revised (BVMT-R): Form 1		X							
Brief Visuospatial Memory Test – Revised (BVMT-R): Form 2								X	
Digit Span (subset of WMS-IV)		X						X	
Symbol Digit Modalities Test (SDMT)		X						X	
Rey Complex Figure Copy (Copy Trial Only)		X						X	
Demographic Questionnaire		X							
Godin-Leisure Time Exercise Questionnaire		X						X	
Primary Care Evaluation of Mental Disorders Health Questionnaire (PHQ-9)		X						X	
Fatigue Symptom Inventory (FSI)		X						X	
Patient's Assessment of Own Functioning Inventory (PAOFI)		X						X	
MRI-Resting state functional MRI and structural MRI.		X						X	
Anthropometric Measurements[1]		X						X	
ECOG	X							X	
2 Day Food Record[2]		X		X		X		X	
Hematology (CBC)[3]	X			X		X		X	
Blood Chemistry (CMP, PT/PTT, LDL)[4]	X			X		X		X	

Research Labs (Oxidative stress, Cytokine)	X							X	
Adverse Events				X		X		X	X
Limited Physical Exam	X[5]							X	
Study Agent/Multivitamin Distribution		X		X		X			
Study Agent Intake & Symptom Logs		X		X		X		X	
Pill Counts				X		X		X	
Follow Up Phone Call									X

[1] Height, Weight, BMI, Waist, & Hip Circumference

[2] Dietary recall forms and instructions will be distributed in person at the screening visit and collected at the baseline, week 4, week 8, and end of treatment visits

[3] Hematology labs do not need to be performed at screening visit if lab results are available within the previous 28 days (+/- 3 days)

[4] Blood Chemistry labs do not need to be performed at screening visit if lab results are available within the previous 28 days (+/- 3 days)

[5] The initial limited physical exam can be done at either screening or baseline.

7.2 Evaluations during Screening Visit

7.2.1 All subjects will have the study fully explained to them and will sign an informed consent document prior to having any invasive procedures performed. They will also be assigned an identification number.

7.2.2 Within 6 months of completing chemotherapy, + or – radiation (+/- 7 days), patients will be screened for cognitive impairment. Evidence of cognitive impairment requires each subject answer in the affirmative: 'do you have problems with memory and attention since having chemotherapy, and do you believe chemotherapy contributed to the problems?'

7.2.3 A limited physical exam will be performed which includes ECOG.
NOTE: The limited physical exam can be done at either screening or baseline.

7.2.4 Concomitant medications, medical history, and current medical condition will be collected.

7.2.5 Mini-Mental State Examination, (MMSE will be administered to screen for and estimate the severity of cognitive impairment in order to determine participant's eligibility to participate in the study.

7.2.6 Blood will be drawn for CBC, CMP, PT/PTT, and LDL and measurement of plasma antioxidant biomarkers or oxidative stress and inflammation.

7.3 Evaluations during Baseline/Randomization Visit

7.3.1 After eligibility has been confirmed based on screening tests using eligibility checklist, subjects will be randomized and supplied with a one-month supply of nutritional supplement (COGNUTRIN) or placebo (n=20/arm) and multivitamin .

7.3.2 Review of completed diet survey and concomitant medications.

7.3.3 Anthropometric measurements will be taken.

7.3.4 The following questionnaires will be completed: Godin-Leisure Time Exercise, Patient's Assessment of Own Functioning Inventory (PAOFI); Personal Health Questionnaire (PHQ-9), and Symptoms of Fatigue and Depression (FSI)

7.3.5 Cognitive testing will be administered (HVLT-R: Form 1, COWA: T1, Color Trails Part 1& 2, Digit Span, SDMT, BVMT-R: Form 1, and Rey Complex Figure Copy

7.3.6 Subjects will also undergo a structural and resting-state functional magnetic resonance Imaging (MRI/fMRI)

Subjects should not be randomized until eligibility has been confirmed. All components of eligibility will be confirmed (inclusion criteria and exclusion criteria).

7.4 Evaluations during Study Intervention

7.4.1 **Weeks 3, 7, 11:** Participants will be contacted reminding them to complete diet survey and to bring completed survey, study agent /symptom logs and all left over study agent (including multivitamin) to their next in person visit scheduled.

7.4.2 **Weeks 4 & 8 (+/- 7 days):** Participants will come in person monthly for the following:

- **7.4.2.1** Review of completed diet surveys, study agent/symptom logs, adverse events and concomitant medications by CRC and verified with CRU RN.
- **7.4.2.2** Collection of any unused nutritional supplement/ placebo and multivitamin for pill counts
- **7.4.2.3** Safety labs (CBC, CMP, PT/PTT, and LDL) will be drawn
- **7.4.2.4** Participant will be supplied with a new one-month supply of nutritional supplement or placebo

7.5 Evaluations at Completion of Study Intervention (3 months, +/- 7 days)

7.5.1 Blood will be drawn for CBC, CMP, PT/PTT, and LDL and measurement of plasma antioxidant biomarkers or oxidative stress and inflammation.

7.5.2 A limited physical exam will be conducted, including anthropometric measurements, ECOG, concomitant medications and adverse event collection by CRC and verified with CRU RN.

7.5.3 Food records and study agent and symptoms logs will be collected and reviewed with subject for clarification.

7.5.4 The following questionnaires will be completed: Godin-Leisure Time Exercise, Patient's Assessment of Own Functioning Inventory (PAOFI); Personal Health Questionnaire (PHQ-9), and Symptoms of Fatigue and Depression (FSI) will be completed.

7.5.5 Cognitive testing will be administered (HVLT-R: Form 2, COWA: T2, Color Trails Part 1& 2, Digit Span, SDMT, BVMT-R: Form 2, and Rey Complex Figure Copy.

7.5.6 Left over study agent will be collected and pill counts done.

7.5.7 Subjects will also undergo a structural and resting-state functional magnetic resonance Imaging (MRI/fMRI).

7.6 Post-intervention Follow-up Period (7 days +/- 3 days)

Subjects will be contacted by telephone 7 (+/-3) days after discontinuing study agent to capture any signs and symptoms occurring since stopping the study drug and changes to concomitant medications. The subject is considered off study after telephone contact is completed.

7.7 Methods for Clinical Procedures:

7.7.1 Demographic Data: At baseline, in addition to the set of intake questionnaires designed to evaluate an expanded set of demographic characteristics, self-reported health, lifestyle activity participation completed at screening, the following data will be collected at baseline (Appendix II). Data regarding menopausal status will also be obtained. (Pre-menopausal, post menopausal and CRA (Chemotherapy related amenorrhea will be defined as without menstrual periods for a period equal to or over 6 months in a patient who was premenopausal at diagnosis).

7.7.2 Anthropometric measurements such as participant's height, weight, BMI, waist and hip circumferences (Appendix III) will be obtained at baseline and at 3 months. These will be measured by a trained CRC using standardized techniques utilized and validated in our previous trials,[104-105] and used to calculate body mass index (Weight kg/ Height m2) and waist: hip ratio. Our group has experience in the use of these methods in this patient population in clinical practice as well as in previous studies.

7.7.3 Physical Activity (PA) will be obtained at baseline and at 3 months using the Godin-Leisure Time Exercise Questionnaire[106] (Appendix IV). They will be reviewed by the study staff to monitor for increase in physical activity and to ensure that subject in not participating in strenuous activities.

7.7.4 Dietary intake (Appendix V) will be assessed at baseline and monthly by conducting random monthly, 2-day, 24-hour dietary recalls (gold standard for collecting dietary data) using a 5-step multipass procedure [107-109] and using the frequently updated NDSR database (for analysis of nutrient composition). At baseline and monthly for up to 3 months, subjects will be requested to complete this instrument and bring it to the scheduled study clinic visit for review and to monitor compliance. Food portion visuals will be provided at baseline in the participant folder to enhance the collection of accurate portion size information. Protocols are established for training dietary interviewers and meticulous quality control procedures are established and supervised by Dr. Kumar. The University of Minnesota Nutrition Data System-Research version (NDS-R) is used for data entry and analysis. This is a microcomputer-based system for collection and analysis of dietary data that prompts the user to describe food intake at the level of detail such as food source, processing method, fat and salt used in preparation, and ingredients that contribute to fat and sodium intake. The nutrient database contains over 19,000 foods, 7,800 brand-name products, and many ethnic foods. Although values are available for 108 nutrients and nutrient ratios, we will specifically analyze average intakes of energy and nutrient density. The database is derived from USDA, food manufacturers, foreign food composition tables, and scientific literature. Dr. Kumar's group is currently using these instruments in the active and proposed chemoprevention trials and has experience working with the data using these instruments. The primary purpose of this instrument will be to (1) verify compliance to restrictions to not over consume other products containing similar agents and (2) to verify consumption of adequate calories, protein and other nutrients during the study. A detailed list of products containing significant amounts of n-3 fatty acids and blueberry anthocyanins will be provided to each subject. Subjects will be instructed to avoid excess intake of these foods.

