A PILOT RANDOMIZED, PLACEBO CONTROLLED, TRIAL OF FERMENTED WHEAT GERM EXTRACT IN WOMEN WITH OVARIAN CANCER

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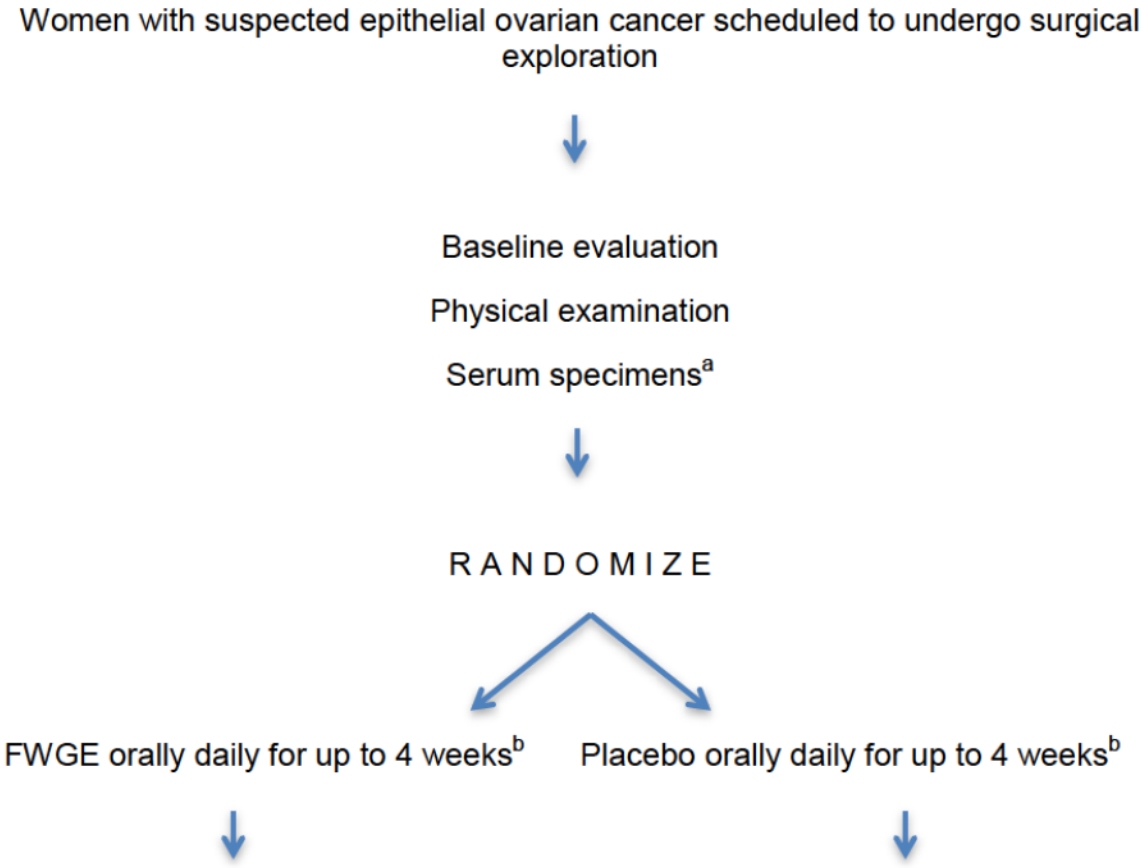
1.0 PROTOCOL SCHEMA



[a]Serum CA125 at enrollment and the day of surgery

[b]Surgery should be completed up to 4 weeks after beginning drug/placebo.

[c]National Cancer Institute's Common Toxicity Criteria

[d]Quality of Life Survey (QoL) – FACT-O

[e]Determine the presence of 2,6-dimethoxy-p-benzoquinone in the serum

[f]Histologic analysis of tissue analyzing for changes in proliferation. Gene microarray assessing for changes in gene expression

2. HYPOTHESIS and OBJECTIVES

2.1 Hypothesis

Fermented Wheat Germ Extract (FWGE) has activity against epithelial ovarian cancer (OVCA). FWGE may increase sensitivity to chemotherapy and may decrease side effects related to chemotherapy. FWGE active components (2,6-dimethoxy-p-benzoquinone) may be detected in serum of women receiving FWGE.

In an effort to generate preliminary data to support a future peer-reviewed grant-funded large phase II clinical trial of adjuvant FWGE in women undergoing therapy for ovarian cancer and to test the safety and tolerability of FWGE, we propose a pilot randomized, placebo-controlled study of FWGE in women with a suspected diagnosis of OVCA. We propose to test the safety and tolerability of FWGE, and to determine if we can detect an active product of FWGE (2,6-dimethoxy-p-benzoquinone) in the serum, and whether short-term therapy with FWGE has an effect on the tumor marker, CA125. We will analyze changes in tissue proliferative assays and gene expression in women who receive FWGE (n=10) and those who receive placebo (n=10).

We recognize that the sample size in the current study will prohibit definitive statistical conclusions to be drawn on our primary objective (to test the safety and tolerability of FWGE), or our secondary objectives (to compare levels of FWGE active product, CA125 levels, proliferation, and gene expression in OVCAs from subjects receiving FWGE versus placebo). The study is neither designed nor powered to develop statistically significant results, rather, we seek to develop preliminary/pilot data that will enable us to pursue a future large phase II, peer-reviewed grant-funded trial of adjuvant FWGE in women undergoing therapy for ovarian cancer. Preliminary data developed in the current proposal will support such grant applications.

