Pharmacokinetics and hepatic safety of EGCG

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1 Acronyms

American College of Obstetricians and Gynecologists	ACOG	Institutional Review Board	IRB
Anti-Mullerian Hormone	AMH	Intramuscular	IM
Aromatase Inhibitors	AI	Intrauterine Insemination	IUI
Assisted Reproductive Technologies	ART	Investigational New Drug	IND
Catechol-O-methyl transferase	COMT	Luteinizing Hormone	LH
Centers for Disease Control	CDC	Methylenetetrahydrofolate reductase	MTHFR
Clinical Report Form	CRF	National Institute of Child Health and Human	NICHD
Clomiphene Citrate	CC	Office for Human Research Protections	OHRP
Data Coordination Center	DCC	Ovarian Hyperstimulation Syndrome	OHSS
Data and Safety and Monitoring	DSMB	Ovarian Stimulation	OS
Epigallocatechin gallate	EGCG	Principal Investigator	PI
Estradiol	E2	Progesterone	P4
Estrogen	E	Protected Heath Information	PHI
Estrogen Receptor	ER	Quality of Life	QOL
Follicle Stimulating Hormone	FSH	Serious Adverse Event	SAE
Health Insurance Portability and Accountability Act	HIPAA	Thyroid-Stimulating Hormone	TSH
Health Related Quality of Life	HRQL	Total and Free Testosterone	T, FT
Human Chorionic Gonadotropin	hCG	Ultrasound	U/S
Human Investigation Committee	HIC	World Health Organization	WHO
Identification	ID		

2 Study Synopsis

2.1 Objectives

The objective of this study is to conduct a randomized clinical trial to determine the pharmacokinetics and hepatic safety of epigallocatechin gallate (EGCG).

MTHFR represents the most studied enzyme in the folate pathway with strong evidence that polymorphisms decrease enzyme activity. Because of the potential effect of EGCG on folate metabolism possibly leading to folate depletion, MTHFR represents a clinically relevant target. We are planning to stratify subjects by wild type MTHFR, MTHFR (C677T), and MTHFR (A1298C). The prevalence of MTHFR mutations can range from 30 to 40% in the American population for C677T and ~50% for A1298C. We will evaluate if green tea catechins may have modulating properties that may increase the risk of folic acid depletion in women expressing known MTHFR polymorphisms. In this protocol we will evaluate if polymorphisms related to folate metabolism increase the risk for folate depletion in women receiving green tea extract.

2.2 Patient Population

The population will consist of 36 women ages ≥18 to ≤40 years (at time of consent). Eighteen women will have intramural and/or subserosal fibroids and 18 women will be without intramural fibroids and/or subserosal fibroids. Participants will be recruited over approximately a 6-month period from the FRIEND Collaborative clinical sites (Johns Hopkins University, University of Illinois at Chicago, University of Chicago and Yale University).

2.3 Study Design

Randomization will be stratified by age (age 18-29, 30-40) and presence of fibroids. The randomization scheme will be coordinated through the data coordination center (DCC).

2.4 Treatment

The 36 women will be randomized to one of the following groups:
1. EGCG daily alone.
2. EGCG daily with clomiphene citrate 100mg for 5 days.
3. EGCG daily with letrozole 5mg for 5 days.

The purpose of studying EGCG in combination with clomiphene citrate or letrozole is to assess the safety of using them together prior to the initiation of the FRIEND study. It is expected that EGCG will decrease fibroid size and decrease humoral activity leading to improved quality of endometrium for implantation. Clomiphene citrate and letrozole are being used for ovulation stimulation as treatment for unexplained infertility. In view of the possibility of interaction and change in toxicity when both are used together for FRIEND study, it was suggested by our pharmacology reviewer to include EGCG with the addition of clomiphene citrate and letrozole for the safety study.

The initial screening visit will include EGCG levels, liver function, folate and estrogen levels. A genetics sample collection, urine pregnancy test and pelvic ultrasound will also be performed after informed consent. Patient will be given the EGCG (Green Tea extract) to start after the screening visit. Patients will be counseled on dietary restrictions while on EGCG.

Visit 1 will occur on menstrual cycle day 3 ± 2 days. EGCG levels over 8 hours, liver function, and urine pregnancy testing will occur at this visit. Patients will be assigned into their groups and provided with their respective treatments at this visit.

Patient will return between days 8-10 of the cycle for Visit 2. EGCG levels over 8 hours, liver function, estrogen, and urine pregnancy testing will occur at this visit.

The patient will return for Visit 3 between days 14-16 for a pelvic ultrasound evaluation of endometrial thickness and ovarian characteristics.

The final study visit (Visit 4) will be approximately 4 weeks after initiation of the study treatment and will involve EGCG levels, liver function, folate, and urine pregnancy test and pill counts.

The 4 lab panels (screening, Visits 1, 2, and 4) will include EGCG and metabolite analyses, liver panel (AST, ALT, total bilirubin, ALP) and estrogen levels, and a urine pregnancy test. The EGCG analyses at visits 1 and 2 will include testing over 8 hours.

2.5 Primary Outcome Parameter

The primary outcome parameter will be differences in pharmacokinetic parameters of EGCG and the major metabolites between the 3 groups.

2.6 Secondary Outcome Parameters

The secondary outcome parameters will include:

- Changes in liver function between the 3 groups.
- Changes in estrogen levels (E2) between the 3 groups.
- Changes in endometrial thickness and ovarian characteristics between the 3 groups.
- Change in the pharmacokinetic parameters of EGCG and metabolites within each group.
- Difference in serum folate levels among wildtype MTHFR, MTHFR (C677T), and MTHFR (A1298C) patients.

2.7 Statistical Analysis

Since the number of patients for this study is minimal and multiple factors are of concern, descriptive statistics will be used, and the study is expected to be under powered to perform any formal statistical inference. The number, mean, standard deviation, median, interquartile range of the level of the pharmacokinetic parameters of EGCG and 2 of the major metabolites at different time points and the difference from screening to Visit 1, Visit 2 or Visit 4, and the changes in liver function, estrogen levels, endometrial thickness, and follicular measurements will be presented for the three treatment groups, and then stratified by age group and/or the presence or absence of uterine fibroid. For comparison, a two-way ANOVA will be used to compare the effects of the three treatments and the presence of fibroids on the above outcome parameters. With a sample size of 36 patients for the three treatment groups, which includes 18 subjects with uterine fibroids and 18 without uterine fibroids, with an alpha of 0.05, the power of the study would be 0.44 and 0.85 for a medium (0.25) or large (0.4) effect size, respectively. There are insufficient preliminary data in the literature to support or not support the reasonableness of these effect sizes. For the comparison of serum folate levels among wildtype MTHFR, MTHFR (C677T), and MTHFR (A1298C) patients, ANOVA (unadjusted) and multivariate linear regression will be used.

2.8 Anticipated Time of Completion

We estimate the study will take approximately 6-12 months to complete. Participants will be recruited from each of the 4 participating FRIEND sites.

2.9 Regulatory Compliance

The DCC is working with The Eunice Kennedy Shriver National Institute of Child Health and Human Development (NICHD), the three participating FRIEND institutions, and the single Institutional Review Board (Johns Hopkins University) to ensure that clinical study data and regulatory requirements are met regarding the Food and Drug Administration (FDA) code for federal regulations. This trial is registered on http://www.clinicaltrials.gov (NCT04177693).

3 Background and Significance

3.1 Overview

Uterine leiomyomas (fibroids) affect 30-50% of reproductive-age women and are a significant cause of infertility. Intramural and submucosal fibroids reduce the likelihood of pregnancy (RR=0.3-0.7) compared to unaffected women. Surgical removal of fibroids can restore fertility; however, recurrence rates are high, and benefits are often temporary. Importantly, serious postoperative consequences such as adhesions can adversely affect a woman's fertility and health in general. Hormonal therapies that induce medical menopause can be used to reduce fibroid size, but these therapies also prevent pregnancy. There is a critical need for effective, non-hormonal, non-surgical fertility treatment options for women with fibroids that may distort the uterine cavity. Our long-term goal is to develop novel non-hormonal treatments for uterine fibroids. Green Tea leaves contain polyphenols and catechins, such as epigallocatechin gallate (EGCG). Studies from our group and others have shown that EGCG is the most abundant and active compound responsible for effects of Green Tea extracts. Notably, EGCG can be taken during pregnancy and there are no known teratogenic effects. EGCG polyphenols inhibit key pathways of tumor growth by modulating signaling pathways involved in cell proliferation, transformation, inflammation, apoptosis, metastasis and invasion. Prior to initiating a randomized, double-blind, multi-center, prospective, clinical trial of EGCG versus placebo in women with uterine fibroids to evaluate birth rate a pharmacokinetic and hepatic safety analysis must be conducted. The pharmacokinetics and hepatic safety of EGCG with clomiphene citrate and letrozole are unknown. A 3-arm study will be conducted in women with and without fibroids to allow comparisons between these groups. The results of this study will be used to confirm hepatic safety for the larger multi-center FRIEND study.

