SCI Protocol #: **SCI 07-001.7**

SCRIHS Protocol #: **07-153**

TITLE: A Phase II Biomarker Trial of Gelatin Encapsulated Extract of American Ginseng Root in Breast Cancer

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Investigational Agent: Gelatin Encapsulated Standardized Lyophilized Water-Extract of American Ginseng root derived from *Panax quinquefolius*, L.

IND Number: **79586**

Protocol History:

Version #: **1.8**

Version Date: **06-05-2018**

Version Number	Amendment Date	Reason for Amendment
1.1	11-12-07	Requests from FDA
1.2	11-13-07	Alter definition of uncontrolled hypertension
1.3	02-15-2008	Clarify method and time point for pregnancy testing.
1.4	03-03-2008	Clarify Inclusion Criteria #10 and Exclusion Criteria #10
1.5	12-16-2008	DRT recommendations
1.6	10-26-2009	Clarify size criteria for Inclusion Criteria #1
1.7	01-26-2011	Addition of angiopoietin-2 biomarker testing, formatting and administrative changes
1.8	06-05-2018	Updated Objectives based on availability of data

SYNOPSIS

PROTOCOL SYNOPSIS	
Study Number	SCI 07-001.1
Title	A Phase II Biomarker Trial Of Gelatin Encapsulated Extract Of American Ginseng Root (LEAG) In Breast Cancer.
Clinical Phase	Phase II
Objectives	
Primary	To analyze the serum and *in vivo* tissue biomarker response of breast cancer tumor and surrounding normal breast epithelial cells to preoperative treatment with gelatin encapsulated lyophilized water-extract of American Ginseng root (LEAG).
Secondary	To compare before and after administration of LEAG responses on a proteinomic panel of inflammatory mediators.
	To establish a base of comparison of biomarkers between breast cancer patients and the database of women with increased breast cancer risk in the Surrogate Endpoints and Lifestyle Factors (SELF) study.
Study Agent	New chemical entity (NCE): A gelatin encapsulated standardized lyophilized water-extract of American Ginseng root derived from Panax *quinquefolius*, L. (LEAG) (250 mg per capsule). Treatment dosage is 1 gram per day (4 capsules per day).
Study Population	A total of 25 to 50 female patients age $\geq$ 18.
	New diagnosis of breast cancer with biopsy showing invasive or non-invasive breast cancer (DCIS) at least 0.5cm greatest diameter on imaging.
	Surgical patients undergoing lumpectomy, subtotal, or total mastectomy are eligible.
Design	Single Center, Non-Randomized, Unblinded, Investigator Initiated Phase II biomarker Trial.
Intervention	A total of 1 gram of LEAG per day for a minimum of 5 days and a maximum of 14 days.
	Four 250 mg capsules taken with food in the morning.
Treatment Group	**Surgical resection alone.** For 5 to 14 days preceding their scheduled surgical procedure, the patient will be treated with a total of 1 gram of LEAG per day. Subjects must complete a minimum of 5 days of treatment with LEAG prior to day of surgery.

Study Evaluations	The diagnostic core biopsy specimen will be compared to the operative specimen for the following:
Primary Objective Evaluations	**Proliferation Markers:** Immunohistochemistry for ER, PR, Ki67, cyclin D1, COX-2
	Angiogenesis Marker: Angiopoetin 2
Secondary Objective Evaluations	
	Evaluate changes in inflammatory mediator proteinomic panel (adiponectin, CRP, HGF, IGF-1, IGF-1R, IL-10, IL-12p40, IL-1b, IL-1ra, IL-2, IL-23, IL-4, IL-6, IL-8MCP-1, TGFb1, TNFa).
	Establish a comparison of biomarkers between breast cancer patients and the database of women with increased breast cancer risk in the SELF study.
	Collect anthropometric data (height, weight, body composition, waist-hip ratio), dietary history, exercise habits, insulin resistance blood panel (HOMA index, leptin, ghrelin, and adiponectin) and Gail Score.
Hypothesis	LEAG will have an anti-proliferative effect on human breast tumors and benign breast epithelia. LEAG may favorably alter biomarkers of inflammation and the metabolic syndrome.
Safety Considerations	All patients will be monitored for toxicity related to therapy. **Adverse events will be collected through the post-surgery visit and all related or possibly related adverse events for 30 days following discontinuation of LEAG therapy or until resolution or stabilization of the adverse event.** Discontinuation of treatment will be considered in patients experiencing excessive toxicity.
Statistical Considerations	High variability among tumors in the patient population is anticipated. Each patient will serve as her own control; pre-treatment biopsy tissue will be compared to post-treatment surgical tissue. Descriptive statistics including the mean, median, range, standard deviation, interquartile range, skewness, and kurtosis will be calculated for each measure. Paired t-tests, or Wilcoxon signed-rank tests, depending on the distributional characteristics of the variables, will be used to assess changes in biomarkers from biopsy to surgery. In order to examine the inter-relationships between the anthropometric data, the cytological markers, serum ginsenoside concentrations, and a panel of inflammatory serum proteins, data visualization techniques will be employed. Correlation coefficients will be used to summarize linear relationships between continuous measures. Regression techniques will be used to model nonlinear relationships. All statistical procedures will be performed in consultation with the Division of Statistics and Research Consulting at SIU School of Medicine.

Timeline	Patient enrollment and data collection are expected to be complete within 72 months.

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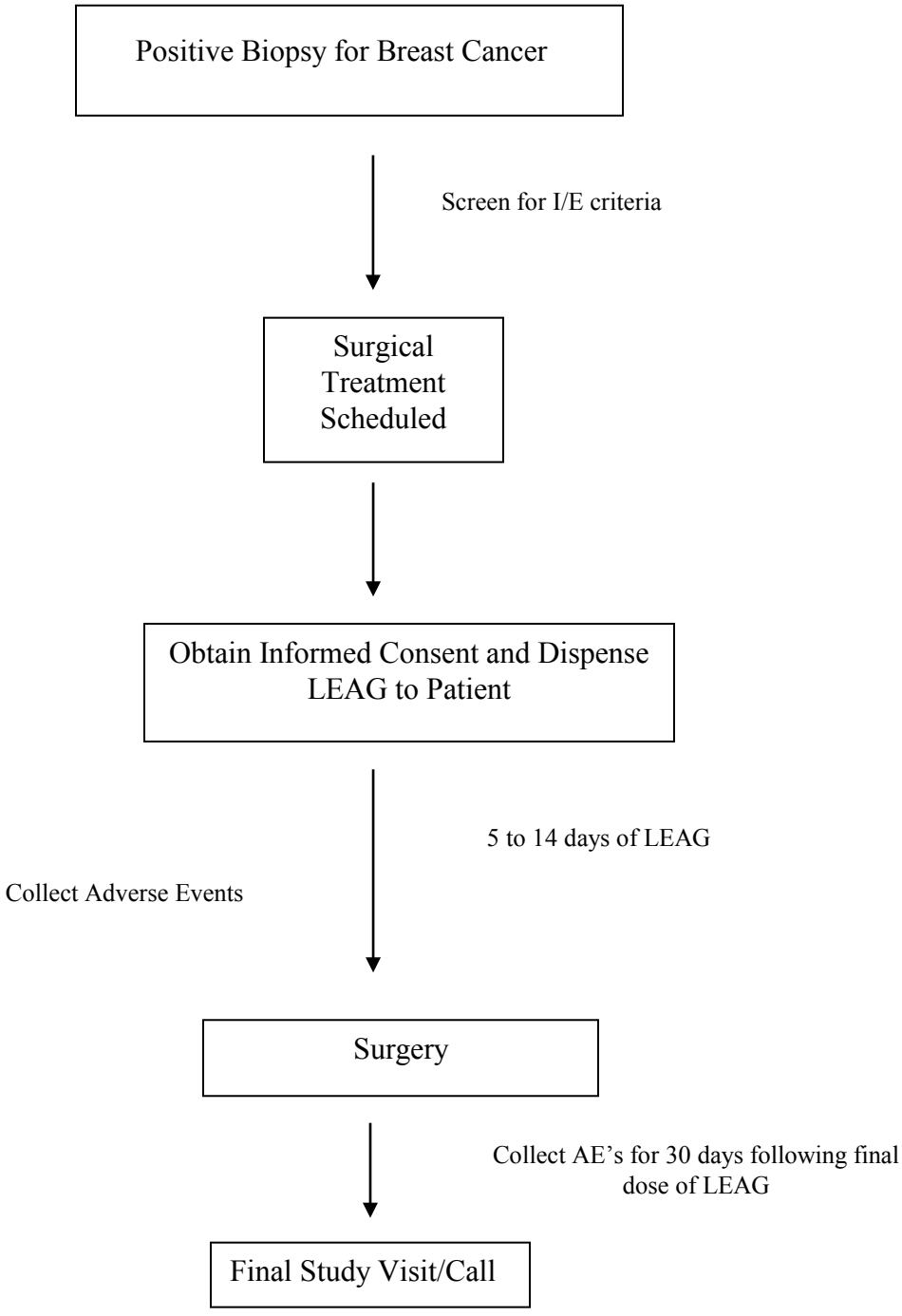
STUDY SCHEMA



1.0 OBJECTIVES

1.1 Primary Objectives

Analyze biomarker *in vivo* response of breast cancer tumors and surrounding normal breast epithelial cells to preoperative treatment with gelatin encapsulated lyophilized water-extract of American Ginseng root (LEAG)**.**

1.1.1 The diagnostic core biopsy will be compared to the operative specimen for effects on the following proliferative and tumor biomarkers: immunohistochemistry for ER, PR, Ki67, cyclin D1, COX-2.