2.2 Objectives

2.21 Primary Pilot Objective: To assess the safety, toxicity and quality of life (QoL) of women with suspected ovarian cancer scheduled to undergo surgery, administered FWGE versus placebo daily for up to 4 weeks.

2.22 Seconday Pilot Objectives:

2.221 To develop pilot data on the impact of FWGE on: the tumor marker CA125, tumor proliferation.

2.222 To develop pilot data on changes in gene expression in ovarian cancer exposed to FWGE versus placebo.

3.0 BACKGROUND AND RATIONALE

3.1 Ovarian Cancer

Ovarian cancer (OVCA) is the 5th leading cause of cancer death among women in the United States and Europe and has the highest mortality of all gynecologic cancers. It is estimated that 21,980 women in the U.S. will be diagnosed with epithelial OVCA in 2014 and 14,270 women will die of the disease [1]. Approximately 75% of cases are diagnosed at an advanced-stage (III/IV) with disseminated intraperitoneal metastasis [2]. Women with OVCA undergo major surgery followed by chemotherapy in an attempt to prolong survival. Although approximately 70% of women will demonstrate a clinical response to this primary therapy, the majority will develop progressive or recurrent disease that is platinum-resistant. Women with platinum-resistant OVCA often demonstrate cross-resistance to most other chemotherapeutic agents. Once platinum-resistance has developed, few active therapeutic options exist and patient survival is generally short-lived. Throughout treatment for OVCA, prolongation of survival and the successful maintenance of quality of life remain important goals. Improving our ability to manage the disease by optimizing the use of existing drugs and/or developing new agents is therefore essential in this endeavor.

3.2 Use of natural products

Up to 90% of patients with cancer use alternative therapies, often herbal or natural products [3,4]. The majority of these products have not been subjected to comprehensive study for efficacy or potential negative interactions with chemotherapy. Natural, non-toxic regimens that enhance standard-of-care therapy and/or prolong survival are highly desirable in management of patients with cancer.

3.3 Fermented Wheat Germ Extract

Fermented wheat germ extract (FWGE, Avemar®) was developed in Hungary in the early 1990's [5]. It is produced by extraction of wheat germ, fermentation of the extract, separation of the fermentation liquid, concentration, and drying. The chemical composition of FWGE is a mixture of molecules including 2-methoxy-p-benzoquinone and 2,6-dimethoxy-p-benzoquinone, which are felt to contribute to its biological properties [6]. FWGE has been available as a dietary supplement in Hungary since 1998 and was approved in 2002 as a "medical nutriment for cancer patients." It is classified by the European Union as a "dietary food for special medical purposes". FWGE has been shown to be safe when taken orally with no toxicity, mutagenicity, or genotoxicity [6].

3.31 In Vitro Studies

FWGE has been evaluated in-vitro and shown to induce apoptosis in some cancer cell types, including: leukemia, breast, colon, testicular, head and neck, cervical, ovarian, gastric, thyroid, melanoma, brain and hepatocellular carcinomas [7-10]. The addition of FWGE to 5-fluorouracil, oxaliplatin or irinotecan chemotherapy has been shown to have no negative impact on effectiveness or toxicity profiles, and in colon cancer cell lines has additive to synergistic effects [7].

With this data suggesting that FWGE has activity against many cancer cell types, we studied the broader spectrum of activity against OVCA.

3.32 Our Ovarian Cancer In Vitro Studies [11]

Our in-vitro data demonstrates that FWGE induces OVCA cell line growth arrest and apoptosis and that it sensitizes OVCA cells to cisplatin-induced cell death. We also have data to suggest that the sensitivity of OVCA cells to FWGE may be determined by a discrete number of molecular signaling pathways.

FWGE induces growth arrest and cell death in ovarian cancer cell lines

We evaluated the effects of FWGE on cell growth arrest in a panel of 12 OVCA cell lines using MTS cell viability assays (Fig 1). Several of these cell lines were paired, mother/daughter, sensitive/resistant lines. Notably, cisplatin-resistance, a surrogate marker of greater chemotherapy-resistance, did not predict FWGE sensitivity. This

suggests the biologic determinants of resistance to chemotherapy agents may differ from those that influence response to FWGE.

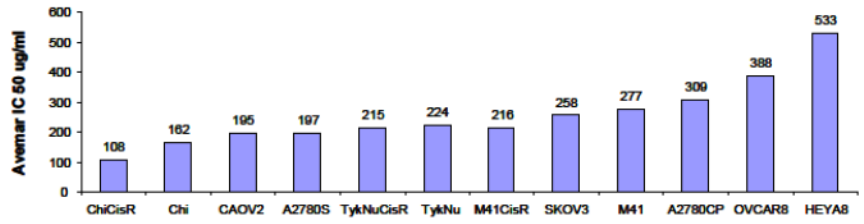


Fig 1. Ovarian cancer cell lines ranked by FWGE sensitivity. FWGE IC50 for each cell line is shown numerically in ug/ml.

FWGE potentiates chemotherapy (cisplatin)-induced cell death

To determine if FWGE and the chemotherapy agent cisplatin work synergistically to induce OVCA cell growth arrest, we evaluated 100 and 200 ug/ml FWGE in combination with cisplatin (190, 390, 780, and 1,560nM). Drug activity was evaluated using the combinational index (CI) value determined by the Chou-Talalay isobologram equation where CI<1, CI=1, and CI>1 indicate synergistic, additive, and antagonistic effects. As shown in **Figure 2** the majority of FWGE/cisplatin combinations showed greater than additive effects while several combinations were strongly synergistic.