7.7.5 Functional markers (Eastern Cooperative Oncology Group: ECOG) [110] (Appendix VI): Functional Markers allow subjects to be monitored for basic functional status and to monitor any functional impairment. Limitations in the activities of daily living have been identified secondary to fatigue and lethargy reported by this patient population. (Screening & 3 months)

7.7.6 Participants will also be provided with **Study Agent Intake and Symptom log** (Appendix VII) which they will be required to complete daily. Study agents and instructions for administration will be distributed to subjects at baseline and monthly during scheduled visits. Each batch will contain a supply for approximately 1 month. The study intake log will require the subject to check that study agent has been taken and will be secured to the symptom monitoring scale to ease completion and to remind patients to monitor symptoms.

7.7.7 Pill counts will be completed at monthly visits prior to providing subject with a new batch of supplements. Working at a flat surface, the research coordinator applies a pair of latex gloves and then pours the

bottle of study agent/placebo or multivitamin on to the pill count tray. Using a gloved hand or sorter the pills are counted individually and returned to the empty bottle. In the event of discrepancy between actual and expected count, the process is repeated.

7.7.8 Adverse events monitoring (Appendix VIII):

All adverse events (AEs) that are reported by the subject, detected during a visit, physical examination, or laboratory work-up will be recorded in the participant's study record and recorded on the CRF. All AEs that occur after the start of the study agent will be recorded on the AE CRF whether or not related to study agent. The following information will be captured for each AE: date reported; verbatim term; CTCAE Term (v 4.0); onset and resolution date; severity grade; attribution to study agent; whether or not the event was reported as an SAE; action taken; whether or not the subject dropped due to the AE; outcome; and comments.

For each subject, AE's will be collected at the screening, Month 1, Month 2, Month 3/EOT, visits as well as at the 7-day post-treatment follow-up call by the clinical research coordinator. AE's collected at the screening visit will be catalogued as baseline symptoms by the clinical research coordinator and the AE log will be reviewed and signed by either the patient's medical oncologist or PI. AE's collected during Month 1 and 2 visits will be reviewed with the patient by the nurse who drew their blood at CRU and the AE log will be signed by the principal investigator. Finally, at the Month 3 visit, the participant's medical oncologist will review the AE log with the patient and sign-off on the reported symptoms; if the medical oncologist is unavailable to sign the AE log at this visit, then the PI will review and sign the form. AE's reported at the 7-day follow-up call will be reviewed and signed by the PI. The participant's reported symptoms at this time point will be documented on a powerchart telephone note by the clinical research coordinator. Serious adverse events (SAE's) are reported to IRB and FDA. If a participant reports an SAE, the PI will immediately be informed and she will then report it to the IRB and FDA. AE logs will be scanned to patients' medical records by the clinical research coordinator once they are off-study.

7.7.9 Assessment of Cognitive Performance.

Cognitive performance will be obtained using a battery of valid and reliable cognitive performance tests has been selected for administration based on the results of preliminary studies and previous research on the impact of cognitive training and nutritional supplementation on cognitive performance in older adults as well as previous research on cognitive deficits in breast cancer survivors. The tests will be used to derive composite indices for the domains of memory, executive functioning, processing speed, visual-spatial ability, and attention/concentration. The assessment of each domain with multiple measures is designed to more fully represent the domain and to minimize the possibility that scores reflect idiosyncratic performance on a single test. The assignment of measures to domains is based on accepted descriptions of their functional demands. We recognize that these measures are not pure tests of a domain and that some measures may draw on multiple domains. Nevertheless, the assignment of measures to domains as organized below can be considered a useful heuristic for characterizing cognitive functioning. To ensure the quality of the data collected, these tests will be administered and scored only by research personnel at Moffitt Cancer center qualified and trained by Drs. Heather Jim and Small before the start of the study and supervised by them during the course of the study.

Cognitive Status. Mini-Mental State Examination, (MMSE) (Appendix IX). This domain will be used to screen for and estimate the severity of cognitive impairment in order to determine participant's eligibility to participate in the study. The MMSE has demonstrated validity and reliability in psychiatric, neurologic, geriatric, and other medical populations.

Estimated Intellectual Ability. The Wechsler Test of Adult Reading (WTAR) [127] (Appendix X) requires participants to pronounce 50 irregular words that cannot be easily decoded phonetically. Previous studies have shown that performance on the NART is highly correlated with general intelligence (factor 'g') as measured by the Wechsler intelligence scales[127]. (Accordingly, the WTAR will be administered to all participants at baseline only to obtain an estimate of overall intellectual ability). WTAR scores will be used for descriptive purposes and as a covariate in all analyses comparing cognitive performance in the study samples.

Verbal Fluency. The Controlled Oral Word Association Test (COWAT) [144] is a measure of verbal fluency and is a subtest of the Multilingual Aphasia Examination (MAE; Benton, Hamsher, & Sivan, 1994). At baseline, the COWA uses the three letter set of F, A, S (Appendix XI)to assess phonemic fluency. Individuals are given 1 min to name as many words as possible beginning with one of the letters. The procedure is then repeated for the remaining two letters. This is then repeated at the end of study using the letters P, R, W (Appendix XII).

Memory. This domain will be assessed with separate measures of visuospatial memory and verbal memory. Visuospatial memory will be assessed using the Brief Visuospatial Memory Test-Revised (BVMT-R) (Appendix XIII). Respondents are presented with six geometric figures printed in a 2 x 3 array. In three learning trials, respondents view the array for 10 seconds and are then asked to draw as many of the figures as possible in their correct location. After a 20 to 25-minute delay, the task is repeated. Then, the respondent is asked to identify the six figures from among 12 figures. These procedures will be used to obtain two summary scores. The immediate recall score represents the number of design features correctly reproduced across the three learning trials. The delayed recall score represents the number of design features correctly reproduced 20-25 minutes following presentation. Form 1 will be used at the baseline visit (Appendix XIV) and Form 2 at the end of treatment visit (Appendix XV). Verbal memory will be assessed using Form 1 of Hopkins Verbal Learning Test-Revised (HVLT-R) [129,143] at baseline (Appendix XVI) and Form 2 at the end of study visit (Appendix XVII). Respondents are given three trials to learn a list of 12 concrete nouns organized into three taxonomic categories. After a 20-25 minute delay in which other tests will be administered, respondents will be given a delayed free recall task. Immediately following the free recall task, respondents will be asked to recognize the original 12 words from a list of 24 words. In total, four measures of memory performance will be derived from this task: immediate recall, delayed free recall, retention (delayed free recall divided by best immediate recall trial), and recognition discrimination index (the total number of true-positive responses subtracted by the total number of false-positive errors, including semantically related and unrelated errors).

Attention/Concentration. This domain will be assessed using the Digit Span (Appendix XVIII) subtest of the Wechsler Memory Scale – Fourth Edition (WMS-III) at baseline and end of study [130] and Part 1 of the Color Trails Test (CTT-1) [131] at baseline and end of study (Appendix XIX). The Digit Span assesses immediate verbal memory and auditory attention. The examiner reads increasingly longer series of numbers and the respondent is required to repeat them in the same order. The examiner then reads additional sequences of numbers and the respondent is required to repeat them in reverse order. The Digit Span subtest yields one score, number of items completed correctly. The CTT is an analogue of the original Trail Making Test without significant influence of language. CTT-1 consists of a page with scattered circles numbered from 1 to 25. Even numbered circles are colored yellow and odd-numbered ones are colored pink. Respondents are instructed to connect the circles in consecutive numeric order with a continuous line as quickly as possible. The alternating colors are not mentioned in the instructions. A total score is determined by recording the number of seconds required to complete the task.
Executive Functioning. This domain will be assessed using Part 2 of the Color Trails Test (CTT-2) [131] (Appendix XX and the Symbol Digit Modalities Test (SDMT) [132] (Appendix XXI) at baseline and end of study. The CTT-2 will be administered immediately following the CTT-1. It consists of a page containing 25 pink circles and 25 yellow circles numbered 1 to 50. Respondents are instructed to connect the circles in consecutive order while alternating colors (1 pink, 2 yellow, 3 pink, etc.). A total scored is determined by recording the number of seconds required to complete the task. Alternate forms will be used at the follow-up assessments. The SDMT requires respondents to write the number that corresponds with each symbol for a series of 110 items in which the symbol but not the number appears. Respondents identify the correct number using a key provided in which a different abstract symbol is matched with a different number. A total score is determined by calculating the number of items correctly completed in 90 seconds.