1.2 Secondary Objectives

1.2.1 To compare before and after administration of LEAG responses on a proteinomic panel of inflammatory mediators.

- Evaluate changes in inflammatory mediator proteinomic panel (adiponectin,CRP,HGF,IGF-1,IGF-1R,IL-10, IL-12p40,IL-1b,IL-1ra,IL-2,IL-23,IL-4,IL-6,IL-8, MCP-1, TGFb1,TNFa).insulin, ghrelin and leptin; measured at baseline and after 5-14 days of treatment.
- Measure angiogensis marker, angiopoetin 2.

1.2.2 In a preliminary manner compare the Gail risk model, anthropomorphic measurements, dietary history, exercise habits, and biomarkers of insulin resistance between breast cancer patients in this study and our database of women with increased breast cancer risk participating in the ongoing Surrogate Endpoints and Lifestyle Factors (SELF) study.

- Collect anthropometric data (height, weight, body composition, waist-hip ratio, BMI), dietary history and exercise habits using the same instruments, as our SELF study (Appendices A, B, and C) at baseline.
- Calculate the homeostasis model assessment (HOMA) index [insulin resistance (HOMA-IR) = fasting insulinemia (mU/mL) × fasting glycemia (mmol/L)/22.5)] (collected fasting on the day of surgery).
- Measure serum leptin, ghrelin, and adiponectin levels (collected fasting on the day of surgery).
- Evaluate Breast cancer risk assessment through calculation of a Gail Score (Appendix D)

2.0 BACKGROUND

2.1 Breast Cancer, Problem and Relevant Cancer Biology

With the aging demographics of this country, breast cancer incidence is expected to double over the next 15 years, bringing prevention efforts to the forefront (1). Current prevention methods include chemoprevention with the selective estrogen receptor modulator, tamoxifen, versus oophorectomy or prophylactic mastectomy. While effective, these measures entail potential adverse effects. Greater than 90% of women with breast cancer risk profiles high enough to consider chemoprevention with tamoxifen decide against taking it. In contrast, there is strong public acceptance of dietary supplements such as soy and green tea which are perceived as safe and health-promoting. An ideal breast cancer prevention agent would be a safe nutritional intervention that could also have additional metabolic benefits. Western women have the highest incidence of breast cancer in the world, and since less than ten percent of breast cancer is attributed to known genetic causes, nutrition and lifestyle are suspected to be significant factors contributing to this risk. The raised incidence of breast cancer in Western countries parallels the prevalence of the so-called metabolic syndrome, the major components of which are obesity, hyperinsulinemia, dyslipidemia, and atherosclerosis. It is an open question whether these two trends are biologically linked (see discussion below). Our study aim is to evaluate whether the gelatin encapsulated lyophilized water-extract of American Ginseng root (LEAG) is a potential breast cancer prevention agent and is able to directly affect both breast cancer cell proliferation in newly diagnosed tumors, as well as positively influence the inflammatory mediators potentially involved in obesity associated pathways that may increase the risk of breast cancer.

2.2 Adiposity, Diet, Diabetes, Hyperinsulinemia and Breast Cancer Risk

There are several lines of epidemiological evidence that Western diet and lifestyle influence the increased rate of breast cancer. Asian women, who have some of the lowest rates of breast cancer in the world, acquire a risk level approximating the national average over several generations after immigrating to the United States (2). The pattern of factors associated with breast cancer incidence in Western countries parallels the major components of the insulin resistance syndrome: hyperinsulinemia, dyslipidemia, hypertension and atherosclerosis. High total energy intake and decreased physical exercise lead to excess adipose tissue, which increases free fatty acids and tumor necrosis factor alpha (TNFa) (3). These two components may be involved in the pathogenesis of hyperinsulinemic glucose intolerance in genetically predisposed individuals as they grow older (4).

It is generally agreed that obese women are at increased risk for postmenopausal, but not pre-menopausal, breast cancer. A plausible mechanism that could link obesity to breast cancer may be that mammary carcinogenesis is promoted in obese post-menopausal women as a result of synergistic activity between the concomitants of hyperinsulinemia, such as insulin-like growth factor (IGF) increase, and increased estrogen concentrations derived from aromatization in adipose tissue. In addition to these facts, it is now proposed that a third mechanism is plausible.

Excess energy intake and resultant hyperglycemia can cause up-regulation of phosphoinositide 3-kinase (PI3-K) signaling via its two downstream effector serine-threonine protein kinases, Akt and the mammalian target of rapamycin (mTOR). Akt and mTOR are known to have oncogenic properties mediated in part by suppression of apoptotic signals and in addition by their effects on cell cycling. In obese humans increased PI3K signaling may lead to mitochondrial damage and be part of the pathway of mammary carcinogenesis inducing ductal hyperplasia and DNA damage through oxidative stress.

The observation that tumors utilize glucose more avidly than normal tissues dates back to 1964 (5) and since then has prompted a number of epidemiologic studies comparing breast cancer rates among subjects with diabetes versus those with normoglycemia. Retrospective studies show a mild positive correlation (RR 1.2-2.0), but researchers admit that confounding variables such as the lower consumption of alcohol among diabetics versus non-diabetics tend to weaken the association (6). While studies to date seem to show a mild increased risk of breast cancer associated with type II diabetes, the potential impact from a prevention perspective is increased by a number of considerations. The prevalence of obesity and type II diabetes in the United States has doubled in the decade since the Atherosclerosis Risk in Communities Study commenced, and even a mild correlation could account for more than 10% of the breast cancer diagnoses in this country per year (5). Persons who are not yet diabetic or obese but who have a predisposition as evidenced by overweight (BMI > 25 but < 30) account for as much as 35% of the U.S. population. The study we are proposing looks at the potential reversibility of the epithelial lesions associated with diet induced obesity and diabetes.

Epidemiological reports are inconsistent on the association between breast cancer risk and the dietary intake of either individual fatty acids or of antioxidant vitamins. The inconsistencies may in part be due to interactions between the two classes of nutrients at the level of the cell membrane, affecting their potential role in mammary carcinogenesis. The effects of specific dietary fatty acids and antioxidant vitamins on experimental mammary cancer systems have been compared with reported epidemiological associations of the same agents with breast cancer risk in humans (7). An increased ratio of n-3 to n-6 polyunsaturated fatty acids (PUFAs) in the diet inhibits the growth of a rat mammary cancer model. There is also evidence that members of the n-3 PUFA series can inhibit the growth of human breast cancer cells both in vitro and in explants. Clinical trials of supplementary n-3 PUFAs in conjunction with a reduced fat intake have been proposed for breast cancer prevention. In particular, it is suggested that n-3 PUFAs have an inhibitory effect on COX-2 activity (8).

2.3 Exercise and Breast Cancer Risk

Multiple epidemiologic studies have evaluated the association between exercise and breast cancer risk. A recent review of 18 cohort and 23 case-control studies found that observations from 26 of these studies including 108,031 breast cancer cases demonstrated that exercise (both occupational and leisure-time) is associated with a 30% reduction in breast cancer risk (9). Further supporting a reduced risk associated with exercise, a significant graded dose-response relationship was found in 16 of the studies (9). Moderate non-vigorous exercise can increase insulin sensitivity and improve dyslipidemia even without change in body weight (10, 11). Thus exercise can serve to counter the negative influences associated with obesity and breast cancer pathogenesis.

2.4 Ginseng has both anti-cancer and anti-diabetic effects

Studies in Dr. Murphy's laboratory have characterized an extract of the American ginseng root that inhibits proliferation of human breast cancer cells in culture and in animal models. Studies from this and other laboratories have shown that American ginseng and ginsenosides may inhibit cancer cell proliferation by inducing gene and protein expression of the cell cycle regulatory protein p21, thus arresting tumor cell cycle progression (12), by inducing cancer cell apoptosis through activation of caspase-3 protease via a *bcl*-2-insensitive pathway and by sensitizing multidrug-resistant tumor cells to chemotherapy (13, 14). Together, these data indicate that the individual ginsenosides may inhibit cancer cell proliferation via different mechanisms of action and in a concentration-dependent manner.

The treatment of human breast cancer cell lines MCF-7 or MDA-MB-231 with a water-extract of powdered North American ginseng root produced a significant decrease in cell proliferation in a dose-dependent and estrogen receptor-independent manner (15). Moreover, in athymic nude mice inoculated with either MCF-7 or MDA-MB-231 cells, treatment with a 1% solution of American ginseng extract in lieu of drinking water produced a $\geq$ 50% reduction in tumor size when compared to water-only controls (16). Recent laboratory studies have determined that ginseng also significantly enhances the efficacy of doxorubicin and tamoxifen on breast cancer cell proliferation *in vitro*, and on tumor growth in nude mice *in vivo*.

Further studies from this laboratory and others, have elucidated the ginsenoside components in ginseng that exhibit the anti-tumor effects (17, 18). Ginsenosides are saponin glycosides unique to and the major biologically active components in ginseng. Over 20 different ginsenosides have been identified and, based on their structural differences; the ginsenosides found in American and Asian ginseng have been categorized into two main classifications: 20(S)-protopanaxadiol and 20(S)-protopanaxatriol. The 20(S)- protopanaxadiol group contains the ginsenosides (i.e., ginsenosides Rc, Rg3, Rh2) that have been shown to have the most potent anti-cancer activity (18) and do not exhibit the estrogenic activity of ginseng extract (19, 20) and other ginsenosides (21, 22). Figure 2 shows the effects of various ginsenosides on cell proliferation. Results clearly show that these ginsenosides inhibit cancer cell proliferation (4), induce cancer cell death (13,

12), and prevent cancer metastasis (23). Thus, it appears that ginsenosides Rc, Rg3, and Rh2 may be responsible for the therapeutic effects of American and Asian ginseng in experimental studies. Ginsenosides Rc and Rh2 have been shown to have the most potent anti-cancer activity (see Figure 1), and do not exhibit the estrogenic activity of ginseng ethanol extracts (21).