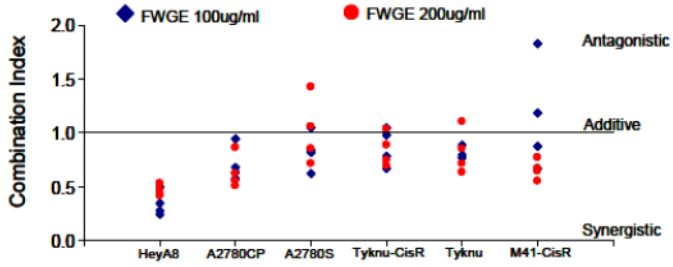


Figure 2. Combinational index (CI) values of FWGE and cisplatin drug activity in OVCA cells. CI values of CI<1, CI=1, and CI>1 indicate synergistic, additive, and antagonistic effects.

Expression of genes and signaling pathways that correlate with FWGE sensitivity in OVCA cells

To better understand the mechanisms that underlie FWGE activity in OVCA cells, Affymetrix gene expression data from the 12 cell lines was correlated with FWGE IC50. Using SAM'S Correlation Test (FDR<20%) identified 40 genes associated with FWGE sensitivity in Table 1. In order to place these genes into a relevant biologic

context, an analysis of biologic pathway relationships was performed using commercially available software (GeneGo Meta-Core™, (genego.com). GeneGo identified 16 pathways represented by genes associated with FWGE sensitivity $p \leq 0.05$. As described above, one of the active components of FWGE is thought to be 2,6-dimethoxy-p-benzoquinone. Analysis of publically available Affymetrix expression data (http://discover.nci.nih.gov/cellminer/loadDownload.do) and NCI60 cell line GI50

ZNF254	ZNF708	COPG2	SHF	tcag7.1228	SLC25A13	ZCCHC17	DYNC2LI1	GPATCH2	FAM133B
ARL5B	FAM117B	NRF1	PHF21A	POLR2J	SOX12	DBF4	FMC1	KLHL7	DUXAP10
LSM8	PHF21A	SLC7A7	SMAD2	SMARCD1	SUZ12P	ZBTB44	ZFP106	TRIM24	RPE
ZNF322A	ZSCAN21	ACVR2B	APOC1	C10orf111	C20orf74	MIDN	PLXND1	AGFG1	CCDC5

Table 1. Genes correlated with FWGE sensitivity.

values for 2,6-dimethoxy-p-benzoquinone (http://dtp.nci.nih.gov/dtpstandard/cancerscreening data/index.jsp), identified 3839 genes representing 267 pathways associated with sensitivity to 2,6-dimethoxy-p-benzoquinone (data not shown). Comparison of signaling pathways correlated with sensitivity to FWGE in OVCA cells and 2,6-dimethoxy-p-benzoquinone in NCI60 cell lines identified 9 common biological pathways

3.33 In Vivo Studies

FWGE has been shown *in vivo* to decrease metastatic spread of cancer, decrease carcinogenesis and to be non-toxic and non-inhibitory when administered with chemotherapy.

Synchronous oral FWGE and 5-fluorouracil intraperitoneal delivery significantly reduced the metastatic spread of c38 colorectal cancer in mice. [12]

In a study on F-344 rats, the administration of azoxymethane induced colon carcinogenesis. When FWGE was fed to the rats prior to the azoxymethane administration, significantly fewer rats developed colon tumors (83% vs 45% $p < 0.001$) [13]

In vivo, FWGE was administered in mice simultaneously with cyclophosphamide,

vinorelbine, or doxorubicin. There was no increased toxicity or decreased antiproliferative activity [14].

3.34 Human Clinical Studies

When taken orally in addition to chemotherapy, FWGE has been shown to decrease adverse events, inhibit cancer cell proliferation and metastatic spread, and increase overall survival in patients with colorectal cancer and melanoma.

One hundred and seventy-six colorectal cancer patients with histologically confirmed (Dukes A–D) colorectal cancer were enrolled. No restrictions were made for prior radiotherapy and/or chemotherapy, but all the patients had to undergo curative surgery at the time of diagnosis. There was no randomization; cohorts were formed based on patient preference. FWGE was taken orally once daily. Patients received adjuvant therapy by physician preference. No grade 3 or 4 adverse events were observed. Patients receiving FWGE had a significant prolongation of progression free survival ($p = 0.0184$) and overall survival ($p = 0.0278$) [15]
A trial performed in pediatric patients with solid malignancies undergoing chemotherapy was conducted to assess whether the combined administration of FWGE with chemotherapy would reduce the incidence of treatment-related febrile neutropenia. Twenty-two patients were enrolled; they were not randomized, but allowed to choose their treatment arm. The patients were matched by diagnosis, stage of disease, age, and gender. During the treatment period, there was no progression of the malignant disease. The frequency of febrile neutropenia was significantly decreased in patients receiving FWGE: 30 febrile neutropenic episodes (24.8%) in the FWGE group versus 46 (43.4%) in the control group ($p < 0.05$) [16]

An open-label, randomized, phase II trial to assess the supportive value of FWGE in patients with stage III melanoma scheduled to receive adjuvant therapy was performed. Post-operatively, patients were randomized to FWGE simultaneous with adjuvant dicarbazine (DTIC) and FWGE continued for 12 months or DTIC alone. Fifty-eight patients were enrolled. Three from each treatment arm withdrew from study, for 52 patients analyzed. Adverse events included: grade1 and 2 nausea/vomiting, and grade 1 diarrhea. There were no grade 3 or 4 adverse events. They noted fewer toxic side effects in patients receiving the FWGE than in those of

the control group. There was a statistically significant improvement in progression-free survival 55.8 months (FWGE) versus 29.9 months (control) p = 0.0137. There was a statistically significant improvement in overall survival of 66.2 months (FWGE) versus 44.7 months (control) p = 0.0298 [17].

