Phase 2 Double-blind, Placebo-controlled Study of the Effects of Muscadine Grape Extract in Men with Prostate Cancer on Androgen Deprivation Therapy
Comprehensive Cancer Center of Wake Forest University; CCCWFU #85417

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Table of Contents

APPENDIX D: Serious Adverse Event (SAE) Notification SOP, see eIRB “Other Documents”

APPENDIX E: Baseline Data Collection Form, see eIRB “Other Documents”

APPENDIX F: Month 3/9 Data Collection Form, see eIRB “Other Documents”

APPENDIX G: Month 6/12 Data Collection Form, see eIRB “Other Documents”

APPENDIX H: PROMIS v1.0 Fatigue 7a, see eIRB “Other Documents”

APPENDIX I: PROMIS v1.2 Global Health, see eIRB “Other Documents”

APPENDIX J: Hot Flash Related Daily Interference Scale, see eIRB “Other Documents”

APPENDIX K: PROMIS v1.0 Sleep Disturbance 8a, see eIRB “Other Documents”

APPENDIX L: PROMIS v2.0 Cognitive Abilities 4a, see eIRB “Other Documents”

APPENDIX M: PROMIS v2.0 Physical Function 10a, see eIRB “Other Documents”

APPENDIX N: Short Physical Performance Battery (SPPB), see eIRB “Other Documents”

APPENDIX O: 6 Minute Walk Test Protocol, see eIRB “Other Documents”

APPENDIX P: Telephone Follow-Up Form, see eIRB “Other Documents”

APPENDIX Q: AE Ascertainment Form (CTCAE v5), see eIRB “Other Documents”

APPENDIX R: Adverse Event (AE) Log, see eIRB “Other Documents”

APPENDIX S: Serious Adverse Event Reporting Form, see eIRB “Other Documents”

APPENDIX T: Disease Progression, see eIRB “Other Documents”

APPENDIX U: Participant Status Change Form, see eIRB “Other Documents”

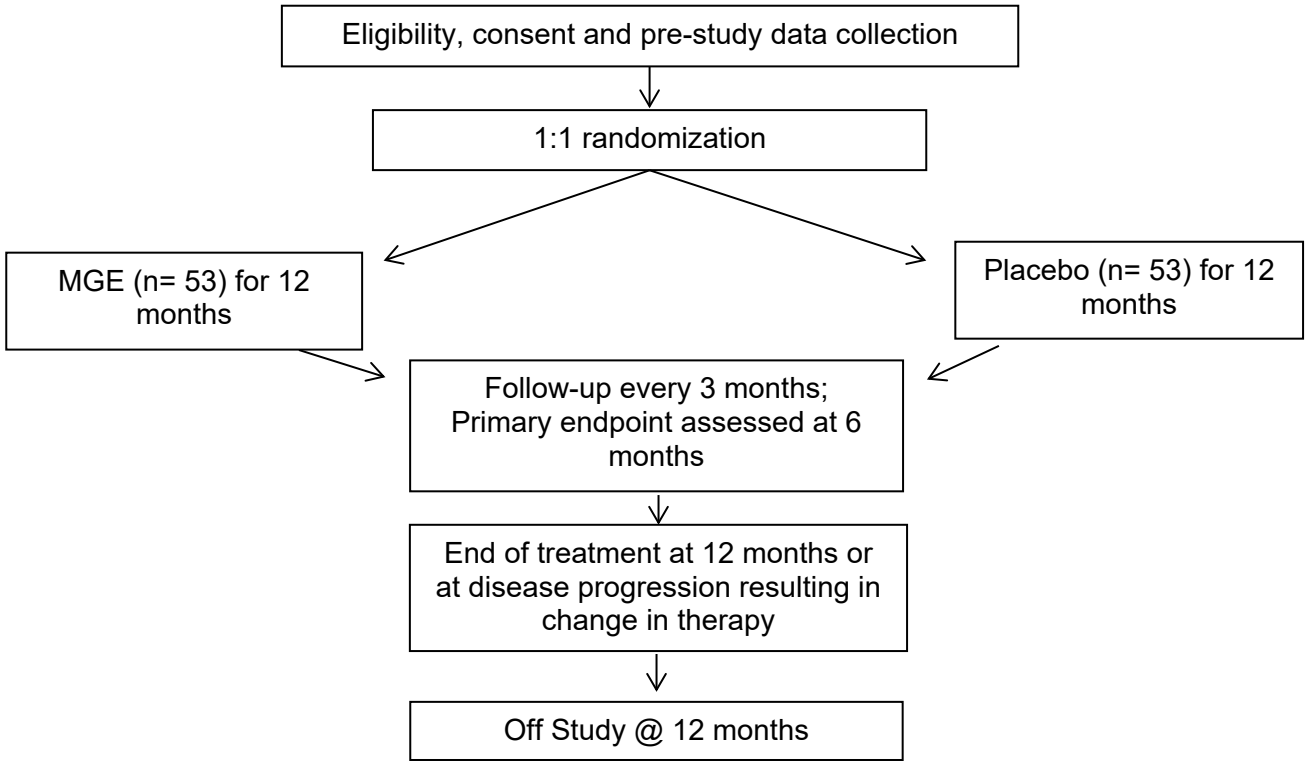
APPENDIX V: End-of-study Follow-up Form, see eIRB “Other Documents”

APPENDIX W: Pre-Toxicity Form, see eIRB “Other Documents”

APPENDIX X: DXA Questionnaire, see eIRB “Other Documents”

APPENDIX Y: Adherence Telephone Script, see eIRB “Other Documents”

SCHEMA


Eligibility, consent and pre-study data collection
1:1 randomization
MGE (n= 53) for 12 months
Placebo (n= 53) for 12 months
Follow-up every 3 months; Primary endpoint assessed at 6 months
End of treatment at 12 months or at disease progression resulting in change in therapy
Off Study @ 12 months

1.0 Introduction and Background

It is estimated that one-third of the more than 7 million deaths from cancer worldwide are 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 or treatment of cancer is attractive, as implementation is relatively easy, even in populations with reduced incomes and resources. Grape extracts or active components isolated from grapes have received attention as chemopreventive or therapeutic 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 or cancer-related side effects, representing an opportunity for novel investigation. This study builds upon promising preclinical and clinical evidence to determine if the addition of a proprietary muscadine grape extract (MGE) to androgen deprivation therapy improves symptoms in men with prostate cancer.

1.1 Preclinical Studies with Muscadine Grape Products

Literature overview: The anti-tumor properties of muscadine grapes have been demonstrated in preclinical models.[4-7] In a mouse model, Porter et al examined the effects of muscadine grape extract on lung tumor formation in the fetus following transplacental exposure to a carcinogen and showed that muscadine exposure through drinking water reduced both tumor proliferation and angiogenesis, leading to a decrease in tumor burden and multiplicity.[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 muscadine products could inhibit 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, 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 data from the investigative team: Preliminary studies from the investigative team (Gallagher/Tallant laboratory) demonstrate that extracts of 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 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 treated for 7 days with increasing concentrations of muscadine grape extracts, 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 used, and similar responses were obtained with grape seed extract compared to grape skin extract.

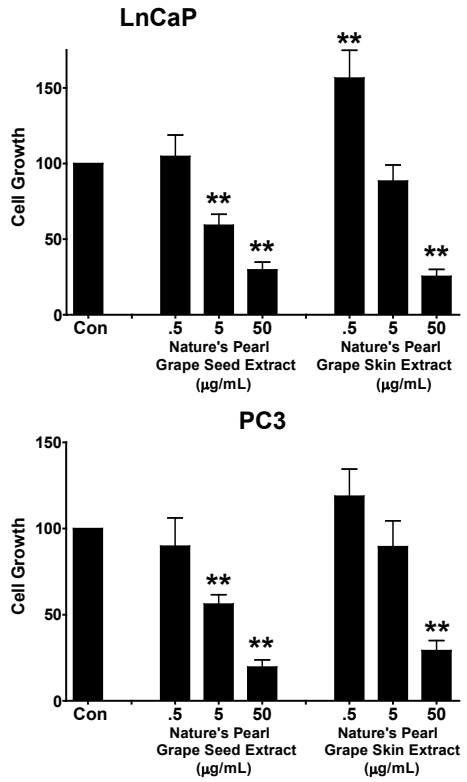


Figure 1. LnCaP or PC3 human prostate cancer cells were incubated with increasing concentrations of either muscadine grape seed or grape skin extracts and cell proliferation was quantified by counting the number of cells per well. The data are presented as the percent of the Control (Con), which was 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 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. Similar to the effects seen in prostate cancer cell lines, muscadine grape extract reduced the proliferation of human breast cancer cells. The

responses were dependent upon the dose of extract used, and similar responses were obtained with grape seed extract compared to grape skin extract.

Because treatment with muscadine grape seeds versus skins showed no significant difference in effect on cancer cell lines, additional preclinical studies were performed with muscadine grape extract (MGE), which contains both seeds and skins.

In vitro and in vivo studies with MGE

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 is the muscadine grape extract (MGE) used in the preclinical studies described below. However, because rodents do not swallow capsules, the MGE powder is reconstituted and added to the drinking water.

MGE reduced the migration of triple negative and HER2 over-expressing breast cancer cells (Collard, Gallagher, and Tallant, presented at 2017 Experimental Biology Annual Meeting). Treatment of murine and human triple negative breast cancer cells with MGE altered the expression of 2196 and 2840 genes, respectively, with 298 genes common between the two cell lines with respect to time of incubation. These genes are involved in numerous cellular processes including proliferation, angiogenesis, energy metabolism, protein processing, cellular structure, DNA replication, DNA repair, and autophagy.

Treatment of triple negative breast cancer cells with MGE was associated with a significant reduction in the hyaluronan-mediated motility receptor (RHAMM), a regulator of both proliferation and migration, as well as phospho-RB which modulates the expression of RHAMM and cyclin D1, a critical component of the cell cycle. MGE inhibited protein kinase B (AKT) signaling in human triple negative breast cancer cells and mitogen activated protein kinase (MAPK) signaling in murine triple negative breast cancer cells, suggesting differential regulation of molecular pathways that control the cyclin D1/phospho-RB/ HMMR pathway in triple negative breast cancer (Collard, Tallant and Gallagher, submitted for presentation at the 2018 American Society of Nutrition annual meeting).

