Phase 1 Study of Muscadine Grape Extract (MGE) in Advanced Malignancy
Comprehensive Cancer Center of Wake Forest University (CCCWFU)
CCCWFU # 01815

Principal Investigators:

Rhonda L. Bitting, MD
Section on Hematology and Oncology
Wake Forest School of Medicine
Medical Center Boulevard
Winston-Salem, NC 27157

Heidi Klepin, MD, MS
Section on Hematology and Oncology
Wake Forest School of Medicine
Medical Center Boulevard
Winston-Salem, NC 27157

Co-Investigators:
Patricia Gallagher, PhD
Hypertension & Vascular Research Center
Wake Forest School of Medicine

Michael M. Goodman, MD
Section on Hematology and Oncology
Wake Forest School of Medicine

Stefan C. Grant, MBBCh, MD, JD, MBA
Section on Hematology and Oncology
Wake Forest School of Medicine

Glenn J. Lesser, MD
Section on Hematology and Oncology
Wake Forest School of Medicine

Susan Melin, MD
Section on Hematology and Oncology
Wake Forest School of Medicine

Boris Pasche, MD
Section on Hematology and Oncology
Wake Forest School of Medicine

W. Jeffrey Petty, MD
Section on Hematology and Oncology
Wake Forest School of Medicine

Mercedes Porosnicu, MD
Section on Hematology and Oncology
Wake Forest School of Medicine

Jimmy Ruiz, MD
Section on Hematology and Oncology
Wake Forest School of Medicine

Paul Savage, MD

Section on Hematology and Oncology
Wake Forest School of Medicine

Steven Sorscher, MD
Section on Hematology and Oncology
Wake Forest School of Medicine

Ann Tallant, PhD
Hypertension & Vascular Research Center
Wake Forest School of Medicine

Christopher Thomas, MD
Section on Hematology and Oncology
Wake Forest School of Medicine

Pierre L. Triozzi, MD
Section on Hematology and Oncology
Wake Forest School of Medicine

George Yacoub, MD
Section on Hematology and Oncology
Wake Forest School of Medicine

Biostatistician: Janet Tooze, PhD
Comprehensive Cancer Center of Wake Forest University

Scott Isom, MS
Comprehensive Cancer Center of Wake Forest University

Study Coordinator: Shannon Golden, MA
Department of Social Sciences & Health Policy
Wake Forest School of Medicine

Katherine Pleasant
Department of Epidemiology & Prevention
Wake Forest School of Medicine

Regulatory Contact: Megan Farmer, MS
Comprehensive Cancer Center of Wake Forest University

Alexandra Bolick
Comprehensive Cancer Center of Wake Forest University

Research Nurses (RN): Jennifer Thomas
Comprehensive Cancer Center of Wake Forest University

Data Manager: Deanna Hissim
Comprehensive Cancer Center of Wake Forest University

Other Team Members:

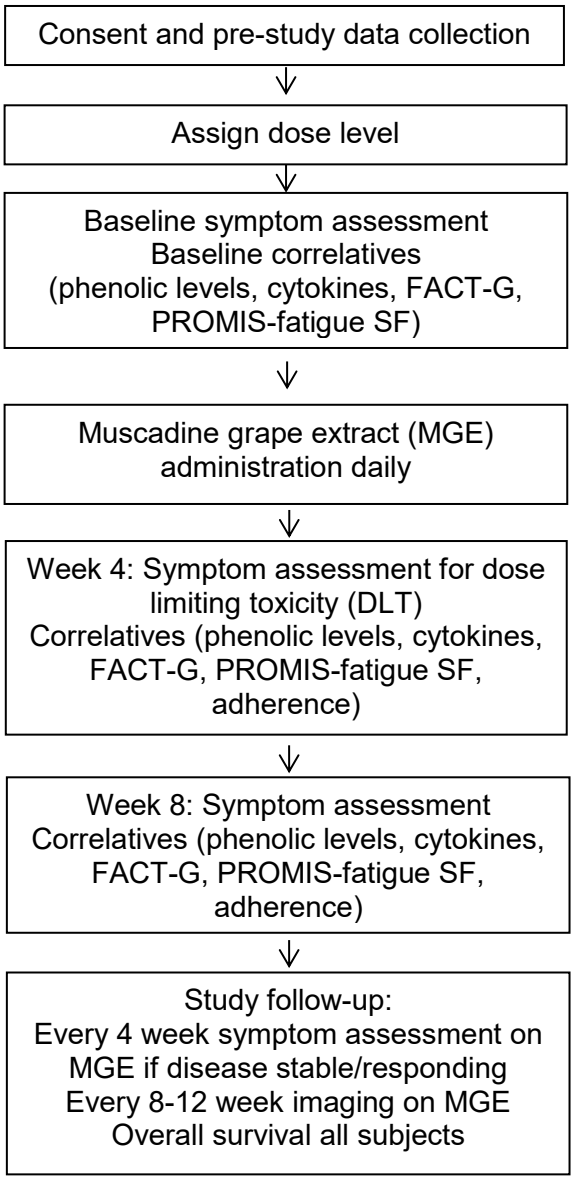
Jasmine Miller
Comprehensive Cancer Center of Wake Forest University

Participating Institutions: CCCWFU
Version Date: 12/22/15
Amended: **10/5/18 (IRB Amendment #26)**
Confidential

Table of Contents

~ Apendices are now separate attachments in the eIRB under *Protocol Document*

SCHEMA


Consent and pre-study data collection
Assign dose level
Baseline symptom assessment
Baseline correlatives
(phenolic levels, cytokines, FACT-G, PROMIS-fatigue SF)
Muscadine grape extract (MGE) administration daily
Week 4: Symptom assessment for dose limiting toxicity (DLT)
Correlatives (phenolic levels, cytokines, FACT-G, PROMIS-fatigue SF, adherence)
Week 8: Symptom assessment
Correlatives (phenolic levels, cytokines, FACT-G, PROMIS-fatigue SF, adherence)
Study follow-up:
Every 4 week symptom assessment on MGE if disease stable/responding
Every 8-12 week imaging on MGE
Overall survival all subjects

1.0 Introduction and Background

It is estimated that one-third of the more than 7 million deaths from cancer worldwide is attributable to potentially modifiable risk factors, with 374,000 deaths preventable through diet modification alone (not including those attributable to obesity or physical inactivity).[1] Diet supplementation for the prevention of cancer is an attractive mechanism to control cancer, since it is easy to implement to a broad population, even populations with reduced incomes and resources. Grape extracts or active components isolated from grapes have received attention as chemopreventive agents based upon their anti-proliferative, anti-inflammatory and anti-oxidant properties.[2]

The muscadine grape (*Vitis rotundifolia*), found in the warm, humid climate of southeast United States, has a distinct phytochemical composition as compared to other grape varieties. The muscadine grape contains a high concentration of anthocyanin 3,5-diglucosides, ellagic acid, ellagic acid precursors, gallic acid, flavan-3-ols and flavonols.[3] Several preclinical studies with muscadine grape products have revealed anti-tumor activity, including inhibition of tumor cell growth and induction of apoptosis.[4-7] Evidence from preclinical trials also suggests that muscadine grape products may decrease systemic inflammation.[8-11] Higher levels of inflammation may not only increase cancer recurrence risk,[12] but also impair quality of life. Higher levels of systemic inflammation are associated with increased fatigue[13], reduced levels of physical activity, adiposity[14,15] and lower levels of physical function[16-18]. By reducing levels of circulating inflammatory markers such as CRP and IL-6, muscadine grape products may improve cancer outcomes by decreasing symptom burden (particularly fatigue).

Despite reports indicating potential anti-tumor activity,[4-6] there are limited clinical studies on the efficacy of muscadine grape products in the prevention or treatment of cancer, representing an opportunity for novel investigations. The current study proposes to build upon promising preclinical and clinical studies to assess the safety and tolerability of a proprietary muscadine grape extract (MGE) formulation, made from muscadine grape skins and seeds and produced by Nature's Pearl, in a Phase 1 clinical trial of patients with metastatic solid tumors who have failed prior standard therapies.