Visual-Spatial Ability. At baseline and end of study, this domain will be assessed using the Rey Complex Figure Copy[133] (Appendix XXII), which asks respondents to reproduce a complicated line drawing. The time it takes a respondent to do so and the number of errors that she makes are used as the basis for scoring. This test has been shown to be sensitive to impairments in visual-spatial ability in breast cancer survivors [7].

Calculation of Domain Scores and Impairment Scores. As in prior research incorporating a non-cancer comparison sample, data from the CA- sample will be used to convert raw scores on each test index into

standardized z scores. A domain score for each of the four domains listed above will be calculated by averaging the z scores of all the tests indices comprising a domain. In addition to calculating continuous scores for each test index and domain, data from the CA- sample will be used to characterize performance on each individual test index as impaired or not impaired. Consistent with previous research [134] , impairment will be defined as ≥1 standard deviation below population norms.

7.7.10 Self-Report Measures (Baseline and End of Study)

7.7.10.1 Depressive Symptomatology. The Primary Care Evaluation of Mental Disorders Health Questionnaire – 9 (PHQ-9)[137] (Appendix XXIII) will be used to assess depressive symptomatology. The PHQ-9 consists of 9 items assessing depression on a 4-point scale (0=not at all, 3=nearly every day). Items are summed for a total score ranging from 0 to 27 with higher scores indicating greater depressive symptomatology. The PHQ-9 is a short self-report questionnaire that shows good sensitivity and specificity to assessment of depressive disorders by mental health professionals in primary care patients [137-138] and cancer patients [139].

7.7.10.2 Fatigue Symptomatology. The Fatigue Symptom Inventory (FSI)[140-141] (Appendix XXIV) is a 14-item scale that assesses the frequency, severity, and disruptiveness of fatigue. Frequency is measured in two ways: the number of days fatigue was experienced in the past week; and the portion of the day on average the respondents felt fatigued. Severity in the past week is measured on an 11-point scale (0 = not at all fatigued; 10 = as fatigued as I could be). Disruptiveness in seven different domains of daily functioning is also evaluated. These domains are assessed on separate 11-point scales (0 = no interference; 10 = extreme interference). Responses to these seven items are summed to provide a total interference score. Previous research has demonstrated the reliability and validity of the FSI with individuals diagnosed with cancer.

7.7.10.3 Subjective Cognitive Function. The Patient's Assessment of Own Functioning Inventory (PAOFI) [142] (Appendix XXV) will be used to assess patients' perceptions of their cognitive functioning. The PAOFI consists of 33 items which assess cognitive functioning on a 6 point scale (1=almost always, 6=almost never). Items are summed to produce four subscales: memory, higher level cognition, language and communication, and motor sensory processing.

7.7.11 Safety Markers: All safety markers will be performed at the Moffitt Cancer Center Clinical laboratories in real time. Non-fasting blood sample will be drawn at the screening visit (unless labs available within 7 days (+/- 3 days), and monthly, using sterile needles for the following studies:

Comprehensive Metabolic Panel (CMP including LDL): Approximately 10 ml of blood will be collected in a speckled red-top SST tube and processed at the clinic site by standing for 20–60 minutes to clot, then spinning for 20 minutes at 3200 rpm. The sample will be used for the CMP with LDL (Moffitt Cancer Center Labs).

Complete Blood Count (CBC): Approximately 2.5 ml of blood collected in lavender top EDTA-containing tubes will be used for CBC. A complete blood count (CBC) will be completed using electronic cell sizing sorting cytometry/microscopy. Tests performed as part of the CBC panel include the following: WBC; RBC; HgB; HCT; MCV; MCH; MCHC; RDW; PLT; MPV; absolute neutrophils; absolute bands; absolute lymphs; absolute monocytes; absolute eosinophils; and absolute basophils (Moffitt Cancer Center Labs).

PT/PTT: Blood will be collected in one full 3.2% sodium citrate (light blue-top) tube. The correct blood to citrate ratio is critical—do not overfill or under fill the tube. Mix by inversion four times. Samples are stable for 24 hours at room temperature. If the sample will be stored longer than 24 hours, centrifuge specimen within one hour of collection at 2500–3000 rpm. Transfer plasma to a plastic screw-top vial and freeze at –20ºC immediately (Moffitt Cancer Center Labs).

7.7.12 Biomarkers of Oxidative Stress. A number of biomarkers of oxidative stress and inflammation have been shown to change with age and disease. For example the isoprostanes are a family of eicosanoidsformed in vivo from the free radical catalyzed peroxidation of arachodonic acid that is independent of cyclooxygenase activity. They appear to be reliable markers of lipid peroxidation. Levels of 8-isoprostane increase with age in some but not all studies, and are reduced by diets high in fruits and vegetables. Ideally measured with GC-mass spectroscopy, isoprostanes can also be measured by ELISA. Other markers of lipid peroxidation include malondiadehyde that can easily be measured, although it may reflect some enzyme dependent peroxidation and inflammation is still a valuable measure for comparison with other studies. Markers of protein oxidation and nitration have also been shown to increase with age and 2 markers routinely measured are protein carbonyls and 3-nitrotyrosine (3-NT). Furthermore, oxidative DNA damage is another marker measured in aging as it is widely thought that oxidative damage to DNA is a significant contributor to age-related disease as well as cancer. Oxidative DNA damage markers are also decreased in humans following diets high in fruits and vegetables. Markers of inflammation are also useful and also show up-regulation with age and disease. Plasma samples will be analyzed for several biomarkers of inflammation and oxidative stress. These methods are in routine use in Dr. Bickford's lab for rodent studies and will be adapted for human plasma: Isoprostanes (8-epi prostoglandin F2α) will be measured by ELISA (Cayman Chemical); 3-NT will also be measured by ELISA and we will make our own plates using routine methods for ELISA with commercially available antiserum (3-NT EIA antiserum from Cayman Chemical). Protein carbonyls are measured by oxy-blot techniques (Chemicon). 8-oxodeoxyguanisine is measured by ELISA (Cayman Chemicals). MDA is measured by HPLC, which is more specific for MDA than the TBAR assay.

One 10 ml of blood will be collected in a green top vacutainer and centrifuged 3000 RPM /10 minutes/ RT to isolate the plasma portion of blood. Approximately 5 ml of plasma will be collected and transferred into 1 ml plasma aliquots. These will be stored in labeled screw cap tubes at -80°C in the Tissue Core of the Moffitt Cancer Center until analysis.

7.7.13 Inflammatory markers-Cytokines will be measured with a panel including IL-1, IL-4, IL-6, IL-8, IL-10, IL-12, GM-CSF, IFN-gamma, TNFα, and CRP (BioRad-Bioplex). All of these biomarkers have been shown to increase with disease, aging and cytotoxic agents and will be used to monitor compliance with the dietary supplementation group and as a covariate in the analysis of cognitive function. The cytokine panel will be completed by Dr. Djeu in her research laboratories.

One 10 ml of blood will be collected in a purple top (EDTA) vacuatiner and centrifuged at 3000 RPM /10 minutes/ RT for isolation of PBMC, plasma and RBC. Approximately 5 ml of plasma will be collected and placed into one labeled screw cap tube and frozen at -80C. The Buffy Coat with adjacent layer of plasma & RBC with be harvested and transferred to 50 cc conical tube with 10ml of PBS. The remaining RBC will be harvested and washed 3 times with PBS and then re-suspended in equal volume of distilled water. Two, 1 ml RBC aliquots will be placed in labeled screw cap tubes and frozen in at -80C. Harvested BC will be harvested on Ficol for PBMC (no cell count required). PBMC will be washed once in PBS and the cells will then be re-suspend in 2ml of Freezing Media (40 ml FBS + 7 ml DMSO + 3 ml RPMI). Two, 1ml aliquots of PBMC will be placed in labeled cryovials, and frozen MR. Once frosty, they will be placed in storage. All samples will be stored in the Tissue Core at Moffitt Cancer enter until analysis.

2 - 10 ml tube will be forwarded to Tissue Core labs at the Moffitt Cancer Center for processing and storage. Once labs specimens for 40 subjects (pre and post intervention) have been collected, they will be transported from Tissue to Dr. Bickford's lab at USF and Dr. Djeu's lab at MCC by the clinical research coordinators, using appropriate containers to preserve the temperature and thus integrity of the specimens for assay of oxidative stress markers.