The MGE-induced reduction in HER2 over-expressing breast cancer was associated with a decrease in phosphorylation and activation of protein kinase B (AKT) which regulates proliferation and migration as well as phosphoinositide-dependent kinase-1 (PDK1), a primary activator of AKT. AKT activation inhibits Forkhead box protein (FOXO1) transcription factor, decreasing expression of negative cell-cycle regulators, including cyclin-dependent kinase inhibitor 1B (p27), resulting in increased proliferation. Treatment with MGE significantly increased FOX01 and p27, suggesting a mechanism for the inhibition of cell cycle progression by MGE. Further, S-phase kinase-associated protein 2 (SKP2) which targets p27 and FOX01 for degradation, was reduced in HER2 over-expressing breast cancer following incubation with MGE, demonstrating multiple molecular mechanisms for the reduction in proliferation in HER2 over-expressing breast cancer (Mackert, Gallagher and Tallant, submitted for presentation at the 2018 American Society of Nutrition Annual Meeting).

As described above, the growth of 2 different prostate cancer cell lines was also significantly reduced by MGE. RNA seq analysis of human prostate cancer cells treated with MGE identified 2768 genes that were altered by the extract, including genes involved in cell proliferation, cell division, mitosis, energy metabolism, protein processing, cellular structure, DNA replication, DNA repair, and autophagy. In addition, 614 genes were common between the prostate cancer cells and the breast cancer cells, suggesting that the extract may regulate common signaling pathways in a variety of cancer cell types. Ongoing studies are interrogating the major pathways that are regulated by MGE in prostate cancer cells.

To determine if MGE reduces the growth of human tumors *in vivo*, nude mice were given MGE in their drinking water. Human breast cancer cells were injected into the mammary fat pad and human prostate cancer cells into the flank. Tumor size was measured. Both breast and prostate tumors measured significantly smaller in mice receiving MGE compared to mice drinking water without MGE, as shown in **Figure 2**.

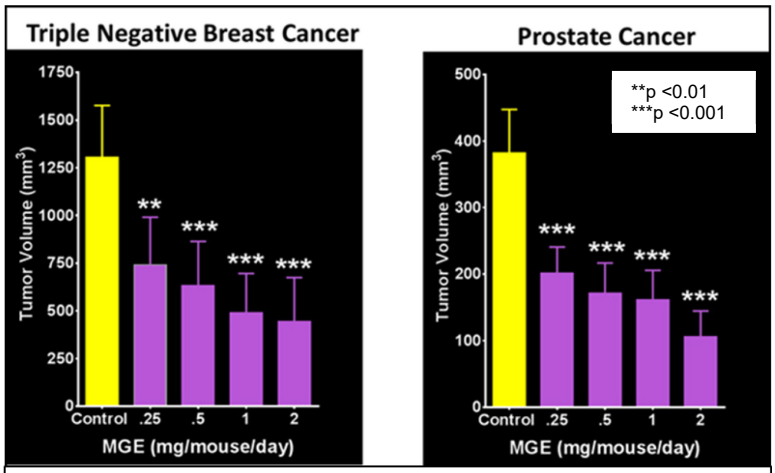


Figure 2. Athymic mice injected with human breast or prostate cancer cells had significantly reduced tumor volume when consuming MGE.

Specifically for the prostate cancer tumors, athymic mice (male, 15-20 g, 5-6 weeks of age) were injected subcutaneously on the lower flank with 1.6 x 10^6 LNCaP cells. When the tumors reached 100 mm^3, the mice were randomized to drinking water with MGE or untreated water. After 5 weeks, the mice were sacrificed and the tumors were weighed. The volume of prostate tumors growing in the flank of nude mice drinking water with MGE was markedly reduced compared to the size of the tumors from control mice (n=6, $p<0.001$). Further, the weight of prostate tumors from mice drinking MGE was also significantly decreased, [0.96 ± 0.14 g (control) as compared to 0.41 ± 0.11 g (MGE)]; a reduction of 57% ($p < 0.05$). Five micron tumor sections were incubated with an antibody to CD34 and tumor vessels were identified by positive immunoreactivity and morphology. In the prostate tumors, there were fewer blood vessels visualized and decreased expression of vascular endothelial growth factor and placental growth factor, suggesting that MGE inhibits angiogenesis (Gallagher and Tallant, presented at AACR 2017 annual meeting). These results demonstrate that MGE reduces prostate tumor growth in a mouse model, by inhibiting angiogenesis, suggesting that MGE may be an effective treatment for prostate cancer.

There is also preclinical evidence that muscadine products decrease systemic inflammation.[8-11] Higher levels of inflammation may not only increase cancer recurrence risk,[12] but also increase fatigue,[13] adiposity[14,15] and decrease physical function.[16-18] By lowering levels of circulating inflammatory markers, treatment with muscadine products may impact these processes. The effect of MGE on exercise was assessed in female hemizygous (mRen2)27 transgenic rats, a well-characterized model of hypertension. The rats were chronically administered MGE (20 mg of total phenolics/kg body weight/day) in the drinking water starting at 14 weeks of age. Control

rats received water only. At 70 weeks of age, exercise tolerance was assessed by treadmill. As shown in **Figure 3**, long-term consumption of MGE did not reduce or exacerbate hypertension, but did increase the exercise capacity, workload performance, and time to exhaustion in female hypertensive rats. This effect was associated with a decrease in 8-OH-deoxyguanosine, a circulating marker of oxidative stress (Duncan AV et al, presented at 2017 Experimental Biology annual meeting). Additional preclinical studies evaluating fatigue and exercise capacity are ongoing. In cardiac cells, MGE increased maximal respiration, spare respiratory capacity and ATP production, suggesting that an increase in energetic capacity and bioenergetics may participate in the enhanced exercise capacity in hypertensive rats.

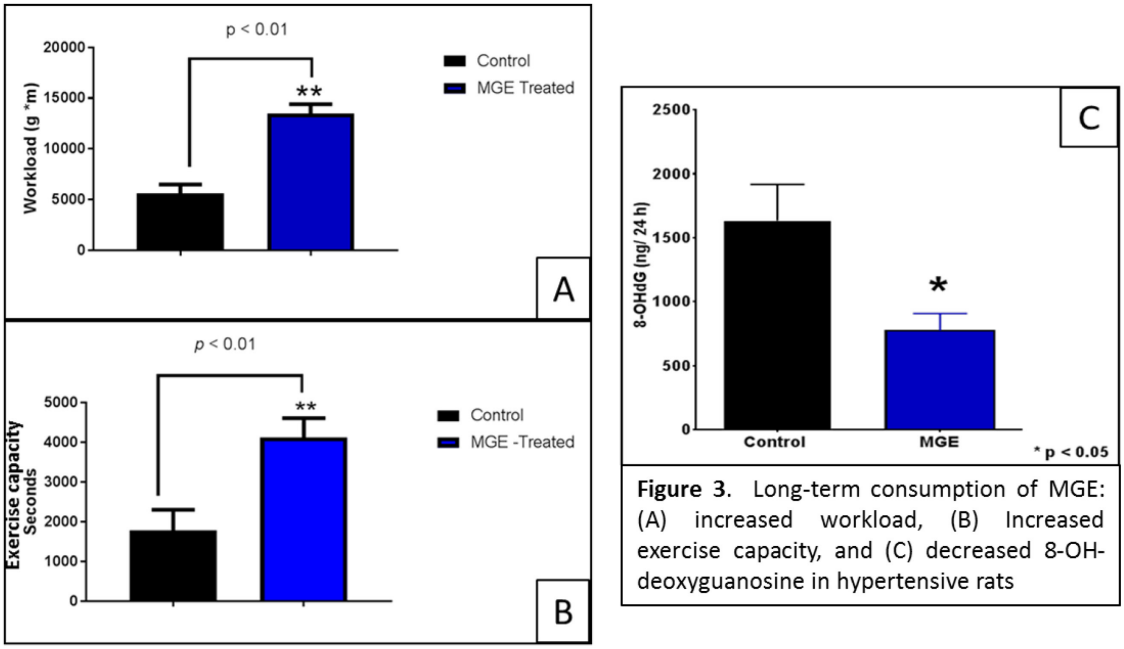


Figure 3. Long-term consumption of MGE: (A) increased workload, (B) Increased exercise capacity, and (C) decreased 8-OH-deoxyguanosine in hypertensive rats

1.2 Preclinical Toxicity Studies with MGE

The primary goal of the rodent toxicity tests with MGE was to identify the range of doses that caused no life-threatening or adverse effects, thereby providing critical information for conduct of clinical trials to assess toxicity in humans with cancer and establishing an estimated clinical margin of safety.

Male C57 black mice (8 weeks of age) were randomized into groups to receive drinking water alone (control) or drinking water with MGE at four escalating doses of MGE, as determined by 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 a non-invasive, small animal VEVO ultrasound imaging system to measure or calculate ejection fraction, fractional shortening, stroke volume, heart rate and cardiac output. 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 for the toxicity studies in mice, based upon the studies in the Gallagher/Tallant laboratory reported above. MGE at 0.25, 0.5, 1 and 2 mg total phenolics/mouse/day was administered to the mice in their drinking water. Total phenolic content was determined using the Folin-Ciocalteau reagent with gallic acid as the standard. Because 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.

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 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, organs 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 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 mg phenolics/mouse/day) was 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:
Little toxicity was observed at any of the doses tested.