1.1 Preclinical Studies

Literature overview: The anti-tumor properties of muscadine grapes have been demonstrated in preclinical models.[4-7] Porter et al examined the effects muscadine grape extract on lung tumor formation in the fetus following transplacental exposure to a polycyclic hydrocarbon. Results indicated that muscadine exposure through drinking water reduces both tumor proliferation and angiogenesis, leading to a decrease in tumor burden and multiplicity in mouse models.[19] Exposure to muscadine grape extract through drinking water similarly reduced breast tumor burden and multiplicity in c-neu mice.[20] In a study by God et al, four varieties of muscadine grape were tested and showed significant inhibition of 2-aminoanthracene mutagenesis, high antioxidant activity, and the ability to inhibit activities of metalloproteinases, implying that these muscadine products could be good inhibitors of carcinogenesis.[6] In colon carcinoma cells, muscadine exposure induced cell death.[4,5] In prostate cancer cell lines, treatment with muscadine grapes induced both cell cycle arrest and apoptosis.[7] One mechanism by which muscadine grapes cause apoptosis is by downregulating the phosphatidylinositol 3-kinase (PI3K)/Akt survival pathway.[7] The PI3K/Akt pathway is a key oncogenic signaling pathway that has been linked to tumorigenesis and resistance to anticancer therapies in a wide variety of tumor types.[21] A study by Burton et al examined the effects of muscadine grape product on bone turnover in prostate and breast cancer cell models overexpressing Snail transcription

factor.[22] They show that Snail regulation can be antagonized by muscadine exposure, leading to decreased cell invasion, migration and bone turnover, and thus suggesting that muscadine grapes could be a promising bioactive treatment for bone metastatic cancer.

Overview of preliminary data from the investigative team: Preliminary studies from the investigative team (Gallagher/Tallant) demonstrate that muscadine grape seeds and skins reduced the proliferation of both human breast and prostate cancer cell lines. The reduction in breast cancer cell growth was associated with a decrease in activated mitogen activated protein (MAP) kinase, a critical enzyme involved in cell proliferation. In addition, muscadine grape liquid extract reduced the tumor burden in a transgenic model of spontaneous breast cancer with a concomitant attenuation in proliferation, tumor blood vessel formation, and tumor-associated fibrosis. These data are described below and provide additional rationale to assess the efficacy of Nature's Pearl proprietary muscadine grape extract (MGE) on cancer outcomes in clinical studies.

Inhibition of Prostate Cancer Cell Proliferation by Muscadine Grape Components

Actively growing human LnCaP or PC3 prostate cancer cells were plated into individual wells of 24 well plates and treated for 7 days with increasing concentrations of extracts from either muscadine grape seeds or skins, to determine whether the extracts reduced prostate cancer cell proliferation. As shown in **Figure 1**, extracts from both grape seeds and skin reduced the proliferation of LnCaP or PC3 human prostate cancer cells. The responses were dependent upon the dose of extract that was used and similar responses were obtained with grape seed extract compared to grape skin extract.

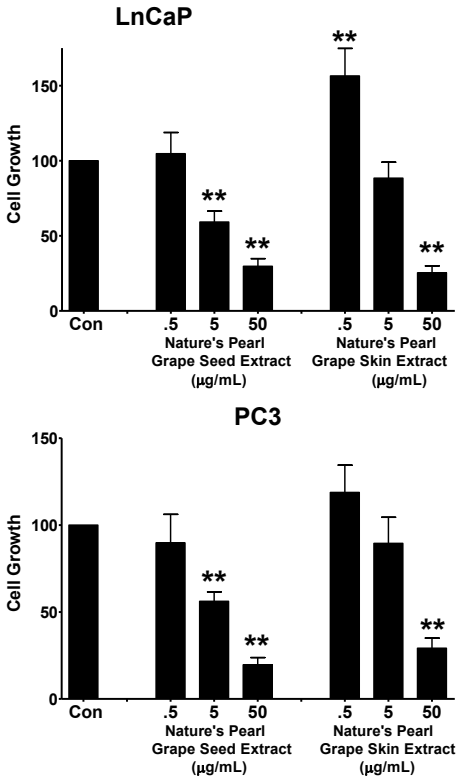


Figure 1. LnCaP or PC3 human prostate cancer cells were incubated with increasing concentrations of either muscadine grape seed or grape skin extracts and cell proliferation was quantified by counting the number of cells per well. The data are presented as the percent of the Control (Con), which was not treated with either extract. n = 12, ** denotes $p < 0.01$.

Inhibition of Breast Cancer Cell Proliferation by Muscadine Grape Components

Actively growing human breast cancer cells—ZR-75-1 ER+ breast cancer cells, MDA-MB-231 or SKBR3 triple negative breast cancer cells--were plated into individual wells of 24 well plates and treated for 7 days with increasing concentrations of extracts from either muscadine grape seeds or skins, to determine whether the extracts reduced breast cancer cell proliferation. As shown in **Figure 2**, extracts from both grape seeds and skin reduced the proliferation of ZR-75-1, MDA-MB-231 and SKBR3 human breast cancer cells. The responses were dependent upon the dose of extract that was used and similar responses were obtained with grape seed extract compared to grape skin extract.

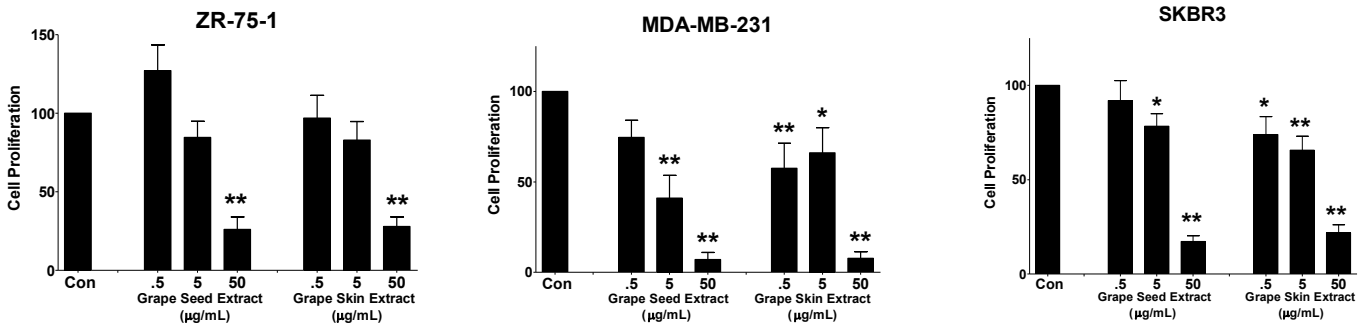


Figure 2. ZR-75-1, MDA-MD-23 or SKBR3 human breast cancer cells were incubated with increasing concentrations of either muscadine grape seed or grape skin extracts and cell proliferation was quantified by counting the number of cells per well. The data is presented as the percent of the Control (Con), which was not treated with either extract. n = 9 for ZR-75-1 cells, 6 for MDA-MB-231 cells and 18 for SKBR3 cells; * denotes $p < 0.05$, ** denotes $p < 0.01$.

Inhibition of MAP Kinase Activity by Muscadine Grape Components

Human MDA-MB-231 or ZR-75-1 breast cancer cells were incubated with 50 µg/mL muscadine grape seed or grape skin extract for increasing periods of time. MAP kinase activity was quantified in cell extracts by Western blot hybridization using an antibody against phospho-ERK1/ERK2 from Cell Signaling. The decrease in phospho-ERK activity was calculated by densitometry using β-actin as a loading control. As shown in **Figure 3**, the extracts from both muscadine grape seeds and grape skins caused a significant reduction in phospho-ERK, suggesting that the extracts significantly reduced proliferation. There was no significant difference in the response to grape seed versus grape skin extracts.

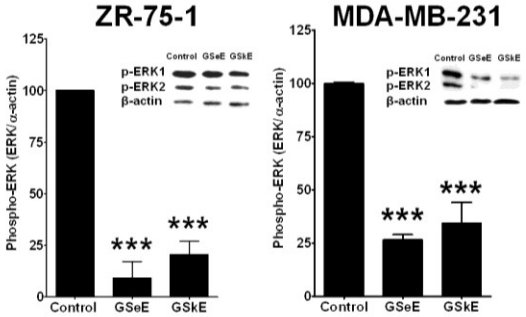


Figure 3. ZR-75-1 and MBA-MD-231 human breast cancer cells were incubated with 50 µg/mL muscadine grape seed extract (GSeE) or muscadine grape skin extract (GSkE) for 4 h and phospho-ERK (both ERK1 and ERK2) were quantified by densitometry using β-actin as a loading control. n = 3-7, *** denotes $p < 0.001$.

Because treatment with muscadine grape seeds versus skins showed no significant difference in effect on cancer cell lines, the additional preclinical studies were performed with a muscadine grape liquid extract containing both seeds and skins.

Reduction in Tumor Growth by Muscadine Grape Liquid Extract in c-neu Mice

The effect of muscadine grape liquid extract (MGLE) was tested on female FVB-Tg(MMTV) NKAMul/J transgenic mice, in which the activated rat Erbb2 (c-neu) oncogene, under the direction of the mouse mammary tumor virus promoter expressed specifically in the mammary gland (c-neu mice), form mammary tumors (the equivalent of human breast cancer) by 6 - 8 months of age. Mice were treated with MGLE beginning at 3 weeks of age (at the time of weaning) and were sacrificed at 7½ months of age. The dose of MGLE was approximately 1 mg of phenolics for a 25 gram mouse (and is equivalent to approximately 10 tablespoons or 148 mL of extract per day for a 70 kg man and an average concentration of 20,000 mg of phenolics/liter of MGLE). The muscadine grape liquid extract was added to the drinking water. Eighteen mice were treated for an average of 220 days (beginning treatment at 21 days of age, at the time of weaning). Ten (10) mice drank regular drinking water (control) and 8 mice drank water containing the MGLE. Although all of the mice developed tumors throughout their mammary tissue, there was a significant difference in the weight of the tumor mass, as shown in **Figure 4**.