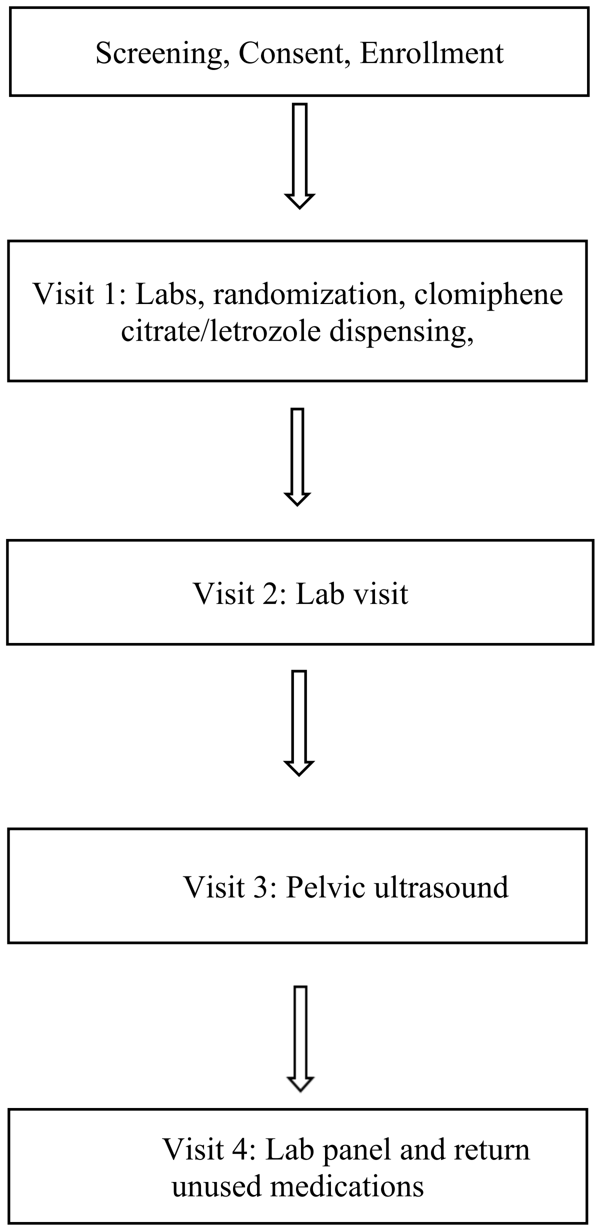
3.2 EGCG (Green Tea extract) and Uterine fibroids

Green Tea leaves contain polyphenols such as catechins or flavin-3-ols that include epicatechin (EC), epigallocatechin (EGC), epigallocatechin gallate (EGCG), and alkaloids. Catechins are the major components of tea phenols and constitute about 30-42% of the dry weight of Green Tea. Studies have shown that the EGCG catechin is the most abundant and active compound responsible for most of Green Tea's role in promoting good health and accounts for the favorable research result cited in the medical literature with the use of Green Tea extracts. EGCG have the highest level of polyphenols and makes it the major anti-oxidant agent in Green Tea. A study conducted by the United States Department of Agriculture reported that Green Tea has potent anti-neoplastic effects against a wide range of human tumor cells (4) EGCG polyphenol have been shown to inhibit key pathways of tumor growth. EGCG appears to block each stage of tumorigenesis by modulating signaling pathways involved in cell proliferation, transformation, inflammation, apoptosis, oxidative stress and invasion (4). Studies by Al-Hendy et al demonstrated increasing levels of Catechol-O-methyl transferase (COMT) in uterine leiomyoma compared to adjacent myometrium and described its important role in fibroid pathogenesis (5) Beside various useful antioxidant and anti-inflammatory effects, EGCG exerts a potent COMT inhibitor effect which also contribute to effective anti-fibroid action (2, 5). Additionally, published work by Dr. Al-Hendy also demonstrated the utility of EGCG for inhibiting fibroid tumor formation in vivo in nude mouse model (3). Dr. Al-Hendy's research team (a member of this consortium) has reported that EGCG acting as anti-uterine fibroid agent through the modulation of multiple signal transduction pathways. The group demonstrated that EGCG works on the gene level, to inhibit the proliferation of Human fibroid cells and induces apoptosis (2). This study also showed an impressive 14-fold increase in the expression of the BMP2 gene in EGCG-treated human fibroid cells than in the untreated control (2). That major increase in the secretion of BMP2 from fibroids by EGCG treatment is likely be able to overcome the fibroid-induced endometrial BMP-resistance through increased binding to the BMP receptors which will lead to improvement in the decidualization of the endometrium and subsequently enhancement of implantation, fertility and pregnancy outcomes. Furthermore, studies also demonstrated that EGCG significantly decreased TGF-β3 production by human fibroid cells. As TGF-β3 is the main cytokine responsible for endometrial BMP-resistance, such effect of EGCG would be expected to lead to improved endometrial receptivity.

3.3 EGCG Clinical trial in women with symptomatic uterine fibroids

To translate the positive EGCG anti-fibroid preclinical findings in human fibroid cell lines and fibroid animal models, the efficacy and safety of EGCG on uterine fibroids burden and quality of life in women with symptomatic uterine fibroids were evaluated in an NIH-funded double-blinded, placebo-controlled randomized clinical trial(NCT 01311869) (1). A total of 39 reproductive-age women (age 18–50 years, day 3 serum follicle-stimulating hormone <12 IU/L) with symptomatic uterine fibroids were recruited for this study. All subjects had at least one fibroid lesion 2 cm3 or larger in volume, as confirmed by transvaginal or transabdominal ultrasonography. The subjects were randomized to oral daily treatment with either 800 mg of decaffeinated EGCG or placebo (800 mg of brown rice) for 4 months, and uterine fibroid volumes were measured at the end of study, also by transvaginal or transabdominal ultrasonography. The fibroid-specific symptom severity and HRQL of these uterine fibroid patients were scored at each monthly visit, using the symptom severity and quality-of-life questionnaires (1). Of the final 39 women recruited for the study, 33 completed all five visits of the study. In the placebo group (n = 11), fibroid volume increased (24.3%) over the study period; however, patients randomized to green tea extract treatment (n = 22) showed significant reduction (32.6%, P = 0.0001) in total uterine fibroids volume. In addition, EGCG treatment significantly reduced fibroid-specific symptom severity (32.4%, P = 0.0001) and induced significant improvement in HRQL (18.53%, P = 0.01) compared to the placebo group. Anemia also significantly improved by 0.7 g/dL (P = 0.02) in the EGCG treatment group, while average blood loss significantly decreased (P = 0.001). No adverse effects, endometrial hyperplasia, or other endometrial pathology were observed in either group (1).

Figure 1. Flowchart



4 Objectives

4.1 Primary Aim

Our goal is to assess the pharmacokinetics and hepatic safety of EGCG in women with and without uterine fibroids.

4.2 Secondary Aim

Not applicable.

4.3 Treatment Design, Study Population, and Study Summary

4.3.1 Treatment Design

This will be a pharmacokinetic trial involving 36 healthy adult female subjects. There will be 18 subjects with uterine fibroids and 18 without uterine fibroids. The randomization scheme will be coordinated through the DCC and the randomization will be stratified for age groups 18-29 and 30-40 and presence of fibroids. The population will consist of 36 women with or without uterine fibroids, age ≥18 to ≤40 years (at time of consent).

The appropriate study candidates will be recruited from the clinics of the 4 FRIEND Collaborative sites after obtaining informed written consent from the female subjects. Recruited participants will meet the inclusion and exclusion criteria detailed below. Monitoring of this trial at all sites will be conducted by the Yale DCC.

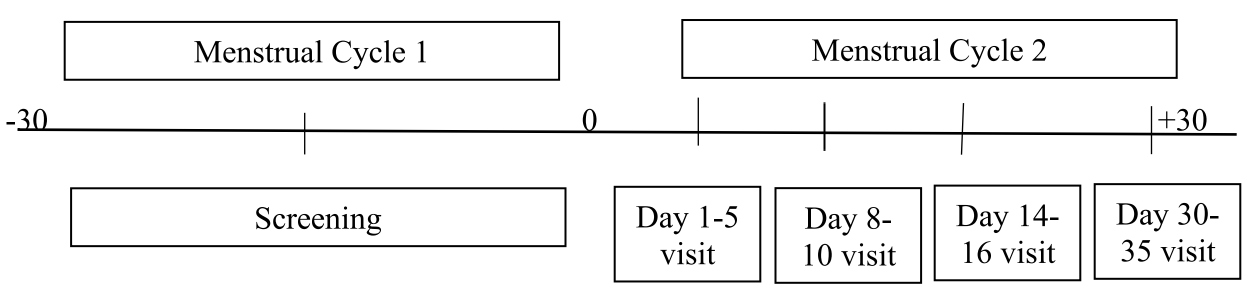
4.3.2 Study Population

Thirty-six (36) patients will be equally randomized via computer-generated randomization schedule to receive:

1. Two capsules of decaffeinated Green Tea extract (close to 800mg EGCG) taken orally on a daily basis for up to 2 months
2. Two capsules of decaffeinated Green Tea extract taken orally on a daily basis for up to 2 months with clomiphene citrate 100mg for 5 days
3. Two capsules) of decaffeinated Green Tea extract taken orally on a daily basis for up to 2 months with letrozole 5mg for 5 days.