1.3 Clinical Studies with Muscadine Grape Products

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 grade 1 adverse gastrointestinal symptoms possibly related to the study agent, including flatulence, soft stools, and burping. No other related adverse events were reported. Six of the 14 patients came off study for disease progression after exposure for a median of 15 months. 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. Compared to placebo, there was no lengthening of the PSA doubling time in prostate cancer patients taking MPX. No additional safety signals were seen. The investigators did identify a subgroup of patients with manganese superoxide dismutase (MnSOD) genotype AA (21% patients) who appeared to benefit, and a larger study in this patient population is planned (Paller et al, presented at 2017 ASCO Genitourinary symposium). MnSOD is an important mitochondrial antioxidant enzyme, and patients with MnSOD AA genotype have been shown to be more sensitive to antioxidant treatment.[26] Treatment with pomegranate extract in a similar patient population was also suggested to benefit patients with the MnSOD AA genotype.[27]

We conducted a phase 1 study of MGE in patients with advanced solid tumors (clinicaltrials.gov identifier NCT02583269). Adult patients with metastatic or unresectable malignancy progressing on standard therapies were assigned to MGE (~160 mg total phenolics/capsule, Piedmont R&D) in a standard 3 + 3 design. Five dose levels were tested (320 to 1600 mg total phenolics/day). Safety and maximum tolerated dose (MTD) were assessed after 4 weeks. Patients were evaluated for response at 8 weeks and continued on MGE if clinically stable. 23 pts (lung n=7; gastrointestinal n=7; genitourinary n=6; other n=3) were treated. Two patients were inevaluable at 4 weeks (1 withdrew, 1 noncompliant). The evaluable cohort (median age 72 years [range 43-86], 48% female, 95% white) was heavily pre-treated. After 4 weeks on MGE, possibly-attributable grade 2-3 adverse events were decreased lymphocytes (4.7%), fatigue (4.7%), and constipation (9.5%), including one dose limiting toxicity for grade 3 constipation requiring hospitalization, most likely attributable to progressive peritoneal disease although contribution from MGE could not be excluded. Dose level 2 was expanded accordingly. Otherwise, MGE was very well tolerated with no other grade 3 or higher adverse events. MTD was not reached. 93% of pts took ≥ 80% of pills prescribed. Median time on therapy was 8.1 weeks (95% CI 7.7, 20.3) with a 7.1 month median OS (95% CI 4.3, 12.2). Quality of life and fatigue levels were stable from baseline to 4 and 8 weeks (p values >0.2). Higher MGE dose was correlated with improvement in self-reported physical function at 8 weeks (r= 0.56, p=0.04). We concluded that MGE is safe and well-tolerated in heavily pretreated and elderly cancer patients, and that the relationship

between MGE and improved physical function warrants further study (Bitting RL et al, abstract submitted for ASCO 2018 annual meeting).

In summary, muscadine grape products have shown anti-cancer and anti-inflammatory activity in preclinical models and have demonstrated a favorable safety profile in preclinical and clinical studies. The proposed study with MGE represents an opportunity to translate promising data with a natural product into a clinical trial in men with prostate cancer.

1.4 Rationale for MGE Dosing

There are many muscadine grape products commercially available. The concentration of phenolics in 9 commercially available muscadine products was tested and compared to MGE. The concentration of phenolics in MGE was more than three times higher than in any other product, at approximately 162 mg phenolics per capsule, compared to approximately 50 mg phenolics/capsule or less. Mass spectrometry was used to further characterize the phenolic components of MGE. MGE had a significantly higher amount of catechin, epicatechin, procyanidin, gallic acid, and ellagic acid compared to the other supplements tested. Resveratrol and quercetin were below the assay detection limit (Duncan et al, presented at 2017 Experimental Biology annual meeting). Ongoing experiments are planned to determine the biologically relevant components of MGE.

The initial studies looking for an anticancer effect of MGE in both breast and prostate cancer mouse models examined 4 different dose levels: 0.25, 0.5, 1 and 2 mg of phenolics per mouse per day. A statistically-significant effect on tumor growth was seen at all 4 dose levels compared to placebo, and while there was a trend toward better efficacy at higher doses, there was no significant difference between the dose levels. An average mouse weighs 0.025 kg, therefore this corresponds to a dose range of 10, 20, 40, and 80 mg of phenolics/kg/day.

A dose 20 mg of phenolics/kg/day was chosen for additional preclinical studies in mice and rats. As described above, these studies show a significant improvement in exercise capacity and workload, along with decreased markers of oxidative stress. This dose is roughly equivalent to 8 capsules of MGE daily. The phase 1 dose-escalation study with MGE in advanced cancer patients described above evaluated patients at and above this dose level. *Based on the preclinical data and the phase 1 study, **8 capsules of MGE per day** was chosen for phase 2 prostate and breast cancer studies, in which both effects on cancer and effects on fatigue will be assessed.*

1.5 Rationale for MGE in Biochemically Recurrent Prostate Cancer

Prostate cancer is the most prevalent non-skin cancer in American men, with >225,000 cases diagnosed annually. Prostate cancer may manifest as localized disease, recurrent disease with rising PSA only, and metastatic disease. Delaying or preventing progression along this spectrum is critical because metastatic prostate cancer is incurable and is the second leading cause of cancer death in men in the United States.

Approximately 1 in 3 men will develop recurrent prostate cancer after definitive local therapy with either surgery or radiation. Biochemical recurrence, which is defined as a rising PSA level after prostatectomy or external beam radiation therapy, heralds the development of local recurrence and/or metastatic disease. However, the clinical course is highly variable. Patients with features suggesting a high risk for local recurrent disease may benefit from curative-intent salvage radiation therapy to the prostate bed. However, even following salvage radiation therapy for PSA-

only disease, many men will again develop biochemical recurrence and eventually metastatic prostate cancer.[28]

Men with biochemically recurrent prostate cancer have a median time from recurrence to prostate cancer death of 16 years.[29] Risk factors for PSA progression despite local therapy include fast PSA kinetics, lower doses of radiation, high Gleason sum, advanced tumor stage, and positive surgical margins.[28,30] Of those, PSA doubling time (PSADT) is the strongest prognostic factor, and PSADT <3 months is strongly associated with prostate cancer mortality, while a PSADT >15 months predicts for a non-prostate cancer cause of death.[31] Therefore, PSADT is often used to risk-stratify patients with biochemical recurrence after local therapy. Patients with a PSADT <3 months are likely to develop metastatic disease within 1 year. In contrast, men with a PSADT >15 months will likely not develop metastatic disease for 15 years. Men with PSADT 3-9 months or 9-15 months have a metastasis-free survival of 4 years and 13 years, respectively.[32]

In patients who relapse after primary therapy for local prostate cancer, the next step in treatment is androgen deprivation therapy (ADT), though the timing of when to begin ADT is controversial, as it is not curative. ADT refers to surgical castration (orchiectomy) or medical castration using a gonadotropin-releasing hormone (GnRH) agonist or antagonist. Many men with biochemically recurrent prostate cancer are observed off treatment until they develop a rapid PSADT or metastatic disease, at which time ADT is initiated. ADT-associated side effects include fatigue, impotence, decrease in bone density, loss of muscle mass, weight gain, cognitive changes, and symptomatic hot flashes. Fatigue is very commonly reported by patients on ADT but has not been rigorously studied. In a small prospective study evaluating ADT-related fatigue, two thirds of patients report a subjective increase in fatigue within 3 months of starting ADT.[33] In patients on a minimum of 6 months of ADT, clinically relevant fatigue (defined as fatigue that limits functioning) is reported by 43% of men.[34] Men on ADT who participate in an exercise program have improvement in fatigue.[35] Exercise has also been shown to improve strength and physical function in men on ADT[36] and is commonly recommended as an adjunct to ADT. Other strategies to mitigate ADT-related side effects have been inconsistent and largely unsuccessful.[37] Because patients initiating ADT are expected to live for many years, it is important to address these ADT-related side effects so that both quantity and quality of life are maintained at the highest possible levels.

As described above, anti-tumor properties of muscadine grapes have been demonstrated in preclinical models. These studies as well as the preliminary results of the investigative team provide the rationale for the treatment of men with prostate cancer with MGE. Also described above, there is evidence that muscadine products decrease systemic inflammation, and therefore may improve fatigue and physical functioning. **The primary goal will be to determine whether treatment with MGE can improve measures of fatigue in men with prostate cancer receiving ADT**. The impact of MGE on PSA progression, quality of life, and physical function will also be examined in this study.

2.0 Objectives

This phase 2 study is a randomized, double-blind, placebo-controlled study evaluating the effect of MGE on fatigue in men with prostate cancer receiving androgen deprivation therapy.

2.1 Primary Objective

To compare levels of fatigue in the MGE group compared to the placebo group at 6 months.

Hypothesis: Subjects receiving MGE will report decreased fatigue (PROMIS-fatigue) at 6 months compared to those receiving placebo.

2.2 Secondary Objectives

2.2.1 To compare levels of fatigue in the MGE group compared to the placebo group at 3, 9, and 12 months.

Hypothesis: Subjects receiving MGE will report decreased fatigue (PROMIS-fatigue) at 3, 9, and 12 months compared to those receiving placebo.

2.2.2 To compare quality of life in men in the MGE group compared to the placebo group.

Hypothesis: Subjects receiving MGE will report better health-related quality of life at 3, 6, and 12 months compared to the placebo group.

2.2.3 To compare physical function, physical fitness, and body composition in men in the MGE group compared to the placebo group.

Hypothesis: Subjects receiving MGE will demonstrate better physical performance (Short Physical Performance Battery), physical fitness (6 min walk), decreased adiposity (dual energy x-ray absorptiometry – DXA), increased bone density (DXA) at 12 months compared to those in the placebo group. DXA will be performed only on participants enrolled at the main campus of the lead site (Wake Forest).

2.2.4 To compare time to PSA progression (from study entry) in men in the MGE group compared to the placebo group.

Hypothesis: Subjects receiving MGE will have a longer time to PSA progression compared to placebo.

2.2.5 To compare progression-free survival (from study entry) in men in the MGE group compared to the placebo group.

Hypothesis: Subjects receiving MGE will have better progression free survival.

2.3 Exploratory Objectives

2.3.1 To examine if differences in levels of fatigue at 6 months by intervention group vary in those participants who report meaningful fatigue at baseline compared to those who did not have meaningful fatigue at baseline.

2.3.2 To examine if differences in levels of fatigue at 6 months by intervention group vary by completing at least 80% of the dose for the full course of

treatment (i.e., completing 12 months of treatment taking ≥80% of pills averaged over the 12 month period) compared to those who did not complete at least 80% of the dose for the full course of treatment.

2.3.3 To compare levels of circulating biomarkers of angiogenesis, inflammation, and oxidative stress at baseline, 6, and 12 months between the MGE and placebo groups.