The plasma collected for assay of oxidative test markers and cytokines will be stored for a maximum of 12 months at -80ºC. All specimens collected for assay of safety markers (CBC, CMP, PT, PTT, and LDL) will not be stored as they will be completed in real time.

7.7.14 Magnetic Resonance Imaging: Structural and Resting-state functional MRI (fMRI):

Self-reported 'chemo-brain' has been validated using neuropsychological tasks and more recently, neuroimaging studies have identified structural [123] and performance differences between patients (i.e. those treated with chemotherapy) and controls [122,124,126]. For example, a recent functional Magnetic Resonance Imaging (fMRI) study has demonstrated that when compared to controls, chemotherapy treated patients show hyporesponsiveness in areas of the brain associated with impaired planning behavior and attentional abilities (i.e. paraphippocampal gyrus, left cuneus, right dorsal striatum, right inferior parietal cortex and left middle temporal gyrus). Specifically, longitudinal assessments of chemotherapy-induced structural changes in cerebral white matter and its correlation with impaired cognitive functioning have been reported in breast cancer patients [145-146]. Differences in diffusion tensor imaging (DTI) white matter (WM) integrity parameters [fractional anisotropy (FA) and mean diffusivity (MD)] between patients and healthy controls were assessed using a voxel-based two-sample-t-test. In comparison with healthy controls, the patient group demonstrated decreased FA in frontal and temporal WM tracts and increased MD in frontal WM [145-146].

Research has demonstrated that an overall diffuse damage to white and gray matter of the brain is likely to disrupt overall brain organization, reducing efficiency of information transfer. Previous studies have suggested that breast cancer survivors show decreased efficiency of neural networks, requiring more functional activation across a range of brain regions to complete certain tasks compared to healthy women [152,155]. The specific pattern of brain changes following breast cancer and chemotherapy, including global versus local deficits, would have important implications for developing the most effective interventions for breast cancer-related cognitive deficits. Elucidation of large-scale brain network configuration in breast cancer may therefore contribute important new information regarding the neurobiologic mechanisms of breast cancer-related cognitive impairment. Research has demonstrated that an overall diffuse damage to white and gray matter of the brain is likely to disrupt overall brain organization, reducing efficiency of information transfer. Previous studies have suggested that breast cancer survivors show decreased efficiency of neural networks, requiring more functional activation across a range of brain regions to complete certain tasks compared to healthy women. [152,155] The specific pattern of brain changes following breast cancer and chemotherapy, including global versus local deficits, would have important implications for developing the most effective interventions for breast cancer-related cognitive deficits. Elucidation of large-scale brain network configuration in breast cancer may therefore contribute important new information regarding the neurobiologic mechanisms of breast cancer-related cognitive impairment[153].

Resting-state fMRI measures spontaneous low-frequency fluctuations in the BOLD signal to investigate the functional architecture of the brain. Spatial patterns of spontaneous fluctuations in blood oxygenation level-dependent (BOLD) signals reflect the underlying neural architecture. The study of the brain network based on these self-organized patterns is termed resting-state functional MRI (fMRI). Resting state functional connectivity MRI (rs-fcMRI), combined with structural connectivity mapping, has proven to be a useful probe for functional alterations in the brain as a consequence of changes in brain state, disease processes, neurodevelopment and aging, pharmacological interventions such as chemotherapy, and genetics 151-155. Additionally, several fMRI studies have demonstrated that some of these self-organizing, resting state networks coincide with brain regions that are found to be deactivated across several fMRI studies where an external stimulus or a cognitive paradigm is applied. Thus, these brain locations are more active at rest than during task performance. Resting state functional magnetic resonance imaging (fMRI) is a reliable, non-invasive method for examining the intrinsic topology of large-scale brain networks 154. Resting state fMRI networks are task-independent and thus less vulnerable to confounds due to performance variance. These observations have led to the hypothesis that the brain remains active in an organized fashion during the resting state, denoted the default mode network (DMN). The default mode hypothesis has been extensively studied, including direct electrophysiological measurement of default network areas, and its changes in different clinical states of consciousness. The concepts and exploration of the DMN has therefore been regarded as "a paradigm shift in functional brain imaging" 147, 150, 148, 149, 153.

Resting state fMRI connectivity studies are sensitive to abnormal global network organization, revealing changes in several clinical populations, including breast cancer patients treated with chemotherapy[153].. Bruno et al (21012) applied resting state functional magnetic resonance imaging and graph theoretical analysis to examine the connectome in breast cancer survivors treated with chemotherapy relative to healthy comparison women.

Compared to healthy females, the breast cancer group displayed altered global brain network organization characterized by significantly decreased global clustering as well as disrupted regional network characteristics in frontal, striatal and temporal areas. Breast cancer survivors also showed significantly increased self-report of executive function and memory difficulties compared to healthy females. These results suggest that topological organization of both global and regional brain network properties may be disrupted following breast cancer and chemotherapy. This pattern of altered network organization is believed to result in reduced efficiency of parallel information transfer. This is the first report of alterations in large-scale functional brain networks in this population and contributes novel information regarding the neurobiologic mechanisms underlying breast cancer-related cognitive impairment [153-154].

We thus propose to evaluate both chemotherapy-induced changes in structural and functional parameters by evaluating (a) differences in cerebral white matter integrity [fractional anisotropy (FA) and mean diffusivity (MD)] maps before and after treatment with COGNUTRIN by using magnetic resonance DTI; (b) we will perform resting state fMRI to determine changes in prefrontal executive functioning in breast cancer patients treated from baseline and post intervention with COGNUTRIN compared to placebo. To date, imaging studies have only focused on assessing structural and functional cognitive disruption induced by chemotherapy. The proposed study aims to replicate these findings and extend them by testing the proposed interventions ability to offset the changes in cognitive performance in breast cancer patients post chemotherapy treatment.

Subjects will be provided prepared, specific and consistent instructions over the intercom prior to start of MRI as follows: “Keep your eyes closed, relax, try not to move, try to stay awake and just let your thoughts wander.” All subjects will be imaged on a 3T scanner with an eight channel phased- array head coil. **Time in scanner for technician getting the imaging optimized for the patient is 5 minutes.**

The resting fMRI will be completed first prior to others. Resting FMRI data will be acquired (Bruno et al, 2012) while participants rest in the scanner with their eyes closed using a T2* weighted gradient echo spiral pulse sequence: relaxation time = 2000 msec, echo time = 30 msec, flip angle = 80° and 1 interleave, field of view = 220, matrix = 64×64, in-plane resolution = 3.125. Number of data frames that will be collected is 216. An automated high-order shimming method based on spiral acquisitions will employed to reduce field heterogeneity. To co register and normalize functional images with a standardized template, a high-resolution, 3 dimension inversion-recovery prepared fast spoiled gradient echo anatomical scan will be acquired: relaxation time: minimum, echo time: minimum, flip: 11 degrees, inversion time: 300 msec, bandwidth: +/−31.25 kHz, field of view: 24 cm, phase field of view: 0.75, slice thickness: 1.5mm, 125 slices, 256×256 at 1 excitation. **The total scan time is estimated at 10 minutes.**

A T1- weighted whole brain 3D-TFE (182 contiguous coronal slices; 250 x 250 mm2 FOV; 4.6 ms TE; 9.7 ms TR; 1.2-mm slice thickness; 256 x 256 matrix; 0.98 x 0.98 x 1.2 mm^3 voxel size). T2-weighted TSE (28 transversal slices; 230 x184 mm^2 FOV; 4-mm slice thickness; 3,000 ms TR; 80 ms TE), and a FLAIR (28 transverse slices; 230 x 183 mm^2 FOV; 125 ms TE; 11,000 ms TR; 2,800 ms IR delay; 4-mm slice thickness; 256 x 256 matrix; 0.65 x 0.87 x 4 mm^3 voxel size) will also be acquired to confirm absence of primary brain pathology as an exclusion criterion. **Scan time 10 minutes**.

A whole brain DTI SE-EPI (diffusion- weighted single shot spin-echo echoplanar imaging) will be acquired with the following scanning parameters: 68 contiguous sagittal slices, 112 x 109 matrix size, 220 x 220 mm^2 FOV, 4,956 ms TR, 55 ms TE, 2.5 parallel imaging factor, 2.2-mm slice thickness, 1.96 x 1.96 x 2.2 mm^3 voxel size. Diffusion gradients will be applied along 45 noncollinear directions with a b-value of 800 s mm-2. Additionally one nondiffusion-weighted (b ¼ 0) set of images will be acquired resulting in a **total scan time of 10.34 min.**

We anticipated a total scan time of 30-45 minutes for both structural and resting functional MRIs, including time to prepare subject.