4.3.3 Study Summary

Recruitment and Prescreening: see Section 5 descriptions following.



Screening visit (anytime in cycle 1):

1. Obtain informed, signed consent.

2. Complete medical/gynecological/menstrual history and physical exam of female study participant.

   - Vital signs, height, weight, hip and abdominal circumference, BMI
   - Pap smear if necessary per current ACOG time-frame guidelines
   - Standard pelvic and breast exam conducted by physician or within past 12 months

3. Perform radiological exams.

   - Pelvic ultrasound measuring uterine and ovarian characteristics or within past 3 months

4. Laboratory tests: EGCG and metabolites, liver function, folate, estrogen levels, genetics sample collection and urine pregnancy test.

5. Dispense Green Tea extract and educate on dietary restrictions while taking Green Tea extract and with the importance of taking food. All patients to start Green Tea extract at this visit.

6. Contraception education. Patients will be counseled to use non-hormonal double-barrier method: a male/female condom plus spermicidal agents, cervical cap, diaphragm or sponge.

7. Concomitant medication collection.

Visit 1 (Day 1-5):
Patient will be seen on menstrual cycle day 3 ± 2 days for clomiphene citrate/letrozole initiation visit

1. Vital signs and weight.
2. Laboratory tests: EGCG levels over 8 hours (0.5,1,2,4,6,8),liver function, folate, estrogen level, and urine pregnancy testing.
3. Concomitant medication collection and pill counts of EGCG.
4. Randomization and dispensation of clomiphene citrate/letrozole.
5. Contraception education. Patients will be counseled to use non-hormonal double-barrier method: a male/female condom plus spermicidal agents, cervical cap, diaphragm or sponge.

Visit 2 (day 8-10):
Patient will return several days after final clomiphene citrate/letrozole administration

1. Vital signs and weight.
2. Laboratory tests: EGCG levels over 8 hours (0.5,1,2,4,6,8), liver function, folate, estrogen level and urine pregnancy testing.

3. Concomitant medication collection and pill counts of EGCG and clomiphene citrate or letrozole as appropriate.
4. Contraception education. Patients will be counseled to use non-hormonal double-barrier method: a male/female condom plus spermicidal agents, cervical cap, diaphragm or sponge.

Visit 3 (day 14-16):

1. Pelvic ultrasound via a transvaginal probe or transabdominal transducer evaluation of endometrial thickness and ovarian characteristics.

Visit 4 (day 30-35):
Patient will return for final study visit.

1. Vital signs and weight, hip and abdominal circumference, BMI
2. Laboratory tests: EGCG and metabolites, liver function, estrogen level, folate, and urine pregnancy test.
3. Concomitant medication collection. Return unused medications and pill counts of EGCG.

Future Studies: One tube of blood will be collected at each visit to be stored for future analyses related to this study.

4.3.4 Inclusion Criteria

1. Healthy women ≥18 to ≤40 years of age with or without uterine fibroids
2. Must use a double-barrier method for contraception

4.3.5 Exclusion Criteria

1. Subjects using Green Tea/EGCG within 2 weeks prior to study enrollment. Matcha (Japanese green tea), maca powder, green tea beverages and all other forms of green tea require a 2-week wash-out. Patients with a detectable EGCG level at the screening visit will be excluded.
2. Known liver disease (defined as AST or ALT>2 times normal, or total bilirubin >2.5 mg/dL).
3. History of alcohol abuse (defined as >14 drinks/week) or binge drinking of ≥ 6 drinks at one time).
4. Subject using hormonal contraceptives: 3-month wash-out is required for all oral or cyclic progestins, GnRH agonists/ antagonists, gonadotropins, injectable contraceptives, oral contraceptives, contraceptive implants and previous use of clomiphene and letrozole.
5. Subjects who are pregnant or breastfeeding
6. Known hypersensitivity to the study drugs
7. Any chronic disease

4.3.6 Study Termination Criteria

Any serious adverse event considered to be related to study treatment (see section 7.3.2.1 and 7.3.5) will be considered reason for termination of assigned intervention; participant will be followed up for cycle outcome and will contribute research data towards intent-to-treat analyses. This includes allergic reactions and liver toxicity. Women with elevation in liver function test with 3 times the upper limit of normal will be terminated from the study and will return to site for repeat testing every 48 hours until resolution.

All reports of liver toxicity will be reviewed by the DSMB (see section 7.3.4). If three (3) or more trial participants developed active liver disease, as defined below, this would be an unexpected significant risk requiring trial termination:

1. Alanine aminotransferase (ALT) or Aspartate aminotransferase (AST) ≥ 8 x upper limit of normal (ULN)
2. ALT or AST ≥ 3 x ULN and total bilirubin (TBL) > 2 x ULN or International Normalized Ratio (INR) > 1.5 or
3. ALT or AST > 3 x ULN with appearance of fatigue, nausea, vomiting, right upper quadrant pain or tenderness, fever, rash, and/or eosinophilia (> 5%).

4.4 Risk/Benefit Assessment

4.4.1 Possible Benefits

No direct benefits to participants.

4.4.2 Possible risks/discomforts

Blood draws: Discomfort, bruising, infection or bleeding at the needle puncture site

Pelvic ultrasound: abdominal or pelvic discomfort

EGCG: Gastrointestinal upset; rare liver toxicity. The literature supports a potential relationship between high-dose Green Tea extract consumption and transient changes in serum liver enzymes. Rare (<5%) and transient liver transaminase elevations have been documented. Hepatotoxicity tended to show a temporal relationship between Green Tea extract consumption and effect onset with Green Tea extract-induced toxicity mainly manifesting after roughly 3-4 months of consumption. Daily intake of 1315 mg of Green Tea catechins containing 843 mg EGCG poses mainly mild, transient hepatic adverse effects. The incidence and severity of hepatotoxicity increased when Green Tea extract or EGCG was administered under fasted conditions, while exposure to Green Tea under fed conditions appears to alleviate such risk.

Clomiphene Citrate: Visual changes (such as blurring of vision, double vision, floaters), abdominal pain, nausea, vomiting, constipation, mood changes, headache, hot flashes, fatigue, abnormal endometrial thickening, multiple pregnancies, formation of ovarian cyst, breast discomfort, abnormal uterine bleeding, and bloating

Letrozole: Fatigue, dizziness, nausea, hot flashes, arthritis pain in your joints, back pain, increased cholesterol levels, formation of ovarian cysts, multiple pregnancies

Other risks: There is also the risk of loss of confidentiality. Filling out the questionnaires may make the subjects uncomfortable or feel some anxiety. There is a risk that information about the subject may become known to people outside of this study.

As this is a NIH (NICHD) funded study, your study information is protected by a Certificate of Confidentiality. This Certificate allows us, in some cases, to refuse to give out your information even if requested using legal means.

It does not protect information that we have to report by law, such as child abuse or some infectious diseases. The Certificate does not prevent us from disclosing your information if we learn of possible harm to yourself or others, or if you need medical help.

Disclosures that you consent to in this document are not protected. This includes putting research data in the medical record or sharing research data for this study or future research. Disclosures that you make yourself are also not protected.

5 Study Design

5.1 Type of Design

This will be a randomized, open-label, multi-center, pharmacokinetics and hepatic safety trial of EGCG with 36 total patients in 3 treatment arms. The randomization scheme will be coordinated through the DCC and the randomization will be stratified for age groups 18-29 and 30-40 and presence of uterine fibroids.

5.2 Rationale for Design

The pharmacokinetics and hepatic safety of EGCG with clomiphene citrate and letrozole are unknown. A 3-arm study will be conducted in women with and without fibroids to allow comparisons between these groups. The results of this study will be used to confirm hepatic safety for the larger multi-center FRIEND study. The pharmacokinetics and hepatic safety of EGCG with clomiphene citrate and letrozole are unknown. A 3-arm study will be conducted in women with and without fibroids to allow comparisons between these groups. The results of this study will be used to confirm hepatic safety for the larger multi-center FRIEND study.

5.3 Recruitment

Hospital/Local Health Care Referrals
Subjects will be recruited at each site from individual practice(s) as well as faculty/resident clinics.

Local Advertisements
Advertisements will be placed in local newspapers and will be continued on a regular basis if response is good.

IRB Approval
It is expressly acknowledged that all informational material that could be construed to be advertising will be approved by the appropriate IRB prior to dissemination.

5.4 Informed Consent

Once subjects have been identified, they will be referred to the site clinical coordinator or his/her designee. The clinical protocol will be explained to potential subjects by the physician investigator or the coordinator depending on the clinical circumstances. Inclusion and exclusion criteria will be reviewed. After the study has been completely explained, they will be given the informed consent documents to review. Some individuals may wish to complete the informed consent process at the time of this discussion. In these cases, the informed consent documents will be signed once all questions are resolved. In other cases, the subjects may wish to take the consent forms home for further consideration. In these cases, the coordinator will set up a tentative timeframe to be back in touch with the subjects. The consents can be signed either with the coordinator or with a physician once all questions have been answered to the satisfaction of the potential patient. A signed informed consent document, approved by the IRB at the study site, will be confirmed on all subjects prior to the baseline evaluation. In order to be eligible for enrollment and randomization, all inclusion and exclusion criteria must be met.