3.35 Summary and Rationale:

Fermented wheat germ extract has been demonstrated in vitro to induce ovarian cancer cell growth arrest and apoptosis. When FWGE is combined with cisplatin chemotherapy it enhances cell killing. In animal studies, FWGE has shown no toxicity and anticancer effects. Observations from human clinical trials demonstrate FWGE to be safe and without serious adverse events. The data also suggest beneficial effects of FWGE on disease progression and survival in patients with melanoma and colorectal cancer.

3.4 Proposal Overview: In this pilot clinical trial, we will evaluate the safety, toxicity and quality of life (QoL) of ten women with ovarian cancer scheduled to undergo surgery, administered FWGE versus ten women receiving placebo daily for up to 4 weeks. We hypothesize that FWGE will be safe and non-toxic and that administration may decrease some of the symptomatology associated with ovarian cancer. As secondary objectives, we will seek to determine whether we can detect differences in levels of 2,6-dimethoxy-p-benzoquinone (one of FWGE's active products), in the serum of women receiving FWGE versus placebo. We will also evaluate the impact of FWGE on the tumor serum marker CA125, as well as histologic tumor proliferation and gene expression in ovarian cancer. As noted above, we recognize that the sample size in the current study will prohibit definitive statistical conclusions to be drawn on our primary objective (to test the safety and tolerability of FWGE), or our secondary objectives (to compare levels of FWGE active product, CA125 levels, proliferation, and gene expression in OVCAs from subjects receiving FWGE versus placebo). The study is neither designed nor powered to develop statistically significant results, rather, we seek to develop preliminary/pilot data that will enable us to pursue a future large phase II, peer-reviewed grant-funded trial of adjuvant FWGE in women undergoing therapy for ovarian cancer. Preliminary data developed in the current proposal will support such grant applications.

3.5 Rationale for the Dosage Used in this Study

The dose of FWGE that has been chosen for the study, is the dose used in each of the three previous clinical trials. This consists of 5.5 grams of FWGE formulated as a water-soluble granulate with added 0.03 grams of stevia sweetener and orange flavorings (taking it to approximately 5.53 grams), dissolved in 150 mL of water, orally once-daily.

This dose has been tolerated well in previous clinical trials with no grade 3 or 4 toxicity, even when combined with chemotherapy.

With regard to length of the intervention, most patients are scheduled for surgery within 4 week interval. We do not want to delay a patient's surgery for the intervention.

4. PATIENT ELIGIBILITY

4.1 Inclusion Criteria

4.11 Women with suspected epithelial ovarian, fallopian tube or primary peritoneal carcinoma scheduled to undergo surgical exploration with no prior treatment for the cancer. Signs of ovarian cancer include, but are not limited to: an elevated CA125, a complex pelvic mass, ascites, and carcinomatosis. These signs are not necessary for suspicion or enrollment in this protocol.

4.12 Age > 18 years and competent to give informed consent

4.13 Patient must have a ECOG Performance sttus of 0, 1, or 2 and a life-expectancy of at least 60 days.

4.14. Patients must have adequate:

4.141 Bone marrow function: Hemoglobin greater than or equal to 9gm/dl. Absolute neutrophil count (ANC) greater than or equal to 1,500/ul, equivalent to Common Toxicity Criteria (CTCAE v4.0) grade 1. Platelets greater than or equal to 100,000/ul.

4.142 Renal function: creatinine less than or equal to 1.5 x institutional upper limit normal (ULN), CTCAE v4.0 grade 1.

4.143 Hepatic function: Bilirubin less than or equal to 1.5 x ULN (CTCAE v4.0 grade 1). SGOT and alkaline phosphatase less than or equal to 2.5 x ULN (CTCAE v4.0 grade 1).

4.15 Study subjects must sign an approved informed consent and authorization permitting release of personal health information.

4.16 Women of childbearing potential must have a negative serum pregnancy test prior to the study entry and be practicing an effective method of birth control during the course of the study, in a manner such that risk of failure is minimized. Prior to study enrollment, women of childbearing potential must be advised of the importance of avoiding pregnancy during trial participation and the potential risk factors for an intentional pregnancy.

4.2 Exclusion Criteria

4.21 Current use of FWGE

4.22 Known allergy to wheat, rice (contained in the placebo), orange or the sweetener, Stevis

4.23 Patient who received neoadjuvant chemotherapy for ovarian cancer

4.24 An upper gastrointestinal or other condition that would impair swallowing or absorption of oral medication

4.25 Any serious illness or medical condition that would not permit the patient to be managed according to the protocol, including, but not to limited, any the following:

- History of significant neurologic or psychiatric disorder (e.g., uncontrolled psychiatric disorders) that would impair the ability to obtain consent or limit compliance with study requirement

- Active uncontrolled or serious infection
- Active peptic ulcer disease

4.26 Uncontrolled hypertension defined as systolic greater than 180 and diastolic greater than 100

5. STUDY MODALITIES

5.1 FWGE

5.11 Formulation: American BioSciences Inc, who is the exclusive manufacturer of FWGE in the Unites States, produces fermented wheat germ extract. See Letter of Support (Appendix II). It is formulated as a granulate. One package contains 5.53 grams of FWGE combined with natural orange flavor and stevia Reb-A sweetener to create an instant drink mix. See compositional analysis (Appendix III).