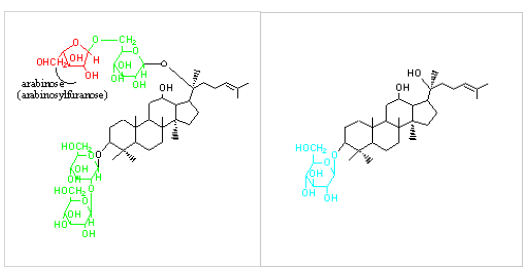


Figure.1 Chemical structure of ginsenosides

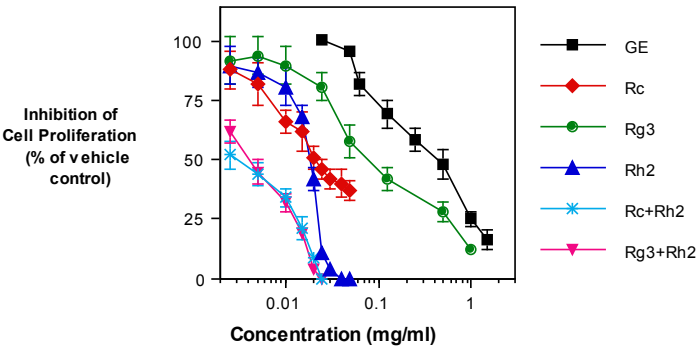


Figure 2. The effect of American ginseng root extract (GE) and ginsenosides, alone and in combination

Recently, ginseng extract (13) and the ginsenosides Rg3 and Rh2 (23) were shown to inhibit cell proliferation and disrupt MAP kinase signaling in a dose-dependent manner in human breast and prostate cancer cells, respectively. Ginseng and ginsenosides also have metabolic effects which may reduce carcinogenesis via the inflammatory and hyperinsulinemic states associated with the metabolic syndrome. American ginseng, in doses of 1 to 3 grams, taken 40 minutes before a glucose challenge test reduced postprandial glycemia in both diabetic and non-diabetic subjects (24, 25). Morever, 20(S)-protopanaxatriol inhibits inducible nitric oxide synthase and COX-2 expression through inactivation of NFκB in RAW264.7 macrophages that were stimulated with endotoxin lipopolysaccharide (26).

An advantage of ginseng therapy is that it remains potent following oral administration. Pharmacokinetic studies have revealed that following oral administration of ginseng, the ginsenosides are absorbed and pass through the system unmetabolized (i.e., Rg1, Rd, Re, Rb1, Rb2, Rc) (27); are hydrolyzed and conjugated with fatty acids into metabolites that become more metabolically stable and retain their activity, and/or are deglycosylated and degraded into metabolites that are either more potent anti-cancer compounds (i.e., protopanaxadiol, protopanaxatriol, IH-901, Compound K or M1) (28, 29), or have no reported anti-cancer activity (i.e., Rh1, Rf1). Interestingly, ginsenosides Rb1 and Rc can be metabolized into the potent anti-cancer ginsenosides Rg3 and Rh2 by acid environment and intestinal flora (30). Ginsenoside Rg3 is rapidly metabolized by the gut into Rh2 and protopanaxadiol (26), both of which possess greater anti-tumor activity than Rg3.

Our existing study, the Surrogate Endpoints and Lifestyle Factors (SELF) trial, has as its goal to analyze the prevalence in women seeking risk assessment for breast cancer, of insulin resistance, central obesity, and proliferative changes in mammary ductal cells, and determine whether these potential surrogate endpoint biomarkers (SEBs) have a positive correlation with breast cancer risk. The findings will be used to refine the relevant biomarkers and perform a power analysis for a 5-year clinical trial with breast cancer as an endpoint. Once these biomarkers are validated, other candidate prevention agents for breast cancer could be incorporated into future trials. Significant correlations between SEB and breast cancer incidence will allow clinicians to identify women who can effectively lower their breast cancer risk, potentially with American ginseng. Therefore we will establish a base of comparison between breast cancer patients and participants in the SELF study by collecting anthropometric data (height, weight, body composition, waist-hip ratio), dietary history, exercise habits, and insulin resistance blood panel (HOMA index, leptin, ghrelin, and adiponectin) and calculated 5 year risk of breast cancer (Gail score) in participating breast cancer patients. Contingent on an observed antiproliferative effect of ginseng on tumor or surrounding normal breast tissue in treated breast cancer patients or on the other surrogate markers of breast cancer risk, a breast cancer prevention trial involving exercise with or without ginseng supplementation will be designed.

2.5 Gelatin Encapsulated Standardized Lyophilized Water-Extract of American Ginseng root derived from *Panax quinquefolius*, L. (LEAG)

Asian ginseng (*Panax ginseng*) and its close relative American ginseng (*Panax quinquefolium*) are perennial aromatic herbs that are widely used in Asian medicine. Ginseng root is used as a tonic thought to increase the body's resistance to stress and fatigue, to increase endurance under heavy physical activity, and to improve well-being in age-related debilitation. Most of the ginseng consumed, even in Asian populations, is American ginseng, and the majority of American ginseng is grown and processed in Wisconsin, with quality and standardization overseen by the Ginseng Board of Wisconsin. Furthermore, in Asian medicine, Asian ginseng (*Panax ginseng*) and American ginseng (*Panax quinquefolium*) are common components in herbals used for cancer prevention and treatment. Indeed, retrospective studies have shown that patients who consumed ginseng on a regular basis experienced cancers at a reduced rate (31), however, breast cancer was not considered. Ginseng has been used medicinally for over 2000 years and there are no substantiated serious adverse effects, and few, if any, non-serious adverse effects. The World Health Organization lists ginseng as a traditional medicine with very low toxicity (32, 33). Clinical trials have also demonstrated an anti-hyperglycemic action of American ginseng. These studies utilized capsules containing dried, ground Ontario-grown *P. qinquefolius* L. root. The ground AG root preparation had an onset of action of 40 minutes (24). They found no significant increase in anti-hyperglycemic action after 40 minutes (24) and no significant increase in anti-hyperglycemic action using 1 gram versus 3 gram dosing.

LEAG is a standardized preparation of lyophilized water-extract of American ginseng root in 250-mg gelatin capsules. The ginseng was purchased through the Ginseng Board of Wisconsin and the lyophilized extract has been certified for percentage of each and total ginsenoside content and screened for safe levels of minerals, metals, and pesticides by ConsumerLab.com, a leading testing service company of dietary supplements (Appendix E).

2.6 Rationale

Studies that target primary prevention are difficult due to the large number of subjects and many years of follow-up that are required. Our strategy is to evaluate the effect of oral ginseng on several proliferative tumor associated biomarkers, as well as inflammatory and obesity related biomarkers.

There is precedent for the use of biomarkers in short-term neoadjuvant therapy of breast cancer. The IMPACT trial compared the preoperative use of tamoxifen with anastrozole alone or in combination in postmenopausal women (n=330) with primary breast cancer (34). Immunohistochemical levels of the proliferation marker Ki-67, estrogen receptor (ER), progesterone receptor (PR), and HER-2 neu were assessed at diagnosis, 2 weeks, and 12 weeks. A reduction in Ki-67 was seen at 2 weeks in the majority of patients (93%, 85%, and 84% of those treated with anastrozole, tamoxifen, and combination, respectively). The percent reduction of Ki-67 by treatment group paralleled the tumor response rate and disease-free survivals of these different treatments, confirming the value of Ki-67 as a biomarker of tumor response to therapy

(35). Because the proliferation marker Ki-67 has been shown to be reduced by as little as two weeks of neoadjuvant therapy (35), we hypothesize that proteins that regulate the cell cycle, inflammation, and cell death can show alteration in expression within one cell cycle and can be detectable in tumor specimens after 5 or more days of treatment.

Targets for cancer prevention are being sought among the numerous regulatory molecules involved in cellular proliferation, differentiation, and DNA repair. A morphological description of breast carcinogenesis is currently based on a model of progression from hyperplasia to DCIS to invasive carcinoma. This is based on both analogy to the colon cancer hyperplasia-dysplasia model (for which many of the genes involved in the progression have been identified) and on surgical and autopsy studies. In a study comparing benign proliferative lesions occurring in the same breast with invasive cancer, 50% of the hyperplastic lesions and 80% of the ductal carcinoma in situ (DCIS) shared at least one LOH with the adjacent cancer (36). In the breast, the process of intraepithelial neoplasia can be shown to contain a spectrum of morphologic findings ranging from ductal hyperplasia to atypical ductal hyperplasia to DCIS. The earliest precursor lesions rarely show overt markers of malignancy such as p53 mutation, but they do differentially express cell cycle regulatory proteins. Immunohistological and RT-PCR studies of proliferative ductal epithelial lesions in breast tissue associated with resected carcinoma show increased cyclin D1 expression, which mediates cell cycle progression, in 72% of DCIS and 27% of atypical ductal hyperplasia (37, 38). In contrast, cyclin D2, which regulates cell cycle arrest, is abundant in finite-lifespan human mammary epithelial cells (HMEC) and benign breast tissue, but is decreased or undetectable in 87% of breast cancer cell lines and primary tumors (39). Recently, cyclooxygenase-2 (COX-2) staining was found to be increased not only in DCIS but in the stroma and benign breast tissue surrounding the lesion (40). COX-2 modulates prostanoid production in the direction of stimulating epithelial proliferation and is upregulated in colon, breast, and other cancers (41). COX-2 upregulation may also be associated with diabetes. Human endothelial cells cultured in high glucose conditions show increased COX-2 mRNA and protein and increases in oxidative stress (42). Though cell proliferation and regulation are obviously important, they are no longer the only targets of therapy. Mechanisms of tumor spread and angiogenesis are also highly studied,

Angiopoietin plays a physiologic role in angiogenesis and lymphangiogenesis and is involved in the maintenance of vascular function and integrity[52]. Four members (Ang 1, Ang 2, Ang 3, and Ang 4) make up this family with 2 receptors, Tie 1 and Tie 2. An increasing number of studies show that angiopoietin-2 correlates with tumor angiogenesis, tumor stage, lymph node invasion and length of survival[51,52,53,54,55,56]. Many cancers, including lung, breast, neuroendocrine, colorectal, and melanomas have associations of high angiopoietin-2 and stage of disease; therefore, signifying high potential as a biomarker of stage and/ or metastasis. This potential is not only due to its broad association with cancer in general but also its ease of analysis in serum. Agents which inhibit angiopoietin's actions would, therefore, also have high potential for use in cancer treatment.