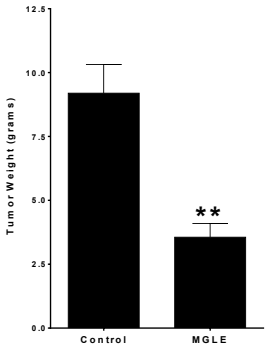


Figure 4. Mice (c-neu) were given MGLE in their drinking water beginning at 3 weeks of age. Control mice drank regular water. The mice were euthanized after 220 days and their mammary tumors were removed and weighed. n = 8-10, ** indicates $p < 0.01$.

Mammary tissue was fixed in 4% formalin, paraffin embedded and sectioned into 5 micron sections, for immunohistochemical analysis. Sections were incubated with an antibody to Ki67, as a measure of cell proliferation. As shown in **Figure 5**, tumors from mice that drank MGLE had a significant decrease in the number of proliferating cells. These results indicate that muscadine grape liquid extract reduces cell proliferation, in agreement with reduction in cell number and MAP kinase activation observed in cultured breast cancer cells.

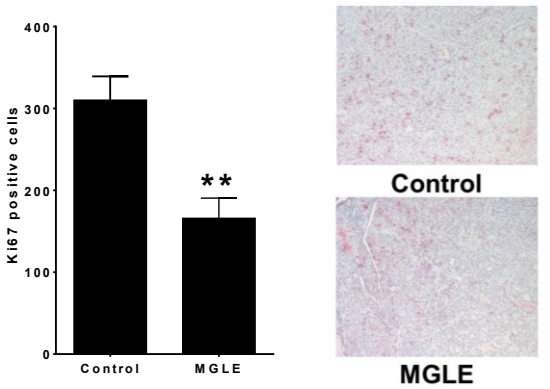


Figure 5. Sections of tumors from mice drinking water (Control) or MGLE were incubated with an antibody to the proliferative marker Ki67. Representative samples are shown to the left. n = 6–8, ** denotes $p < 0.01$.

Tumor sections from c-neu mice drinking regular water (control) or MGLE were incubated with an antibody to the endothelial cell marker, CD34, and vessels were identified by a combination of morphology and positive CD34 immunoreactivity. As shown in **Figure 6**, tumors from mice drinking MGLE had fewer blood vessels than mice drinking regular water, suggesting that the grape extract inhibits angiogenesis.

Interstitial tumoral fibrosis was quantified in breast tumor tissue sections from c-neu mice and stained with picrosirius red, a nonspecific collagen stain. Collagen reaction product was markedly reduced by MGLE administration as compared to controls (**Figure 7**). Treatment with the MGLE reduced interstitial fibrosis by more than 50%, indicating that the extract reduces cancer-associated fibrosis in breast tumors.

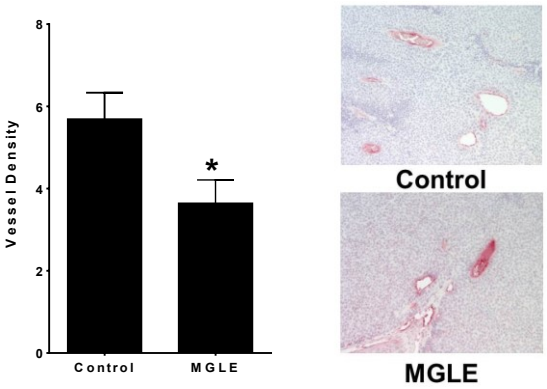


Figure 6. Sections of tumors from Control or MGLE-treated mice were incubated with an antibody to CD34 and blood vessels were identified by positive immunoreactivity and morphology. n = 8-10, ** denotes p< 0.05.

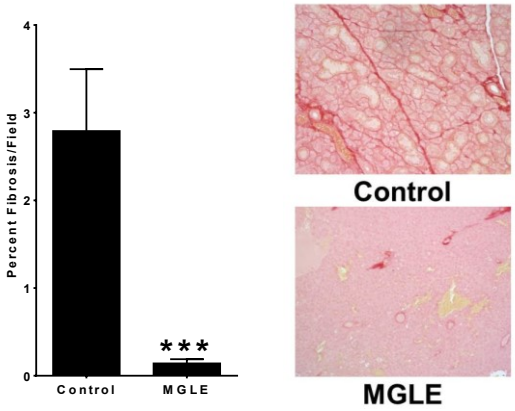


Figure 7. Sections of tumors from Control or MGLE-treated mice were stained with Picrosirius red to measure tissue fibrosis. n = 8-10, *** denotes p < 0.001

1.2 Preclinical toxicity studies with MGE

The animal studies above were performed with muscadine grape liquid extract from Nature's Pearl (Advance, NC), which was added directly to the rodent drinking water. To study the effects of muscadine grape extract in humans, the muscadine grape liquid extract is dried, converted to powder form, and encapsulated. This proprietary product produced by Nature's Pearl is the muscadine grape extract (MGE) used in the preclinical toxicity studies below and in the phase 1 clinical study proposed. However, because rodents do not swallow capsules, the MGE powder is reconstituted and added to the drinking water for the toxicity studies described below.

The primary goal of the rodent toxicity tests on the Nature's Pearl MGE was to identify the range of doses that caused no life-threatening or adverse effects, thereby providing critical information for conduct of a Phase I clinical trial to assess toxicity in humans with cancer and establishing an estimated clinical margin of safety. Toxicity tests on the proprietary MGE that will be used in clinical testing were performed in mice, to assess effects of the extract on the general health of the mice as well as their hearts, liver, lungs and kidneys.

Male C57 black mice (8 weeks of age) were randomized into groups to receive drinking water alone (control) or MGE in the drinking water at four escalating doses of MGE as determined by the measurement of total phenolics. Daily observations of the mice included evaluations for weight loss, diarrhea, dehydration, edema, abdominal enlargement or ascites, progressive dermatitis, rough hair coat or unkempt appearance due to lack of grooming, hunched posture indicative of pain, lethargy or persistent decumbency due to loss of appetite, coughing, labored breathing, nasal discharge, jaundice, cyanosis, pallor/anemia, neurological signs indicated by inappropriate head carriage or shaking of the head, bleeding from any orifice, or any condition interfering with daily activities. Prior to sacrifice, the mice were placed in metabolic cages for 24 hours, and food intake and fecal and urine output were measured to quantify markers of renal damage. During the 4th week of treatment, blood pressure was determined by tail cuff plethysmography in conscious mice and cardiac function was assessed in mice anesthetized with isofluorane, using the non-invasive, small animal VEVO ultrasound imaging system in the M and B modes, to measure or calculated ejection fraction, fractional shortening, stroke volume, heart rate and cardiac output were determined. The mice were sacrificed after one month of treatment; tissues (heart, kidney, lung, liver, spleen and brain) were weighed, fixed in 4% formalin, embedded in paraffin, sectioned at 5 microns and stained with Hematoxylin & Eosin (H & E) for analysis by a veterinary pathologist to assess any gross structural abnormalities.

Four concentrations of the MGE were tested. The range of concentrations to be tested was based upon previous studies in the Gallagher/Tallant laboratory reported above. In those studies, a daily concentration of 1 mg of total phenolics/mouse/day was effective at reducing tumor multiplicity. For the toxicity studies in mice, the four doses of the MGE tested were 0.25, 0.5, 1.0 and 2.0 mg total phenolics/mouse/day, administered to the mice in their drinking water. Total phenolic content was determined using the Folin-Ciocalteau reagent with gallic acid as the standard. Since an average mouse of this age weighs approximately 25 g or 0.025 kg, this corresponds to a dose range of 10, 20, 40 and 80 mg total phenolics/kg/day. Based upon an average mass of 70 kg for a patient, this would correspond to 700, 1400, 2800, and 5600 mg phenolics/day. The proprietary Nature's Pearl muscadine grape extract capsule contains approximately 162 mg total phenolics/capsule, this would correspond to taking 5, 10, 21 and 42 capsules/day.

Results

During the 4 week administration of the MGE at the four doses, there were no observations of diarrhea, dehydration, edema, abdominal enlargement, loss of hair coat, reduced grooming, or decreased level of activity, appetite, drinking or breathing. There were no indications of pallor, jaundice or cyanosis or any neurological signs of discomfort. There was no bleeding or discharge. These results suggest that the mice were free from any pathology that affected their normal daily activities or health.