2.3.4 To compare phenolic levels and circulating phenolic metabolites at baseline, 6, and 12 months between the MGE and placebo groups.

2.3.5 To correlate PSA changes with changes in circulating biomarkers of angiogenesis, inflammation, oxidative stress, phenolic levels, and phenolic metabolites.

2.3.6 To assess for the manganese superoxide dismutase genotype AA at baseline and to correlate genotype with fatigue and PSA levels at 6 months.

2.3.7 To bank blood for the future study of prostate cancer-related biomarkers such as mitochondrial function, circulating exosomes, and tumor-derived microRNA at baseline, 6, and 12 months.

2.3.8 To examine if differences in levels of fatigue at 6 months by intervention group vary by ADT alone compared to those taking ADT plus at least one additional androgen-pathway directed therapy.

3.0 Patient Selection

3.1 Inclusion Criteria

3.1.1 Men age ≥18 years who are fluent in English.

3.1.2 Histologically confirmed prostate adenocarcinoma.

3.1.3 Prior surgical castration or active ongoing use of androgen deprivation therapy (ADT) with expectation by the treating physician that patient would remain on ADT for the upcoming 12 months. ADT in the setting of definitive radiation therapy permitted. Concurrent treatment with androgen pathway inhibitors (examples include enzalutamide, abiraterone, darolutamide, apalutamide) permitted.

3.1.4 Normal organ and marrow function (labs within 30 days prior to study entry) as defined below:

White blood cell count	≥3,500/mcL (or 3.5 ($x10^3$))
Platelet count	≥75,000/mcL (or 75 ($x10^3$))
Hemoglobin	≥9 g/dL
Total bilirubin	≤2.5 X institutional upper limit of normal
AST(SGOT)/ALT(SGPT)	≤2.5 X institutional upper limit of normal
Creatinine	≤2.5 X institutional upper limit of normal

3.1.5 Able to ambulate (use of assist device is acceptable).

3.1.6 Able to cooperate with study-related activities.

3.1.7 The effects of MGE on the developing human fetus are unknown. Men must agree to use adequate contraception (barrier method of birth control; abstinence) prior to study entry and for the duration of study participation.

3.1.8 Ability to understand and the willingness to sign an IRB-approved informed consent document (either directly or via a legally authorized representative).

3.2 Exclusion Criteria

3.2.1 Symptomatic metastatic disease requiring medical treatment (i.e. painful metastases to bone).

3.2.2 Prostate cancer-related surgery or radiation within 60 days prior to study entry.

3.2.3 Documented rise in PSA (defined as rise of > 0.5 ng/mL) while on current prostate cancer therapy, determined by PSA values, at least one of which must be during the 6 months prior to study entry. PSA values must be at least 7 days apart.

3.2.4 Planned cessation of ADT or planned use of cytotoxic chemotherapy (i.e. docetaxel) within 12 months after study entry.

3.2.5 Ongoing use of any other investigational cancer-directed agents.

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

3.2.7 Inability to swallow oral medications.

3.2.8 Malabsorption due to bowel resection or gastrointestinal disease leading to uncontrolled diarrhea, or persistent nausea or vomiting requiring daily antiemetic therapy for symptom management within the past week.

3.2.9 Uncontrolled intercurrent illness, including but not limited to: active infection, symptomatic congestive heart failure, unstable angina pectoris, cardiac arrhythmia, or psychiatric illness/social situations that would limit compliance with study requirements.

3.3 Inclusion of Women and Minorities

Men of all races and ethnicity who meet the above-described eligibility criteria are eligible for this trial. Women do not get prostate cancer and are therefore excluded from participation.

4.0 Screening, Registration, and Randomization Procedures

4.1 Screening

Study coordinators will pre-screen appropriate patients to assess eligibility via IRB-approved HIPAA waiver. Patients who are eligible will be contacted by phone. Study coordinators will explain the study to potential participants to determine their interest and willingness. If a patient is likely eligible, but there is not adequate information available to confirm eligibility, the patient will be contacted for further assessment. If the patient has documentation of the necessary laboratory studies within the specified window that does not appear in the medical record, the study coordinator will obtain and review these documents. If the patient needs additional studies in order to assess eligibility, the study coordinator will arrange to obtain the necessary studies. Signed consent for eligibility screening will be obtained prior to performing the additional studies. If the patient meets eligibility criteria, the study coordinator will arrange for the baseline study visit, which will include informed consent, registration, randomization, and day 1 study activities.

4.2 Registration (Lead site)

Patients **must** be registered prior to the initiation of treatment. The following steps will be followed to register a patient:

1) Obtain informed consent
2) Complete the Eligibility Checklist in OnCore (Wiser)
3) Register the patient in Wiser – this will alert the cancer center registrar, who will then review the signed informed consent and confirm the eligibility.

After registration is complete, Wiser will assign the participant a study number and assign him to a study arm (randomize).

Patients **will not** be registered without all required supporting documents.

Note: For any screening activities performed at an outside institution, the source documentation must be provided.

4.3 Randomization

After written informed consent has been obtained and the patient registered and randomized in Wiser, the study site will obtain a unique patient identifier, which will stay the same throughout the entire study. Patients who give consent but are not registered and/or randomized for any reason will be documented as a Screen Failure.

Patients will be randomized with equal probability to one of the two treatment arms using Wiser. Balance in treatment assignment will be achieved using blocks. Randomization will be carried out via computer generated random assignment within Wiser stratified by study site. All patients must commence treatment within 14 calendar days from date of randomization.

4.4 Registration and randomization (Participating sites)

Central registration for this study will take place at Wake Forest Baptist Medical Center (WFBMC). To complete registration and enroll participants from other institutions, the study coordinator will contact the designated research coordinator at WFBMC.

The following documents must be submitted prior to participant enrollment:

- the completed eligibility checklist
- the signed informed consent
- supporting source documentation for eligibility questions
- registration & ethnicity forms

Participating sites will not share the following data of individuals, screened or enrolled, with the lead site: names, medical record numbers, dates of birth, social security numbers, addresses, phone numbers, email addresses.

If the participant meets all criteria and all source documentation is received, the research coordinator will send the completed registration documents back to the site. The participant will be enrolled at WFBMC and assigned a unique study ID. This unique study ID will be relayed back to staff at participating centers via email and will serve as the confirmation of enrollment.

4.5 Blinding

This is a double-blinded study. Only the investigational pharmacists and the statisticians will unblinded. The blind will be maintained until the study is complete.

4.6 Multicenter Guidelines

Principal Investigator: the PI is responsible for performing the following tasks:

- Coordinating, developing, submitting, and obtaining approval for the protocol as well as its subsequent amendments.
- Assuring that all participating institutions are using the correct version of the protocol.
- Taking responsibility for the overall conduct of the study at all participating institutions and for monitoring the progress of the study.
- Participating sites will enroll a minimum of 40 patients to the trial.
- Reviewing and ensuring reporting of Serious Adverse Events (SAE)
- Reviewing data from all sites.

Lead Site: Wake Forest Baptist Medical Center is responsible for performing the following tasks:

- Ensuring that IRB approval has been obtained at each participating site prior to the first patient registration at that site, and maintaining copies of IRB approvals from each site.
- Managing central patient registration.
- Collecting and compiling data from each site.
- Establishing procedures for documentation, reporting, and submitting of AEs and SAEs to the PI and all applicable parties.
- Facilitating audits by securing selected source documents and research records from participating sites for audit, or by auditing at participating sites.
- The lead site is the central location and coordinating center for the collection and maintenance of documentation of adverse events and is responsible for submitting adverse event reports to the PI promptly. The lead site will maintain documentation of all adverse event reports for each participating site. Adverse event reports submitted to the lead site must be signed and dated by the participating site's PI. The lead site will provide appropriate forms to be used by all participating sites for reporting adverse events. See section 8.3 and Appendix D for details on this process.

Participating Sites: Participating sites are responsible for performing the following tasks:

- Following the protocol as written, and the guidelines of Good Clinical Practice (GCP).
- Submitting data, identifiers redacted, to the lead site for oversight and monitoring.
- Registering all patients with the lead site by submitting patient registration form and signed informed consent promptly.
- Providing sufficient experienced clinical and administrative staff and adequate facilities and equipment to conduct a collaborative trial according to the protocol.
- Maintaining regulatory binders on site and providing copies of all required documents to the lead site.
- Collecting and submitting data according to the schedule specified by the protocol.
- Participating sites are responsible for reporting adverse events to their IRB according to its specific requirements and to the lead site

5.0 Study Outcomes and Study Measures

5.1 Primary Outcome

5.1.1 **Fatigue** at 6 months is the primary outcome for this trial. The PROMIS Fatigue 7a Short-Form assesses the experience (3 items) and impact (4 items) of fatigue during the past week. In the PROMIS initiative, fatigue is divided into the experience of fatigue (frequency, duration, and intensity) and the impact of fatigue (upon physical, mental, and social activities). Item responses are rated on a five-point scale ranging from "never" to "always" and are summed for a total score and transformed to a T-score metric, which has a mean of 50 and a standard deviation of 10. Higher scores indicate more fatigue. The PROMIS Fatigue Short-Form 7a has shown robust reliability and validity across multiple

samples.[38-42] Complete reliability and validity information on the PROMIS Fatigue can be found on the Assessment Center website (www.assessmentcenter.net). Recommendations for classifying fatigue based on the T scores are as follows: <50 normal; 50-59 mild; 60-69 moderate; ≥70 severe.[43]

All patients will be evaluable for the primary outcome from the time of randomization.

5.2 Secondary Outcomes

5.2.1 Quality of life

General Quality of Life will be measured using the Patient Reported Outcomes Measurement Information System© (PROMIS©) Global Health Short Form (SF), a 10-item instrument representing multiple domains.[44] Items assess self-reported measures of general aspects of physical, mental and social health in adults. Raw scores are summed within each sub-domain and converted to T-scores using a conversion table. T-score distributions are standardized such that a 50 represents the average for the US general population, and the standard deviation around that mean is 10 points. Higher scores indicate better general health than the general population.