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5.12 Administration: FWGE is dissolved in 150ml water and taken orally daily.

5.13 Adverse effects

The clinical safety experience of FWGE is derived from trials in which FWGE has been used as a single agent or in combination with chemotherapeutic agents. FWGE has not been associated with any grade 3 or 4 toxicities

5.14 Storage: FWGE is to be maintained at <80 degrees F

5.15 Quality assurance of the manufacturing of FWGE in the U.S.

5.151 The purchased wheat germ and yeast, which are the raw materials of FWGE production, are certified for human consumption. The origin of the wheat germ and yeast, used in the fermentation, are all U.S. certified.

5.152 After each batch of FWGE has been freeze-dried, it is tested for hazardous contaminates, such as toxic elements, including heavy metals, and for toxic microorganisms, such as E.Coli, Listeria, etc. Once, the lyophilizate has been found free from toxic contaminants, and has been qualified as microbiologically safe (such as complete lack of toxic micriorganisims, etc.), it Is standardized to ensure batch-to-batch chemical compositional homogeneity and biological efficacy-related reproducibility.

5.153 For this purpose, a characteristic high performance liquid chromatography (HPLC) fingerprint UV chromatogram of FWGE is compared with the HPLC fingerprint UV chromatogram of an established standard marker "core" of FWGE (reference pattern). As a result of a 2-years research collaboration between the manufacturer and an NCI-designated Cancer Center in the U.S., the marker "core" of the FWGE has been developed and validated to possess the characteristic biological effects of FWGE, including testing by *in vitro* antiproliferative assays using human cancer cell lines; kinase panel tests; robust gene expression assays by quantitative proteomic analysis, using isobaric tag for relative and absolute quantitation labeling technique with electrospray mass spectrometry; and by laboratory animal cancer experiments. The said reference pattern of the standard marker "core" of FWGE has been established to comprise peaks characterized by retention times and relative retention quotients, respectively.

The said fingerprint of the representative sample of the FWGE batch must be identical with the reference pattern. (Chemical standardization/Qualitative chemical assay.)

5.154 The quality of the marker "core" in each FWGE batches must also meet a fixed reference value. (Chemical standardization/Quantitiative chemical assay.)

5.155 FWGE batches are also characterized by quantitative biological assays according to *in vitro* anticancer protocols. Each and all batches must show the required quantitative biological efficacy. (biological standardization.)

5.2 Placebo

5.21 Formulations: American BioSciences Inc, who is the exclusive manufacturer of FWGE in the Unites States, produces the placebo. See Letter of Support (Appendix II). It is formulated as a granulate. One package contains cooked,

dried and pulverized, white and black rice, 5.53 grams, combined with natural orange flavor and stevia Reb-A sweetener, to create an instant drink mix. See compositional analysis placebo (Appendix IV).

5.22 Administration: FWGE Placebo is a dissolved in 150ml water and taken orally daily.

5.23 Supplier: American BioSciences Inc

5.24 Storage: to be maintained at <80 degrees F

5.3 Drug Accountability: The investigator, or a responsible party designated by the investigator, will maintain a careful record of the receipt, disposition, and return of all drugs received from American Biosciences Inc.

5.4 Drug Dispensing: FWGE and placebo will be in unidentified packages. A random number table will be used to assign patients to the FWGE versus placebo group. The key will be held by the investigational pharmacy and is not revealed to the researchers or patients until the study is complete.

5.4 Unblinding: This is a double-blind study. The treating physician and/or patient may be informed as to which regimen the patient was assigned if either of two situations arises: serious adverse reaction occurs or study completion. In the case of an adverse reaction, the treating physician must contact the Study Chair to obtain permission to break the blind. The Study Chair will notify the Pharmacy which in turn will notify the treating physician. The patient will be considered off study treatment at that time.

6. TREATMENT PLAN AND ENTRY PROCEDURE

Prior to enrollment, approval will be obtained from the scientific review committee and the institutional review board.

6.1 Patient Entry and Registration

6.11 An informed consent and personal health information release will be signed by each patient enrolled.

6.12 All eligibility requirements indicated in Section 4.0 must be satisfied.

6.2 Treatment plan

6.21 All subjects will undergo:

6.211 history and physical examination within 28 days of study entry.

6.212 laboratory evaluation: complete blood count, differential, comprehensive metabolic panel and CA125 test prior to starting protocol therapy and preoperatively.

6.213 serum pregnancy test (within 7 days of starting study drug), for women of child-bearing potential.

6.214 Evaluation for National Cancer Institute's Common Toxicity Criteria, all grades at the time of enrollment, then weekly and the day of surgery.

6.215 Quality of life questionnaire (FACT-O) at the time of enrollment, then weekly and the day of surgery (Appendix I).

6.22 Patients will be randomized to treatment with either FWGE or placebo and assigned a blinded treatment number corresponding to one of the two treatment groups. The pharmacy will label the appropriate blinded drug specifically for the patient. A daily supply of drug will be given to the patient from the study pharmacy. The amount will be determined by the time until the surgical procedure is scheduled. Neither patients nor treating physicians will be informed of patients "treated/control" status.

Participants will undergo their planned surgery within 4th week after entry into the study. However, if there are delays, patients can continue to take drug/placebo

for a time period no greater than 2 months. If surgery has not occurred after this 2-month period, the patient will be removed from the study.
Participants will take the study drug up until the day prior to surgery. A separate consent form will be obtained for the surgical procedure.

6.23 The primary tumor tissue obtained during the subject's surgery will be transferred to the pathology department for processing.

6.231 Formalin Fixation

Tissue will undergo formalin fixation then embedment in paraffin; tumor tissue will be stained using the H&E process and will be examined by light microscopy. The tumor will also be stained by immunohistochemical (IHC) stain for proliferation marker Ki-67 (Anti-Ki67 primary antibody, catalog number 790-2910, Ventana, Tucson, AZ, USA). The proliferation index will be calculated by percent of nuclear staining of the tumor for tumor tissue treated with FWGE versus placebo and the result will be compared. The reading pathologist will be blinded to the treatment group.

6.232 Fresh frozen

An additional specimen, approximately 30mg, will be flash frozen.

6.233 RNA extraction and Affymetrix gene expression analysis

A total of 20 fresh frozen primary OVCA tissues (10 FWGE- and 10 placebo-treated) will be used for RNA extraction. Tissues (~30mg) will be submerged in lysis buffer. Tissues are then pulverized in BioPulverizer H tubes (Bio101) using a Mini-Beadbeater (Biospec Products). Tubes are spun briefly to pellet the garnet mixture and reduce foam. The lysates are then transferred to a new 1.5 ml tube using a syringe and 21 gauge needle, followed by passage through the needle 10 times to shear genomic DNA. Total RNA will be collected using the Qiagen RNeasy Mini kit according to the manufacturer's instructions. RNA quality will be checked on an Agilent Bioanalyzer to assess quality of RNA via the 28S:18S ribosomal RNAs.

Microarray analysis: 10 µg of total RNA will be used to develop the targets for Affymetrix microarray analysis and probes will be prepared according to the manufacturer's instructions. Briefly, biotin-labeled cRNA is produced by in vitro transcription, fragmented, and hybridized to customized Human Affymetrix U133-Plus 2.0 gene chip arrays at 45° C for 16 hours and then washed and stained using the GeneChip Fluidics.

Method of statistical analysis: The expression value will be calculated and normalized using iterative rank-order normalization (IRON) [18]. IRON has been shown to perform better than both MAS5 and RMA and is not model based like RMA. Batch effects will be checked by using PCA; if present, they will be removed using COMBAT [19]. The 54,675 probesets will be screened to remove control probesets, probesets with a small variance, and probesets expressed at low levels.

6.24 Blood will be drawn at the time of surgery and sent for CA125 and a red top tube will be drawn and sent to the laboratory for 2,6, DMBQ testing

6.25 2,6-DMBQ determination

2,6-DMBQ will be determined in human plasma by high-performance liquid chromatography tandem mass spectrometry. The method will be validated per ICH/FDA guidelines and chromatography will be conducted similarly as previously published [20]. Plasma samples will be prepared for chromatographic injections by solid-supported liquid extraction (SLE). Calibration and quality control (QC) samples will be made by adding known amounts of 2,6-DMBQ to blank plasma. All calibration, QC, and unknown patient samples will be prepared in 96 well-plate format. Aliquots of human plasma and an internal standard will be added to a Biotage Isolute ® SLE plate. A non-polar solvent, similar to the previously published method, will be used to extract and elute the 2,6-DMBQ to a collection plate. The eluate will be evaporated under a gentle stream of air and wells will be reconstituted with mobile phase for injection. Samples will be injected into a Thermo Accela/TSQ Quantum LC/MS/MS system (Thermo Scientific, San Jose, CA). Analytes will be separated using a C18 column. Column eluate will enter the mass spectrometer via electrospray ionization and

multiple reactions monitoring (MRM) will be employed for identification and quantitation. Peaks will be detected and integrated utilizing Thermo LC Quan software. Calibration curves will be generated for each run and patient sample concentrations back-calculated from the corresponding regression line.

6.26 Quality of Life (QoL)

Patients receiving FWGE may experience a positive or negative affect on QoL relative to those who receive placebo.

The measures used in this study to assess QoL are the self-administered Functional Assessment of Cancer Therapy questionnaire for ovarian cancer (FACT-O) a 39-question assessment. Patients will be asked to complete these questionnaires at the following time points during their participation in the study:

i. At the time of enrollment
ii. Weekly
iii. Prior to surgery

Construct and content

The Functional Assessment of Cancer Therapy scale developed for ovarian cancer (FACT-O) is a tool that provides a general QoL score. It consists of 3 subscales: physical well-being (7 items), social/family well-being (7 items), emotional well-being (6 items), functional well-being (7 items), and the Ovarian Cancer subscale (12 items). This questionnaire has been found to be valid and reliable [21].

Descriptive statistics from the baseline QoL data will be calculated. These will include descriptions of the distribution of QoL scores (mean, standard deviation, median, etc.). For all patients the baseline scores will be calculated using the questionnaire completed at the time of enrollment.

Differences in FACT-O scores between patients receiving FWGE and placebo:

A paired t-test will be used to test the null hypothesis that there is no difference between baseline PF scores and the PF scores weekly and prior to surgery for those patients randomized to FWGE versus placebo.

7. TREATMENT MODIFICATIONS

7.1 It is expected that the dose of FWGE used in this study will be well tolerated; therefore there will be no dose modification. Specimens will be collected from all patients who undergo surgery even if they do not complete the full course of medication.

7.2 Criteria for removal from study:

- 7.31 Patient refusal to continue treatment.
- 7.32 Patient non-adherence to study plan.
- 7.33 Pregnancy
- 7.34 Patient does not have surgery within 2 months of starting FWGE or placebo.
- 7.35 Grade 3 or 4 allergic reaction to FWGE or placebo

8. STUDY PARAMETERS & SERIAL OBSERVATIONS

The patient will be evaluated at the time of enrollment. Quality of life and toxicity will be monitored weekly. The final assessment will occur on the day of surgery. No further surveillance will be performed for this trial.

8.1 Observations and Tests

The following observations and tests are to be performed and recorded:

	Baseline	Weekly	On the day of surgery
History & Physical Exam	x		x
Performance Status	x		x
WBC, Diff, Hgb, Hct, Platelets	x		x
Chemistries: Na, K, Cl, CO2, BUN, creatinine, glucose, bilirubin(T/D), SGOT (AST), SGPT (ALT), Alk Phos,	x		x
CA-125	x		x
2,6 DMBQ			1
Toxicity Assessment Grade 1-4 AEs	2	2	2
QoL Survey	3	3	3
Cancer tissue for paraffin embedded and frozen			x

1. drawn in a red top tube
2. National Cancer Institute's Common Toxicity Criteria – CTC version 4
3. Quality of Life Survey (QoL) – FACT-O

9. EVALUATION CRITERIA

The evaluation criteria for this study are primarily safety, toxicity, and QoL. Secondary assessments are disease response based on CA125, apoptosis and tumor proliferation, 2,6 DMBQ serum levels, and molecular changes as indicated below.

9.1 Safety, Toxicity and Quality of Life: will be assessed as reviewed in section 6.

9.2 Response Criteria

9.21 Evaluation of Biomarkers

Biomarker-based response involves assessing the patient's longitudinal CA125 values. The definition of CA125 response is based on the Gynecologic Cancer Intergroup (GCIG 2005) criteria. CA125 response will be determined and compared between FWGE and placebo treated groups

9.22 2,6-DMBQ determination

2,6 DMBQ serum presence will be determined in FWGE versus placebo-treated groups.

9.23 Proliferation

Immunohistochemistry detection of Ki-67 will be carried out in OVCA obtained from women following treatment with FWGE or placebo. The percentage of positively stained cells relative to total cells in each section will be determined and compared between treatment groups.

10. DURATION OF STUDY

Duration of the intervention, including treatment and surgery will be completed within 4 weeks after beginning of study. If a patient's surgery is delayed, the maximum time a patient will be on this study is 2 months.

11. STUDY MONITORING & REPORTING PROCEDURES

11.1 Definition of Adverse Events (AE)

An adverse event is the development of an untoward medical occurrence, undesirable medical condition, or recurrence or deterioration of a pre-existing medical condition subsequent to exposure to FWGE. The adverse reaction must be reported to Moffitt Cancer Center IRB. This study will utilize the Common Terminology Criteria for Adverse Events version 4.0 (CTCAE v4.0) for toxicity and adverse event reporting. A copy of the CTCAE v 4.0 can be downloaded from the CTEP home page http://ctep.cancer.gov/reporting/ctc.html

11.2 Definition of Serious Adverse Events (SAE)

A serious adverse event is any untoward medical occurrence that:

- Results in death,
- Is life-threatening (defined as an event in which the patient was at risk of death at the time of the events; it does not refer to an event which hypothetically might have caused death if it were more severe),
- Requires inpatient hospitalization or causes prolongation of existing hospitalization.
- Results in persistent or significant disability/incapacity,
- Overdose

All grade 4 and 5 adverse events are by definition serious, and must be reported within 24 hours of being aware of the event, with follow-up reporting of outcome. Grade 2 or 3 adverse events that require or prolong hospitalization, that result in persistent or significant disability, or that are events that jeopardize the patient and require intervention to avoid their becoming fatal, life-threatening, or disabling are also serious and must be reported as soon as the investigator becomes aware of the event.

Collection of complete information concerning grade 3-4 SAEs is extremely important. Full description of each event will be followed. Thus, follow-up information which becomes available as the SAE evolves, as well as supporting documentation (e.g., hospital discharge summaries and autopsy reports), should be collected subsequently, if not available at the time of the initial reports, and within 24 hours sent using the same procedures as the initial SAE reports.

12.0 STATISTICAL CONSIDERATIONS

12.1 Study Design: This study is a randomized, double-blind, placebo-controlled, trial of the effect of FWGE on women with epithelial ovarian cancer scheduled to undergo surgery. As noted above, we recognize that the sample size in the current study will prohibit definitive statistical conclusions to be drawn on our primary objective (to test the safety and tolerability of FWGE), or our secondary objectives (to compare levels of FWGE active product, CA125 levels, proliferation, and gene expression in OVCAs from subjects receiving FWGE versus placebo). The study is neither designed nor powered to develop statistically significant results, rather, we seek to develop preliminary/pilot data that will enable us to pursue a future large phase II, peer-reviewed grant-funded

trial of adjuvant FWGE in women undergoing therapy for ovarian cancer. Preliminary data developed in the current proposal will support such grant applications.

12.2 Primary outcome variable: The principal parameters to be collected, analyzed and reported will be:

- patient and disease characteristics: age, race, co-morbidities, cancer histologic type and stage. Optimal versus suboptimal surgical cytoreduction.
- differences in toxicity
- differences in quality of life

For each measure, Student's t-test will be used to identify differences between FWGE- versus placebo-treated OVCAs.

12.3 Secondary outcome variables for placebo versus FWGE:
-the presence of 2,6-dimethoxy-p-benzoquinone in the serum
-the change in the tumor marker CA125,
-tumor proliferation as measured by Ki-67.

For each measure, Student's t-test will be used to identify differences between FWGE- versus placebo-treated OVCAs.

12.4 Secondary outcome variables for genomic changes in ovarian cancer tissue for placebo versus FWGE.

Expression values will be calculated using the robust multi-array average (RMA) algorithm implemented in the Bioconductor (http://www.bioconductor.org) extensions to the R statistical programming environment.

Student's t-test will be used to identify differentially expressed genes in comparisons between FWGE- versus placebo-treated genomic data. Differentially expressed genes will analyzed using GeneGO MetaCore™ software to identify represented biologic pathways.

12.5 Accrual, Sample Size, and Study Duration: The study plan is a one-stage, randomized, double-blind, clinical trial. Patients will be randomized with equal

probability to receive either FWGE or placebo. The study will accrue 20 patients. It is anticipated that the accrual of this many patients will be achieved in 18 months. Less than 10% of the patients registered are expected to be lost due to follow-up or any other reason, so 20 accruals should allow for 18 evaluable cases.

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FACT-O (Version 4)

Below is a list of statements that other people with your illness have said are important. **Please circle or mark one number per line to indicate your response as it applies to the <u>past 7 days</u>.**

	PHYSICAL WELL-BEING	**Not at all**	**A little bit**	**Some -what**	**Quite a bit**	**Very much**
GP1	I have a lack of energy	0	1	2	3	4
GP2	I have nausea	0	1	2	3	4
GP3	Because of my physical condition, I have trouble meeting the needs of my family	0	1	2	3	4
GP4	I have pain	0	1	2	3	4
GP5	I am bothered by side effects of treatment	0	1	2	3	4
GP6	I feel ill	0	1	2	3	4
GP7	I am forced to spend time in bed	0	1	2	3	4

	SOCIAL/FAMILY WELL-BEING	**Not at all**	**A little bit**	**Some -what**	**Quite a bit**	**Very much**
GS1	I feel close to my friends	0	1	2	3	4
GS2	I get emotional support from my family	0	1	2	3	4
GS3	I get support from my friends	0	1	2	3	4
GS4	My family has accepted my illness	0	1	2	3	4

GS5	I am satisfied with family communication about my illness	0	1	2	3	4
GS6	I feel close to my partner (or the person who is my main support)	0	1	2	3	4
Q1	*Regardless of your current level of sexual activity, please answer the following question. If you prefer not to answer it, please mark this box* ☐ *and go to the next section.*					
GS7	I am satisfied with my sex life	0	1	2	3	4

Please circle or mark one number per line to indicate your response as it applies to the <u>past 7 days</u>.

	<u>EMOTIONAL WELL-BEING</u>	**Not at all**	**A little bit**	**Some -what**	**Quite a bit**	**Very much**
GE1	I feel sad	0	1	2	3	4
GE2	I am satisfied with how I am coping with my illness	0	1	2	3	4
GE3	I am losing hope in the fight against my illness	0	1	2	3	4
GE4	I feel nervous	0	1	2	3	4
GE5	I worry about dying	0	1	2	3	4
GE6	I worry that my condition will get worse	0	1	2	3	4

	FUNCTIONAL WELL-BEING	Not at all	A little bit	Some -what	Quite a bit	Very much
GF1	I am able to work (include work at home).....	0	1	2	3	4
GF2	My work (include work at home) is fulfilling.....	0	1	2	3	4
GF3	I am able to enjoy life.....	0	1	2	3	4
GF4	I have accepted my illness	0	1	2	3	4
GF5	I am sleeping well	0	1	2	3	4
GF6	I am enjoying the things I usually do for fun	0	1	2	3	4
GF7	I am content with the quality of my life right now.....	0	1	2	3	4

Please circle or mark one number per line to indicate your response as it applies to the <u>past 7 days</u>.

	ADDITIONAL CONCERNS	Not at all	A little bit	Some -what	Quite a bit	Very much
O1	I have swelling in my stomach area.....	0	1	2	3	4
C2	I am losing weight.....	0	1	2	3	4
C3	I have control of my bowels.....	0	1	2	3	4
O2	I have been vomiting	0	1	2	3	4
B5	I am bothered by hair loss	0	1	2	3	4
C6	I have a good appetite	0	1	2	3	4

C7	I like the appearance of my body.....	0	1	2	3	4
BMT5	I am able to get around by myself.....	0	1	2	3	4
B9	I am able to feel like a woman.....	0	1	2	3	4
O3	I have cramps in my stomach area.....	0	1	2	3	4
BL4	I am interested in sex	0	1	2	3	4
BMT7	I have concerns about my ability to have children	0	1	2	3	4

MCC 17883

Protocol Revision 06/09/2017 – Summary of Changes

- Changed minimum timeline needed for FWGE and surgery