The mice were weighed at the end of the treatment period, to determine the effect of the MGE on total body weight. The weight of the mice at the end of the 4-week treatment period was similar with the three lowest concentrations of the MGE compared to the control group. Mice treated with the highest concentration of MGE had a small but significant decrease in total body weight (from 28.6 ± 1.2 grams to 25.0 ± 1.3 grams, which represents less than 15% of their total body weight). However, there was no effect on food intake, fecal output or urine volume compared to the untreated mice, measured during the last week of treatment, suggesting little effect of the MGE on eating and drinking behaviors.

At the time of sacrifice, various organs (heart, lung, liver, kidney, spleen and brain) were removed and weighed and the organ weights of mice treated with each dose of MGE were compared to the untreated control group. A portion of each organ was fixed in formalin, sectioned and stained with H & E, for assessment by a veterinary pathologist. In addition, the heart and kidney were assessed by echocardiography and radioimmunoassay of urinary proteins, respectively, as a further measure of heart and renal function. There was no difference in the weight or structure of the hearts of mice treated with increasing doses of MGE. Cardiac parameters including ejection fraction, fractional shortening, cardiac output, stroke volume and heart rate were all similar in mice treated with MGE compared to untreated Control mice. There was no difference in the weight or structure of the lungs of mice treated with MGE compared to the untreated control mice. The weight of the livers of mice treated with the two lowest MGE (0.25 and 0.5 mg phenolics/mouse/day) and the highest MGE (2.0 mg phenolics/mouse/day) concentrations were no different than the weight of the livers of the untreated mice. However, the weight of the livers of mice in the 1.0 mg phenolics/mouse/day) were slightly increased compared to the control. Spleen weight of mice treated with MGE was not significantly different than untreated control mice and the spleens of mice were generally within normal limits with one mouse in the highest MGE treatment group having some extra medullary hematopoiesis. The weight of the brains of mice treated with MGE was not significantly different than untreated mice. The brains were subdivided into four regions—cortex, cerebellum, hypothalamus and brainstem. All brain regions examined were within normal limits.

Conclusions from toxicity studies and corresponding clinical doses:
Little toxicity was observed at any of the doses tested. Therefore, the level of phenolics used to treat the mice suggests that this level of phenolics and corresponding numbers of capsules will likely be tolerated by patients. However, based upon patient compliance, a recommended dose range for patients would include 2, 4, 6, 8 and 10 capsules/day (corresponding to approximately 5, 9, 14, 18 and 23 mg phenolics/kg/day).

1.3 Clinical Studies

Muscadine grape skin extract has been evaluated in clinical trials to a limited extent in the setting of cardiovascular disease[23] and diabetes.[24] In a randomized, double-blind, placebo-controlled crossover trial, 50 adults with coronary disease or ≥1 cardiac risk factor received muscadine grape seed supplementation (1300 mg daily) and placebo for 4 weeks each, with a 4-week washout, and no adverse events were reported.[23] Similarly, a randomized study of non-diabetic and type 2 diabetic participants using 150 mL of muscadine grape juice, muscadine grape wine, and dealcoholized muscadine grape wine over a 28-day period had no adverse events reported.[24]

A muscadine grape product has also been examined in a phase 1/2 trial of men with biochemically recurrent prostate cancer.[25] The phase 1 portion of the study evaluated increasing doses of pulverized muscadine grape skin in a product called MPX (Muscadine Naturals Inc, Clemmons NC) in cohorts of two patients, with six patients at the highest dose of 4,000 mg (n=14). Dose selection was based on unpublished preclinical work in animal studies. Four patients experienced adverse gastrointestinal symptoms possibly related to the study agent, including grade 1 flatulence, grade 1 soft stools, and grade 1 burping. No other related adverse events were reported and one patient reported improvement of chronic constipation. Six of the 14 patients came off study for disease progression (five metastatic, one rising PSA) after exposure for a median of 15 months.

One patient came off study for myasthenia gravis that was unrelated to treatment. Seven patients remained on the study. The lack of dose-limiting toxicities led to the selection of 4,000 mg/d as the highest dose for further study. Median within-patient PSA doubling time increased by 5.3 months (non-significant, P=0.17). Based on the favorable safety profile and a possible lengthening of PSA doubling time, the phase 2 portion of this study was expanded to 125 patients randomized to placebo, 500 mg/d, or 4000 mg/d MPX arms. Recruitment was completed in October 2014 with results pending.

In summary, muscadine grape products have shown anti-cancer activity in preclinical models and have demonstrated a favorable safety profile in preclinical and clinical studies. This Phase 1 study using MGE represents an opportunity to translate promising preclinical data supporting the anti-cancer properties of a natural product into clinical trials.

2.0 Objectives

2.1 Primary Objective

2.1.1 To determine the safety and MTD of MGE after 4 weeks of administration for patients with metastatic solid tumors.

2.2 Secondary Objectives

2.2.1 To monitor adverse events/toxicity every 4 weeks while on treatment

2.2.2 To evaluate change in phenolic levels (total and component, blood and urine) from baseline to 4 and 8 weeks on MGE

2.2.3 To evaluate change in serum cytokines and growth factors from baseline to 4 and 8 weeks on MGE

2.2.4 To observe the response rate of MGE in patients with metastatic cancer.

2.2.5 To assess overall and progression-free survival in patients with metastatic cancer receiving MGE.

2.2.6 To assess global quality of life (FACT-G) and fatigue (PROMIS-fatigue short form) in cancer patients taking MGE.

2.2.7 To assess adherence to MGE treatment.

3.0 Patient Selection

3.1 Inclusion Criteria

3.1.1 Patients must have histologically confirmed solid tumor malignancy (excluding primary brain tumor) that is metastatic or unresectable and have failed standard therapies. Patients are also eligible if patients declined (or if their physicians determined them unsuitable for) standard therapy options.

3.1.2 ≥ 18 years of age

3.1.3 The effects of MGE on the developing human fetus are unknown. Women of child-bearing potential and men must agree to use adequate contraception (hormonal or barrier method of birth control; abstinence) prior to study entry and for the duration of study participation. Should a woman become pregnant or suspect she is pregnant while participating in this study, she should inform her treating physician immediately.

3.1.4 Ability to understand and the willingness to sign an IRB-approved informed consent document.

3.1.5 ECOG performance status ≤2.

3.1.6 Patients must have adequate organ and marrow function as defined below:
- absolute neutrophil count ≥1000/mcL
- platelets ≥50,000/mcL
- total bilirubin within normal institutional limits
- AST(SGOT)/ALT(SGPT) ≤2.5 X institutional upper limit of normal
- creatinine clearance ≥40 mL/min

3.1.7 Stable supplement usage for >2 weeks prior to starting and agrees not to change while on this study.

3.1.8 Life expectancy ≥ 3 months

3.1.9 Patients who declined standard therapies or whose physicians determined they were not suitable for standard therapy options are eligible.

3.2 Exclusion Criteria

3.2.1 Patients who have had chemotherapy or radiotherapy within 2 weeks (6 weeks for nitrosoureas or mitomycin C) prior to entering the study or those who have not recovered from adverse events due to agents administered more than 2 weeks earlier.

3.2.2 Patients may not be receiving any other investigational cancer-directed agents.

3.2.3 History of allergic reactions attributed to compounds of similar chemical or biologic composition to MGE.

3.2.4 Patients unable to take oral medications or those with history of malabsorption due to bowel resection or gastrointestinal disease.

3.3.5 Patients with uncontrolled diarrhea or persistent nausea/vomiting requiring daily antiemetic therapy for symptom management within the past 21 days

3.2.6 Uncontrolled intercurrent illness including, but not limited to ongoing or active infection, symptomatic congestive heart failure, unstable angina

pectoris, cardiac arrhythmia, or psychiatric illness/social situations that would limit compliance with study requirements.

3.2.7 Pregnant women are excluded from this study because of potential for teratogenic or abortifacient effects with using MGE. Because there is an unknown but potential risk for adverse events in nursing infants secondary to treatment of the mother with MGE, breastfeeding should be discontinued.

3.2.8 Patients with primary brain tumors are excluded due to lack of data on MGE crossing the blood brain barrier. Patients with metastatic brain tumors may be enrolled.

3.3 Inclusion of Women and Minorities

Men and women of all races and ethnicities who meet the above-described eligibility criteria are eligible to participate in this study.

The study consent form will also be provided in Spanish for Spanish-speaking participants. We anticipate enrolling between 2 and 30 participants on this study. Based on CCCWFU population estimates, we expect approximately 40% of participants to be women. Translating this to our maximum sample size estimate of 30, we plan to enroll at least 12 women if we reach our maximum accrual. We do not expect the percentage of Hispanic/Latino or racial minority cancer patients eligible for this study to be higher than the percentage of Hispanic or racial minority new cancer patients seen at CCCWFU (1.7% and 14.4%, respectively); therefore, we plan to enroll at least 4 racial minority and 1 Hispanic/Latino patient if we reach our maximum accrual.

Should we not meet or exceed these estimates, the PI will engage the Cancer Center Health Equity Advisory Group to discuss strategies to enhance recruitment in these target populations.

4.0 Registration Procedures

Patients **must** be registered prior to the initiation of treatment.

You must perform the following steps in order to ensure prompt registration of your patient:

1. Complete the Eligibility Checklist (Appendix B)
2. Complete the Protocol Registration Form (Appendix A)
3. Alert the Cancer Center registrar by phone, *then* send the signed Informed Consent Form, Eligibility Checklist and Protocol Registration Form to the registrar, either by fax or e-mail. Protocol Registration is open from 8:30 AM - 4:00 PM, Monday-Friday.
   Contact Information:
   Protocol Registrar PHONE (336) 713-6767
   Protocol Registrar FAX (336) 713-6772
   Protocol Registrar E-MAIL (registra@wakehealth.edu)
4. Fax/e-mail ALL eligibility source documents with registration. Patients **will not** be registered without all required supporting documents.

Note: If labs were performed at an outside institution, provide a printout of the results. Ensure that the most recent lab values are sent.

To complete the registration process, the Registrar will:

- assign a patient study number
- assign the patient a dose
- register the patient on the study

5.0 Study Outcomes and Study Measures

5.1 Primary Outcome

5.1.1 The primary outcome measure will be dose limiting toxicity (DLT) assessed by SAEs in patients with metastatic cancer receiving MGE under the proposed regimen at 4 weeks.

5.2 Secondary Outcomes

5.2.1 Adverse events/toxicity at weeks 4, 8 and every 4 weeks thereafter if remain on treatment

5.2.2 Identification of measured phenolic levels (total and component phenolics) at baseline,4, and 8 weeks

5.2.3 Evaluate change in systemic cytokines, growth factors from baseline to 4 and 8 weeks including but not limited to: TNFα, IL6, IL6R, IL8, IL1α, IL1β, IGF-1, VEGF

5.2.4 Overall response rate (CR, PR, and SD) at 8 weeks.

5.2.5 PFS and OS in patients with metastatic cancer receiving MGE under the proposed regimen.

5.2.6 Quality of life in cancer patients taking MGE using the Functional Assessment of Cancer Therapy–General (FACT-G) measure and fatigue, as measured by the PROMIS Fatigue-Short Form.

5.2.7 Adherence to MGE treatment, as assessed by patient-reported medication logs and pill count every 4 weeks while on treatment.

6.0 Treatment Plan

6.1 Study-Related Activities

Measures / Forms	Pre-Study[a]	Baseline	Week 2[h]	Week 4[j]	Week 8[k]	Every 4 weeks[k]	Off-study
Informed consent	X						
Demographics	X						
Medical history	X			X	X	X	
Concurrent meds	X			X	X	X	
Physical exam	X			X	X	X	
Vital signs	X			X	X	X	
Weight		X					
Height		X					
ECOG Performance Status	X			X	X	X	
Pill Diary		X		X	X	X[i]	
Adherence assessment			X	X	X		
Quality of life and fatigue assessments		X		X	X	X	
Tumor measurement by imaging[b]	X				X	X[b]	
Tumor markers (if applicable)[c]		X		X	X		
CBC w/diff, platelets	X	X		X	X	X	
Serum chemistries[d]	X	X		X	X	X	
B-HCG[e]	X						
INR check[f]			X				
Phenolic levels (blood and urine)		X		X	X		
Research labs (cytokines and growth factors)		X		X	X		
Adverse event evaluation		X	X	X	X	X	
Vital status[g]							X

[a] Pre-study imaging listed in table must be completed **within** 42 days prior to registration. Additional pre-study activities need not be repeated if performed within 10 days of starting study drug.
[b] CT imaging or other imaging modality will be obtained at baseline (within 42 days of registration) and at 8 weeks (± 7 days). Imaging will then be q12 weeks (± 28 days) for as long as the patient remains on study. CT, PET/CT, or MRI are acceptable imaging modalities and all imaging will be considered per standard of care for patients on active cancer treatments.
[c] Tumor-specific markers may include but are not limited to testosterone level and PSA for prostate cancer patients, CEA for colorectal patients, and CA19-9 for pancreatic cancer patients as performed per standard of care.
[d] Albumin, alkaline phosphatase, total bilirubin, bicarbonate, BUN, calcium, chloride, creatinine, glucose, potassium, total protein, SGOT[AST], SGPT[ALT], sodium.
[e] Serum pregnancy test (women of childbearing potential).
[f] Patients taking warfarin will have INR checked within 2 weeks of starting MGE. This can be performed locally.
[g] Vital status will be assessed by chart review or phone every 6 weeks (± 14 days) after patient comes off study.
[h] Phone contact will occur 2 weeks after each clinic visit (± 3 days) until the Week 8 visit is complete.
[i] Patients will not be asked to complete a pill diary after 6 cycles (24 weeks).
[j] Week 4 study appointments will be scheduled at day 28 ±3 days.
[k] Study appointments at Week 8 and after will be scheduled 28 days after the prior appointment, ± 7 days

6.2 Treatment Administration

Treatment will be administered on an outpatient basis. Reported adverse events and potential risks are described in Section 8.0. No investigational or commercial agents or therapies other than those described below may be administered with the intent to treat the patient's malignancy.

Dose-Escalation Schedule	
Dose Level	**Dose of MGE**
Level 1	1 pill po BID
Level 2	2 pills po BID
Level 3	3 pills po BID
Level 4	4 pills po BID
Level 5	5 pills po BID

6.2.1 Definition of Dose-Limiting Toxicity (DLT)

Dose-limiting toxicity (DLT) will be defined as grade 3 gastrointestinal side effects attributable to treatment or any grade 4 or 5 adverse event attributable to treatment as per the Common Terminology Criteria for Adverse Events (CTCAE) 4.0 criteria. Any adverse event attributable to treatment and resulting in permanent discontinuation of MGE will be considered a DLT. Toxicity will be assessed at 4 weeks. Any DLTs that occur 1 day (day 29) after the 4 week period will be evaluated and considered as potentially attributable to the administration of MGE during the first 4 weeks. The washout period for this drug is 24 hours. Dose escalation will proceed within each cohort as follows:

Number of Patients with DLT at a Given Dose Level	Escalation Decision Rule
0 out of 3	Enter 3 patients at the next dose level.
$\geq$ 2	Dose escalation will be stopped. This dose level will be declared the maximally administered dose (highest dose administered). Three (3) additional patients will be entered at the next lowest dose level if only 3 patients were treated previously at that dose.
1 out of 3	Enter at least 3 more patients at this dose level. • If 0 of these 3 patients experiences DLT, proceed to the next dose level. • If 1 or more of this group suffer DLT, then dose escalation is stopped, and this dose is declared the maximally administered dose. Three (3) additional patients will be entered at the next lowest dose level if only 3 patients were treated previously at that dose.
$\leq$1 out of 6 at highest dose level below the maximally administered dose	This is generally the recommended phase 2 dose (Maximum Tolerated Dose [MTD]). At least 6 patients must be entered at the recommended phase 2 dose.

6.2.2 Dose modification in the event of toxicity

In the event of dose-limiting toxicity, the patient will be removed from the study and the escalation decision rules defined above will be followed. There will be no dose-modifications on study. Either the patient or the treating physician can chose to discontinue MGE at any time. If necessary, MGE can be held for up to 7 days in the first month and for up to 21 days subsequently, as described in Section 6.5.1.

6.3 Study procedures

6.3.1 Recruitment

6.3.1.1 Potentially eligible patients will be recruited from the Wake Forest Comprehensive Cancer Center

6.3.1.2 The research nurse (RN) will be contacted to review eligibility and to consent eligible patients in person.

6.3.1.3 The RN will obtain baseline surveys, clinical information and ensure research labs are drawn and processed per protocol. Pill dairy will be provided at each visit.

6.3.1.4 Study drug (30-day supply) will be provided to participant by the RN.

6.3.2 Follow-up Assessments

6.3.2.1 Study coordinator will contact participants 2 weeks after each clinic visit until week 8 to assess medication adherence and ascertain any potential adverse events.

6.3.2.2 The RN will review AE with the patient at each follow-up visit in clinic, perform pill count, obtain clinical information per protocol and administer surveys at 4 and 8 weeks per protocol. RN will obtain study labs are each visit and provide 30-day supply of MGE at prescribed dose level.

6.4 General Concomitant Medication and Supportive Care Guidelines

Patients should receive *full supportive care*, including transfusions of blood and blood products, antibiotics, antiemetics, etc., as clinically indicated. Anti-inflammatory or narcotic analgesics may be offered as needed. Medications considered necessary for the patient's well-being may be given at the discretion of the managing physician, i.e., chronic treatments for concomitant medical conditions, as well as agents required for life-threatening medical problems, etc. The reason(s) for treatment, dosage, and dates of treatment should be recorded on the flow sheets.

Participants on warfarin should have their INR checked within 2 weeks of starting MGE to ensure there is no clinically significant interaction. Further blood work to monitor warfarin levels are at the discretion of the treating physician per usual care.

6.5 Duration of Therapy

In the absence of treatment delays due to adverse events, treatment may continue until one of the following criteria applies:

- Disease progression, either by standard imaging or clinical parameters per the judgment of the treating physician,
- Intercurrent illness that prevents further administration of treatment,
- Unacceptable adverse event(s),
- Patient decides to withdraw from the study, or
- General or specific changes in the patient's condition render the patient unacceptable for further treatment in the judgment of the treating physician.

6.5.1 Missed doses

If a dose of MGE is missed, the patient will be instructed to take their next dose as scheduled. Missed doses should not be added to the next dose.

6.5.1.1 Missed doses during first 28 days

If the patient is unable to take their dose of MGE **for more than 7 consecutive days** due to unforeseen circumstances unrelated to drug toxicity, then the subject will not be evaluable for the primary outcome and will be removed from the study. Participants who come off study prior to 28 days for reasons other than toxicity will be evaluated for DLT during the washout period (24 hours, per section 6.2.1).

6.5.1.2 Missed doses after first 28 days

If treatment must be held for any reason, it must be restarted **within 21 days** or the patient will be removed from the study.

6.6 Duration of Follow Up

Patients removed from the study for unacceptable adverse events will be followed until resolution or stabilization of the adverse event. Patients who are removed or withdraw from the study for any reason other than toxicity will be followed for vital status only after the 24 hour washout period. Patients will be followed every 6 ± 2 weeks until death for monitoring survival study endpoints.

7.0 Measurement of Effect

Although response is not the primary endpoint of this trial, patients with measurable disease will be assessed by the treating physician, based on the clinical radiologic report and clinical status, per usual care. For the purposes of this study, patients will be reevaluated with imaging 8 weeks (± 7 days) after study initiation and then every 12 weeks (± 28 days) while on MGE.

7.1 Definitions

Evaluable for toxicity (primary outcome): All patients will be evaluable for toxicity from the time of their first treatment with MGE. Per section 6.5.1, patients who miss 7 consecutive days of treatment in the first 28 days for reasons other than toxicity are not evaluable and will be removed from the study and replaced.

Inevaluable for objective response: When no imaging/measurement is done at all at a particular time point, the patient is not evaluable (NE) at that time point.

Measurable disease: Measurable lesions are defined as those that can be accurately measured in at least one dimension (longest diameter in the plane of measurement is to be recorded) with a minimum size of 10 mm by CT scan, 10 mm caliper measurement by clinical exam, or 20 mm by chest X-ray.

Non-measurable lesions: Non-measurable lesions include: bony metastases, leptomeningeal disease, ascites, pleural or pericardial effusion, inflammatory breast disease, lymphangitic involvement of skin or lung, abdominal masses or organomegaly identified by physical exam that is not measurable by reproducible imaging techniques.

Progression-Free Survival is defined as the duration of time from the start of treatment to the time of progression or death. Patients will be followed until disease progression and death.

Overall Survival is defined as the duration of time from the start of treatment to date of death.

7.2 Methods for Evaluation of Measurable Disease

The same method of assessment and the same technique should be used to characterize each identified and reported lesion at baseline and during follow-up whenever possible. Measurements will be obtained from usual care imaging and assessed by the PI(s) in confirmation with the physician of record.

- CT is the best currently available and reproducible method to measure lesions selected for response assessment. MRI and PET/CT are also acceptable.
- Lesions on a chest X-ray may be considered measurable lesions if they are clearly defined and surrounded by aerated lung. However, CT is preferable.
- Clinical lesions will only be considered measurable when they are superficial and ≥10 mm in diameter as assessed using calipers. For the case of skin lesions, documentation by color photography, including a ruler to estimate the size of the lesion, is recommended.
- Tumor markers alone will not typically be used to define response. If markers are initially above the upper normal limit, they must normalize for a patient to be considered in complete response.
- Cytology and histology can be used in rare cases (e.g., for evaluation of residual masses to differentiate between Partial Response and Complete Response or evaluation of new or enlarging effusions to differentiate between Progressive Disease and Response/Stable Disease).

8.0 Adverse Events List and Reporting Requirements

8.1 Adverse Event List for MGE

Expected: Based on previous studies, only minimal AEs are expected, including flatulence, diarrhea, nausea, dyspepsia, and abdominal cramping.

8.2 Adverse Event Characteristics

- **CTCAE term (AE description) and grade:** The descriptions and grading scales found in the revised NCI Common Terminology Criteria for Adverse Events (CTCAE) version 4.0 will be utilized for AE reporting. All appropriate treatment areas should have access to a copy of the CTCAE version 4.0. A copy of the CTCAE version 4.0 can be downloaded from the CTEP web site (http://ctep.cancer.gov).
- **'Expectedness'**: AEs can be 'Unexpected' or 'Expected' (see Section 8.1 above) for expedited reporting purposes only.
- **Attribution** of the AE:
  - Definite: The AE **is clearly related** to the study treatment.
  - Probable: The AE **is likely related** to the study treatment.
  - Possible: The AE **may be related** to the study treatment.
  - Unlikely: The AE **is doubtfully related** to the study treatment.
  - Unrelated: The AE **is clearly NOT related** to the study treatment.

8.3 STRC SAE Reporting Requirements

The Safety and Toxicity Reporting Committee (STRC) is responsible for reviewing SAEs for CCCWFU Institutional studies as outlined in Appendix C. STRC currently requires that all unexpected 4 and all grade 5 SAEs on these trials be reported to them for review. All CCCWFU Clinical Research Management (CRM) staff members assisting a Principal Investigator in investigating, documenting and reporting an SAE qualifying for STRC reporting are responsible for informing a clinical member of the STRC as well as the entire committee via the email notification procedure of the occurrence of an SAE.

8.4 WFUHS IRB AE Reporting Requirements

Any unanticipated problems involving risks to subjects or others and adverse events shall be promptly reported to the IRB, according to institutional policy. Reporting to the IRB is required regardless of the funding source, study sponsor, or whether the event involves an investigational or marketed drug, biologic or device. Reportable events are not limited to physical injury, but include psychological, economic and social harm. Reportable events may arise as a result of drugs, biological agents, devices, procedures or other interventions, or as a result of questionnaires, surveys, observations or other interactions with research subjects.

All members of the research team are responsible for the appropriate reporting to the IRB and other applicable parties of unanticipated problems involving risk to subjects or others. The Principal Investigator, however, is ultimately responsible for ensuring the prompt reporting of unanticipated problems involving risk to subjects or others to the IRB. The

Principal Investigator is also responsible for ensuring that all reported unanticipated risks to subjects and others which they receive are reviewed to determine whether the report represents a change in the risks and/or benefits to study participants, and whether any changes in the informed consent, protocol or other study-related documents are required.

Any unanticipated problems involving risks to subjects or others occurring at a site where the study has been approved by the WFUHS IRB (internal events) must be reported to the WFUHS IRB within 7 calendar days of the investigator or other members of the study team becoming aware of the event.

Any event, incident, experience, or outcome that alters the risk versus potential benefit of the research and as a result warrants a substantive change in the research protocol or informed consent process/document in order to insure the safety, rights or welfare of research subjects.

9.0 Pharmaceutical Information

Muscadine grape extract is a nutraceutical compound.

A list of the adverse events and potential risks associated with the investigational agent administered in this study can be found in Section 8.0.

9.1 Drug Accountability

Drug accountability logs will be provided and maintained by the Wake Forest Investigational Drug Services for the investigative agent (MGE) used under this protocol. These logs shall record quantities of study drug received and quantities dispensed to patients, including lot number, date dispensed, patient identifier number, protocol number, dose, quantity returned, balance remaining, and the initials of the person dispensing the medication.

9.2 Muscadine Grape Extract (MGE)

Product description: MGE will be supplied by Nature's Pearl (Advance, North Carolina). The muscadine grapes are pressed and processed on site per good manufacturing practice (FDA Code of Federal Regulations 21). The extract is dried and converted to powder at Pharmachem Laboratories, Inc (Kearny, New Jersey). The MGE is encapsulated with a vegetable capsule of hypromellose obtained from CapsCanada (Pompano Beach, Florida), bottled, and labeled at Nature's Pearl. Quality control testing for antimicrobial contamination (aerobic count, yeast and mold, E.coli/coliform, staph aureus, enterobacteriaceae, and salmonella) and phenolic levels occurs numerous times during the production process.

Storage requirements: Capsules should be stored at room temperature.

Route of administration: MGE is available for oral administration.

10.0 Laboratory Studies

10.1 Phenolic assays

Total and component phenolics will be measured from blood and urine at baseline, 4 weeks, and 8 weeks if the patient remains on MGE. Blood assays will require approximately 10ml of blood. For the polyphenolic analysis[26] one tube of blood at each time point will be collected in tubes containing EDTA and ascorbic acid and stored at -80°C for an indefinite period of time. The blood sample will be thawed on ice and used to measure polyphenols by ultra-high performance liquid chromatography (UPLC) coupled to mass spectroscopy detection (UPLC-MS).

A spot urine collection will be collected at baseline, 4 weeks, and 8 weeks if the patient remains on treatment with MGE. The urine will be collected into a urine collection cup and mixed with ascorbic acid; 20 mL aliquots will be stored at -80°C for an indefinite period of time. The urine will be thawed on ice and used to measure polyphenols by UPLC-MS.

10.2 Systemic cytokines and growth factor assays

Approximately 10ml of blood will be collected in a tube that does not contain EDTA (silicone-coated, red-top tube) at baseline, 4 weeks, and 8 weeks (if the patient remains on MGE treatment) to measure cytokines and growth factors (including but not limited to TNFα, IL6, IL6R, IL8, IL1α, IL1β, IGF-1, VEGF), using the Cira multiplex solid phase immunoassay platform from Aushon and the Human Cytokine 2 and Angiogenesis Factor 1 Arrays[27-29], as well as individual ELISA's for CRP, TGFβ1, IGF-1. The blood will be allowed to clot. Serum will be collected and samples will be stored at -80°C for an indefinite period of time.

11.0 Data Management

Forms/Data collected	Collection Time Point	Data Management
Informed consent document	Pre-study	ORIS/WakeOne
Protocol Registration & Race Verification Forms	Pre-study	ORIS
Adverse Event Log (Toxicity including CBC with differential, platelets, serum chemistries)	Each monthly clinic visit	ORIS (labs to be pulled from EPIC)
Labs (CBC with differential, platelets, serum chemistries)	Baseline and monthly clinic visit	ORIS (labs to be pulled from EPIC)
Treatment Response Evaluation	Monthly clinic visit	REDCap
End of Treatment Evaluation of Best Response	Off-study	REDCap
Baseline Data Collection Form	Baseline	REDCap
Follow-up Data Collection Form	Monthly clinic visit	REDCap
Correlative Research Labs Data Collection Form	Baseline, week 4, week 8	REDCap
Telephone Follow-up Form	Weeks 2 & 6	REDCap
FACT-G	Baseline and monthly	REDCap
PROMIS-Fatigue	Baseline and monthly	REDCap
Daily Pill Dairy	Monthly clinic visit	REDCap
Off Study Documentation Form	Off-study	REDCap
Follow-Up Form for Survival	Every 6 weeks	ORIS

12.0 Statistical Considerations

12.1 Analysis of Primary Objective

The primary objective of determining the safety and MTD of MGE will be completed by monitoring toxicities and using the dose escalation plan detailed above.

To determine the maximum tolerated dose (MTD) of MGE in patients with metastatic cancer, we will use a standard 3+3 Phase 1 dose escalation statistical design. All MGE dose escalations conducted will be escalated according to the dose escalation scheme (see Section 6.2). The number of patients in each cohort will be 3. If no patients in any cohort develop a dose-limiting toxicity (DLT), dose escalation will continue in cohorts of 3 patients up to the maximum dose. However, if a DLT is observed in 1 of 3 patients at any dose level, the cohort of that dose level will be expanded to a maximum of 6 patients. If 1 of the 6 patients has a DLT, the dose escalation procedure will continue in 3 patients for each subsequent cohort. However, once a DLT is observed in a total of 2 patients at any dose level, dosing of MGE in patients at that dose level will stop immediately, even though the total number of patients at the last cohort may be as few as 2. Dose escalation is considered to be complete. The dose level that induces a DLT in 2 or more patients is considered to be above MTD, and the dose level immediately below the dose level that induced a DLT in ≥2 patients is considered the MTD for MGE if ≤1 participants of 6 patients at that level have a DLT.

In the event that the MTD is not observed after the 5 dose levels listed in Section 6.2 have been tested, the investigators will consider stopping additional dose escalation. The decision will be based on the actual dose level tested, the extent of anti-tumor efficacy observed, and the type of toxicity observed, etc.

12.2 Analysis of Secondary Objective

12.2.1 To monitor toxicity at 4, 8, and every 4 weeks thereafter on MGE

To analyze objective 2.1.1, we will summarize any expected toxicities, any laboratory based toxicities, and any grade 3 or higher gastrointestinal toxicities and any grade 4 or higher toxicities using frequency tables overall and by week.

12.2.2 To evaluate change in phenolic levels (total and component, blood and urine) from baseline to 4 and 8 weeks

To analyze objective 2.2.2, we will fit a mixed model ANOVA model with phenolic level as the outcome and time (baseline, 4, and 8 weeks) as a fixed effect and subject as a random effect. We will use contrasts to estimate the changes from baseline to week 4 and week 8.

12.2.3 To evaluate change in serum cytokines and growth factors from baseline to 4 and 8 weeks on MGE

To analyze objective 2.2.3, we will fit a mixed model ANOVA model with the outcome of interest (cytokine or growth factor) as the outcome and time (baseline, 4, and 8 weeks) as a fixed effect and subject as a random effect.

We will use contrasts to estimate the changes from baseline to week 4 and week 8.

12.2.4 To observe the response rate of MGE in patients with metastatic cancer.

To analyze objective 2.2.4, we will characterize response at 8 weeks using frequency table. We will also characterize the best response at the end of treatment with MGE using a frequency table.

12.2.5 To assess overall and progression-free survival in patients with metastatic cancer receiving MGE.

To analyze objective 2.2.5, we will summarize overall survival and progression-free survival using the Kaplan-Meier method. We will calculate median survival rates and associated 95% confidence intervals. Patients will be censored at date of last contact.

12.2.6 To assess global quality of life and fatigue in cancer patients taking MGE.

Quality of Life and fatigue will be monitored every 4 weeks until the end of the study. To analyze objective 2.2.6, we will fit a mixed model ANOVA model with phenolic level as the outcome and time (baseline, every 4 weeks) as a fixed effect and subject as a random effect. We will use contrasts to estimate the changes from baseline to each follow-up time period.

12.2.7 To assess adherence to MGE treatment.

To analyze objective 2.2.7, we will summarize the percent of pills taken at the end of every 4 week period *i* using the formula:

$$Percent\ of\ pills\ taken_i = \frac{(Dose\ Level\ \times 7\ \times 4)_i - Pill\ Count_i}{(Dose\ Level\ \times 7\ \times 4)_i}.$$

In the formula above, pill count will be calculated from counting the number of pills returned as well as by summarizing the patient's pill diary. The percent of pills taken will be summarized by dose level using the mean and 95% confidence interval.

12.3 Power and Sample Size

The study will enroll a minimum of 2 participants and a maximum of 30.

12.4 Estimated Accrual Rate and Study Length

We anticipate accruing at least 2 patients per month on this protocol and thus expect that accrual for this Phase 1 trial will be completed in less than 16 months, and that the MTD can be determined within 1 month of the last accrual. Patients will be followed until death.

12.5 Interim Analysis Plan

This is a standard 3+3 design with monitoring throughout the study and stopping rules as defined above.

References

1. Danaei G, Vander Hoorn S, Lopez AD, Murray CJ, Ezzati M. Causes of cancer in the world: comparative risk assessment of nine behavioural and environmental risk factors. *Lancet.* Nov 19 2005;366(9499):1784-1793.

2. Yang J, Xiao YY. Grape phytochemicals and associated health benefits. *Critical reviews in food science and nutrition.* 2013;53(11):1202-1225.

3. Lee JH, Johnson JV, Talcott ST. Identification of ellagic acid conjugates and other polyphenolics in muscadine grapes by HPLC-ESI-MS. *Journal of agricultural and food chemistry.* Jul 27 2005;53(15):6003-6010.

4. Yi W, Fischer J, Akoh CC. Study of anticancer activities of muscadine grape phenolics in vitro. *Journal of agricultural and food chemistry.* Nov 2 2005;53(22):8804-8812.

5. Mertens-Talcott SU, Lee JH, Percival SS, Talcott ST. Induction of cell death in Caco-2 human colon carcinoma cells by ellagic acid rich fractions from muscadine grapes (Vitis rotundifolia). *Journal of agricultural and food chemistry.* Jul 26 2006;54(15):5336-5343.

6. God JM, Tate P, Larcom LL. Anticancer effects of four varieties of muscadine grape. *Journal of medicinal food.* Mar 2007;10(1):54-59.

7. Hudson TS, Hartle DK, Hursting SD, et al. Inhibition of prostate cancer growth by muscadine grape skin extract and resveratrol through distinct mechanisms. *Cancer Res.* Sep 1 2007;67(17):8396-8405.

8. Noratto GD, Angel-Morales G, Talcott ST, Mertens-Talcott SU. Polyphenolics from acai (Euterpe oleracea Mart.) and red muscadine grape (Vitis rotundifolia) protect human umbilical vascular Endothelial cells (HUVEC) from glucose- and lipopolysaccharide (LPS)-induced inflammation and target microRNA-126. *Journal of agricultural and food chemistry.* Jul 27 2011;59(14):7999-8012.

9. Bralley EE, Hargrove JL, Greenspan P, Hartle DK. Topical anti-inflammatory activities of Vitis rotundifolia (muscadine grape) extracts in the tetradecanoylphorbol acetate model of ear inflammation. *Journal of medicinal food.* Dec 2007;10(4):636-642.

10. Ghanim H, Sia CL, Korzeniewski K, et al. A resveratrol and polyphenol preparation suppresses oxidative and inflammatory stress response to a high-fat, high-carbohydrate meal. *The Journal of clinical endocrinology and metabolism.* May 2011;96(5):1409-1414.

11. Sandhu AK, Gu L. Antioxidant capacity, phenolic content, and profiling of phenolic compounds in the seeds, skin, and pulp of Vitis rotundifolia (Muscadine Grapes) As determined by HPLC-DAD-ESI-MS(n). *Journal of agricultural and food chemistry.* Apr 28 2010;58(8):4681-4692.

12. Wu Y, Zhou BP. Inflammation: a driving force speeds cancer metastasis. *Cell Cycle.* 10/15/2009 2009;8(20):3267-3273.

13. Panju AH, Danesh A, Minden MD, Kelvin DJ, Alibhai SM. Associations between quality of life, fatigue, and cytokine levels in patients aged 50+ with acute myeloid leukemia. *Support.Care Cancer.* 5/2009 2009;17(5):539-546.

14. Gourineni V, Shay NF, Chung S, Sandhu AK, Gu L. Muscadine grape (Vitis rotundifolia) and wine phytochemicals prevented obesity-associated metabolic complications in C57BL/6J mice. *Journal of agricultural and food chemistry.* Aug 8 2012;60(31):7674-7681.

15. Gourineni V, Shay NF, Chung S, Sandhu AK, Gu L. Correction to Muscadine Grape (Vitis rotundifolia) and Wine Phytochemicals Prevented Obesity Associated Metabolic Complications in C57BL/6J Mice. *Journal of agricultural and food chemistry.* Oct 20 2014.

16. Brinkley TE, Leng X, Miller ME, et al. Chronic inflammation is associated with low physical function in older adults across multiple comorbidities. *J.Gerontol.A Biol.Sci.Med.Sci.* 4/2009 2009;64(4):455-461.

17. Penninx BW, Abbas H, Ambrosius W, et al. Inflammatory markers and physical function among older adults with knee osteoarthritis. *J.Rheumatol.* 10/2004 2004;31(10):2027-2031.

18. Penninx BW, Kritchevsky SB, Newman AB, et al. Inflammatory markers and incident mobility limitation in the elderly. *J.Am.Geriatr.Soc.* 7/2004 2004;52(7):1105-1113.

19. Porter BE, Moore JE, Miller MS, Tallant EA, Gallagher PE. Muscadine grape extract reduces lung carcinogenesis in female mice exposed to 3-methylcholanthrene in utero [abstract]. Paper presented at: American Association for Cancer Research Annual Meeting. 2015; Philadelphia; Abstract #4563.

20. Gallagher PE, Tallant EA. Prevention of breast tumor growth by an extract from the muscadine grape. Paper presented at: American Association for Cancer Research Annual Meeting. 2015; Philadelpha; Abstract # 2825.

21. Courtney KD, Corcoran RB, Engelman JA. The PI3K pathway as drug target in human cancer. *Journal of clinical oncology : official journal of the American Society of Clinical Oncology.* Feb 20 2010;28(6):1075-1083.

22. Burton LJ, Smith BA, Smith BN, et al. Muscadine Grape Skin Extract (MSKE) can Antagonize Snail-Cathepsin L-Mediated Invasion, Migration and Osteoclastogenesis in Prostate and Breast Cancer Cells. *Carcinogenesis.* Jun 10 2015.

23. Mellen PB, Daniel KR, Brosnihan KB, Hansen KJ, Herrington DM. Effect of muscadine grape seed supplementation on vascular function in subjects with or at risk for cardiovascular disease: a randomized crossover trial. *Journal of the American College of Nutrition.* Oct 2010;29(5):469-475.

24. Banini AE, Boyd LC, Allen JC, Allen HG, Sauls DL. Muscadine grape products intake, diet and blood constituents of non-diabetic and type 2 diabetic subjects. *Nutrition (Burbank, Los Angeles County, Calif.).* Nov-Dec 2006;22(11-12):1137-1145.

25. Paller CJ, Rudek MA, Zhou XC, et al. A phase I study of muscadine grape skin extract in men with biochemically recurrent prostate cancer: Safety, tolerability, and dose determination. *Prostate.* May 27 2015.

26. Biasutto L, Marotta E, Garbisa S, Zoratti M, Paradisi C. Determination of quercetin and resveratrol in whole blood--implications for bioavailability studies. *Molecules (Basel, Switzerland).* Sep 2010;15(9):6570-6579.

27. Liu Y, Starr MD, Bulusu A, et al. Correlation of angiogenic biomarker signatures with clinical outcomes in metastatic colorectal cancer patients receiving capecitabine, oxaliplatin, and bevacizumab. *Cancer medicine.* Apr 2013;2(2):234-242.

28. Tran HT, Liu Y, Zurita AJ, et al. Prognostic or predictive plasma cytokines and angiogenic factors for patients treated with pazopanib for metastatic renal-cell cancer: a retrospective analysis of phase 2 and phase 3 trials. *Lancet Oncol.* Aug 2012;13(8):827-837.

29. Uronis HE, Cushman SM, Bendell JC, et al. A phase I study of ABT-510 plus bevacizumab in advanced solid tumors. *Cancer medicine.* Jun 2013;2(3):316-324.

Appendix A – Protocol Registration Form

See separate attachment in the eIRB under *Protocol Document*.

Appendix B – Subject Eligibility Checklist
See separate attachment in the eIRB under *Protocol Document*

Appendix C – Mandatory STRC SAE Reporting Guidelines
See separate attachment in the eIRB under *Protocol Document*

Appendix D – Treatment Response Evaluation
See separate attachment in the eIRB under *Protocol Document*.

Appendix E – End of Treatment Evaluation of Best Overall Response
See separate attachment in the eIRB under *Protocol Document*.

Appendix F – FACT-G
See separate attachment in the eIRB under *Protocol Document.*

Appendix G – PROMIS-Fatigue SF
See separate attachment in the eIRB under *Protocol Document.*

Appendix H – Daily Pill Diary
See separate attachment in the eIRB under *Protocol Document*

Appendix I – Baseline Data Collection Form
See separate attachment in the eIRB under *Protocol Document*

CBC results will be drawn directly into the database from ORIS. Do not abstract them from the medical record. Results will include WBC, RBC, Hemoglobin, Hematocrit, MCV, Neutrophils, Lymphocytes, Platelets.

Serum chemistry results will be drawn directly into the database from ORIS. Do not abstract them from the medical record. Results will include Albumin, Alkaline phosphatase, Total bilirubin, Bicarbonate, BUN, Calcium, Chloride, Creatinine, Glucose, Potassium, Total protein, SGOT[AST], SGPT[ALT], Sodium.

Cancer-specific tumor marker values will be drawn directly into the database from ORIS. Do not abstract them from the medical record.

Appendix J – Follow-Up Data Collection Form
See separate attachment in the eIRB under *Protocol Document*

CBC results will be drawn directly into the database from ORIS. Do not abstract them from the medical record. Results will include WBC, RBC, Hemoglobin, Hematocrit, MCV, Neutrophils, Lymphocytes, Platelets.

Serum chemistry results will be drawn directly into the database from ORIS. Do not abstract them from the medical record. Results will include Albumin, Alkaline phosphatase, Total bilirubin, Bicarbonate, BUN, Calcium, Chloride, Creatinine, Glucose, Potassium, Total protein, SGOT[AST], SGPT[ALT], Sodium.

Cancer-specific tumor marker values will be drawn directly into the database from ORIS. Do not abstract them from the medical record.

Appendix K – **Correlative Research Labs Data Collection Form**
See separate attachment in the eIRB under *Protocol Document*

Appendix L – **Telephone Follow-Up Form**
See separate attachment in the eIRB under *Protocol Document*

Appendix M – **Off Study Documentation Form**
See separate attachment in the eIRB under *Protocol Document*

Appendix N – **Follow-Up Form for Survival**
See separate attachment in the eIRB under *Protocol Document*

Appendix O – **Adverse Event Log**
See separate attachment in the eIRB under *Protocol Document*

Appendix P – Physician Order Form for Study Drug
See separate attachment in the eIRB under *Protocol Document*