Quality of life will also be assessed by the Hot Flash Related Daily Interference Scale (HFRDIS). HFRDIS is a 10-item scale that assesses the degree to which hot flashes interfere with a variety of daily activities and quality of life. Interference is rated on an 11-point scale (0=not interfere; 10=completely interfere). The validity of the HFRDIS has been demonstrated in breast cancer survivors and healthy women.[45] HFRDIS has also been used in prostate cancer patients receiving ADT.[46]

Sleep disturbance will be measured using the PROMIS Sleep Disturbance (SD) SF 8a.[47,48] The PROMIS-SD items assess self-reported perceptions of sleep quality, sleep depth, and restoration associated with sleep. This includes perceived difficulties and concerns with getting to sleep or staying asleep, as well as perceptions of the adequacy of, and satisfaction with, sleep. The PROMIS-SD SF is not disease-specific and assesses sleep disturbance over the past seven days. It has been tested and exhibited validity evidence (e.g., expected associations, discrimination among known groups) in a wide range of populations including, but not limited to, parents in neonatal ICU,[49] individuals with neurological conditions,[50] patients with pelvic pain,[51] and head and neck cancer patients.[52] Each question has five response options ranging in value from one to five. The lowest possible raw score is 8; the highest possible raw score is 40. Raw scores are converted to a standardized T-score; final scores are represented by the T-score with a mean of 50 and a standard deviation of 10. Higher scores indicate more sleep disturbance.

Cognitive abilities will be measured using the PROMIS Cognitive Abilities SF 4a, which assesses patient-perceived functional abilities related to mental acuity, concentration, and memory. The PROMIS Cognitive Abilities SF is not disease-specific and assess abilities over the past seven days. It has been successfully validated in adult medical outpatients,[53] and elderly individuals.[54] It has also been proven comparable to the FACT-Cog often used with cancer patients.[55]. Raw scores are converted to a standardized T-score; final scores are represented by the T-score with a mean of 50 and a standard deviation of 10. Higher scored indicate more cognitive ability.

5.2.2 Physical function, physical fitness, and body composition

Self-reported physical function will be measured using the PROMIS Physical Function 10a SF, which is designed to assess self-reported capability rather than actual performance of physical activities.[42,56] Capabilities include the function of upper extremities, lower extremities, and central regions, as well as instrumental activities of daily living. The form consists of 10 items, is universal rather than disease-specific, and assesses current function rather than function over a specified time period. Studies show PROMIS physical function short forms are valid and reliable in multiple patient populations including but not limited to prostate cancer, other cancers, and rheumatoid arthritis.[57-59] Raw scores are summed within each sub-domain, and converted to T-scores using a conversion table. T-score distributions are standardized such that a 50 represents the average for the US general population, and the standard deviation around that mean is 10 points. Higher scores indicate better physical function general health than the general population.

Physical performance will be objectively assessed using the Short Physical Performance Battery (SPPB). This validated measure comprises a short walk, repeated chair stands, and balance test. Each performance measure is scored ranging from 0-4 (0 = unable to complete; 4 = highest performance level), with total sum score range from 0-12. Lower score on the SPPB have been associated with increased risk of disability, hospitalization and worse survival among older adults with and without cancer.[60-62]

Submaximal (6-minute walk) exercise capacity will be measured to assess physical fitness. The 6-minute walk[63] is a measurement that is easy to administer in a clinical setting, feasible, and accurately assesses submaximal exercise capacity. The 6-minute walk is an independent predictor of mortality and is correlated with peak V02 testing.[64]

Body composition: ADT has been associated with changes in body composition, specifically a decrease in lean body mass and an increase in adiposity.[65] Whole body lean mass, fat mass, and bone mass will be measured in participants, enrolled at the main campus of lead site only (Wake Forest), by duel energy X-ray absorptiometry (DXA).[66] BMI will be calculated from height and weight.

5.2.3 **PSA progression** and **progression-free survival**

PSA will be measured at screening, 6, and 12 months while patient is on MGE/placebo, but all PSA measurements obtained per standard-of-care over 12 months (from initiation of MGE/placebo) will be captured.

PSA progression is defined as two serial increases in PSA from time of randomization (at least two weeks apart) and a PSA ≥0.2 ng/mL in prostatectomy patients and ≥2.0 ng/mL in radiation patients (based on local lab measurements). The first date of increasing PSA at or above these thresholds is considered the PSA progression date. Patients will be followed for up to 12 months from study initiation for PSA progression. Participants will be censored at time of loss to follow-up, or at the end of study, or at disease progression (defined as radiographic progression, need for new anticancer therapy, or death).

Progression-free survival is defined as the time from randomization to disease progression or death. Participants are censored if lost to follow-up or, at the end of 12 months, if no event has occurred. Radiographic evidence of disease progression, initiation of new systemic therapy by the treating physician, or death is considered disease progression regardless of PSA. Radiographic assessment of the prostate cancer will be per the discretion of the treating physician.

5.3 Exploratory Outcomes

5.3.1 Biomarkers of angiogenesis, inflammation, and oxidative stress include but are not limited to 8-OH deoxyguanosine.

5.3.2 Phenolic levels and metabolites in the blood and in the urine will be assessed using mass spectrometry.

5.3.3 Manganese superoxide dismutase genotype AA will be assessed using germline DNA derived from white blood cells at baseline.

6.0 Treatment Plan

6.1 Study-Related Activities

Measures / Forms	Pre-Study[a]	Baseline	Week 2	Week 6	Week 12[b] (3 mo)	Week 16	Week 20	Week 24[b] (6 mo)	Week 36[b] (9 mo)	Week 48[b,c] (12 mos)	Off-study (12 mos)
Informed consent	X										
Medical and oncologic history	X										
Concomitant medications	X	X			X			X	X	X	
Vital signs		X						X		X	
ECOG performance status		X									
Adverse event assessment		X			X			X	X	X	
Drug dispensing[d]		X			X			X	X		
Pill count					X			X	X	X	
ADT administration status[e]	X				X			X	X	X	
Telephone follow-up[f]			X	X		X	X				
Survival status[c,g]											X
Standard-of-care Evaluations											
INR level, for patients on warfarin[m]				X							
Research Evaluations											
Body composition (height, weight, DXA – DXA in Wake main campus participants only)[n]		X								X	
Physical function & fitness (SPPB, 6-min walk)		X						X		X	
CBC[i]	X							X		X	
Serum chemistries[i]	X							X		X	
Serum PSA[j]	X							X		X	
Serum testosterone[k]		X						X		X	
(Partially fasting) labs for phenolic levels (blood and urine)[k]		X						X		X	
(Partially fasting) labs for correlative studies[l] (blood and urine)		X						X		X	
DNA[l] (correlative)		X									
PROMIS Fatigue survey		X			X			X	X	X	
Other patient-reported outcomes: PROMIS Global QOL, HFRDIS, PROMIS-SD, PROMIS Cog, PROMIS physical function		X			X			X		X	

[a] Labs to assess eligibility must be completed within 30 days prior to the first dose of MGE/placebo.

[b] Follow-up study visits will occur every 12 weeks and will have a ±14 day allowance window which includes not just the visit itself, but any of the activities at that visit. For example, if labs cannot be collected at the actual study visit, the ±14-day window permits a second attempt at lab collection; all activities, however, must be completed in the ±14-day window from the expected visit date. At the lead site, these visits will occur in the Clinical Research Unit and may not be coupled with MD visits. At the discretion of the study team, due to COVID-19, follow-up visits may occur in whole or in part by phone, followed by the pills being shipped to participants who remain on treatment.

[c] PSA and disease status will be assessed for up to 12 months per review of treating physician notes or phone call as needed.
[d] Study drug (MGE or placebo) will be dispensed as a 90-day supply at each visit.
[e] Confirmation of continuous ADT dosing while on MGE/placebo. Androgen deprivation therapy (ADT) will be given per the treating physician. Any GnRH agonist or antagonist is permitted and dosing needs to be continuous for 1 year while on study (given within 42 days of due date). Surgical castration is also allowed.
[f] Adherence will be assessed at each study visit. In addition, participants will be contacted by phone to assess adherence as follows, ±3 days: Wk2, Wk6, Wk16, Wk20. At the Wk6 call, we will also assess potential adverse events. Monthly adherence after the 6M time point will continue only for participants who return >125 pills at the 6M visit.
[g] Survival status will be assessed by chart review or phone every 6 months for up to 12 months from study enrollment.
[h] <deleted>
[i] Safety laboratory assessments must be done within 30 days of first dose of MGE/placebo and then on the study visits indicated. These labs include:
Complete blood count (CBC): WBC count, platelet count, hemoglobin, and hematocrit.
Serum chemistries: creatinine, aspartate aminotransferase (AST), alanine aminotransferase (ALT), total bilirubin, and alkaline phosphatase.
[j] PSA levels will be obtained at screening, 6, and 12 months on study. In addition, all standard-of-care PSA measurements will be recorded for 12 months after MGE/placebo initiation.
[k] Testosterone levels will be obtained at baseline, 6, and 12 months on study.
[l] Phenolic levels and other research labs will be obtained at baseline, 6 months, and 12 months (±14 days). Patients are asked to avoid coffee, tea, fruit juice, fruit, chocolate or wine after midnight prior to blood draw. Blood for manganese superoxide dismutase genotype will be collected at baseline only.
[m] Participants on warfarin should have their INR checked within 4 weeks (+/- 14 days) of starting MGE to ensure there is no clinically significant interaction. This can be performed locally. Further blood work to monitor INR levels are at the discretion of the treating physician per usual care. We will verify that an INR check was performed (if needed) during the call at 6 weeks.
[n] Only subjects participating at WFUHS main campus will participate in the DXA scan.

6.2 Treatment Administration

Treatment will be administered on an outpatient basis. No investigational or commercial agents or therapies, other than those described below, may be administered with the intent to treat the patient's malignancy.

Patients will be randomized 1:1 basis to either MGE or placebo. The study is double-blind and the patients will take 4 capsules by mouth twice daily.

6.2.1 Investigational agent

This phase 2 study uses a specific formulation of muscadine grape extract (MGE) produced by Piedmont Research & Development Corporation from a powdered extract obtained from NPC Corporation, formerly known as Nature's Pearl (Advance, NC). The same product was used for a phase 1 study in advanced cancer patients (FDA IND 128937; clinicaltrials.gov NCT02583269). Each capsule will contain 500mg of MGE, which corresponds to approximately 165 mg total phenolics per capsule.