5.5 Randomization/Treatment Initiation

Thirty-six (36) patients will be equally randomized via computer-generated randomization schedule to receive: 1) 800 mg of decaffeinated EGCG taken orally on a daily basis with food for up to 2 months; 2) 800 mg of decaffeinated EGCG taken orally on a daily basis with food for up to 2 months with clomiphene citrate 100mg for 5 days; 3) 800 mg of decaffeinated EGCG taken orally on a daily basis with food for up to 2 months with letrozole 5mg for 5 days.

Treatment assignments will be randomized for age groups and presence of fibroids by a varying-block-size design. The DCC statisticians will generate the randomization scheme for the study.

5.6 Physical Exam

A physical exam with standard pelvic and breast exam will be performed on all patients by a study physician. Height, weight and waist and hip circumferences will be recorded to the nearest 0.1 cm, 0.1 kg and 1 cm, respectively. Waist will be measured at the level of the umbilicus and hip circumference will be measured at the widest diameter. Participants will be weighed while dressed in light clothing, without shoes. Weight will be collected at each cycle initiation visit; however, height, waist and hip circumferences are only collected at the screening and end of study drug visits. Blood pressure will be determined in the right arm in the sitting position. Large cuff will be used as necessary. Blood pressure will be assessed at each visit. Elevated blood pressures (≥160/100) will be repeated following acclimation to the study environment. All patients aged 21 and older should have had a normal Pap smear in accordance with current ACOG guidelines. If not, one should be performed at the screening exam. Patients with cytological abnormalities will need to have these resolved prior to study entry.

5.7 Transvaginal Ultrasound Exam

An ultrasound exam will be performed with a transvaginal probe. For subjects who cannot tolerate a transvaginal probe, a transabdominal ultrasound evaluation will be performed. The following measures will be obtained at screening: uterine dimensions, leiomyoma presence and size, other uterine abnormalities, endometrial thickness, ovarian size in three dimensions, the size of the largest ovarian follicle, follicle count in the largest plane and ovarian morphology. Ovarian size is determined by measuring the largest plane of the ovary in two dimensions and then turning the vaginal probe 90 degrees and obtaining a third measurement. Endometrial thickness is the largest anterior-posterior measurement of the endometrium in the sagittal plane. Ovarian volume is determined by the formula for a prolate ellipsoid (length x width x height x $\pi/6$)(Pache, Hop et al. 1991). If the patient has had no prior test of tubal patency, this may be the desired time to perform a sonohysterogram to determine tubal patency. Only endometrial thickness and ovarian follicular measurements will be measured at visit 2.

5.8 Laboratory Exam

Lab tests are found in Table 1 below. Both local and central labs will be utilized. The EGCG and metabolite analyses will be conducted in the lab of Dr. Jeremy Johnson at the University of Illinois. Dr. Jeremy Johnson is the pharmacokinetics core leader for UICentre, the academic drug discovery initiative at the University of Illinois at Chicago.

Table 1. Screening labs of study women

LOCAL LABORATORY BLOOD TESTS	CENTRAL LABORATORY BLOOD TESTS

Hormones	Liver	EGCG and metabolites
Estrogen	Total Bilirubin	EGCG
	ALT/AST	EGC
	Alkaline Phosphatase	ECG
		4′-*O*-methyl-epigallocatechin
		Genetic polymorphisms related to folate metabolism

5.9 Schedule of Evaluations

Study procedure	Cycle 1	Cycle 2			
	Screening	Visit 1 (Day 1 -5)	Visit 2 (Day 8 -10)	Visit 3 (Day 14-16)	Visit 4 (Day 30 - 35)
Informed consent	X				
Inclusion/exclusion criteria	X				
Demographic information	X				
Medical history	X				
Concomitant medications	X	X	X		X
Gynecological history	X				
Menstrual history	X				
Vital signs and weight	X	X	X		X
Height	X				
Hip and abdominal circumference	X				X
Physical examination	X				
Pap smear	X*				
Pelvic and breast exam	X**				
Genetics sample, laboratory test	X				
Pelvic ultrasound	X‡			X	
EGCG levels and metabolites, laboratory test	X	X	X		X
Liver function, laboratory test	X	X	X		X
Folate, laboratory test	X				X
Estrogen levels, laboratory test	X	X	X		X
Contraceptive counseling	X	X	X		
Dispense EGCG	X				
Pill counts of EGCG		X	X		X
Randomization and dispensation of clomiphene citrate or letrozole		X			

Pill counts of clomiphene citrate and letrozole			X		
Urine pregnancy test	X	X	X		X

*If necessary per ACOG guidelines
**if not done within past 12 months
‡ if not done within past 3 months

6 Data Analysis

6.1 Data Analysis

Data will be collected prospectively by designated research personnel at each study site, supervised by the site PI. Individual data will be entered into a web-based data management system created by the DCC, using only a study ID number.

6.2 Primary Outcome Measurements

The primary outcome parameter will be differences in pharmacokinetic parameters of EGCG and 2 of the major metabolites between the 3 treatment groups.

6.3 Secondary Outcome Measurements

The secondary outcome parameters will include:

- Changes in liver function between the 3 groups.
- Changes in estrogen levels (E2) between the 3 groups.
- Changes in endometrial thickness and ovarian characteristics between the 3 groups.
- Change in the pharmacokinetic parameters of EGCG and metabolites within each group.

6.4 Difference in serum folate levels among wildtype MTHFR, MTHFR (C677T), and MTHFR (A1298C) patients. Statistical Analyses

Since the number of patients for this study is minimal and multiple factors are of concern, descriptive statistics will be used, and the study is expected to be under powered to perform any formal statistical inference. The number, mean, standard deviation, median, interquartile range of the level of the pharmacokinetic parameters of EGCG and 2 of the major metabolites at different time points and the difference from screening to Visit 1 or Visit 2 or Visit 4, and the changes in liver function, hormone levels, endometrial thickness and follicular measurements will be presented for the three treatment groups, and then stratified by age group and/or the presence or absence of uterine fibroid. Patients with a detectable EGCG level at the screening visit will be excluded. For comparison, a two-way ANOVA will be used to compare the effects of the three treatments and the presence of fibroids on the above outcome parameters. With a sample size of 36 patients for the three treatment groups, and 18 subjects with uterine fibroids and 18 without uterine fibroids, with an alpha of 0.05, the power of the study would be 0.44 and 0.85 for a medium (0.25) or large (0.4) effect size, respectively. There are insufficient preliminary data in the literature to support or not support the reasonableness of these effect sizes. For the comparison of serum folate levels among wildtype MTHFR, MTHFR (C677T), and MTHFR (A1298C) patients, ANOVA (unadjusted) and multivariate linear regression will be used.

7 Technical Aspects

7.1 Study Agent Preparation

We propose to purchase EGCG from Beehive Botanicals. Beehive Botanicals, Inc. has the capacity to formulate and manufacture vegetable capsules containing 200mg of Green Tea extract. Beehive Botanicals is NSF certified as a cGMP facility. Per Beehive SOPs, all incoming material is third party tested for identity and potency to confirm compliance with Certificates of Authentication (COA). The material per the COA has a shelf life of 3 years in correct holding conditions. Clomiphene citrate and letrozole will be obtained through generic pharmaceutical companies.

The study coordinator at each site will be responsible for distributing the supply drug kits, verifying pill/vial counts, and determining that there are adequate medication supplies for existing patients and new patients to be randomized. The PI will ultimately be accountable for administration and accountability of the medication used in this study.

7.2 Concomitant Medications

An inquiry into concomitant medications will be made at the screening visit as well as each subsequent visit.

7.3 Adverse Experience Reporting

7.3.1 Adverse Events

7.3.1.1 Definition of Adverse Events (AE)

Adverse event means any untoward medical occurrence associated with the use of an intervention in humans, whether or not considered intervention-related (21 CFR 312.32 (a)).

7.3.1.2 Definition of Serious Adverse Events (SAE)

An adverse event (AE) or suspected adverse reaction is considered "serious" if, in the view of either the investigator or sponsor, it results in any of the following outcomes: death, a life-threatening adverse event, inpatient hospitalization or prolongation of existing hospitalization, a persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions, or a congenital anomaly/birth defect. Important medical events that may not result in death, be life-threatening, or require hospitalization may be considered serious when, based upon appropriate medical judgment, they may jeopardize the participant and may require medical or surgical intervention to prevent one of the outcomes listed in this definition.

7.3.2 Classification of an Adverse Event

7.3.2.1 Severity of Event

The following guidelines will be used to describe severity.

- **Mild** – Events require minimal or no treatment and do not interfere with the participant's daily activities.

- **Moderate** – Events result in a low level of inconvenience or concern with the therapeutic measures. Moderate events may cause some interference with functioning.
- **Severe** – Events interrupt a participant's usual daily activity and may require systemic drug therapy or other treatment. Severe events are usually potentially life-threatening or incapacitating. Of note, the term "severe" does not necessarily equate to "serious".

7.3.2.2 Relationship to Study Intervention

All adverse events (AEs) must have their relationship to study intervention assessed by the clinician who examines and evaluates the participant based on temporal relationship and his/her clinical judgment. The degree of certainty about causality will be graded using the categories below. In a clinical trial, the study product must always be suspect.

- **Definitely Related** – There is clear evidence to suggest a causal relationship, and other possible contributing factors can be ruled out. The clinical event, including an abnormal laboratory test result, occurs in a plausible time relationship to study intervention administration and cannot be explained by concurrent disease or other drugs or chemicals. The response to withdrawal of the study intervention (dechallenge) should be clinically plausible. The event must be pharmacologically or phenomenologically definitive, with use of a satisfactory rechallenge procedure if necessary.
- **Probably Related** – There is evidence to suggest a causal relationship, and the influence of other factors is unlikely. The clinical event, including an abnormal laboratory test result, occurs within a reasonable time after administration of the study intervention, is unlikely to be attributed to concurrent disease or other drugs or chemicals, and follows a clinically reasonable response on withdrawal (dechallenge). Rechallenge information is not required to fulfill this definition.
- **Potentially Related** – There is some evidence to suggest a causal relationship (e.g., the event occurred within a reasonable time after administration of the trial medication). However, other factors may have contributed to the event (e.g., the participant's clinical condition, other concomitant events). Although an AE may rate only as "possibly related" soon after discovery, it can be flagged as requiring more information and later be upgraded to "probably related" or "definitely related", as appropriate.
- **Unlikely to be related** – A clinical event, including an abnormal laboratory test result, whose temporal relationship to study intervention administration makes a causal relationship improbable (e.g., the event did not occur within a reasonable time after administration of the study intervention) and in which other drugs or chemicals or underlying disease provides plausible explanations (e.g., the participant's clinical condition, other concomitant treatments).
- **Not Related** – The AE is completely independent of study intervention administration, and/or evidence exists that the event is definitely related to another etiology. There must be an alternative, definitive etiology documented by the clinician.

7.3.2.3 Expectedness

The study PI will be responsible for determining whether an AE is expected or unexpected. An AE will be considered unexpected if the nature, severity, or frequency of the event is not consistent with the risk information listed in the package insert, or in the consent form.

7.3.3 Time Period and Frequency for Event Assessment and Follow-up

The occurrence of an AE or serious adverse event (SAE) may come to the attention of study personnel during study visits and interviews of a study participant presenting for medical care, or upon review by a study monitor.

All AEs including local and systemic reactions not meeting the criteria for SAEs will be captured on the appropriate case report form (CRF). Information to be collected includes event description, time of onset, clinician's assessment of severity, relationship to study product (assessed only by those with the training and authority to make a diagnosis), and time of resolution/stabilization of the event. All AEs occurring while on study must be documented appropriately regardless of relationship. All AEs will be followed to adequate resolution.

Any medical condition that is present at the time that the participant is screened will be considered as baseline and not reported as an AE. However, if the study participant's condition deteriorates at any time during the study, it will be recorded as an AE.

Changes in the severity of an AE will be documented to allow an assessment of the duration of the event at each level of severity to be performed. AEs characterized as intermittent require documentation of onset and duration of each episode.

Site personnel will record all reportable events with start dates occurring any time after informed consent is obtained until 7 (for non-serious AEs) or 30 days (for SAEs) after the last day of study participation. At each study visit, the investigator will inquire about the occurrence of AE/SAEs since the last visit. Events will be followed for outcome information until resolution or stabilization.

7.3.4 Adverse Event Reporting

Adverse events deemed non-serious will be recorded throughout study participation from the start of study drug through one week after the last dose of study medication and reported to the DCC. Non-serious adverse events will be reported to the DSMB quarterly.

Supervising Institutional Review Boards (IRBs) will be notified by local investigators of adverse events occurring at their institution according to their reporting requirements. Investigators also will notify the medical monitor and DCC within 24 hours after knowledge of a death or of an event that is life-threatening, that results in hospitalization or prolongation of hospitalization, or that involves a persistent or significant disability or incapacity. Study staff will collect information regarding the treatment provided, outcome, and presumed relationship to study, and a narrative description of the event. The study staff will provide updated reports to the DCC as new information becomes available. DCC personnel will review the data and query the clinical sites for clarification and additional information. In addition, the DCC will provide the information to the Data and Safety Monitoring Board (DSMB) as part of their safety review.

The DCC will provide study-wide summary statistics (not filtered by treatment group) of adverse events to the single IRB annually and upon request to all sites on an annual basis for submission to their IRBs.

7.3.5 Serious Adverse Event Reporting

All serious adverse events (SAEs) that occur from randomization through thirty days after the last dose of study medication must be reported or if the patient is pregnant, 6 weeks following delivery.

The site PI will report the SAE by completing and signing the Serious Adverse Event Report Form [available in the "Study Forms" section of the FRIEND website], and then emailing the document in PDF format to dcc.c2s2@mailman.yale.edu. Subjects will be identified by study number only. No other identifying information will be included on the form. The site PI must determine and record on the SAE form whether the SAE is unanticipated or anticipated, and if it is related, possibly related, or unrelated to participation in the research. The site coordinator will enter the information into the DCC database. The DCC will inform the sIRB within 24 hours of after knowledge of a SAE.

The Safety Surveillance team, consisting of the DCC and lead PI of the protocol, will analyze the SAE to determine if it meets the criteria listed in the OHRP 45CFR46 and/or FDA 21CFR312.32 & 3.14.80.

These determinations will dictate timeframes for sites' submission to the DCC, and the DCC's submission to the DSMB (Table 2):

Table 2. Types of Serious Adverse Events and their reporting requirements

TYPE	SITE	DCC
Unanticipated and related/possibly related SAE, fatal or life-threatening	Report to DCC within 1 business day of discovery	Notify DSMB by end of next business day of receiving site report
Other unanticipated and related/possibly related SAE	Report to DCC within 1 business day of discovery	Notify DSMB 5 business days of receiving site report
Anticipated and related/possibly related SAE	Report to DCC within 5 business days of discovery	Notify DSMB 5 business days of receiving site report
Unrelated SAE (anticipated or unanticipated	Report to DCC within 10 business days (no more than 3 weeks) of discovery	Notify DSMB within 10 business days (no more than 3 weeks) of receiving site report

Upon receiving notification of an SAE, the DSMB will review it via a closed-session email or conference-call discussion. The DSMB will send a report to the DCC within two weeks; reports for life-threatening SAEs will be submitted in one week. The DSMB report will include: statement indicating what related information the DSMB reviewed; the review date; the DSMB's assessment of the information reviewed; and the DSMB's recommendation, if any, for the DCC.
The DCC will then record the DSMB report and disburse. The DCC will forward reportable events to all FRIEND investigators and NIHCD. The Protocol PI will evaluate the frequency and severity of the SAEs and determine if modifications to the protocol and consent form are required. Site PIs will report the SAE to their site IRB according to local IRB requirements. For more information, please see the FRIEND/DSMB Communication Procedure.

7.4 Unanticipated Problems

7.4.1 Definition of Unanticipated Problems (UP)

The Office for Human Research Protections (OHRP) considers unanticipated problems involving risks to participants or others to include, in general, any incident, experience, or outcome that meets all of the following criteria:

- Unexpected in terms of nature, severity, or frequency given (a) the research procedures that are described in the protocol-related documents, such as the Institutional Review Board (IRB)-approved research protocol and informed consent document; and (b) the characteristics of the participant population being studied;
- Related or possibly related to participation in the research ("possibly related" means there is a reasonable possibility that the incident, experience, or outcome may have been caused by the procedures involved in the research); and
- Suggests that the research places participants or others at a greater risk of harm (including physical, psychological, economic, or social harm) than was previously known or recognized.

7.4.2 Unanticipated Problem Reporting

The investigator will report unanticipated problems (UPs) to the reviewing Institutional Review Board (IRB) and to the Data Coordinating Center (DCC)/lead principal investigator (PI). The UP report will include the following information:

- Protocol identifying information: protocol title, PI's name, and the IRB project number;
- A detailed description of the event, incident, experience, or outcome;
- An explanation of the basis for determining that the event, incident, experience, or outcome represents an UP;
- A description of any changes to the protocol or other corrective actions that have been taken or are proposed in response to the UP.

The following UPs require prompt reporting:

- Breaches of confidentiality involving risks;
- Data and Safety Monitoring Board (DSMB) reports an interim analysis altering the risk/benefit profile by identification of increased risk;
- Protocol deviations, violations, or other accidental or unintentional changes to the protocol or procedures involving risks or with the potential to recur;
- Unapproved changes made to the research to eliminate an apparent immediate hazard to a subject;
- Other problem or finding (e.g., loss of study data or forms) that an investigator or research staff member believes could influence the safe conduct of the research.

To satisfy the requirement for prompt reporting, UPs will be reported using the following timeline:

- UPs that are serious adverse events (SAEs) or require prompt reporting will be reported to the IRB and to the DCC/study sponsor according to the timeline laid out for other SAEs in Table 2.

Any other UP will be reported to the IRB and to the DCC/study sponsor at time of continuing review of the investigator becoming aware of the problem.

7.5 Data Collection and Management (including quality assurance/compliance measures)

7.5.1 Data Entry and Forms

Case Report Forms (CRFs) will be developed as the protocol is developed. All CRFs will be paper forms. Site coordinators will be responsible for timely CRF completion and data entry of the CRFs into the designated electronic data entry (EDC) system. CRF completion should occur at the time of the visit. Lab results sections should be completed within two weeks of results. Each site is asked to ensure that all data is entered into the EDC system within three weeks of the study visit.

7.5.2 Data Security

For security purposes, PHI, including patient names and addresses, will be locked and secured at the participating sites, and data will be linked through a unique identification number, which will be assigned after a patient is screened or enrolled. Access will be limited to authorized individuals (21 CFR 11.10(d)).

7.5.3 Data Quality Control

The site PI will be responsible for ensuring that study related tests are performed by competent personnel. The criteria for determination of competency may vary between sites in the study. Attempts will be made to standardize protocols whenever possible to minimize inter-site variation.

7.6 Study Monitoring

A monitoring plan that satisfies the Guideline for Monitoring of Clinical Investigations of the National Cancer Institute will be used. A Project Manager from the DCC will lead this effort, and report findings to the DCC PI. The Project Manager will have full knowledge of the study protocol, Manuals of Procedures, and is familiar with the database system and is trained to review patient charts. The Project Manager will be responsible for training and supervising other personnel.

Once personnel at participating site are trained to recruit patients, the Project Manager will be sent to the site to help initiate the study according to the study protocol, and to ensure that the clinical site meets the scientific, clinical, and regulatory requirements. For example, the Project Manager will review all signed and dated forms required by the FDA (such as financial disclosure forms), the curriculum vitae and certifications of the investigators and personnel, CRF training, and the written IRB approval of the protocol and consent form.

The on-site monitor will return to the clinical site after a defined number of patients are recruited (can be as early as the recruitment of the 2nd patient) or a certain time period has passed, depending on the duration of the protocol execution. The schedule of visits will be discussed and agreed upon by the Steering Committee and we anticipate that the Project Manager will visit each participating site at least once.

During the site visit, the clinical sites should provide to the monitor a space and access to all relevant records including medical records and regulatory binders, and there would be immediate verbal feedback provided to the site after original source documents are compared to entries in the

CRF. The clinical sites must agree to cooperate with the monitor to ensure that any problems detected in the course of these monitoring visits are resolved. The on-site monitor will conduct an audit of a random sample of entered information against the source documents, a review of all regulatory documents, a review of all informed consents, and a review of all pharmacy logs. The clinical site PI and coordinator should be available to meet the monitor during the visit. The monitor will review electronic data from all sites, providing a method for identifying systematic errors or problems.

To assure Good Clinical/Laboratory Practice, the monitor will control adherence to the protocol at the clinical sites and evaluate the competence of the personnel at the clinical sites including the ability to obtain written informed consents and record data correctly. The monitor will inform the DCC PI, the Steering Committee, and NICHD regarding problems relating to facilities, technical equipment, or medical staff. A thorough written report will follow each site-visit and will include a detailed itemization of discrepancies and items requiring follow-up or reconciliation. This report will also be forwarded to the Steering Committee for review. The monitor will be responsible for maintaining regular contact between the investigators in the clinical sites. When the study ends, the monitor will also visit the clinical site to provide assistance for close-out.

7.7 Data and Safety Monitoring Board

An independent Data and Safety Monitoring Board (DSMB) will review and interpret data generated from the FRIEND sites and to review protocols prior to their implementation. Its primary objectives are to ensure the safety of study subjects, the integrity of the research data and to provide FRIEND with advice on the ethical and safe progression of the study. The DSMB advises on research design issues, data quality and analysis, and research participant protections for each prospective and on-going study. A copy of the DSMB Charter can be found in the appendix.

The DSMB members are appointed by the FRIEND consortium in accordance with established NIH and NICHD policies. DSMB members are experts in and represent the following fields: biostatistics, epidemiology, infertility, gynecology and ethics. The DCC Coordinator is responsible for scheduling regular committee meetings, recording all meeting minutes and summarizing the committee recommendations for the FRIEND consortium. Steering Committee members are prohibited from attending closed sessions of the DSMB. Open sessions may be attended by Steering Committee members or Chairperson when requested by the DSMB.

7.8 Reporting

Administrative Reports will be prepared by the DCC, and they include monthly and quarterly reports to the FRIEND Collaborative group on accrual, data quality and study compliance and reports presented in the packet produced for each Steering Committee and DSMB. Statistical reports include reports to the FRIEND Collaborative group from the data analysis, and special reports for scientific manuscripts.

Statistical Reports will be generated in SAS. Reports are provided for DSMB reviews, and for final analysis of study results in preparation for scientific publications. The content of the interim reports will be very complete and will serve as the template for the final report of each study, which in turn will form the basis of the publication of the results. Our proposed reports to the DSMB would include the following: a protocol description and history; accrual rates; site performance in terms of accrual; eligibility; protocol violations; data accuracy and minority representation; patient characteristics by treatment and site; and the rate of adverse experiences.

7.9 Obligation of the Investigator

One of the requirements for certification of a site to begin participant activities will be IRB approval. Johns Hopkins will act as the IRB of record, and the protocol and informed consent template, including future amendments, will be submitted to the sIRB. Protocol amendments and changes to the consent forms will be distributed from the DCC via numbered memos. Approval documents and consents will be made available to the sites. Reporting local and study-wide adverse events will be done according to the sIRB Maintenance/Retention of site records. In order to comply with Good Clinical Practice (GCP) requirements, the investigators must maintain the master patient log that identifies all patients entered into the study for a period of two years after the study ends so that the subjects can be identified by audit. The PI must maintain adequate records pertaining to subjects' files and other source data for a minimum of 5 years after completion of the study.

7.10 Regulatory Requirements

The DCC will work with NICHD staff by providing them with clinical study data, reports, and other support as required for AE Reporting and IND submissions. The Project Managers will work with NICHD colleagues in meeting all regulatory requirements including compliance with ICH and HIPAA requirements, FDA code for federal regulations (Title 21), and IND applications. For example, the DCC Project Managers will register this clinical trial in a timely manner with ClinicalTrials.gov via a web-based data entry system called the Protocol Registration System (PRS).

7.11 Protocol Amendments

Given the scope of this trial, the protocol is not expected to be revised once the recruitment starts. However, if any changes do occur, Protocol amendments and changes to the consent forms will be distributed from the DCC via numbered memos. Approval documents and consents will be made available to the sites. Reporting local and study-wide adverse events will be done according to the sIRB.

7.12 Overall Policy

The publications policy proposes guidelines for publications that originate from our collaborative FRIEND consortium. Decisions concerning publications shall be determined by consensus (majority vote) of the collaborating principal investigators (or designate) noted below as the "Consortium". This policy is designed to promote prompt, exact, quality publications and presentations of Network studies with appropriate academic recognition of those with significant contributions. Protocols are classified into three types: 'Main Study' (which may include major and minor publications), 'Ancillary Study', and 'Pilot Study'. Additionally, there may be publications from concepts or ideas generated by the FRIEND ("Related Publications") or from other groups utilizing FRIEND data and/or specimens "Outside Studies" (those utilizing data and/or specimens from the FRIEND studies). Abstract submissions to national meetings will also follow the publications policy below. The progress of publications (including presentations) will be a standing agenda note on all phone conferences and meetings. The FRIEND consortium will make the final disposition regarding disputes with respect to analysis request approval, prioritization, presentation, authorship and/or manuscript submission.

7.13 Main Study

A Main Study is a consortium study designed prospectively by an investigator independent of other studies. At the end of each Main Study, a primary analysis resulting in the primary manuscript and a number of secondary analyses is produced based on the research questions stated in the protocol. The Protocol leader is the primary author of the primary analysis. A main study can generate major (related to the major hypotheses) and minor publications (relating to secondary hypotheses).

7.13.1 Major Publications

A major publication is defined as one reporting results of the major hypotheses tested. (For example, does hMG/IUI increase cyclic fecundity in couples with unexplained or male factor infertility?).

1. Authorship: Publications will include the names of investigators from each FRIEND site and the DCC. Each FRIEND and DCC will have up to two authors per publication, ordinarily the PI and the Co-PI, but this may at times involve another investigator who has contributed to the study at their site, in lieu of the PI or Co-PI. The principal investigator at each FRIEND site will be responsible for submitting the names of the two authors from that unit and designating them as either the primary and secondary authors of the unit. No more than 2 authors may represent a FRIEND site. An ancillary site may only have 1 investigator.
2. First Author: The lead investigator initiating the protocol will be the first author. The first author would always be expected to prepare the initial draft of the manuscript. The author will prepare the first draft of the manuscript in a timely fashion after receiving all the relevant data analyses from the DCC. The primary author will circulate the final draft to all authors prior to submission, with a timely turnaround of comments from other authors expected. Final decision of the manuscript content will be determined by the consortium. In the event that the initiating protocol investigator declines first authorship or fails to meet the timeline determined by FRIEND (as determined by majority vote) and monitored monthly, the next FRIEND investigator in the rank order of authors (described below) will be the first author.
3. Authorship Order: All authorships are expected to meet reasonable criteria as set forth by the International Committee of Medical Journal Editors. Uniform requirements for manuscripts submitted to biomedical journals. http://www.icmje.org. Updated February 2006. Accessed April 4, 2007. The overall authorship order will be 1) the primary author, 2) FRIEND investigators, 3) a DCC investigator, additional outside investigators with a limit of one author per site, and end with the DCC PI.

Authorship Order Category	**Description**
1	Lead Investigator of the Protocol (N =1)
2	Primary FRIEND Investigators of the Protocol (N = 6); DCC investigator (N = 1)
3	Other Investigators (N to be determined)
4	DCC PI (N = 1)

The authorship order of the FRIEND Consortium and outside sites will be based upon subject recruitment, data accuracy and promptness of data report according to the chart below:

Investigative Sites	# Subjects Rank	Accuracy Rank	Total Rank	Authorship Order
A	1	4	5	3
B	2	7	9	6
C	3	1	4	2
D	4	2	6	4
E	5	3	8	5
F	6	5	11	7
G	7	6	13	8

Data accuracy will be ranked according to the rate of missing or false data entries/randomized subject at each site. Inquires that show data was accurately entered will not count against this rate of data inaccuracy. Each site's PI will be responsible to document the contributions to the study of that site's authors. In the event the journal editor requires fewer authors even after written documentation of the authors' contribution has been provided, the steering committee will vote on the authorship order which will include at a minimum the Lead Investigator and PI of the DCC (or his/her designate) in the positions listed above. The other authors will be referenced in the footnote and listed in the title page.

4. Acknowledgement Section: The acknowledgement section will include other investigators and study personnel who contributed substantially to the study by site, as well as members of the Data and Safety Monitoring Board. The designation will list the initials of the individual followed by their highest degree (e.g. C. L. Gnatuk, J.A. Ober, R.N., etc.) Significant contributions include but are not limited to protocol review, initiation and participation at each site, subject recruitment and enrollment, study conduct, data analysis, and preparation of the manuscript.

7.13.2 Minor Publications

Minor studies are defined as those in which the hypotheses would not be the main elements of consortium studies, but in which the study data base would be utilized to test secondary hypotheses. (One example would be testing whether metformin use spares the dose of clomiphene resulting in lower dose needs.) Ideas for "minor studies" will, in general, be proposed by a single individual, who would direct all efforts leading to publication and representation. The results from minor studies would be handled similarly to those from major studies. The "Protocol" is defined as the Concept Protocol/study design of the hypothesis resulting in the publication.

Authorship will follow the Major publications guidelines above with the exception that the individual leading the minor study would be the first author, followed by the ranked primary FRIEND investigators involved in developing the Concept Protocol. The Lead Investigator of the minor publication can propose additional investigators who contributed to the study, whose inclusion in the authorship will be voted on by the FRIEND consortium (majority vote of the consortium required for inclusion in authorship). Centers may wish to withdraw inclusion from authorship of publications of minor studies in which only data are contributed, and this will be the decision of the individual site PI.

7.13.3 Ancillary Study

An Ancillary Study is an observational study, conducted as a supplement to a Main Study. By definition, an Ancillary Study involves all or a subset of patients enrolled in a Main Study. An Ancillary Study does not involve any additional participants. To be defined as an Ancillary Study, there must be a need for collection of additional data not already collected in the Main Study. An Ancillary Study may also be designed by another consortium investigator, who would serve as the lead investigator and primary author of the paper. Ancillary Studies may be a "single-center" or "multi-center".

A "single-center" Ancillary Study is a study in which all data are collected, stored and analyzed at a single center. The center bears the additional cost of such a study. The study requires approval of the FRIEND consortium. The center conducting the study is responsible for the analysis and reporting of the results. Abstracts and manuscripts resulting from data from the single-center Ancillary Study are not subject to the FRIEND Publications Policies.

A "multi-center" Ancillary Study is defined as one for which data or material (such as specimens) are collected at more than one center, or additional funds for conduct of the study are provided by the FRIEND and the DCC provides data analysis. Multi-center Ancillary Studies require the approval of the FRIEND consortium.

Authorship will be as per Major publications above with the exception that the individual leading the ancillary study and writing the paper would be the first author, followed by ranked FRIEND primary investigators, etc. A center not participating in the ancillary study would not receive authorship unless by majority vote of the steering committee.

7.13.4 Related Publications

A related publication is one that has had significant input from members of the FRIEND consortium at formal meetings in terms of study significance and design. It is distinct from an ancillary publication in that a related publication reports on a study, concept or new methodology that has not been subjected to formal DSMB review and approval. Generally, "Related Publications" will arise from ideas and studies discussed with the FRIEND consortium, but not voted upon to become formal protocols.

The investigator who initiates, conducts and writes the study and those who (s)he names will be the sole authors. The authors should acknowledge the contribution of the FRIEND consortium in the author line of the publication according to the format of the journal.

7.13.5 Outside Studies

Outside studies will result from the sharing of data and/or specimens with investigators whose protocols have been approved by the steering committee, and who comply with all components of those policies. All publications will acknowledge the assistance of the FRIEND consortium in making the database available on behalf of the project. In addition, however, a disclaimer will need to be included stating, "the contents of this report represent the views of the authors and do not represent the views of the FRIEND consortium."

7.13.6 Presentations

FRIEND data should be presented before national organizations by the lead investigators of Main Studies, Ancillary, and Pilot studies. Organizations that might be appropriate include the American Society of Reproductive Medicine, the Society for Gynecologic Investigation, and

the American College of Obstetricians and Gynecologists. All presentations will be approved by the P & P committee. Once data are published in at least abstract form, all members of the FRIEND consortium can cite them publicly in lectures.

However, investigators should avoid citing specific numbers in review articles and chapters, for this could jeopardize peer review publication. Authorship, First Author, and Author Order are as described for Major Publications, and if there is an authorship limit to the abstract we will follow the plan above under Major Publications. Oral and poster presentations, including those resulting from secondary analyses at professional societies, must list all authors and participating institutions.

8 References

1. Roshdy E, Rajaratnam V, Maitra S, Sabry M, Allah AS, Al-Hendy A. Treatment of symptomatic uterine fibroids with green tea extract: a pilot randomized controlled clinical study. International journal of women's health. 2013;5:477-86.
2. Zhang D, Al-Hendy M, Richard-Davis G, Montgomery-Rice V, Rajaratnam V, Al-Hendy A. Antiproliferative and proapoptotic effects of epigallocatechin gallate on human leiomyoma cells. Fertility and sterility. 2010;94(5):1887-93.
3. Zhang D, Al-Hendy M, Richard-Davis G, Montgomery-Rice V, Sharan C, Rajaratnam V, et al. Green tea extract inhibits proliferation of uterine leiomyoma cells in vitro and in nude mice. American journal of obstetrics and gynecology. 2010;202(3):289.e1-9.
4. Khan N, Mukhtar H. Tea polyphenols for health promotion. Life sciences. 2007;81(7):519-33.
5. Zhang D, Rajaratnam V, Al-Hendy O, Halder S, Al-Hendy A. Green tea extract inhibition of human leiomyoma cell proliferation is mediated via catechol-O-methyltransferase. Gynecologic and obstetric investigation. 2014;78(2):109-18.

9 Appendix J: Investigator Signature of Agreement

Investigator Signature of Agreement

Title:

Version:

Principal Investigator:

I, *[Insert PI's name]*, the Principal Investigator for *[Insert Institute Name]*, hereby certify that I have read and agree to conduct this study in accordance with this protocol on behalf all FRIEND Investigators and research staff from my site.

____ ____________________
Signature Date

9 Appendix K: DSMB Charter

DATA AND SAFETY MONITORING BOARD (DSMB) CHARTER

Purpose and Responsibilities of the DSMB

The members of the Data and Safety Monitoring Board (DSMB) identified in this Charter for the FRIEND study are responsible for safeguarding the interests of study participants, assessing the safety and efficacy of all study procedures, and shall monitor the overall conduct of the FRIEND trial. This Committee will serve as an independent advisory group to the Director of NICHD, and is required to provide recommendations about starting, continuing, and stopping the FRIEND study.

This Committee is responsible for identifying mechanisms to complete various tasks that will impact the safety and efficacy of all study procedures, and overall conduct. The table below identifies the key areas where oversight is necessary and the ways in which the Committee for the FRIEND study will complete those tasks.