The present study was designed to explore the changes brought about by LEAG in breast cancer tumors and surrounding normal breast epithelial cells. Various tumor biomarkers, as well as

inflammatory mediators, will be examined in tissue following LEAG treatment. Positive trends and/or results in this study will be the basis for future studies utilizing LEAG.

3.0 PATIENT SELECTION

3.1 Eligibility Criteria

1. Patients with a cytologically confirmed diagnosis of breast cancer with a biopsy showing invasive or non-invasive breast cancer (DCIS) at least 1.0 cm greatest diameter on imaging (biopsy must have been performed within 6 months of screening visit).
2. Surgical patients, undergoing lumpectomy, subtotal or total mastectomy.
3. Age $\geq$ 18 years of age.
4. Female.
5. Patients must have tissue blocks available from diagnostic biopsy.
6. Negative pregnancy test, medical history of surgical sterilization, or 1 year post-menopausal. The effects of LEAG on the developing human fetus at the recommended therapeutic dose are unknown. For this reason women of child-bearing potential must agree to use adequate contraception (hormonal or barrier method of birth control; abstinence) prior to study entry and for the duration of study participation.
7. Patient must be willing to forgo surgery for a minimum of 5 days.
8. The ability and willingness to sign a written consent.
9. Patients currently being treated for hypertension must be on a stable dose of medication for the past 30 days.
10. Diabetic patients must be well controlled (HbA1c < 8.5 within past 60 days or documented fasting blood glucose < 140 mg/dl for three consecutive days). Known diabetics who have experienced episodes of symptomatic hypoglycemia in the last 6 months are also considered poorly controlled and will be excluded from study participation.
11. ECOG status of $\leq$ 2 or Karnofsky $\geq$ 60% (Appendix F).

3.2 Exclusion Criteria

1. Patients with previous or concurrent malignancy, excluding non-melanotic skin cancer. Malignancies treated and without recurrence or symptoms of recurrence within the last 3 years are acceptable.
2. Patients with evidence of distant metastatic disease.
3. Patients who have had chemotherapy, biologic or radiotherapy within 6 months of biopsy.
4. Patient usage of herbal supplements or alternative medications not approved by the FDA within 1 week of starting study drug. LEAG or related ginseng products, and combination products containing ginseng should be discontinued within 6 weeks of starting study drug.

5. Patients with a history of allergic reactions attributed to compounds of similar chemical or biologic composition to LEAG, including other products claiming to contain ginseng in any form.
6. Patients with a history of a chronic inflammatory process, including, but not limited to, rheumatoid arthritis and lupus. This includes patients on concurrent chronic systemic steroids or anti-inflammatory medications.
7. The subject has active bleeding (i.e. gastrointestinal ulcer with bleeding) or a pathological condition that carries a high risk of bleeding, in the investigator's opinion.
8. Patients with any swallowing dysfunction leading to difficulty taking the investigational therapy are ineligible for the study.
9. Uncontrolled intercurrent illness including, but not limited to, ongoing or active infection, symptomatic congestive heart failure, unstable angina pectoris, cardiac arrhythmia, or psychiatric illness/social situations that would limit compliance with study requirements. HIV-positive patients on combination antiretroviral therapy are ineligible because of the potential for pharmacokinetic interactions with LEAG. Appropriate studies will be undertaken in patients receiving combination antiretroviral therapy when indicated.
10. Patients with poorly controlled diabetes (control indicated with HbA1c $\leq$ 8.5 within past 60 days or documented fasting blood glucose < 140 mg/dl for three consecutive days). Known diabetics who have experienced episodes of symptomatic hypoglycemia in the last 6 months are also considered poorly controlled and will be excluded from study participation.
11. Patients with uncontrolled hypertension (SBP > 140 mmHg or DBP > 90 mmHG). Should a subject's blood pressure on initial presentation fall above defined parameters for hypertension two additional blood pressure measurements will be performed at least 15 minutes apart. The initial blood pressure and two additional measurements will be averaged. If the average of the three measurements remains above the defined parameters for hypertension, the patient will be excluded. If the average of the three measurements falls below the defined parameters for hypertension, the patient will be considered to meet eligibility.
12. Pregnant or breast feeding women. Women must be willing to use birth control throughout study duration.
13. Patients on other investigational medications or having been treated with an investigational agent within 6 weeks prior to biopsy.
14. Patients currently receiving coumadin therapy or who have been treated with coumadin within the 2 weeks prior to biopsy.
15. Patients taking monoamine oxidase inhibitors.

3.3 Inclusion of Women and Minorities

Women and members of all races and ethnic groups are eligible for this trial.

4.0 REGISTRATION AND ENROLLMENT PROCEDURES

4.1 General Guidelines

To be eligible for registration to this study, the patient must meet each inclusion criteria listed on the eligibility checklist and none of the exclusion criteria should apply. To be registered, the patient must have the ability to understand and the willingness to sign the written informed consent.

4.2 Registration Process

- Contact the Clinical Trials Office (CTO) at 217-545-7929 with patient information: name, physician, and diagnosis.
- CTO staff will screen patients' charts for eligibility. The CTO must have the following information, at minimum, to determine eligibility: past medical history information, diagnostic biopsy report, radiologic exams, and staging information.
- If the patient is eligible following chart review: CTO staff will meet with patient to review the Informed Consent Form and proceed in obtaining written consent.

4.3 Criteria for Enrollment

- Confirmation that patient has met all of the Inclusion Criteria and none of the Exclusion Criteria.
- Patient has voluntarily signed informed consent document.

5.0 TREATMENT PLAN

5.1 Screening Procedures

5.1.1 History and physical reviewed and recorded, including performance status.
5.1.2 Concomitant medications reviewed and recorded.
5.1.3 Vital signs reviewed and recorded.
5.1.4 Inclusion and exclusion criteria will be reviewed.
5.15 Review and record available HbA1c status (within past 60 days) or fasting blood glucose values (three consecutive days below 140 mg/dl)
5.1.6 Informed consent process
5.1.7 Pregnancy test, if required

5.2 Study Visit 1

5.2.1 Obtain tissue blocks for immunohistochemisty from core biopsy

- Proliferation Markers: immunohistochemistry for ER, PR, Ki67, cyclin D1, Cox-2, and angiopoietin 2.
- Cytotoxicity Markers: cyclin D2 and TUNEL
- Angiogenesis Markers: angiopoetin 2

5.2.2 Fasting lab work will be obtained.

Serum for levels of gRc and gRh2, inflammatory mediator proteinomic panel (IL1b, IL6, IL8, MCP-1, TNFa, HGF, NGFb, Insulin, and Leptin), pregnancy test (if not already done at screening visit) if woman is of child-bearing potential, HOMA index, leptin, ghrelin, and adiponectin.

5.2.3 Questionnaires will be completed

Dietary, Tecumseh and Minnesota Activity Questionnaires

A. Tecumseh and Minnesota Occupational and Leisure Time Activity Questionnaires: Both occupational and leisure time exercise exposure for the prior 12 months will be measured by the Tecumseh and Minnesota occupational and leisure time activity questionnaires. The validity and reliability of the Tecumseh Occupational Physical Activity Questionnaire (43) has been documented by Ainsworth (44, 45) (Appendix A). Leisure time physical activity will be measured by the Minnesota Leisure Time Physical Activity Questionnaire (Appendix B) which has also been previously tested for validity and reliability and is a well-accepted measurement tool (43). Of note, the use of the Tecumseh and Minnesota Occupational and Leisure Time Surveys allow for calculation of energy expenditure. Specifically, each activity recorded on the survey will be assigned a calculated metabolic equivalent (MET) value based on the published compendium of physical activity (46). This MET value will be multiplied by the number of hours spent in each activity per week (i.e. METs x hrs/wk). This variable (METs x hrs/wk) will then be analyzed for the study group as a continuous variable and in quartiles.

B. National Cancer Institute Dietary History Questionnaire: The current proposal will utilize the Dietary History Questionnaire (DHQ) developed by the Risk Factor Monitoring and Methods Branch (RFMMB) of the National Cancer Institute. The *Eating at America's Table Study* conducted by the RFMMB compared their new DHQ with the previous standard Block and Willet food frequency questionnaires (FFQs). The DHQ performed best of all in a randomized sample of 1,640 men and women (47). The DHQ is a self-administered questionnaire that represents a refinement over the RFMMB Block FFQ, with

improved comprehension across various economic and ethnic groups and increased attention to dietary constituents of interest in disease prevention: fiber, energy, carotenoids and vitamin A, E and C (Appendix C). The questionnaire will be entered into an ASCII text file and analyzed using Diet*Calc Version 1.2 free downloadable software. Energy intake (kcal/kg), refined carbohydrate, fat, and fruit and vegetable intake (% of total intake) will be analyzed as continuous variables and by quartiles.

5.2.4 Anthropometric data will be collected

Body measurements including height and waist-hip ratio and body composition.

A. Waist-hip ratio: Over light clothing with a measuring tape in inches: waist girth (W) is measured at the minimum circumference between the costal margin and the iliac crest; hip girth (H) is measured at the greatest circumference at the level of the greater trochanters. The ratio of W/H is normal for females if less than 0.85.

B. Body composition: Body composition will be estimated by bioelectric impedance analysis performed with a hand-held device (Omrons HBF-360 body Fat Analyzer).