A matching placebo will be supplied by Clinical Encapsulation Services (CES) (Schenectady, NY), an FDA-registered facility, registration number 16691092818. The capsules will contain 500 mg of microcrystalline cellulose

tinted to match MGE via Colorcon® or Chromatech, and will be encapsulated with a vegetable capsule of hypromellose obtained from ACG (South Plainfield, NJ) or Capsugel (Charlotte, NC) of the same size as the MGE capsules. Both the MGE and matching placebo will be bottled and labeled by CES.

6.2.2 Other Agent

Androgen deprivation therapy (ADT) is to be started prior to initiation of MGE/placebo. Any form of medical or surgical castration is permitted. If medical castration is used, the dosing should be continuous without interruption for the 12 months of treatment with MGE/placebo. For the purpose of this study, continuous dosing is defined as ADT given within 6 weeks (42 days) of ADT due date. Intermittent dosing ADT after 12 months on study is acceptable, per the discretion of the treating physician. Androgen pathway inhibitors such as enzalutamide, abiraterone, darolutamide, and apalutamide, are not considered ADT. Therefore, ceasing use of inhibitors is not a reason to come off-study as long as ADT continues.

6.3 General Concomitant Medication and Supportive Care Guidelines

Patients should receive *full supportive care*, including antibiotics, antiemetics, transfusions of blood and blood products, 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 treating physician, i.e., chronic treatments for concomitant medical conditions, as well as agents required for life-threatening medical problems.

Participants on warfarin should have their INR checked within 4 weeks of starting MGE to ensure there is no clinically significant interaction. This can be performed locally. Further blood work to monitor INR levels are at the discretion of the treating physician per usual care.

6.4 Study assessments

All study-related activities at the lead site will occur at the Wake Forest Baptist Medical Center Clinical Research Unit and will not be coupled with standard-of-care clinic visits. At the discretion of the study team, in response to COVID-19, follow-up visits may occur in whole or in part by phone, followed by the pills being shipped to participants who remain on treatment. Participants will receive a $10 gift card at the completion of each study assessment (for up to $50 total). This gift card serves to cover all or part of their travel expenses in attending study appointments.

6.4.1 Vitals signs, performance status

Vital sign assessment consists of pulse, blood pressure, respiration rate, temperature.

Performance status will be assessed at baseline using the ECOG performance status scale.

6.4.2 Laboratory assessments (safety)

The following laboratory studies will be obtained at specified intervals to assess subject safety while on study:

- Complete blood count (CBC): Total white blood cell count (WBC), hemoglobin, hematocrit, and platelet count.
- Blood chemistry: Creatinine, total bilirubin, alkaline phosphatase, AST (SGOT), ALT (SGPT).
- Participants on warfarin should have their INR checked within 4 weeks of starting MGE to ensure there is no clinically significant interaction. This can be performed locally. Further blood work to monitor INR levels are at the discretion of the treating physician per usual care.

Critical laboratory values will be reported to the PI and to the treating physician.

6.4.3 Laboratory assessments (other)

The following laboratory studies will be obtained at specified intervals while on study:

- Prostate specific antigen (PSA). All PSA measurements obtained, both per study requirements and per standard-of-care, will be recorded during the 12 months on study.
- Testosterone level
- Correlative research labs (refer to **section 10.0**)

6.4.4 Imaging

Imaging may be performed as clinically indicated per the discretion of the treating physician, as per standard-of-care.

6.4.5 Body composition and physical function assessment

Height and weight will be obtained at specified intervals. Whole body lean mass, fat mass, and bone mass will be measured by duel energy X-ray absorptiometry (DXA). DXA will be performed only on participants who enroll at the main campus of lead site (Wake Forest).

Physical performance will be objectively assessed using the Short Physical Performance Battery (SPPB). This validated measure comprises a short walk, repeated chair stands, and balance test. Submaximal (6-minute walk) exercise capacity will be measured to assess physical fitness.

6.4.6 Patient reported outcomes

The PROMIS fatigue survey will be administered at baseline, 3, 6, 9, and 12 months. Other patient-reported outcomes will be administered at baseline, 3 months, 6 months, and 12 months as outlined in the study calendar (see section 6.1).

6.5 Duration of Study Intervention

Treatment with MGE/placebo will continue for 12 months or until one of the following criteria applies:

- Prostate cancer disease progression requiring a new cancer therapy
- Intercurrent illness that prevents further administration of treatment;
- Unacceptable adverse event(s) attributable to MGE/placebo;
- Patient decides to stop ADT or opts for intermittent ADT therapy prior to 12 months of continuous ADT (see section 6.2.2). No longer taking androgen pathway inhibitors is permissible;
- Patient misses more than 28 consecutive days of treatment for any reason;
- Changes in the patient's condition render the patient unacceptable for further treatment in the judgment of the treating physician;
- Patient decides to withdraw from the study; or
- Patient is noncompliant, determined at the discretion of the principal investigator in conjunction with the treating physician.

6.6 Duration of Follow Up

Patients will be followed for toxicity/adverse events for 72 hours after the last dose of the study drug and while on study per Table 6.1.

Patients will be instructed to inform the study staff of any medical concerns that arise during the first 3 days after stopping the study drug for any reason. The study team will assess for toxicity/adverse events and determine attribution. Of note, a 24-hour washout period was adequate in the Phase 1 protocol with the same drug (CCCWFU #01815).

Patients removed from the study intervention for unacceptable adverse events will be followed until resolution or stabilization of the adverse event. All participants who discontinue taking the study drug (see section 7.1) will be asked to continue study visits per protocol for the duration of the year. If they are unable or unwilling to come to the CRU as planned, study staff will attempt to collect fatigue and other QOL data in lieu of study visits as long as consent is not withdrawn. All participants will be followed for the study outcomes until the end of the study.

Patients will be followed for up to 12 months from study initiation for disease progression or death.

6.7 Criteria for Removal from Study Intervention

All participants will be expected to complete the 6 month study visit, unless they withdraw consent or are medically unable, even if they have stopped MGE/placebo. All participants will be asked to also complete the 12 month study visit, but if they are unable or unwilling, they will be contacted by phone and asked to complete the survey-based data.

Participant will be removed from study intervention when any of the criteria listed in **section 6.5** applies.

6.8 Procedures for removal from study treatment

If a participant discontinues his MGE/placebo or is removed from the study intervention for any reason, he will be seen at the next scheduled follow-up. He will be asked to return all pills and to complete the study-related activities.

7.0 Dosing Delays/Dose Modifications

7.1 Missed doses

If a dose of MGE/placebo is missed, the participant will be instructed to take his next dose as scheduled. Missed doses should not be added to the next dose.

If treatment must be held for any reason, it must be restarted **within 28 days,** or the participant will be removed from the study intervention. Unused pills will be collected at the next study visit. No more pills will be dispensed if 28 consecutive days are missed.

At each study visit, participants will be sent home with blank calendar pages for the upcoming 3 months, where they can mark am/pm doses as they are taken. Participants will be contacted by phone at Weeks 2, 6, 16, 20 (± 3 days) to assess adherence between study visits (see Appendix Y). After the 6-month study visit, adherence calls will cease unless the participant returns ≥125 pills at the 6M study visits. In that event, calls will continue until the 9-month visit. After the 9-month study visit, adherence calls will cease unless the participant returns ≥125 at the 9-month visit. Adherence data from phone calls will be kept in the study file.

7.2 Dose modification

There will be no dose-modifications on study. Either the patient or the treating physician can choose to discontinue MGE/placebo at any time. If necessary, MGE can be held for up to 28 days.

Participants or physicians who notify the study team of potential study drug-related toxicities will be contacted by the study PI for further information. Participant will permanently discontinue the study drug for any attributable grade 4 toxicity. Otherwise, appropriate supportive care recommendations will be made, and the study team will follow up with the participant to make sure toxicity has resolved to grade 0-1. If the participant continues to experience grade 2 or 3 toxicity, he will hold the study drug for up to 27 days, and the study team will again follow up with the participant to ensure toxicity has resolved to grade 0-1. If the toxicity persists beyond 27 days and is possibly attributable to the study drug, the study drug will be permanently discontinued. If the toxicity resolves to grade 0-1, the participant will restart the study drug. If the same grade 2 or higher possibly-attributable toxicity returns despite appropriate supportive care measures (e.g. diarrhea despite use of anti-diarrheal medication), the participant will permanently discontinue the study drug.

8.0 Adverse Events List and Reporting Requirements

8.1 Adverse Event List

8.1.1 MGE/placebo

Expected: Based on previous studies, minimal AEs are expected, which may include flatulence, diarrhea, nausea, dyspepsia, constipation, and abdominal cramping.

8.1.2 Androgen deprivation therapy

Surgical or medical castration can cause side effects due to changes in the levels of testosterone. All patients will be receiving ADT, and this is not the intervention being studied.

Expected side effects of ADT include but are not limited to: fatigue, reduced or absent libido, impotence, hot flashes, breast tenderness or growth of breast tissue, decreased bone density, anemia, decreased mental acuity, loss of muscle mass, weight gain. Patients receiving medical castration may also experience injection site tenderness or swelling. These side effects will not be considered study-related adverse events.

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 5.0 will be utilized for AE reporting. All appropriate treatment areas should have access to a copy of the CTCAE version 5.0. A copy of the CTCAE version 5.0 can be downloaded from the CTEP web site (http://ctep.cancer.gov).

'Expectedness': AEs can be 'Unexpected' or 'Expected' (see Section 8.1.1 above) for expedited reporting purposes only.

Attribution of the AE:

Definite: The AE **is clearly related** to MGE/placebo.
Probable: The AE **is likely related** to MGE/placebo.
Possible: The AE **may be related** to MGE/placebo.
Unlikely: The AE **is doubtfully related** to MGE/placebo.
Unrelated: The AE **is clearly NOT related** to MGE/placebo.

8.3 STRC SAE Reporting Requirements

The Safety and Toxicity Reporting Committee (STRC) is responsible for reviewing SAEs for WFBCCC institutional studies as outlined in Appendix D. STRC requires that all unexpected 4 and all grade 5 SAEs on these trials be reported to them for review. All WFBCCC Clinical Research Management (CRM) staff members assisting a PI 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.