8 CRITERIA FOR EVALUATION AND ENDPOINT DEFINITION

8.1 Primary Endpoint

The primary efficacy endpoint is to generate effect sizes for changes in the number of impaired neuropsychological tests. The planned intervention period is three months. Neuropsychological outcomes will be examined using a 2 (group; COGNUTRIN, placebo) X 2 (time; baseline, three-months) repeated measures ANOVA to examine differences in the number of impaired neuropsychological tests. Age, premorbid cognitive functioning, depression and fatigue will be evaluated for association with neurocognitive performance and entered as covariates in statistical analyses if a marginally significant association ($p<.10$) is observed.

The primary safety endpoint is incidence and severity of AEs occurring during intervention with either COGNUTRIN or placebo. All AEs that are reported by the subject, detected during a visit, physical examination, or laboratory work-up will be recorded in the participant's medical record and recorded on the CRF. All AEs that occur after the informed consent is signed will be recorded on the AE CRF whether or not related to study agent.

The sample size proposed in the current protocol will not provide sufficient statistical power, but information regarding effect sizes will be gathered and used as motivation for future clinical trials. We estimate that a minimum evidence of improvement in cognitive function with the 3 month intervention as indicated by a score of 1 standard deviation or more above population norms on the Hopkins Verbal Learning Test – Revised, Controlled Oral Word Association Test, or Color Trails Test Part 1 or 2 will be considered adequate to justify progression to a larger study.

8.2 Secondary Endpoints

The secondary endpoint will be improvement in subjective cognitive functioning as well as structural and resting state functional changes in MRI.

In addition we will examine the role of the cytokines and anti-oxidant biomarkers as mediators of reductions in the number of impaired cognitive tests.

We will also examine if other factors such as fatigue, depression, physical and functional activities are affected by COGNUTRIN compared to placebo.

The sample size proposed in the current protocol will not provide sufficient statistical power, but information regarding effect sizes will be gathered and used as motivation for future clinical trials.

8.3 Off Agent Criteria

Participants may stop taking study agent for the following reasons:

- after completing the protocol-prescribed intervention
- at the discretion of the investigator
- AE or serious adverse events (SAE) requiring dose interruption
- inadequate agent supply
- noncompliance with study requirements
- medical contraindication.

8.4 Off Study Criteria

If the subject never took study medication (*e.g.*, does not meet eligibility criteria or withdraws consent prior to randomization) the only activity required to remove the subject from the study is to complete the Off Study CRF. If the subject was randomized to a treatment arm and received study medication, post-intervention procedures according to the schedule of events should be completed to the extent possible.

- not meeting randomization (eligibility) criteria
- the protocol intervention and any protocol-required follow-up period is completed
- experiencing an AE or SAE

- lost to follow-up
- taking a concomitant medication
- medical contraindication prohibiting further study participation
- discovery of a pre-existing condition not observed at baseline eligibility
- withdrawal of consent
- protocol non-compliance *(i.e.,* visit schedule, dose administration, *etc*.)
- at the discretion of the investigator
- death

Post-intervention procedures according to the schedule of events will be completed to the extent possible.

9 SPECIMEN MANAGEMENT

9.1 Blood Samples

All samples once drawn must be processed and stored appropriately until pick-up by the designated courier to transport to the Moffitt Cancer Center clincial laboratories. All safety labs will be forwarded to the Moffitt clinical labs in real time (CBC/CMP/PT/PTT). 2 - 10 ml tube will be forwarded to Tissue Core labs at the Moffitt Cancer Center for processing and storage. Once labs specimens for 40 subjects (pre and post intervention) have been collected, they will be transported from Tissue to Dr. Bickford's lab at USF and Dr. Djeu's lab at MCC by the clinical research coordinators, using appropriate containers to preserve the temperature and thus integrity of the specimens for assay of oxidative stress markers.

10 REPORTING ADVERSE EVENTS (AEs)

DEFINITION: An adverse event is any unfavorable and unintended sign (including an abnormal laboratory finding), symptom, or disease temporally associated with the use of a medical treatment or procedure that may or may ***not*** be considered related to the medical treatment or procedure. An AE is a term that is a unique representation of a specific event used for medical documentation and scientific analyses.

A list of AEs that have occurred or might occur (Reported AEs and Potential Risks) can be found in § 6.2, Pharmaceutical Information as well as the Investigator Brochure.

10.1 Adverse Events

10.1.1 Reportable AEs

All AEs that are reported by the subject, detected during a visit, physical examination, or laboratory work-up must be recorded in the participant's medical record and recorded on the CRF. All AEs that occur after the start of study agent must be recorded on the AE CRF whether or not related to study agent.

10.1.2 Data Elements

- AE reported date
- AE Verbatim Term
- CTCAE Term (v 4.0)
- Event onset date and event ended date
- Severity grade
- Attribution to study agent (relatedness)
- Whether or not the event was reported as a Serious AE (SAE)
- Action taken with the study agent
- Whether or not the subject dropped due to the event
- Outcome of the event
- Comments

10.1.3 Severity of AEs

Identify the AE using the NCI Common Terminology Criteria for AEs (CTCAE) version 4.0. The

CTCAE provides descriptive terminology and a grading scale for each AE listed. A copy of the CTCAE can be found at http://evs.nci.nih.gov/ftp1/CTCAE/CTCAE_4.03_2010-06-14_QuickReference_8.5x11.pdf

AEs will be assessed according to the CTCAE grade associated with the AE term. AEs that do not have a corresponding CTCAE term will be assessed according to their impact on the participant's ability to perform daily activities as follows:

Grade	Severity	Description
1	Mild	Asymptomatic or mild symptoms, clinical or diagnostic observations only; intervention not indicated
2	Moderate	Minimal, local or noninvasive intervention indicated; limited age-appropriate instrumental ADL*
3	Severe	Severe or medically significant but not immediately life-threatening; hospitalization or prolongation of hospitalization indicated; disabling; limiting self care ADL**
4	Life threatening	Life threatening consequences; urgent intervention indicated
5	Fatal	Death related to AE

* Instrumental ADL refer to preparing meals, shopping for groceries or clothes, using telephone, managing money, etc.

** Self care ADL refer to bathing, dressing and undressing, feeding self, using the toilet, taking medications, and not bedridden.

10.1.4 Assessment of relationship of AE to treatment

Relationship of the AE to study drug will be classified as one of the following: not related, unlikely, possibly, probably, definitely. All AEs will be considered due to drug (AEs classified as unlikely, possibly, probably or definitely) unless clearly not related to therapy. The severity and seriousness of each AE will be assessed by the site investigators.

10.1.5 Follow-up of AEs

All AEs, including lab abnormalities that in the opinion of the investigator are clinically significant, will be followed until resolution, if possible. If an AE persists more than 30 days after the subject goes off study, the subject will be referred to their personal physician.

10.2 SERIOUS ADVERSE EVENTS (SAEs)

DEFINITION: ICH Guideline E2A and Fed. Reg. 62, Oct. 7, 1997 define SAEs as those events, occurring at any dose, which meet any of the following criteria:

- Results in death
- Is life threatening (*Note: the term life-threatening refers to an event in which the patient was at risk of death at the time of the event; it does not refer to an event which hypothetically might have caused death if it were more severe).*
- Requires inpatient hospitalization or prolongation of existing hospitalization
- Results in persistent or significant disability/incapacity
- Is a congenital abnormality/birth defect
- Events that may not meet these criteria, but which the investigator finds very unusual and/or potentially serious, will also be reported in the same manner.

Contact Dr. Kumar by phone or e-mail within 24 hours of knowledge of the event. A SAE regardless of relatedness or expectedness will be reported to the Principal Investigator by telephone within 24 hours of the PI/study team becoming aware of the event.

FDA Notification by Sponsor: Dr. Kumar will then notify the FDA by telephone or by facsimile transmission of any unexpected fatal or life-threatening experience associated with the use of the drug as soon as possible but no later than seven calendar days after the initial receipt of the information.

IRB Notification by Investigator: Reports of all SAEs (including follow-up information) will be submitted to the all IRBs per state and federal guidelines. Copies of each report and documentation of IRB notification and receipt will be kept in the Clinical Investigator's binder. AEs and/or laboratory abnormalities identified in the protocol as critical to safety evaluations will also be reported the IND sponsor.

MCC and all participating organizations will comply with applicable regulatory requirements at the participating institution related to reporting SAEs to the IRB.

Follow-up of SAE:

Site staff should send follow-up reports as requested when additional information is available or to resolve queries. Additional information should be entered on the SAE form in the appropriate format. Follow-up information should be sent to the Principal Investigator as soon as possible. SAEs will be followed until resolved or stable, especially for those related to the study agent.

11 STUDY MONITORING

11.1 Data Management

The Data Management team will consist of the PI, Biostatistician, and Data Entry Manager at MCC, Tampa, FL. As part of our efforts to ensure subject confidentiality, all names and other data, which might uniquely identify a subject, will be expunged from this data set. The database will be password protected and only the data entry person and project manager will have access to it. The computer that will house the database is part of a network that is backed up daily. The network also makes use of the latest in firewall technology to prevent unauthorized access or tampering and keeps up-to-date virus protection on each computer as well as the network.

Computer files will be backed on regular intervals. Informed consent documents will be kept in files in an area designated by the principal investigator.

The safety of the study will be monitored by the Principal Investigator according to the monitoring plan proposed in this Pilot clinical trial. The investigator will allocate adequate time for such monitoring activities. The investigator will also ensure that the monitor or other compliance or quality assurance reviewer is given access to all the above noted study-related documents and facilities (*e.g.*, pharmacy, diagnostic laboratory, *etc*.), and has adequate space to conduct the monitoring visit.

Internal Monitoring: Data will be captured in Oncore, Moffitt's Clinical Trial Database. The Case Report Forms will be reviewed by Moffitt's Internal Monitors, periodically throughout the conduct of this trial. The monitoring will include source data verification, utilizing research subject's medical records.

11.2 Case Report Forms

Participant data will be collected using protocol-specific case report forms (CRFs).

11.3 Source Documents

Original documents (*i.e.,* informed consent forms) will be treated as source documentation for this clinical trial.

11.4 Data and Safety Monitoring

Toxicities will be monitored continuously through the trial by the IRB, Moffitt and FDA. The PI and Biostatistician along with the MDs in the study will be responsible for reviewing AEs after 20 subjects have completed the study. If 50% of patients randomized to the treatment arm experience grade III or greater adverse events, we will discontinue this study.

11.5 Sponsor or FDA Monitoring

The investigator will permit study-related monitoring, auditing, and inspection by the IRB and government regulatory bodies (FDA) of all study-related documents (*e.g.*, source documents, regulatory documents, laboratory data, data collection instruments, *etc.*).

11.6 Record Retention

Records for all participants will be retained, at a minimum, for two years after the approval of a New Drug Application (NDA) or as otherwise, directed by the Sponsor. The sponsor will be notified prior to the planned destruction of any materials. The records should be accessible for inspection and copying by authorized persons of the Food and Drug Administration.

12 STATISTICAL CONSIDERATIONS

12.1 Study Design/Endpoints

The central hypothesis for this Pilot clinical trial is that adults who receive COGNUTRIN will exhibit reduction in the number of impaired cognitive tests, as compared to adults on the placebo.

12.2 Sample Size/Accrual Rate

In this pilot trial, we plan to accrue 40 evaluable subjects. This is a feasibility clinical trial in preparation to inform the design of a well-powered randomized clinical trial.

12.3 Randomization and Stratification

During the baseline/randomization visit, subjects will be randomized using block randomization. The current design will ensure equal number of subjects in each arm receiving radiation therapy post chemotherapy and concurrent endocrine therapy.

12.4 Primary Endpoint

The primary efficacy endpoint is to generate effect sizes for changes in the number of impaired neuropsychological tests. The planned intervention period is three months. Neuropsychological outcomes will be examined using a 2 (group; COGNUTRIN, placebo) X 2 (time; baseline, three-months) repeated measures ANOVA to examine differences in the number of impaired neuropsychological tests. Age, premorbid cognitive functioning, depression and fatigue will be evaluated for association with neurocognitive performance and entered as covariates in statistical analyses if a marginally significant association ($p<.10$) is observed.

The primary efficacy endpoint is to generate effect sizes for changes in the number of impaired neuropsychological tests. The planned intervention period is three months. Neuropsychological outcomes will be examined using a 2 (group; COGNUTRIN, placebo) X 2 (time; baseline, three-months) repeated measures ANOVA to examine differences in the number of impaired neuropsychological tests. Age, premorbid cognitive functioning, depression and fatigue will be evaluated for association with neurocognitive performance and entered as covariates in statistical analyses if a marginally significant association ($p<.10$) is observed. The sample size of 20 patients per arm will allow us to generate useful information for future studies, in terms of anticipated effect size. Although this pilot study is not designed to be fully powered, the current sample size will allow us to detect an effect size difference of .40 for a one-tailed paired sample t-test, on account of the matched groups, at power of .80 and an alpha of .05. Moreover, the sample sizes will enable us to detect a group X time interaction of .23, with an alpha of .05, power of .80, and a stability coefficient of .5.

12.5 Secondary Endpoint(s)

12.5.1 Is the number of impaired neuropsychological tests in the COGNUTRIN supplementation group mediated by changes in cytokines and anti-oxidant markers? In order to examine the mediation of the intervention group effects on number of impaired neuropsychological tests by changes in biomarkers, we will adopt a bootstrapping approach to this analysis. Baron and Kenny's approach to regression-based statistical mediation. In this case, the 95% confidence intervals around the bootstrapped indirect estimate will be examined to evaluate whether the mediation relationship is statistically significant. Again, the analyses here are focused on generating information regarding the size of the effects, rather than statistical significance.

12.5.2 We thus propose to evaluate both chemotherapy-induced changes in structural and functional parameters by evaluating (a) differences in cerebral white matter integrity [fractional anisotropy (FA) and mean diffusivity (MD)] maps before and after treatment with COGNUTRIN by using magnetic resonance DTI; (b) we will perform resting state fMRI to determine changes in the connectome in breast cancer patients treated from baseline and post intervention with COGNUTRIN compared to placebo. To date, imaging studies have only focused on assessing structural and functional cognitive disruption induced by chemotherapy. The proposed study aims to replicate these findings and extend them by testing the proposed interventions ability to offset the changes in cognitive performance in breast cancer patients post chemotherapy treatment.

12.5.3 Evaluate the effects of COGNUTRIN on changes in subjective cognitive function. We will evaluate the effect of COGNUTRIN on changes in subjective cognitive function; Compliance will be evaluated by standard pill counts, diet records and plasma levels of anthocyanins and erythrocyte n-3 index. Any unusual patterns in compliance will be noted and apparent discrepancies between pill counts or diet records and plasma levels by will be summarized as well. The collected data will be entered through screens created by the Research IT analyst in ONCORE, the Research Data Management Systems used at the MCC.

12.5.4 Evaluate the effects of COGNUTRIN on changes in symptoms of fatigue and depression.
Additionally, we will evaluate the effect of COGNUTRIN on changes in symptoms of fatigue and depression. Medication use such as antidepressants, tranquillizers and anxiolytics will also be obtained at baseline and

throughout the study. As with age and fatigue, we will evaluate the effect of these medications for association with neurocognitive performance and entered as covariates in statistical analyses if a marginally significant association ($p<.10$) is observed.

12.6 Evaluation of Toxicity

The primary safety endpoint is incidence and severity of AEs occurring during intervention with either COGNUTRIN or placebo. All participants will be evaluated for toxicity from the time that the informed consent is signed up to the end of the intervention according to the NCI CTCAE version 4.0. Standard assessment of toxicity will also include interviews, clinical evaluation, and monitoring of laboratory values. All toxicity data will be summarized by category and grade in a standard fashion. Proportions experiencing most common types of toxicity in this trial will be estimated and exact 95% CIs calculated based on the exact binomial distribution for these proportions will be reported. Compliance will be evaluated by standard pill counts, diet records and plasma levels of anthocyanins and erythrocyte n-3 index. Any unusual patterns in compliance will be noted and apparent discrepancies between pill counts or diet records and plasma levels by will be summarized as well.

12.7 Evaluation of Response

All participants included in the study will be assessed for response to intervention, even if there are major protocol deviations. We estimate that a minimum evidence of improvement in cognitive function with the 3 month intervention as indicated by a score of 1 standard deviation or more above population norms on the Hopkins Verbal Learning Test – Revised, Controlled Oral Word Association Test, or Color Trails Test Part 1 or 2 will be considered adequate to justify progression to a larger study.

13 ETHICAL AND REGULATORY CONSIDERATIONS

13.1 Form FDA 1572

Prior to initiating this study, the Principal Investigator at the participating organizations will provide a signed Form FDA 1572 stating that the study will be conducted in compliance with regulations for clinical investigations and listing the investigators at each site that will participate in the protocol.

13.2 Other Required Documents

- Signed and dated current (within two years) CV or biosketch for all investigators listed on the Form FDA 1572 for the PI and all co-investigators.
- Current medical licenses for all investigators listed on Form FDA 1572 for the PI and co-investigators
- Signed receipt of Investigator Brochure
- Delegation of Responsibility form
- IRB approval of protocol, informed consent, recruitment materials

13.3 Informed Consent

All potential study participants will be given a copy of the IRB-approved Informed Consent to review. The investigator or other research staff will explain all aspects of the study in lay language and answer all questions regarding the study. If the participant decides to participate in the study, he will be asked to sign the Informed Consent document. The subject is considered enrolled in the study from the time the informed consent document is signed. The study agent(s) will not be released to a participant who has not signed the Informed Consent document. Subjects who refuse to participate or who withdraw from the study will be treated without prejudice.

Participants must be provided the option to allow the use of blood samples, other body fluids, and tissues obtained during testing, operative procedures, or other standard medical practices for further research purposes. A separate signature area is required to allow participants to opt out of allowing tissue to be used for further research.

The informed consent document must be reviewed and approved by the IRB at each organization at which the protocol will be implemented prior to study initiation. Any subsequent changes to the informed consent must be approved by each organization's IRB for approval prior to initiation.

13.4 Confidentiality

Information about the study subjects will be kept confidential and managed according to the requirements of the Health Insurance Portability and Accountability Act of 1996 (HIPAA). Additionally, subject will not be identified by name, only subject identification numbers will appear on specimens and questionnaires.

13.5 Other

This study is to be conducted according to US and international standards of Good Clinical Practice (FDA Title 21 part 312 and International Conference on Harmonization guidelines), applicable government regulations and Institutional research policies and procedures.

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MCC# 17089

Amendment 11.0; 07 October 2015

Pilot Trial of COGNUTRIN in Breast Cancer Survivors

Principal Investigator: Nagi Kumar, PhD, RD, FADA

Summary of Changes:

4.2 Criteria for exclusion

- Corrected actual creatinine clearance of >60 to <60.

4.3 Anticipated problems with subject recruitment and retention

- Increased payment to participants from $25 to $50 for each visit to cover travel expenses.
- Added that participants traveling more than 100 miles from their home to reach the research site will receive an additional $30 for transportation costs.

MCC# 17089

Amendment 10.0; 25 February 2015

Pilot Trial of COGNUTRIN in Breast Cancer Survivors

Principal Investigator: Nagi Kumar, PhD, RD, FADA

Summary of Changes:

4.2 Criteria for exclusion

- Added the following:

  Subjects with metabolic disorders (a) who are otherwise eligible, (b) treated for hypothyroidism by their primary MD with Synthroid (levothyroxine) and (c) with the approval of the Moffitt treating oncologist will not be excluded from the study.
- Added "(ex. tissue expanders)" as an example of ferromagnetic material that would prohibit MRI imaging.

7.7.8 Adverse events monitoring (Appendix VIII)

- Added the following:

  For each subject, AE's will be collected at the screening, Month 1, Month 2, Month 3/EOT, visits as well as at the 7-day post-treatment follow-up call by the clinical research coordinator. AE's collected at the screening visit will be catalogued as baseline symptoms by the clinical research coordinator and the AE log will be reviewed and signed by either the patient's medical oncologist or PI. AE's collected during Month 1 and 2 visits will be reviewed with the patient by the nurse who drew their blood at CRU and the AE log will be signed by the principal investigator. Finally, at the Month 3 visit, the participant's medical oncologist will review the AE log with the patient and sign-off on the reported symptoms; if the medical oncologist is unavailable to sign the AE log at this visit, then the PI will review and sign the form. AE's reported at the 7-day follow-up call will be reviewed and signed by the PI. The participant's reported symptoms at this time point will be documented on a powerchart telephone note by the clinical research coordinator. Serious adverse events (SAE's) are reported to IRB and FDA. If a participant reports an SAE, the PI will immediately be informed and she will then report it to the IRB and FDA. AE logs will be scanned to patients' medical records by the clinical research coordinator once they are off-study.

MCC# 17089

Amendment 9.0; 22 January 2015

Pilot Trial of COGNUTRIN in Breast Cancer Survivors
Principal Investigator: Nagi Kumar, PhD, RD, FADA

Summary of Changes:

7.7.9 Assessment of Cognitive Performance (pages 21-22)

- Clarified the procedures in detail for memory testing.

November 20, 2014

Janelle Perkins, Pharm.D., Chairperson
USF Institutional Review Board

Re: Summary of Changes
IRB#: Pro00009858
Title: Pilot Trial of COGNUTRIN in Breast Cancer Survivors – MCC #17089

Attached, you will find a tacked and clean copy of an amendment to the above application. The evaluations during each study visit have been updated to reflect the current standards and evolving methods in the research literature. Modification of faculty in the study is also included based on turnover.

The following are included with this submission:

A. Tracked copy of the currently approved protocol, reflecting changes below
B. Clean copy of the amended protocol

Summary of changes and rationale:

- **Cover Page:** Updated to reflect addition of new staff
- **Schema:** Updated to reflect the following cognitive tests are now conducted at the screening visit instead of baseline:
  - HVLT-R
  - COWA
  - Color Trails Part 1 & 2
- **Schema:** Updated to reflect different versions of cognitive testing forms used at different visits
- **Schema:** Addition of post intervention phone call 7 +/- 3 days after end of treatment visit
- **Schema:** Updated diet survey distribution during intervention
- **Table of Contents:** Updated to reflect correct protocol page numbering
- **Protocol Section 7:**
  - Updated clinical assessments and procedures to reflect changes mentioned above
  - removal of concomitant medication collection at weeks 2, 3, 5, 6, 7, 9, 10 & 11 as patient is not contacted at this time points
  - Section was redone to provide clarity regarding procedures and specific time points
  - Appendix numbers updated to reflect changes in updated version of appendices
- **Protocol Section 9.1:** Updated blood sample collection to provide clarity regarding where samples will be kept

Thank you,

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July 16, 2014

Janelle Perkins, Pharm.D., Chairperson
USF Institutional Review Board

Re: Summary of Changes
IRB#: Pro00009858
Title: Pilot Trial of COGNUTRIN in Breast Cancer Survivors – MCC #17089

Attached, you will find a tracked and clean copy of an amendment to the above application. Due to the complicated nature of breast cancer and the treatments associated with this disease, the inclusion criteria has been revised to allow sufficient time for patients to have any necessary surgeries (i.e. breast reconstruction) after completing chemotherapy +/- radiation. Therefore the inclusion criterion has been revised as follows:

- Completed neoadjuvant or adjuvant treatment with anthracyclines and/or taxanes + or –Radiation therapy within past 6 months (+/- 7 days) (subjects on concurrent endocrine therapy (TAM, Aromatase inhibitors are also eligible to participate as this is standard of care for this patient population)

Please feel free to contact me if you have any questions regarding these changes.

Thank you,

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May 5, 2014

Janelle Perkins, Pharm.D., Chairperson
USF Institutional Review Board

Re: Summary of Changes
IRB#: Pro00009858
Title: Pilot Trial of COGNUTRIN in Breast Cancer Survivors – MCC #17089

Attached, you will find a tacked and clean copy of an amendment to the above application. Inclusion/exclusion criteria have been modified to include the most current standards and evolving methods.

Since this clinical trial is a feasibility trial, it is aimed at providing information regarding (Section 2.0 of the protocol):

- Available patient population
- Likelihood of patients agreeing to participate in the clinical trial
- Potential modifications to clinical trial design and eligibility criteria to enhance enrollment

We have screened over 90 potentially eligible subjects in the past 30 days and have not identified any subject to be eligible for this clinical trial, although this group of subjects report cognitive impairment. With the constantly evolving changes in treatment options for this subject population as well as the timing of chemotherapy, surgery and radiation therapy along with concurrent endocrine therapy, our team has observed that the criteria of inclusion has to be revised accordingly and has made the following changes in the criteria for inclusion:

- **Schema:** Updated to reflect changes in eligibility criteria
- **Protocol Section 3.0:** Screening updated to reflect changes to inclusion criteria
- **Protocol Section 4.0:** Screening updated to reflect changes to inclusion criteria
- **Protocol Section 4.1:** Inclusion criteria now includes women 30-70 years of age, cases with Stage I-IIIB Breast Cancer, treatment with neoadjuvant or adjuvant anthracyclines or taxanes, treatment completed within the past 60 days
- **Protocol Section 4.2:** Exclusion criteria updated to reflect patients with Stage IIIC or IV
- **Protocol Section 7.6.12:** Clarification regarding biomarker collection and processing

Please feel free to contact me if you have any questions regarding these changes.