5.2.5 Performance Status based on Karnofsky Scale (Appendix F).

5.2.6 Gail score will be calculated (Appendix D).

Gail score: Age, race, age at menarche, age at first live birth, number of first degree relatives with breast cancer, number of breast biopsies and presence of atypical ductal hyperplasia will be entered into a Gail score calculation program. The resulting score represents a patient's 5-year cumulative risk of breast cancer. A risk greater than 1.66% is the threshold for chemoprevention trials.

5.2.7 Study drug dispensed

Four 250 mg capsules of LEAG will be dispensed for every day of treatment expected (5-14 days) based on the scheduled surgery date.

5.3 Treatment Regimen

5.3.1 Patients will be treated with ginseng for 5 to 14 days prior to surgery. The final dose of LEAG will be administered the day prior to surgery as surgical patients are asked to abstain from food and drink (excluding water) over night prior to surgery.

5.3.2 Patients will take four, 250mg tablets daily.

5.3.3 Patients will be given a drug diary to record dates of ginseng therapy in order to monitor compliance.

5.3.4 Because ginseng has a rare risk of lowering the blood sugar level, patients with diabetes (as defined by the American Diabetes Association) will be encouraged to use their home blood glucose machine to monitor their blood sugar twice a day during treatment and report any readings less than 60. Patients without a home monitoring device will be instructed to report any signs or symptoms of hypoglycemia such as lightheadedness, sweating or confusion. Patients will be provided a diary to track their twice daily glucose checks.

5.3.5 Because ginseng has been reported to alter blood pressure and heart rate, patients being treated for hypertension will be encouraged to report any changes of blood pressure greater than 20 mmHg increase from baseline. Patients should be encouraged to have blood pressure readings daily. Patients will be provided with a diary (Appendix I) and a blood pressure monitoring system to record daily blood pressure measurements. If preferred, patients may use their own home monitoring device or use a monitor found at a local drug store.

5.4 Interim Phone Call

All patients will be contacted within 48-72 hours by telephone (or in-person if more convenient for patient) of starting medication to assess any adverse events. Any adverse events will be reported to the principle investigator for review and assessment.

5.5 Day of Surgery Visit

5.5.1 Repeat fasting lab work.

5.5.2 Obtain tissue samples during scheduled surgery.

5.5.3 Review and complete drug accountability and calculate compliance. Collect Ginseng medication diary and any unused study drug.

5.5.4 Obtain vital signs.

5.5.5 Complete performance status evaluation.

5.5.6 Review and complete adverse event log.

5.5.7 Review and record concomitant medications.

5.5.8 Collect, review and record blood pressure and/or blood sugar diaries (Appendix I), if indicated.

5.6 Follow-up Post Surgery Visit

5.6.1 Adverse events will be collected through the post-surgery follow-up visit and all related or possibly related adverse events for at least 30 days following the last dose of LEAG and continue to be followed until all adverse events have resolved or stabilized (Grade 2 or less by CTCAE vs. 3.0).

5.6.2 Obtain vital signs and perform physical exam.

5.6.3 Performance status will be completed.

5.6.4 Duration of follow up

Patients will be followed until the post-surgery follow-up visit (generally 10-20 days) following surgical resection. All related or possibly related adverse events will be followed for 30 days from the final dose of LEAG or until resolution or stabilization (Grade 2 or less) of adverse event. Patients removed from study for unacceptable adverse events will be followed until resolution or stabilization of the adverse event.

5.7 Study Role-Out: Additional Procedures for the First 5 Patients

Additional procedures will be conducted to evaluate the effects of LEAG on platelet function. The first 5 patients enrolled will have platelet function assessed prior to surgery with an interim analysis of platelet function and bleeding complications prior to additional patient enrollment to determine if platelet function should be evaluated in all study patients. After 5 patients have completed the study protocol a safety meeting will convene to determine if pre-operative assessment of platelet function is needed in all study patients. The following procedures have been added for the first five patients.

5.7.1 Added Exclusion Criteria

- Role-out patients taking aspirin, other anti-coagulants, or anti-platelet aggregate are excluded at this time. Platelet Function Assay -100 may be affected by anti-coagulant use.

5.7.2 Added Pre-LEAG Administration Laboratory Test

- Platelet Function Assay-100 test (2 citrate tubes of blood prior to taking LEAG)

5.7.3 Added post-LEAG but pre-surgical laboratory test

- Repeat Platelet Function Assay-100 test prior to surgery (2 citrate tubes of blood drawn day of surgery)

5.7.4 Interim Analysis of Platelet Function Assay completed following completion of 5 subjects. All PFA-100 results were within normal limits. Recommendations from Data Review Team state that further Platelet Function Assay testing is not necessary.

6.0 GINSENG THERAPY

6.1 Summary

- LEAG, 1 gram (4 capsules) by mouth daily taken with food in the morning.
- All patients will receive a minimum of 5 days and a maximum of 14 days of treatment with LEAG for treatment prior to surgical therapy.
- The final dose of LEAG will be taken the day prior to surgery to satisfy the fasting recommendation prior to surgery.

6.2 Study Agent

The investigational agent is a clear gelatin capsule containing 250 mg of lyophilized water-extract of American Ginseng root. The lyophilized extract is a white powdery substance that can be seen through the clear gelatin capsule. The capsule is intended for oral use and does not contain any active or inactive substances other than the lyophilized water-extract of American Ginseng root. For additional information on general use of all types of ginseng, please see the Customized Monograph published by Clinical Pharmacology (Appendix G) and the Investigator's Brochure.

6.3 Preparation

No preparation needed.

6.4 Mechanism of Action

The major active components of ginseng are the ginsenosides found within the compound. Ginsenosides, also called panaxosides, are triterpenoid saponin glycosides. Asian ginseng contains up to 13 different ginsenosides which may have opposing actions on one another. Small animal tests have indicated that the active components of ginseng can prolong exercise time,

stimulate protein synthesis, inhibit platelet aggregation, stimulate the proliferation of hepatic ribosomes, prevent stress-induced ulcers, and increase the activity of interferons (clinical pharmacology). The antiplatelet effect appears to be a result of the activity of panaxynol and ginsenosides Ro, Rg1, and Rg2. Enhanced nitric oxide production in the lung, heart, and kidney, as well as the corpus cavernosum has been proposed as a mechanism of action. Various glycans have demonstrated a hypoglycemic effect in normal mice (clinical pharmacology). The ginsenosides found within the gelatin encapsulated lyophilized water-extract of American Ginseng root used in this study are as follows:

Ginsenosides per gram of Gelatin Encapsulated lyophylized water-extract of American Ginseng Root

Ginsenoside	mg
Rg1	2.38
Re	16.3
Rf	0
Rb1	25
Rc	4.60
Rb2	0.81
Rd	7.75
Total	40.04

6.5 Administration Guidelines

Patients will be instructed verbally on the administration of gelatin encapsulated lyophilized water-extract of American Ginseng root.

The study agent will be self-administered in an open-label, unblinded manner to all patients enrolled in the study. During the treatment period, patients will receive 1 gram (4 capsules) of LEAG each day. Capsules should be taken at approximately the same time daily. To reduce the insomnia and hypoglycemic effects, LEAG administration is recommended with breakfast.

Patient must complete at least 5 days of treatment with LEAG prior to day of surgery. LEAG will be dispensed to the patient on the last clinic visit prior to the scheduled date of surgery. Treatment with study medication will be initiated 5 to 14 days prior to surgery and end the night before surgery. **If the patient is not seen in clinic the day that study drug is to be initiated, a reminder telephone call will be made to the patient.** A capsule count will be taken the day of surgery.

Patients will be given a drug diary to record dates of ginseng therapy in order to monitor compliance.

6.6 Duration of Therapy

In the absence of treatment delays due to adverse event(s), treatment may continue for 14 days or until one of the following criteria applies:

- Intercurrent illness that prevents further administration of treatment,
- Unacceptable adverse event(s) or toxicity of grade 3 or higher,
- Patient decides to withdraw from the study, or
- General or specific changes in the patient's condition that render the patient unacceptable for further treatment in the judgment of the investigator.

6.7 Dose Reductions

Reported adverse events and potential risks are described in Section 7. If LEAG therapy is discontinued prior to surgery, blood levels of LEAG will not be drawn on the day of surgery but the breast tissue will be studied for immunohistochemistry, in situ TUNEL, and oxidative stress as described. Dose reductions of LEAG are not permitted.

6.8 Storage and Accountability

All study material should be stored at room temperature and out of direct light. LEAG will be supplied to the patients in white opaque bottles with child resistant closures. The study agent required for completion of this study has been provided by Dr. Murphy's laboratory at Southern Illinois University. The Clinical Research Office will document receipt and dispensation of study agent on the accountability log (Appendix H) in accordance with regulations.

Agent Inventory Records – The investigator, or a responsible party designated by the investigator, must maintain a careful record of the inventory and disposition of the American Ginseng. Dispensation of drug to each individual patient must be recorded on the accountability log (Appendix H).

6.9 Availability

The American Ginseng root has been obtained from the Ginseng Board of Wisconsin and analyzed for content by consumerlab.com. The extraction process was performed by Dr. Murphy's Laboratory at Southern Illinois University prior to analysis. American Ginseng will only be available through the Clinical Research Office of the Simmons Cancer Institute at SIU

School of Medicine. The investigational agent will be stored at room temperature in a locked cabinet.

7.0 Risks/ Precautions

7.1 Contraindications/Precautions

- Ginseng is generally well tolerated when taken orally. Various sources indicate that long term use should be limited to 3 months.
- The most common side effects reported include headache, nervousness/anxiety, sleep disturbances, and gastrointestinal disturbances.
- Estrogenic effects, such as breast tenderness and vaginal bleeding have been reported though not well substantiated.
- Reports of changes in blood pressure and blood sugar levels indicate that individuals being treated for hypertension and/or diabetes should use ginseng with caution.
- Ginseng has been reported to interact with coumadin. Use with coumadin should be closely monitored.

7.2 Concomitant Medications

Because there is a potential for interaction of American Ginseng with other concomitantly administered drugs, the case report form must capture the concurrent use of all other drugs, over-the-counter medications, and alternative therapies.

All medications currently being taken by the subject (including over the counter medications and herbal supplements) need to be entered into the Concomitant Medication Log for each subject. Concomitant medications will be monitored from enrollment into the study through the post-surgery visit. The information collected will include dates of administration, dosage, and form.

7.3 Prohibited Concomitant Medications

7.3.1 Products Containing Ginseng

- All products containing or claiming to contain any form of ginseng product including oral and external application products are prohibited from use during this study and for six weeks prior to initial biopsy.

7.3.2 Coumadin

- Due to a report concerning the possible interaction between coumadin and ginseng, patients currently being treated with coumadin are ineligible for the study.

All other medications, prescription, and over the counter, that affect platelets and/or clotting functions should be used with caution.

7.3.3 Herbal Supplements and Alternative Medications not approved by the FDA.

- All Herbal Supplements and Alternative Medications not approved by the FDA including oral and external application products are prohibited from use during this study and for six weeks prior to initial biopsy.

7.3.4 Aspirin, other anti-coagulants, and anti-platelet aggregates

- Aspirin and other anti-coagulants are only prohibited in the first 5 role-out patients until the effects of LEAG on platelet function can be assessed.

8.0 ASSESSMENT INSTRUMENTS

8.1 Gail score (Appendix D)

Age, race, age at menarche, age at first live birth, number of first degree relatives with breast cancer, number of breast biopsies and presence of atypical ductal hyperplasia will be entered into a Gail score calculation program (Appendix D) (National Cancer Institute Breast Cancer Risk Assessment Tool online calculator at www.cancer.gov/bcrisktool/). The resulting score represents a patient's 5-year cumulative risk of breast cancer. A risk greater than 1.66% is the threshold for chemoprevention trials.

8.2 Tecumseh Occupational and Minnesota Leisure Time Activity Questionnaires (Appendices A and B)

Both occupational and leisure time exercise exposure for the prior 12 months will be measured by the Tecumseh and Minnesota occupational and leisure time activity questionnaires. The validity and reliability of the Tecumseh Occupational Activity Questionnaire (43) has been documented by Ainsworth (44, 45). Leisure time physical activity will be measured by the Minnesota Leisure Time Activity Questionnaire which has also been previously tested for validity and reliability and is a well-accepted measurement tool (43). Of note, the use of the Tecumseh and Minnesota Occupational and Leisure Time Surveys allow for calculation of energy expenditure. Specifically, each activity recorded on the survey will be assigned a calculated metabolic equivalent (MET) value based on the published compendium of physical

activity (46). This MET value will be multiplied by the number of hours spent in each activity per week (i.e. METs x hrs/wk). This variable (METs x hrs/wk) will then be analyzed for the study group as a continuous variable and in quartiles.

8.3 National Cancer Institute Dietary History Questionnaire (Appendix C)

The current proposal will utilize the Dietary History Questionnaire (DHQ) developed by the Risk Factor Monitoring and Methods Branch (RFMMB) of the National Cancer Institute. The *Eating at America's Table Study* conducted by the RFMMB compared their new DHQ with the previous standard Block and Willet food frequency questionnaires (FFQs). The DHQ performed best of all in a randomized sample of 1,640 men and women (47). The DHQ is a self-administered questionnaire that represents a refinement over the RFMMB Block FFQ, with improved comprehension across various economic and ethnic groups and increased attention to dietary constituents of interest in disease prevention: fiber, energy, carotenoids and vitamin A, E and C. The questionnaire will be entered into an ASCII text file and analyzed using Diet*Calc Version 1.2 free downloadable software. Energy intake (kcal/kg), refined carbohydrate, fat, and fruit and vegetable intake (% of total intake) will be analyzed as continuous variables and by quartiles.

9.0 LABORATORY TESTS AND METHODS

9.1 Blood Collection

Collection of blood for inflammatory mediators and HOMA index must occur within 2 weeks of treatment. Participants will have blood drawn prior to starting ginseng and on the day of surgery.

A. Fasting Blood Draw (NPO after midnight):

1. Draw 40 cc blood from a vein into vacutainer tubes: 6mL in EDTA, 6mL in Na Heparin, 5mL in clot activator tube, and 24mL in serum tubes. (Serum tubes to be refrigerated (4˚C) until centrifuged, and then serum will be aliquoted into 1ml cryotubes).

2. An additional 2 tubes of blood with citrate will be drawn on the first five patients for the Platelet Function Assay-100. – See section 5.7.4

3. Serum for ginsenoside levels and inflammatory proteins stored in -80˚C freezer in lab prior to shipping to Carbondale laboratory.

4. Analyze serum for HOMA index, leptin, ghrelin, adiponectin, and angiopoietin.

5. Analyze serum for gRc and gRh2, IL1b, IL6, IL8, MCP-1, TNFa, HGF, NGFb, insulin, leptin.

B. Inflammatory Serum Protein Panel (LINCOplex) and Serum Adipokine Panel

Serum levels of TNFa and IL6 are elevated in patients who are obese or hyperinsulinemic and play an important role in the pathogenesis of type II diabetes. These serum markers also declined in insulin-resistant individuals who participated in an exercise program (48). In a study of adipokine expression in cancer patients, breast and colorectal cancer patients had blood plasma levels of insulin, TNF-alpha and breast epithelial expression of estrogen and progesterone receptors higher than controls. In the same study, breast cancer patients, but not colorectal cancer patients, had plasma levels and adipose tissue expression of leptin significantly higher than controls, associated with elevated values of estrogen and progesterone-receptors (49). These serum markers can be assayed simultaneously in a multiplex immunoassay (human adipokine panel B, LINCO, St. Louis, MO) using Luminex technology. The assay will be performed by the SIU Cancer Institute Molecular Core Facility. Serum adipokine levels of breast cancer patients before and after ginseng treatment will be compared. Serum will be collected and stored at -80°C until the assay is performed.

High serum angiopoietin-2 levels have been associated with tumor burden, stage of disease, and metastatic spread. Pre-LEAG serum levels will be compared with post-LEAG serum levels of enroll patients. ELISA assays will be use to batch the samples and perform angiopoiten-2 analysis.

C. High performance Liquid Chromatography-Mass Spectrophotometry

The concentrations of G2, grh in serum will be measured using liquid chromatography followed by tandem mass spectrometry. This combination is able to provide excellent sensitivity, speed and offers positive compound identification through the use of multiple reaction monitoring (MRM). Prior to analysis, samples of gRc and gRh2 will be analyzed using tandem mass spectrometry to determine the characteristic fragmentation pattern of each compound. During analysis, the mass spectrometer will be set up to monitor only signals corresponding to the molecular weights of the two compounds of interest. The instrument will then fragment each compound and indicate whether the fragmentation pattern matches the previously recorded one. This method is used often for monitoring specific compounds in complex samples, such as the detection of steroid use in athletes. It provides a positive identification through accurate mass measurement followed by a characteristic fragmentation pattern. Absolute quantification is calculated by either measuring a calibration curve with known samples or by using an internal standard.

D. Risks and side effects for Blood Tests

Likely	Unlikely
• Slight discomfort and/or pain when drawing blood • Bruising at the site • Minor bleeding at the site (just after sample is drawn)	• Infection at the site • Fainting

E. Immunocytochemistry

Unstained paraffin-embedded sections from the diagnostic biopsy and the resected specimen including tumor and histologically normal breast epithelium will be subjected to immunostaining using a streptavidin-peroxidase technique. Briefly, permeabilized sections will have endogenous peroxidase activity blocked with 1X blocking solution (Kirkegaard & Perry Laboratories, Gaitersburg, MD) and the antigen unmasked by incubating in 10mM citrate buffer, pH 6.0 for 5 min at 95° C and rinsing with TBS. Nonspecific binding of the secondary antibody will be blocked with normal goat serum for 15 min, then sections will be incubated with a 1:50 dilution of rabbit anti-human COX-2 polyclonal antibody (Santa Cruz Biotechnology, Inc., Santa Cruz, CA), or cyclin D1 (Labvision). Immunoperoxidase staining will be performed using a biotin/strepavidin peroxidase complex (HistoMark™ Kirkegaard & Perry Laboratories, Gaitersburg, MD) and DAB substrate (Research Genetics, Huntsville, AL). Sections will be counterstained with hematoxylin. Fetal kidney tissue will be used as a positive control, and the primary antibody will be replaced with normal rabbit serum as a negative control. The ER, PR, and Ki67 staining will be done in the pathology department of the hospital where the diagnostic biopsy was performed, in order to optimally compare baseline and treated tumor.

F. In situ TUNEL

Unstained paraffin-embedded sections from the diagnostic biopsy and the resected specimen including tumor and histologically normal breast epithelium will be assayed for apoptosis by terminal deoxynucleotidyl transferase-mediated dUTP-biotin nick end-labeling (TUNEL). 5 μm sections will be deparaffinized and rehydrated in ethanol followed by an antigen retrieval step consisting of boiling in 0.01 M sodium citrate buffer for 2 min. Sections will be incubated with proteinase K (6.2 μg/ml; Boehringer Mannheim, Indianapolis, IL) followed by TdT (300 enzyme units/ml, Pharmacia Biotec, Piscataway, NJ) and Bio-14-dATP (0.94nM, GIBCO-BRL, Life Technologies). Biotinylated ATP will be detected using the ABC staining method (Vector Laboratories). As a positive control, slides will be pre-treated with DNase (20K units/ml; Sigma Biosciences, St. Louis, MO). Cells will be counted as TUNEL-positive if their nuclei

fluoresce and display typical apoptotic morphology with chromatin condensation. The number of apoptotic cells will be defined as the number of TUNEL-positive cells per field after counting 20 sequential fields at 250X.

G. Assessment of oxidative stress by MDA Assay

This assay will be used to determine the amount of lipid peroxidation by the malondialdehyde (MDA) levels as described by Ohkawa (50). Approximately 1 gm of tumor and normal breast tissue will be harvested, weighed and homogenized with a glass homogenizer in 1:10 wt:wt PBS. BHT will be added to the homogenates to arrest further oxidation and the homogenates will be stored at -80°C. The homogenates will then be analyzed for thiobarbituric acid reactive substances (TBARS) using commercially available assay kit from Zeptometrix. Briefly, mammary homogenate will be added to 8.1% SDS and vortexed. Thiobarbituric acid will then be added and placed in 95° C water bath for 60 minutes. The samples will be cooled to room temperature and centrifuged at 3000 rpm for15 minutes. Supernatants from experimental samples and MDA standards will be measured by fluorescence excitation at 530 nm and emission at 550 nm.

9.2 Pathology Studies

9.2.1. Tissue Needed

- 10gm segment of grossly normal tissue processed for paraffin embedded slides
- 10gm segment of tumorous tissue processed for paraffin embedded slides
- Pathology slides stained for ER, PR, Ki67 and inked for margins
- Unstained sections processed for SIU lab for cyclin D1 and cyclin D2, COX-2, and TUNEL

9.2.2 Procedure

On the day of surgery, the breast tissue will be submitted to the pathologist and inked for margins per routine. The tissue will be sectioned and margins studied per routine. From the grossly normal breast tissue that would be otherwise discarded, a 20 gm segment will be frozen for oxidative stress MDA assay, and a 10 gm segment will be processed for paraffin-embedded slides. The tumor will be processed as paraffin-embedded slides per routine diagnostic stains. Tumor and normal breast

slides will be stained for ER, PR, and Ki-67 in the pathology dept of the hospital where the surgery takes place. Unstained paraffin sections of tumor and normal breast will be allocated to Dr. Peralta's lab for immunohistochemical staining for cyclin D-1, cyclin D-2, COX-2 and TUNEL. These sections will be cut from tissue remaining in paraffin blocks after adequate diagnostic studies have been completed.

10.0 CRITERIA FOR REMOVAL FROM STUDY

Patients will be removed from study when any of the criteria listed in Section 7.3 applies. The reason for study removal and the date the patient was removed must be documented in the Case Report Form.

Noncompliance
Patient noncompliance of study medication will be defined as the patient voluntarily taking fewer than 80% of their assigned study medication. For example, out of 10 days of therapy the patient would need to miss at least 3 doses to be noncompliant. Temporary discontinuation of the investigational product by a physician's order due to adverse events or disease complication does not constitute non-compliance.

11.0 ADVERSE EVENTS: LIST AND REPORTING REQUIREMENTS

11.1 Adverse Event Characteristics

- **CTCAE term (AE description) and grade:** The descriptions and grading scales found in the revised NCI Common Terminology Criteria for Adverse Events (CTCAE) version 3.0 will be utilized for AE reporting. All appropriate treatment areas should have access to a copy of the CTCAE version 3.0. A copy of the CTCAE version 3.0 can be downloaded from the CTEP web site (http://ctep.cancer.gov).

- **"Expectedness"**: AEs can be 'Unexpected' or 'Expected'

- **Attribution** of the AE:
  - Definite – The AE *is clearly related* to the study treatment.
  - Probable – The AE *is likely related* to the study treatment.
  - Possible – The AE *may be related* to the study treatment.
  - Unlikely – The AE *is doubtfully related* to the study treatment.
  - Unrelated – The AE *is clearly NOT related* to the study treatment.

11.2 Adverse Event (AE) Reporting

An adverse event is any untoward medical occurrence in a patient that does not necessarily have

a causal relationship with the study intervention. An AE can, therefore, be any unfavorable and unintended sign (including an abnormal laboratory finding), symptom or disease temporarily associated with the use of a medical treatment or procedure (attribution of unrelated, unlikely, possible, probable, or definite).

Adverse events will be reported once treatment with the study agent begins to the Chairperson of the Springfield Committee for Research Involving Human Subjects (SCRIHS) at Southern Illinois University School of Medicine, 801 N. Rutledge Street, Springfield, IL 62702, telephone number: 217/545-7936.

Submission requirements of AE's, as defined by the SCRIHS procedure manual, will be strictly adhered to.

Adverse events that are expected and outlined in the pre-op discussion and/or listed in the surgical consent as related to surgery or anesthesia will not be reported.

11.3 Safety Reporting Requirements for IND Holders

In accordance with 21 CFR 212.32, sponsor-investigators of studies conducted under an IND must comply with following safety reporting requirements:

1. Expedited IND Safety Reports:

    - Seven Calendar-Day Telephone or Fax Report

      The Sponsor-Investigator is required to notify the FDA of any fatal or life-threatening adverse event that is unexpected and assessed by the investigator to be possibly related to the use of LEAG. An unexpected adverse event is one that is not already described in the Investigator Brochure. Such reports are to be telephoned or faxed to the FDA within seven calendar days of first learning of the event. Each telephone call or fax transmission (see fax number below) should be directed to the FDA new drug review division in the Center for Drug Evaluation and Research or in the product review division for the Center for Biologics Evaluation and Research, whichever is responsible for the review of the IND.

    - 15 Calendar-Day Written Report

      The Sponsor-Investigator is also required to notify the FDA and all participating investigators, in a written IND Safety Report, of any serious, unexpected AE that is considered possibly related to the use of LEAG. An unexpected adverse event is one that is not already described in the Investigator Brochure.

Written IND Safety Reports should include an Analysis of Similar Events in accordance with regulation 21 CFR § 312.32. All safety reports previously filed with the IND concerning similar events should be analyzed. The new report should contain comments on the significance of the new event in light of the previous, similar reports.

Written IND safety reports with Analysis of Similar Events are to be submitted to the FDA and all participating investigators within 15 calendar days of first learning of the event. The FDA prefers these reports on a MedWatch 3500A Form but alternative formats are acceptable (e.g. summary letter).

FDA fax number for IND Safety Reports:
1-800-FDA-0178 (1-800-332-0178)

MedWatch 3500A Reporting Guidelines

In addition to completing appropriate patient demographic and suspect medication information, the report should include the following information within the Event Description (Section 5) of the MedWatch 3500A form:

- Treatment regimen (dosing frequency, combination therapy, administration dates);
- Protocol description (and number, if assigned);
- Description of event, severity, treatment, and outcome, if known;
- Supportive laboratory results and diagnostics; and
- Investigator's assessment of the relationship of the adverse event to each investigational product and suspect medication.

11.4 IND Annual Reports

In accordance with the regulation 21 CFR § 312.32, the Sponsor-Investigator shall within 60 days of the anniversary date that the IND went into effect, submit a brief report of the progress of the investigation. Please refer to the Code of Federal Regulations, 21 CFR § 312.32 for a list of the elements required for the annual report.

12.0 STUDY CALANDER

	Screening	Study Visit One	Follow-up Phone Call	Study Visit Two	Study Visit Three
Informed Consent	X				
LEAG[A,]		X----------X			
Surgery				X	
Demographics	X				
Medical History and Physical	X				X
Vital Signs	X	X		X	X
Concurrent Medications	X	X----------X			
Height	X				
Weight	X				
Body mass Index		X			
Performance Status	X	X		X	X
Waist Hip Ratio		X			
Body Composition		X			
Gail Model Risk Assessment		X			
NCI Dietary Questionnaire		X			
Tecumseh Questionnaire		X			
Minnesota Questionnaire		X			
Fasting Blood Draw for Laboratory Studies[B]		X		X	
Platelet Function Assay-100[G]		X		X	
Adverse Event Evaluation		X----------X			
Radiologic Evaluation[C]	X				
Tissue Collection	X			X	
Pregnancy test[D]	X				
HbA1c/ Blood glucose[E]	X				
Diary[F]					
Capsule Count[H]				X	

A: LEAG must be taken for a minimum of 5 days and no more than 14 days. Final dose to be taken day prior to surgery. Patients are required to keep a Ginseng medication diary.

B: Patients must be NPO after midnight. Draw 40 cc of blood from a vein into vacutainer tubes: 6mL in EDTA, 6mL in Na Heparin, 5mL in clot activator tube, and 24mL in serum tubes.. Refrigerate serum tubes at 4˚C until centrifugation for serum collection and storage.

C: All radiologic evaluations must be completed within 6 weeks of treatment. (mammogram, ultrasound, and MRI are acceptable)

D: Urine pregnancy test for patients of child bearing potential.

E: Only required in diabetic patients. If an HbA1c has not been conducted within 60 days, a fasting blood glucose finger stick of < 140 mg/dl for 3 consecutive days will be allowed as a substitute for verification of control.

F: Patient must be given a diary for blood pressures and/ or blood glucose by finger stick if they are being treated for hypertension or have been diagnosed with diabetes, respectively. Diaries need only be kept while patient is actively on LEAG (5-14 days). Diaries to be returned to research nurse on day of surgery.

G: The Platelet Function Assay-100 test is only conducted on the first 5 patients enrolled. Draw 2 – 2.7mL citrate tubes.

H: Patients are required to keep a Ginseng medication diary. The diary and pill bottle should be returned the day of surgery.

13.0 DEFINITIONS OF MEASUREMENT

13.1 Diagnostic Disease Parameters for Inclusion in Study

- Measurable disease

  Measurable lesions are defined as those that can be accurately measured in at least one dimension (longest diameter to be recorded) as ≥0.5 cm with conventional techniques (mammography, MRI, ultrasound). All tumor measurements must be recorded decimal fractions of centimeters.

- Non-measurable disease

  All lesions <0.5 cm using mammography, MRI or ultrasound, are considered non-measurable disease.

  All measurements should be taken and recorded in metric notation using a ruler or calipers. All baseline evaluations should be performed as closely as possible to the beginning of treatment and never more than 6 weeks before the day of surgery.

- Mammogram

  Lesions on mammogram films are acceptable as measurable lesions when they are clearly defined and surrounded by fatty breast tissue.

- Ultrasound (US)

  Ultrasound measurement of tumor is an adjunct to the clinical measurement of palpable breast tumors and lymph nodes and to the mammographic measurement of tumor. When the clinically palpable lesion is larger than the US image, the clinical measurement is primary.

- Magnetic Resonance Imaging (MRI)

14.0 DATA REPORTING / REGULATORY CONSIDERATIONS

14.1 Adverse Events

Adverse event lists, guidelines, and instructions for AE reporting can be found in Section 11.0 (Adverse Events: List and Reporting Requirements).

14.2 Institutional Review Board Approval

This protocol, the informed consent document, and relevant supporting information must be submitted to the IRB for review and must be approved before the study is initiated. In addition, any advertising materials must be approved by the IRB. The study will be conducted in accordance with U.S. FDA, applicable national and local health authority, and IRB requirements.

The Principal Investigator is responsible for keeping the IRB apprised of the progress of the study and of any changes made to the protocol as deemed appropriate, but in any case, the IRB approval must be updated at least once a year. The Principal Investigator must also keep the IRB informed of any significant adverse events.

Investigators are required to promptly notify their respective IRB of all adverse drug reactions that are both serious and unexpected. This generally refers to serious adverse events that are not already identified in the Investigator Brochure and that are considered possibly or probably related to the study drug by the investigator. Some IRBs may have other specific adverse event requirements to which investigators are expected to adhere. Submit IRB information to:

Erin Campbell
Southern Illinois University School of Medicine
801 N. Rutledge
Springfield, IL 62702
Phone: 217/545-7602
E-mail: ecampbell@siumed.edu

14.3 Sponsor and Investigator Requirements

Sponsors are responsible for selecting qualified investigators, providing them with the information they need to conduct an investigation properly, ensuring proper monitoring of the investigation(s), ensuring that the investigation(s) is conducted in accordance with the general investigational plan and protocols contained in the IND, maintaining an effective IND with respect to the investigations, and ensuring that the FDA and all participating investigators are promptly informed of significant new adverse effects or risks with respect to the drug. Please refer to the Code of Federal Regulations, Title 21, Subpart D, 312.50 through 312.69 at:

http://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfcfr/CFRSearch.cfm.

14.4 Study Initiation

Before the start of this study the following documents must be on file at the Clinical Research Office:

- U.S. Food and Drug Administration (FDA) Form 1572, signed by the Principal Investigator.
    - The names of any sub-investigators must appear on this form. Investigators must also complete all regulatory documentation as required by local regulations.
- Current curricula vitae and license of the Principal Investigator.
- Final Protocol and ICF
- Written documentation of IRB approval of protocol and ICF (identified by title and date of approval) for each site.
- Written documentation from the FDA assigning an IND number to the trial and the approval to begin the study.

14.5 Study Completion

The following data and materials are required to be on file at the Clinical Research Office before a study can be considered complete or terminated:

- Copies of protocol amendments and IRB approval/notification, if appropriate.
- Copies of the IRB final report/termination document, documentation of submission to the IRB and to the FDA.
- A summary of the study prepared by the Principal Investigator (study report, manuscript and/or abstract).
- All regulatory documents (e.g., updated curriculum vitae for each Principal Investigator, updated U.S. FDA Form 1572 for each site).

14.6 Informed Consent

The informed consent documents must be signed by the patient, or the patient's legally authorized representative, before his or her participation in the study. The case history for each patient shall document that informed consent was obtained prior to participation in the study. A copy of the informed consent documents must be provided to the patient or the patient's legally authorized representative. If applicable, they will be provided in a certified translation of the local language.

Original signed consent forms must be filed in the site study binder or in each patient's study file.

14.7 Data Collection

Patient medical information obtained by this study is confidential, and disclosure to third parties other than those noted below is prohibited. Upon the patient's permission, medical information may be given to his or her personal physician or other appropriate medical personnel responsible

for his or her welfare.

Data generated by this study must be available for inspection upon request by representatives of the U.S. FDA, local health authorities, and their authorized representative(s), collaborators and licensees, and the site, if IRB.

All study related data will be kept and locked in the Clinical Research Office at Southern Illinois University School of Medicine. Submission of all study assessments, mammography, blood tests, and body composition results along with all case report forms should be submitted to the Clinical Research Office (CRO) to be placed in the patients study chart in a timely manner. The case report form completion guidelines are as follows:

Retention of Records

U.S. FDA regulations (21 CFR §312.62[c]) require that records and documents pertaining to the conduct of this study and the distribution of investigational drug, including CRFs, consent forms, laboratory test results, and medication inventory records, must be retained by the Principal Investigator for two years after marketing application approval. If no application is filed, these records must be kept two years after the study is discontinued and the U.S. FDA and the applicable national and local health authorities are notified.

Confidentiality/ Data Collection

Patients will be assigned a unique patient identification number upon enrollment. Study data and results will be kept confidential. No personal identification will be used in any publications resulting from this study. Results of the baseline and follow-up assessments will be shared with the subjects and they can request any data to be sent to their primary care physician. All of the data will be collated into a database that will be coded so that individual subjects identities will be known only to the investigators.

Analysis of Safety

With respect to the safety goals of this study, toxicities specific to this therapy are not known at this time. Therefore, patients will be monitored for unexpected toxicities and if any serious side effects are observed, an independent monitoring committee will examine the data and evaluate the appropriate course of the study. At the conclusion of the study, all unexpected toxicities will be summarized and reported.

To evaluate a potential effect of LEAG on platelet function, a Platelet Function Assay-100 test will be conducted on the first five patients enrolled. Platelet function will be assessed prior to LEAG administration and prior to surgery on the day of surgery. All adverse events will be

monitored for 30 days beyond the last dose of LEAG, including bleeding complications. Following completion of the study protocol by 5 patients the Data Safety Monitoring Committee will review the adverse events associated with LEAG administration paying particular attention to platelet function. The committee will make a recommendation on continued monitoring of platelet function or its removal from required study procedures for the remainder of patients enrolled. – See section 5.7.4

14.8 Compensation

No benefits will be promised and no rewards for participation will be stated. Subjects will not receive payment for participating in this study. All medical care will be charged in the usual way. LEAG will be provided by the Simmons Cancer Institute at Southern Illinois University School of Medicine. Initial and follow-up specialized blood tests strictly required for the study and study medication will be provided free of cost.

15.0 PARTICIPATING INSTITUTIONS

SOUTHERN ILLINOIS UNIVERSITY SCHOOL OF MEDICINE
MEMORIAL MEDICAL CENTER
ST. JOHN'S HOSPITAL

16.0 STATISTICAL CONSIDERATIONS

Statistical Methods

High variability among tumors in the patient population is anticipated. Each patient will serve as her own control; pre-treatment biopsy tissue will be compared to post-treatment surgical tissue. Descriptive statistics including the mean, median, range, standard deviation, interquartile range, skewness, and kurtosis will be calculated for each measure. Paired t-tests, or Wilcoxon signed-rank tests, depending on the distributional characteristics of the variables, will be used to assess changes in biomarkers from biopsy to surgery. In order to examine the inter-relationships between the anthropometric data, the cytological markers, serum ginsenoside concentrations, and a panel of inflammatory serum proteins, data visualization techniques will be employed. Correlation coefficients will be used to summarize linear relationships between continuous measures. Regression techniques will be used to model nonlinear relationships. All statistical procedures will be performed in consultation with the Division of Statistics and Research Consulting at SIU School of Medicine.

16.1 Sample Size/Accrual Rate

This study is expected to accrue 25 to 50 patients based on the time frame of treatment (5-14 days). Accrual will take approximately 3 years.

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APPENDIX A

Tecumseh Occupational Physical Activity Questionnaire

APPENDIX B

Minnesota Leisure-Time Physical Activity Questionnaire

APPENDIX C

Dietary History Questionnaire

APPENDIX D

Gail Score Calculation

APPENDIX E

Certificate of Analysis

APPENDIX F

Performance Status Criteria

ECOG Performance Status Scale		Karnofsky Performance Scale	
Grade	Descriptions	Percent	Description
0	Normal activity. Fully active, able to carry on all pre-disease performance without restriction.	100	Normal, no complaints, no evidence of disease.
		90	Able to carry on normal activity; minor signs or symptoms of disease.
1	Symptoms, but ambulatory. Restricted in physically strenuous activity, but ambulatory and able to carry out work of a light or sedentary nature (e.g., light housework, office work).	80	Normal activity with effort; some signs or symptoms of disease.
		70	Cares for self, unable to carry on normal activity or to do active work.
2	In bed <50% of the time. Ambulatory and capable of all self-care, but unable to carry out any work activities. Up and about more than 50% of waking hours.	60	Requires occasional assistance, but is able to care for most of his/her needs.
		50	Requires considerable assistance and frequent medical care.
3	In bed >50% of the time. Capable of only limited self-care, confined to bed or chair more than 50% of waking hours.	40	Disabled, requires special care and assistance.
		30	Severely disabled, hospitalization indicated. Death not imminent.
4	100% bedridden. Completely disabled. Cannot carry on any self-care. Totally confined to bed or chair.	20	Very sick, hospitalization indicated. Death not imminent.
		10	Moribund, fatal processes progressing rapidly.
5	Dead.	0	Dead.

APPENDIX G

Clinical Pharmacology Customized Monograph

APPENDIX H

Drug Accountability Log

APPENDIX I

Patient Diaries