See section 6.6 and Appendix D for details.

Data Safety and Monitoring
The protocol is being conducted at additional sites external to WFBH. In conjunction with the STRC at WFBH, the principal investigator (PI) is responsible for overall monitoring of the data and safety of study participants at all sites. The PI will assure that the study is conducted, recorded, and reported in accordance with the protocol and applicable regulations. Data for safety and SAEs will be monitored on an ongoing basis through regular study team meetings, including data from all sites involved.

For external sites, SAEs, whether or not considered drug-related, should be reported by the study team to the lead site within 24 hours of discovery. The process is as follows:

- Research nurse or study team member becomes aware of the SAE.
- Within 24 hours of the SAE discovery:
    - Site follows its own SAE reporting protocol, notifies the site PI, and documents the event in entirely within 24 hours if its discovery.
    - Participant's treating physician reviews data and attributes SAE as necessary, acknowledging SAE using their template.
    - Research nurse or study team member notifies WFBMC study staff as soon as possible [redacted] of SAE. All necessary information is provided at that time for WFBMC staff to complete required STRC reporting documentation as soon as possible (within 24 hours) of becoming aware of the SAE.
- WFBMC study staff notifies WFBMC PI immediately.
- WFBMC study staff notifies STRC immediately, per STRC protocol (Appendix D).
- WFBMC study staff completes data entry of the event into the SAE Reporting Form in Wiser immediately.
- VA provides source documentation and completed Serious Adverse Event Reporting Form (Appendix S).

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 unanticipated problems involving risks to subjects or others occurring at another site conducting the same study that has been approved by the WFUHS IRB (external 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.

AEs will only be collected from the AE Ascertainment Form administered to participants per protocol. No AEs will be collected via screening review of the medical record. Specifically, routine review of oncology notes will not be required, but if an AE is noted in review of notes such as an ED note or an inpatient note, then it will be captured and the details of that AE will be obtained by reviewing all appropriate notes in the medical record. We will follow all the standard reporting procedures for SAEs including unexpected hospitalizations and/or other issues that arise between study visits as discussed in section 8.3 and Appendix D.

9.0 Confidentiality and Privacy

Confidentiality will be protected by collecting only information needed to assess study outcomes, minimizing to the fullest extent possible the collection of any information that could directly identify subjects, and maintaining all study information in a secure manner. To help ensure subject privacy and confidentiality, only a unique study identifier will appear on the data collection form. Any collected patient identifying information corresponding to the unique study identifier will be maintained on a linkage file, store separately from the data. The linkage file will be kept secure, with access limited to designated study personnel. Following data collection subject identifying information will be destroyed (state the anticipated time the data will be destroyed, e.g. three years after closure of the study, and the method of destruction), consistent with data validation and study design, producing an anonymous analytical data set. Data access will be limited to study staff. Data and records will be kept locked and secured, with any computer data password protected. No reference to any individual participant will appear in reports, presentations, or publications that may arise from the study.

10.0 Pharmaceutical Information

Muscadine grape extract is a nutraceutical compound.

A list of the adverse events and potential risks associated with the investigational agents administered in this study can be found in Section 8.

10.1 Pharmaceutical Accountability

Patients will be instructed to return their pill bottles and remaining pills at each study visit. Drug accountability logs will be maintained by the Wake Forest Investigational Drug Services for the investigative agent (MGE/placebo) 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. At non-Wake Forest sites, the site pharmacist will be responsible for collecting the quantity of pills returned at each study visit.

10.2 Muscadine Grape Extract (MGE)

Product description: This phase 2 study uses a specific formulation of muscadine grape extract (MGE) produced by Piedmont Research & Development Corporation from a powdered extract obtained from NPC Corporation, formerly known as Nature's Pearl (Advance, NC). The same product was used for a phase 1 study in advanced cancer patients (FDA IND 128937; clinicaltrials.gov NCT02583269).

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, bottled, and labeled at Clinical Encapsulation Services (CES) (Schenectady, NY). Quality control testing for microbial contamination (aerobic count, yeast and mold, E.coli/coliform, staph aureus, enterobacteriaceae, and salmonella) and phenolic levels occurs numerous times during the production process. Stability testing for both microbial contamination and phenolic levels will continue at regular intervals until the study is complete.

Storage requirements: Capsules should be stored at room temperature.

Route of administration: MGE is available for oral administration.

10.3 Placebo

Product description: Placebos will be supplied by Clinical Encapsulation Services (CES) (Schenectady, NY), an FDA-registered facility, registration number 16691092818. The capsules will contain microcellulose tinted to match MGE and will be encapsulated with a vegetable capsule of hypromellose of the same size as the MGE capsules. Placebo will be bottled and labeled by CES. Quality control testing for microbial contamination (aerobic count, yeast and mold, E.coli/coliform, staph aureus, enterobacteriaceae, and salmonella) and phenolic level (to ensure no cross contamination) occurs during the production process. Stability testing for microbial contamination and content uniformity will continue at regular intervals until the study is complete.

Storage requirements: Capsules should be stored at room temperature.

Route of administration: Placebo is available for oral administration.

11.0 Correlative/Special Studies

11.1 **Biomarkers of angiogenesis, inflammation, and oxidative stress** (blood & urine)

To be collected at baseline, 6, and 12 months. Approximately 10 mL of blood will be collected into an EDTA tube to measure cytokines and growth factors, including but not limited to HGF, VEGF, and 8-OH deoxyguanosine. Plasma will be collected and samples will be stored at -80°C indefinitely until batched analysis. At least 20 mL of urine will also be collected for 8-OH deoxyguanosine levels and stored at -80°C indefinitely until batched analysis.

11.2 **Phenolic assays** (blood & urine)

To be collected at baseline, 6, and 12 months. Total and component phenolics will be measured from blood and urine. Blood assays will require approximately 10 mL of blood. For the polyphenolic analysis[67] one tube of blood at each time point will be collected in tubes containing EDTA 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, 6 months, and 12 months. The urine will be collected into a urine collection cup and mixed with ascorbic acid; 20 mL aliquots will be stored at -80°C. The urine will be thawed on ice and used to measure polyphenols by UPLC-MS.

11.3 **Plasma, PBMC, and whole blood specimen banking**

Blood will be collected at baseline, at the 6 month visit, and at the 12 month visit for the future study of cancer-related biomarkers such as mitochondrial function, circulating exosomes, and tumor-derived microRNA. Approximately 10 mL of blood will be drawn into a 10 mL EDTA tube. 3 mL of whole blood will be removed and stored at −80°C without additional processing. The remaining 5-7 mL will be separated into peripheral blood mononuclear cells (PBMC) and plasma by gradient centrifugation. All samples are aliquoted and stored at −80°C until further use. The lead site will perform the laboratory analysis. Neither the local sites nor the patients will receive information regarding individual subjects' results.

11.4 **Manganese superoxide dismutase genotype**

Peripheral blood will be collected into a single 10 mL EDTA tube at baseline for analysis of the manganese superoxide dismutase genotype. Deidentified samples will be processed in the Tallant-Gallagher lab for DNA extraction.

The DNA will be stored at -80°C, then batched to Dr. James Eshleman at Johns Hopkins for analysis.

James R. Eshleman, MD, PhD
Molecular Diagnostics Laboratory Johns Hopkins University School of Medicine
Room 344, Cancer Research Building-II
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12.0 Data Management

Item/Form	Location
Informed consent document	OnCore/ WakeOne
Protocol registration & race/ethnicity verification	OnCore
Study visit data collection forms: Baseline, forms for months 3, 6, 9, 12	REDCap
Labs (CBC, serum chemistry, testosterone, PSA and tumor markers)	REDCap/ WakeOne
Correlative labs (blood/urine for phenolics and inflammatory markers)	REDCap
Adverse Event Ascertainment and Pre-Toxicity Forms	OnCore
PROMIS surveys (fatigue, global health, sleep disturbance, cognitive abilities, physical function)	REDCap
HFRDIS	REDCap
Physical function & fitness forms (SPPB, 6-minute walk test)	REDCap
DXA scans (main campus of lead site only)	OnCore
Telephone survey @ Wk6 only	REDCap

12.0 Statistical Considerations

This two-group, randomized study will have one primary comparison of interest: comparison of the MGE arm to the placebo arm. We will use an intention-to-treat approach by collecting data from participants on outcome measures (if possible) even if they discontinue the study intervention.

12.1 Analysis of Primary Objective (fatigue)

The primary objective of this study is to test the hypothesis that men with prostate cancer in the MGE group have less fatigue at 6 months than men in the placebo group. We will use an ANCOVA approach to compare the levels of fatigue at 6-months, adjusted for the baseline value. The ANCOVA approach is more efficient than an ANOVA to compare the intervention groups.[68] Although we do not anticipate that the groups will differ due to randomization, we will compare participant characteristics between groups and include them in the model to adjust for any group differences, if necessary, using a general linear model.

12.2 Analysis of Secondary Objectives

12.2.1 *To compare levels of fatigue in men in the MGE group compared to the placebo group at 3, 9, and 12 months.*

We will model fatigue over time (at 3, 6, 9, and 12 months) using a general linear mixed model (GLMM) ANCOVA approach with adjustment for the baseline value and necessary covariates to explore trajectories of change over time using linear contrasts to estimate group effects over time and with covariates, if necessary.

12.2.2 *To compare quality of life in men in the MGE group compared to the placebo group.*

We will use a general linear mixed model (GLMM) ANCOVA approach with adjustment for the baseline value and necessary covariates to explore trajectories of change over time using linear contrasts to estimate group effects over time and with covariates, if necessary to compare the levels of health-related quality of life, hot flashes, sleep disturbance, and cognition at 6-months, adjusted for the baseline value and any covariates reflecting group differences at baseline if applicable.

12.2.3 *To compare physical function, physical fitness, body composition in men in the MGE group compared to placebo group.*

We will test the hypotheses that the MGE group will have better physical performance, physical fitness, and decreased adiposity compared to the placebo group. As above, we will model the trajectory of change over time by group using an ANCOVA GLMM approach.