Thank you,

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MCC# 17089

Pilot Trial of COGNUTRIN in Breast Cancer Survivors

Principal Investigator: Nagi Kumar, PhD, RD, FADA

Amendment 5.1, 04/08/2014

Summary of Changes:

SCHEMA (page 3)

- Revised to correct the questionnaires that will be completed at each visit.

7 CLINICAL EVALUATIONS AND PROCEDURES (pages 24-26)

- Added window (+/- 7 days) to week 4, week 8, and week 12 visits.

March 12, 2014

Janelle Perkins, Pharm.D., Chairperson
USF Institutional Review Board

Re: Summary of Changes
IRB#: Pro00009858
Title: Pilot Trial of COGNUTRIN in Breast Cancer Survivors – MCC #17089

Enclosed, please find a copy of an amendment to the above application. This modification reflects no substantive changes in procedure. Specific methods as well as inclusion/exclusion criteria have been modified to include the most current standards and evolving methods in the research literature. Modification of faculty in the study is also included based on turnover.

The following are included with this submission:

A. Tracked copy of the currently approved protocol, reflecting changes below
B. Clean copy of the amended protocol

Summary of changes and rationale:

- **Schema & Protocol Section 3.0:** Clarification regarding the inclusion of women on concurrent endocrine therapy; Updated to reflect that all patients will now receive resting-state functional MRI in addition to structural MRI
- **Protocol Section 2.1:** Specific Aim 3 updated to reflect the use of resting-state functional MRI in addition to structural MRI in order to evaluate changes in imaging markers
- **Protocol Sections 4.0 & 4.1:** Clarification regarding the inclusion of women on concurrent endocrine therapy
- **Protocol Section 4.2:** Clarification regarding the parameters for determining the exclusion of women with renal or liver disease
- **Protocol Sections 5.1.2, 5.6 & 6.1.2:** The removal of specific drug bottle descriptions in order to reflect that all study agents are bottled and labeled per investigational drug use regulations
- **Protocol Section 6.5:** Clarification regarding which labs determine abnormal liver function and the adverse event grades that will result in discontinuation of the study agent
- **Protocol Section 7.1:** Updated to reflect that all patients will now receive resting-state functional MRI in addition to structural MRI; Removal of attention tasks during MRI
- **Protocol Section 7.3:** Updated to reflect that all patients will now receive resting state functional MRI in addition to structural MRI
- **Protocol Section 7.6.6:** Removal of additional 5 days of study agent being supplied to patient , agents were bottled based on a 30 day supply
- **Protocol Section 7.6.10:** Functional MRI has been replaced with resting-state

functional MRI, therefore working memory tasks to be performed in MRI has been removed from protocol

- **Protocol Section: 7.6.15:** Functional MRI methods were replaced with resting-state functional MRI methods
- **Protocol Section 12.5.2:** Secondary endpoint changed to reflect the use of a resting-state functional MRI versus a functional MRI
- **Protocol Section 14.0:** References updated to include those used to support the use of resting-state functional MRI
- **Appendices:** Updated with the Teleform version of questionnaires

Please feel free to contact me if you have any questions regarding these changes.

Thank you,

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January 30, 2014

Janelle Perkins, Pharm.D., Chairperson
USF Institutional Review Board

Re: Summary of Changes
IRB#: Pro00009858
Title: Pilot Trial of COGNUTRIN in Breast Cancer Survivors – MCC #17089

Enclosed, please find a copy of an amendment to the above application. This modification reflects no substantive changes in procedure. Specific methods as well as inclusion/exclusion criteria have been modified to include the most current standards and evolving methods in the research literature. Modification of faculty in the study is also included based on turnover.

The following are included with this submission:

A. Tracked copy of the currently approved protocol, reflecting changes below
B. Clean copy of the amended protocol

Summary of changes and rationale:

- **Schema:** Updated to reflect changes regarding functional MRI (fMRI)
- **Schema:** Updated to reflect removal of weekly phone calls
- **Protocol Section 2.0:** Objective 3 updated to reflect changes regarding fMRI

Rationale for Change: Although fMRI is one of the variables proposed in this clinical trial, there has been several installation issues with regard to the fMRI equipment in association with the MRI equipment current used in structural MRI studies. We anticipate that this will require several months before the equipment can be installed that ensures safety of the MRI. Since funding was received in July of 2013, we have been in the process of identifying MRI equipment where the functional MRI equipment can be added without compromising the safety to the subjects/other patients. We have learned that there will be further delay in the availability of the fMRI. The delay in the availability of the functional MRI has serious implications on (a) bioavailability of the agent we have developed for this clinical trial; (b) cost of reproducing another batch of agent to match the current batch for consistency of components and (c) the funding for this clinical trial. Based on these major concerns, we have amended the proposal to include all other primary and secondary variables that we proposed to monitor in this pilot trial and will perform the functional MRI in the second 10 pairs of subjects (10 from the Treatment arm 10 from the placebo arm).

- **Protocol Section 7.**1: Removal of weekly phone calls

Rational for Change: Subjects will see a Nurse Practitioner (NP) at monthly visits for a limited physical exam assessing any adverse events. Subjects are instructed to contact coordinator, PI or physician should any adverse events occur prior to monthly visit.

- **Protocol Section 7.3:** Addition of limited physical exam with NP to assess adverse events

Please feel free to contact me if you have any questions regarding these changes.

Thank you,

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MOFFITT CANCER CENTER

August 21, 2013

Janelle Perkins, Pharm.D., Chairperson
USF Institutional Review Board

Re: Summary of Changes
IRB#: Pro00009858
Title: Pilot Trial of COGNUTRIN in Breast Cancer Survivors – MCC #17089

Enclosed, please find a copy of an amendment to the above application. This modification reflects no substantive changes in procedure. Specific methods as well as inclusion/exclusion criteria have been modified to include the most current standards and evolving methods in the research literature. Modification of faculty in the study is also included based on turnover.

The following are included with this submission:

A. Tracked copy of the currently approved protocol, reflecting changes below
B. Clean copy of the amended protocol
C. Updated study materials

Summary of changes and rationale:

- Cover Page: Addition of Dr. Hatem Soliman as a co-investigator
- Cover Page: Addition of Dr. David Evans as co-investigator (Assistant member)
- Cover Page: Deletion of Kate Jan Van Rensberg (Applied Research Scientist)
- Schema: Updated to reflect changes made to eligibility criteria
- Protocol Section 3.0: Clarification of study plan summary
- Protocol Section 4.0: Updated to reflect current NCCN guidelines (Version 3.2013), which state "chemotherapy and endocrine therapy when used as adjuvant therapy should be given sequentially with endocrine therapy following chemotherapy. Available data suggest that sequential or concurrent endocrine therapy with radiation therapy is acceptable". Based on these revised guidelines, we have revised the inclusion and exclusion criteria. The rationale for these revisions is to ensure that the current pilot trial includes a homogenous and representative population of patients who are treated using the standard guidelines and who will be potentially benefitted the most with an intervention with COGNUTRIN
- Protocol Section 4.1: Inclusion criteria of age updated to be more representative of the population being studied.
- Protocol Section 4.1: Allowable treatments updated to allow for endocrine therapy and radiation therapy as this is standard of care in this population
- Protocol Section 4.2: Addition of Stage IIIB and IIIC as exclusion criteria
- Protocol Section 4.2: Exclusion criteria of advanced diabetes clarified to state insulin dependent diabetes
- Protocol Section 4.2: Exclusion criteria updated to include patients with medical history of concussions as this could skew study results of MRI Brain

- Protocol Section 6.2: Administration of Lovaza has been updated to reflect 2, 1 gm capsules will be taken BID for a total of 4 gm daily
- Protocol Section 6.5(c): Risks due to MRI updated to include section on possible psychological distress posed by MRI's confining environment.
- Protocol Section 7.6.9: Removal of the administration of Attention Task and Executive Functioning in MRI and instead using various Working Memory Task parameters
- Protocol Section 7.6.12: Collection of biomarkers will now be done in 10ml blood in green top tubes rather than purple top tubes to allow for consistency is sample collection
- Protocol Section 7.6.15: MRI scanner will no longer be completed on Moffitt's 1.5T scanner, instead they will now be done on University Diagnostic Institute's (UDI) 3T scanner
- Protocol Section 7.6.15: The addition of Diffusion Tensor Imaging (DTI) to measure changes in white matter tracts across sections
- Protocol Section 7.6.15: The addition of resting-state fMRI data collection period
- Protocol Section 12.3: Randomization will now include block randomization to ensure an equal number of subjects in each arm receiving radiation post chemotherapy and concurrent endocrine therapy

Please feel free to contact me if you have any questions regarding these changes.

Thank you,

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