Basic Responsibility of DSMB	Method DSMB for FRIEND will use to complete task
Familiarize themselves with the study protocol	· Review study protocols and informed consent forms.
Monitor adverse events	· Adverse Event: Review quarterly progress reports prepared by the DCC. · Serious Adverse Events: Review report submitted by the DCC within one week of the event if life-threatening or fatal, or within two weeks otherwise. · The DSMB will submit a report of their review to the DCC Coordinator within 7 business days if the SAE is life-threatening or fatal, or within two weeks otherwise.
Monitor data quality	· Conduct interim evaluations of the data.
Oversee participant recruitment and enrollment	· Review interim progress reports prepared by the DCC.
Develop an understanding of the Study's risks and benefits	· Review study protocols and related literatures. · Review interim reports of subject accrual and outcome measures provided by the DCC. · Assess the need to perform further in-depth evaluation of the benefits and risks of the study after reviewing each report.
Ensure the proper reporting occurs	· Review and approve the meeting and reporting schedule listed in Sections 5 and 6 of this DSMB charter.

Contacts

NICHD
Esther Eisenberg, MD, Project Scientist

Data Coordination Center (DCC)
Heping Zhang, PhD, DCC Principal Investigator
Hao Huang, DCC Data Manager and Statistician

Janet Sun, MS, Biostatistician
Siobhan Thompson, MPH, Project Manager/Clinical Research Associate
Donna DelBasso, DCC Sr. Administrative Assistant

Lead Investigator(s)

The Data Manager at the DCC will prepare the DSMB reports. The DCC Project Director will review all DSMB reports prior to submission to the DSMB. The DCC Data Manager will not be blind to treatment condition.

DSMB Members, Organizational Chart, & Communications

Members
The DSMB for the FRIEND study is comprised of the members listed in the table below. In addition, their high-level roles and responsibilities are identified in the table.

<table>
<tr><th>Name of Member</th><th>Role on DSMB</th><th>High Level Responsibilities</th></tr>
<tr><td>David S. Guzick, M.D., Ph.D.</td><td>Chair of DSMB Voting member</td><td>· Chair the DSMB discussion and prepare written recommendations to FRIEND.
· Ensure the safety of study subjects, the integrity of the research data.
· Provide FRIEND with advice on the ethical and safe progression of studies conducted.
· Advises on research design issues, data quality and analysis, and research participant protection for each prospective and on-going study.</td></tr>
<tr><td>Peter S. Bernstein, MD, MPH</td><td>Voting member</td><td rowspan="5">· Ensure the safety of study subjects, the integrity of the research data.
· Provide FRIEND with advice on the ethical and safe progression of studies conducted.
· Advises on research design issues, data quality and analysis, and research participant protection for each prospective and on-going study.</td></tr>
<tr><td>Katherine Burns, Ph.D.</td><td>Voting member</td></tr>
<tr><td>Robert W. Rebar, M.D.</td><td>Voting member</td></tr>
<tr><td>Ming T. Tan, Ph.D.</td><td>Voting member</td></tr>
<tr><td>Kimberly L. Thornton, M.D.</td><td>Voting member</td></tr>
</table>

Conflict of Interest and Compensation

It is extremely important that all members of the DSMB state any real or apparent conflicts of interests at the onset of the study. Members of the DSMB shall read the NICHD Clinical Research Guidance Document regarding Conflict of Interest (COI) and provide their signed summary of any COI for the study, at its onset, to the DCC. A table summarizing any COI within the DSMB is provided in the Appendix.

Prior to each meeting, all members of the FRIEND DSMB will have an opportunity to state whether they have developed any new conflicts of interest since the meeting. As a new COI is identified it must be documented in the table in the Appendix and a new signed summary of the COI should be provided to the DCC for record-keeping purposes.

If a new conflict is reported, the DCC will determine if the conflict limits the ability of the DSMB member to participate in the discussion.

All DSMB members will be compensated for their role in supporting the committee. Compensation will include an honorarium for meeting attendance and any travel costs.

Meeting Schedule

DSMB meetings will be conducted quarterly. However, the DSMB may hold a meeting at any time in accordance with their mission.

Report Schedule and Content

The type of reports (full or brief) is indicated below, followed by a description of the contents of each type.

DSMB Report	Report Submission Date	Type of Report
1.		Brief
2.		Brief
3.		**Full**
4.		Brief
5.		**Full**
6.		Brief
7.		**Full**
8.		Brief

Brief DSMB reports will include the following summaries:

- overall actual versus projected enrollment accrual
- overall randomization update
- overall study drop-out rate
- serious adverse events

- primary outcome measures update

Full DSMB reports will include the following summaries:

- recruitment update (number screened) overall and by site
- enrollment update (enrolled defined as randomized to a treatment) overall and by site
- accrual status including actual enrollment compared to projections overall and by site
- randomization update (i.e., number assigned to each treatment arm)
- study drop-out rate for enrolled patients (number, reason, time point) overall and by site)
- pre-specified subset of baseline demographic data for enrolled patients
- safety data, adverse events, and serious adverse events
- number of case report forms expected
- number/percentage of expected case report forms received – overall and by site
- number of case report forms that are query clean
- primary outcome measures update

Efficacy Outcome Summary

An interim analysis is planned at the mid-point of the study to assess the overall pregnancy rate (i.e., the three groups combined) while maintaining blinding of treatment assignment in order to assess whether this pregnancy rate approximates the predicted rate of 30-40%, and whether additional subjects may be needed to meet power targets. Since treatment assignments will not be identified, it is not thought that adjustments in significance levels will be required.

Appendix: Summary of COI within the DSMB

DSMB Member Name	Date Submitted Signed COI	Was a COI Identified?	Will the Member Remain Part of the Committee?

Conflict of Interest Statement

I, ______________________, assuming the role of ______________________________

(insert role, for example: DSMB member)

for the ____

(insert project or study name)

agree to the following statements.

□ I agree to:

- protect the interests and safety of study participants;
- uphold the integrity of the research process including data collection and analysis to be as free from bias and preconception as I am able;
- adhere to the highest scientific and ethical standards, to comply with all relevant regulations and to eliminate or disclose, during my involvement with the proposed clinical research project, any real or apparent conflicts of interest.

In addition:

□ I declare that I, my spouse or dependent children, or organization with which I am connected, do/does not have any financial interest in the ___________________ study, where financial interested is defined by the DHHS, as anything of monetary value, including but not limited to, salary or other payments for services (for example, consulting fees or honoraria); equity interests (for example, stocks, stock options or other ownership interests); and intellectual property rights (for example, patents, copyrights and royalties from such rights).
The financial interest term does not include various items which can be found in The Federal regulation, PHS, DHHS Part 50--Policies of General Applicability; Subpart F- *Responsibility of Applicants for Promoting Objectivity in Research for Which PHS Funding Is Sought.*
For Federal employees, financial interests that are allowable and require disclosure are:

Financial Interest Disclosure<u>: Financial interest that require disclosure are stock holdings in pharmaceutical firms, medical device manufacturers, and biotechnology companies</u>
Allowable Financial Interests<u>: In a company that produces a product that is being evaluated by a study, participants may hold up to $15,000 of stock: and, up to an aggregate of $25,000 of the stock of that company and its competitors who produce that (or a similar) product. As an alternative to individual stock holdings, participants may hold up to an aggregate of $50,000 in sector mutual funds-including pharmaceutical/health care sectors.</u>

<u>For holdings in excess of these *de minimus* levels, a conflict of interest analysis needs to be conducted by NIH regarding the holding, the company producing the product being evaluated under the study, and its competitors, and, if a conflict exists, could lead to the need to withdraw from the study.</u>

□ I agree to not withhold any data related to the ______________________ study or to interfere with the analysis or publication of the study's results.

□ I will not engage in activities that could be viewed as real or apparent COI, including but not limited to:

> □ having a part-time, full-time, paid, or unpaid employee status of any organizations that are: (a) involved in the study under review; (b) whose products will be used or tested in the study under review, or whose products or services would be directly and predictably affected in a major way by the outcome of the study;
>
> □ being an officer, member, owner, trustee, director, expert advisor, or consultant of such organizations;
>
> □ being a current collaborator or associate of the principal investigator (applicable to potential members of data safety and monitoring boards);
>
> □ having a scientific interest beyond that required for my role, where scientific interest is defined as having influence over the protocol, the study design, conducting the study analysis or any reporting related to the investigation (applicable to potential members of data safety and monitoring boards).

Data and Safety Monitoring Board (DSMB)
Confidentiality Agreement

I agree to serve as a DSMB member for the FRIEND (Reproductive Medicine Collaborative Consortium: a randomized placebo-controlled trial of EGCG to improve fertility in women with uterine fibroids) consortium for which Yale University Collaborative Center for Statistics in Science serves as the Data Coordination Center (DCC).

As a DSMB member I understand that I will be provided with and have access to documents submitted by the NICHD or the DCC as they relate to study protocols, Registries or other consortium-related materials, including proprietary and confidential information.

I shall not disclose any confidential information (oral or written) unless required to do so by law. Confidential documents may be distributed to an administrative assistant, who is not permitted to share the materials with anyone other than me.

I agree that I will not distribute or publish the study records. I further agree that I shall not make use of consortium materials except for the express purpose of advising the Consortia and the NICHD.

I have read this agreement and agree to abide by its terms.

Name (Print or Type): ____

Signature: _____________________________________ Date: ______________