12.2.4 *To compare time to PSA progression in men in the MGE group compared to the placebo group.*

Participants will be censored at time of loss to follow-up, or at the end of study, or at disease progression (defined as radiographic progression, need for new anticancer therapy, or death). We will describe the time to PSA progression from the time of randomization using the Kaplan-Meier method by group. We will use a log-rank test to compare time to PSA progression between the two groups. If there are differences between the two groups at baseline, we will use Cox regression with adjustment for appropriate covariates to compare the two groups.

12.2.5 *To compare progression-free survival in the MGE group compared to the placebo group*.

Participants will be censored at time of loss to follow-up or end of study. We will use the Kaplan-Meier method to estimate progression free survival in the two groups. We will use a log-rank test to compare progression-free survival between the two groups. If there are differences between the two groups at baseline, we will use Cox regression with adjustment for appropriate covariates to compare the two groups.

12.3 Analysis of Exploratory Objectives

12.3.1 *To examine if differences in levels of fatigue at 6 months by intervention group vary in those participants who report meaningful fatigue at baseline compared to those who did not have meaningful fatigue at baseline.*

To explore whether baseline fatigue is related to differences in level of fatigue at 6 months by intervention group, we will perform the analysis described for the primary objective for subgroups of baseline fatigue,

defined as described in Section 5.1.1. Categories may be collapsed depending on the distribution of participants across categories.

12.3.2 *To examine if differences in levels of fatigue at 6 months by intervention group vary by completing at least 80% of the dose for the full course of treatment (i.e.,completing 12 months of treatment taking ≥80% of pills averaged over the 12 month period) compared to those who did not complete at least 80% of the dose for the full course of treatment.*

To explore whether intervention adherence is related to differences in level of fatigue at 6 months by intervention group, we will perform the analysis described for the primary objective for the subgroups of those who complete 12 months of treatment taking ≥80% of pills averaged over the 12 month period compared to those who do not.

12.3.3 *To compare levels of circulating biomarkers of angiogenesis, inflammation, and oxidative stress at baseline, 6, and 12 months between the MGE and placebo groups.*

We will model the trajectory of change over time by group using an ANCOVA GLMM approach with contrasts to make comparisons at the time points of interest.

12.3.4 *To compare phenolic levels and circulating phenolic metabolites at baseline, 6, and 12 months between the MGE and placebo groups.*

We will model the trajectory of change over time by group using an ANCOVA GLMM approach with contrasts to make comparisons at the time points of interest.

12.3.5 *To correlate PSA changes with changes in circulating biomarkers of angiogenesis, inflammation, oxidative stress, phenolic levels, and phenolic metabolites.*

Change in all measures from baseline to 6 months and from baseline to 12 months will be calculated and correlated using Pearson correlations. We will also consider modeling change in PSA as a function of change in the other variables using GLMM.

12.3.6 *To assess for the manganese superoxide dismutase genotype AA at baseline and to correlate genotype with fatigue and PSA levels at 6 months.*

To assess the impact of the manganese superoxide dismutase genotype AA, we will enter it into our model for the primary aim as a main effect and as its interaction with group (MGE or placebo) and time.

12.3.7 *To examine if differences in levels of fatigue at 6 months by intervention group vary by ADT alone compared to those taking ADT plus at least one additional androgen-pathway directed therapy.*

To explore whether changes in fatigue at 6 months differ in patients taking ADT alone compared to ADT plus one additional androgen-pathway directed therapy, we will perform the analysis described for the primary objective for subgroups ADT use (ADT alone vs. ADT plus at least one other androgen-pathway directed therapy). We will also examine including the ADT use predictor in the model described for the primary objective.

12.4 Power and Sample Size

With 53 men per group, we will have 87% power to detect a difference in the means between the two groups of 0.50 SD at 6 months using an ANCOVA with a 0.05 two-sided significance level, assuming a 15% drop out rate (N=45 per group) and correlation of r=0.65 between baseline and 6 months. This difference is consistent with the minimally important difference, which is the difference in a score that is considered large enough to reflect a clinically meaningful difference. For cancer patients on the PROMIS fatigue scale, the interquartile range of the minimally important difference was estimated to be 0.39 to 0.65, with a median of 0.52. This range of effect sizes corresponds to a difference in the T-score of 3.0-5.0 points, with a median of 4 points; 0.50 SD corresponds to a T-score of 4.0 points, assuming an SD of 8.0.[43]

12.5 Estimated Accrual Rate

We estimate accruing 4 patients/month, with accrual expected to be completed in 48 months.

12.6 Estimated Study Length

Patients will be followed for a total of 12 months, thus the study will be completed 12 months after the last patient is accrued.

12.7 Interim Analysis Plan

There will be no interim analysis.

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APPENDICES

The following Appendices have been separated and **moved to the Other Documents section of the eIRB** in order to make access and administration easier.

Appendix A & B = Protocol Registration Form/Race & Ethnicity Verification Form
Appendix C = Eligibility Checklist **(script remains within this protocol document)**
Appendix D = SAE Notification SOP
Appendix E = Baseline Data Collection Form
Appendix F = 3 mo/ 9 mo Data Collection Form
Appendix G = 6 mo/12 mo Data Collection Form
Appendix H-M = Surveys (PROMIS and HFRDIS)
Appendix N & O = Physical Function Measures (SPPB & 6MWT)
Appendix P = Telephone Survey
Appendix Q = AE Ascertainment Form
Appendix R = AE Adverse Event Log
Appendix S = SAE Reporting Form
Appendix T = Disease Progression Form
Appendix U = Status Change Form
Appendix V = End of Study Follow Up Form
Appendix W = Pre-Toxicity Form
Appendix X = DXA Questionnaire
Appendix Y = Adherence Telephone Script

Appendix C - Subject Eligibility Checklist Script for Eligibility Screen by Phone
Text in ALL CAPS is for the recruiter only and should not be read aloud.

A. Hi, my name is _____ from Wake Forest Baptist Health. I got your name from Dr. _____ because s/he thought you might be a good candidate for our study about prostate cancer. Do you have a few minutes for me to tell you about the study and ask a few questions?

IF YES, PROCEED TO B.
IF NO: Is there a better time for me to contact you? IF YES, MAKE NOTES AND RECONTACT. IF NO, MARK AS REFUSAL ON PAGE 2 OF THE SCREENER AND END THE CALL.

B. Great, thanks! This study is for men with prostate cancer who are receiving androgen deprivation therapy (ADT). The goal of the study is to possibly help with side effects related to ADT. First we need to make sure everyone we enroll meets the criteria specified by the study. If you qualify and agree to participate, you will be randomized to one of two study groups. Both groups take 4 pills twice a day for a year, but in one group the pills are muscadine grape extract, and in the other group, it is a placebo, or 'sugar pill.' We are testing to see if the muscadine grape extract helps prostate cancer patients in any way, particularly with fatigue.

   The study will ask everyone to attend a study appointment every 3 months for a year, totaling 5 appointments. All 5 study appointments will involve you completing surveys and at 3 appointments we will collect blood and urine ask you to do physical function tests which involve walking a short distance. At 2 visits we will do a body scan called a "dexa". If you qualify, we will provide many more details about these procedures before you begin the study. Are you still interested; Can I ask you some questions to see if you might qualify?

IF YES, PROCEED TO C.
IF NO: I understand. Can I answer any questions for you or provide more information that might help you reconsider? IF YES, DO SO. AFTERWARDS SAY, "Do you think you might be interested in the study?" IF YES say, "Great, now let's see if you qualify" AND PROCEED TO C. IF STILL NO, MARK AS REFUSAL ON PAGE 2 OF THE SCREENER AND END THE CALL.

C. Now I am going to ask you some questions about your health history. Answer them as best you can. PROCEED TO PAGE 1 OF THE ELIGIBILITY SCREENER AND COMPELTE THE OUTSTANDING ITEMS. IF LABS ARE NEEDED AFTER SCREENING, PROCEED TO D. IF LAB VALUES WERE CONFIRMED PRIOR TO THE CALL, GO TO E.

D. Unfortunately, I still don't know if you are eligible because we need to check some bloodwork. Can I schedule you for an appointment here at Wake Forest Baptist Health? If you come in and consent to give some blood, we can use those results to figure out whether you will qualify for the study.

IF YES, SCHEDULE HIM OR DISCUSS THE PLAN FOR SCHEDULING HIM IN SECTION F.
IF NO, ask "Can I answer any questions for you or provide more information that might help you reconsider?" IF YES DO SO. AFTERWARDS SAY, "Can I schedule you for a blood draw now?"

IF STILL NO, MARK AS A REFUSAL ON PAGE 2 OF THE SCREENER AND END THE CALL.

E. DETERMINE IF ELIGIBILE OR INELIGIBLE.

IF **ELIGIBLE**: Thank you for answering all my questions! It looks like you qualify for our study! Let me tell you what will happen at the first study visit and during the year. USE CONSENT FORM TO DESCRIBE THE FIRST VISIT AND SUBSEQUENT VISITS. ASK IF HE HAS QUESTIONS; ANSWER THEM. THEN SCHEDULE THE BASELINE VISIT; SEE SECTION F. MARK ELIGIBLE ON PAGE 2 OF THE ELIGIBILITY SCREENER. IF HE REFUSES AT THIS POINT, ASK Can I answer any questions for you or provide more information that might help you reconsider?" IF YES, DO SO. AFTERWARDS SAY, "Can I schedule your first study appointment?" IF YES, SCHEDULE THE APPOITNMENT; SEE SECTION F. IF STILL NO, MARK AS A REFUSAL ON PAGE 2 OF THE SCREENER, THANK HIM AND END THE CALL.

IF **INELIGIBLE**: Thanks for answering all my questions. Unfortunately you do not qualify for our study because our study requires…..DESCRIBE WHY HE CAN'T ENROLL. THANK HIM AGAIN AND END CALL. MARK INELIGIBLE ON PAGE 2 OF ELIGIBILITY SCREENER.

IF **UNCERTAIN:** Thank you for answering all my questions. I will take this information to the doctor in charge and she will let me know if you qualify based on your answers. *If you qualify,* I will call you back to schedule an appointment. Do you have any questions for me before I go? ANSWER QUESTIONS; THANK HIM; CONSULT WITH PI.

F. SCHEDULING PLAN/NOTES FROM DISCUSSION HERE: