Pilot study of muscadine grape extract to improve fatigue among older adult cancer survivors (FOCUS)
Wake Forest Baptist Comprehensive Cancer Center
WFBCCC #98320
ClinicalTrials.gov: NCT04495751

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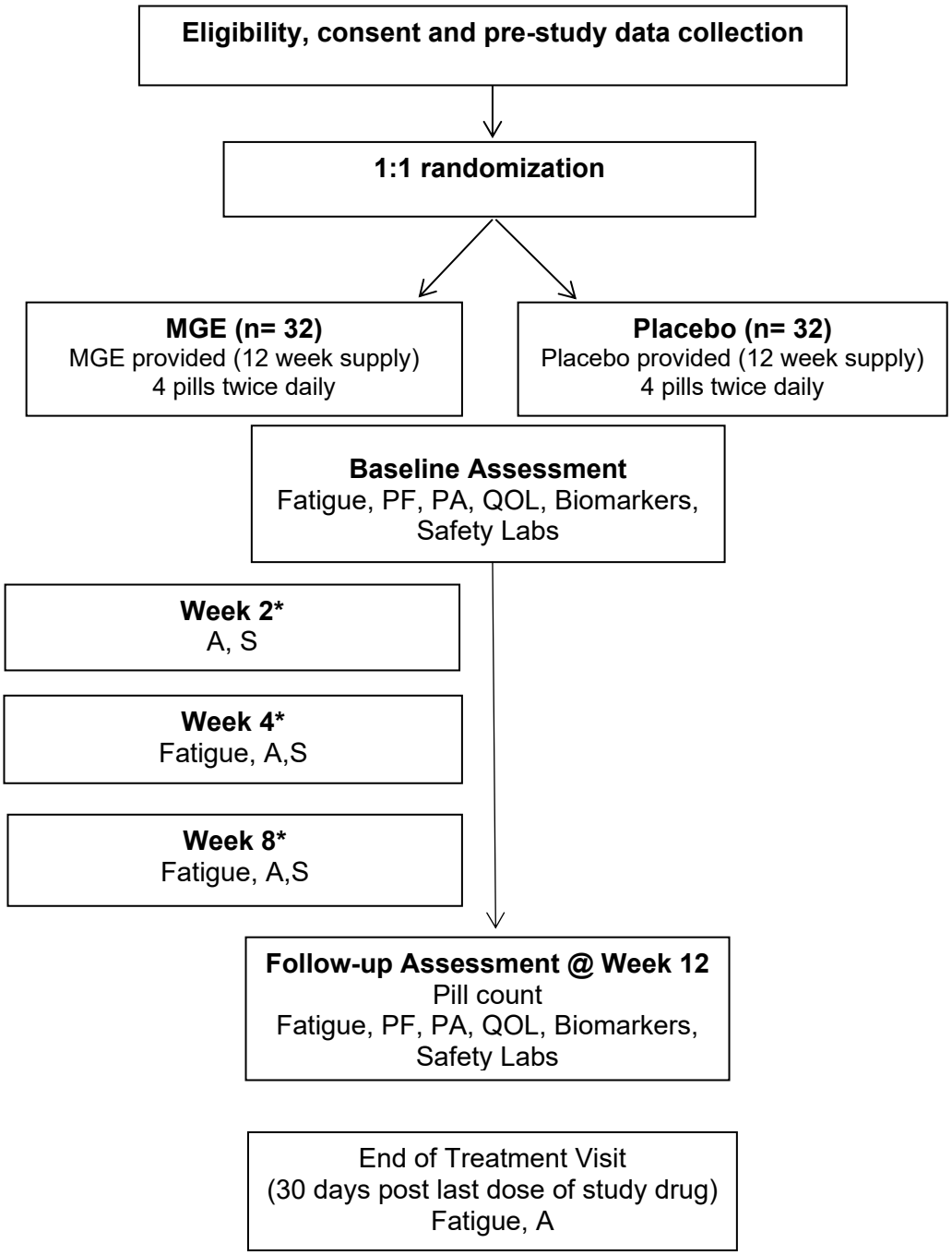
***NOTE*:** Appendices/Forms for use by study coordinators or CRU and site nursing staff were moved to the Other Documents section of the eIRB application.

Schema

Prospective double blind placebo controlled trial of 64 patients age 65 and over with a history of cancer who self-report fatigue. After baseline assessment, eligible subjects will be randomized and provided with proprietary MGE pills or placebo to take twice daily for 12 weeks (4 capsules twice daily). Subjects will receive phone call follow-up for toxicity assessment and adherence review at weeks 2, 4, 8. PROMIS Fatigue will also be collected on week 4 and 8 calls. Follow-up assessment will be completed at 12 weeks and a post-treatment assessment at 16 weeks.



Legend: MGE= muscadine grape extract; PF= physical function testing (SPPD, PAT-D, 6-minute walk); PA=physical activity QOL=quality of life/symptoms; A=adherence, S=symptom review; *collected by phone

1.0 Introduction and Background

Fatigue is the most prevalent symptom among cancer survivors and is commonly associated with aging.[1] Over 40% of community dwelling adults over age 65 reported prevalent fatigue in a recent national survey.[1] Older cancer survivors are therefore at particularly high risk of experiencing persistent fatigue which increases the risk of developing disability. Fatigue is associated with sedentary behavior, decline in physical activity and physical function, poor quality of life and contributes to the phenotype of frailty.[2-7] This cascade of symptoms and behaviors has negative long-term effects including increased risk of cardiovascular events, decreased muscle mass, increased health care utilization, and mortality risk.[5,8-11] Decreasing fatigue could have a significant impact on maintenance of functional independence and overall health for a large proportion of older adults.

Despite the common nature of the symptom, there are few pharmacologic interventions shown to improve fatigue. Treatments for fatigue after exclusion of modifiable medical causes include physical activity, mind body interventions and psychosocial interventions.[12] Despite evidence to support these strategies, these interventions can be resource intensive and difficult for most adults to maintain, particularly older adults. There are no well-tolerated FDA approved medications for treatment of chronic fatigue symptoms. Identifying well-tolerated, efficacious, and cost effective pharmacologic interventions to reduce fatigue is of high priority for the scientific community and of great interest to older adults.[13]

Nutritional interventions and nutraceuticals represent appealing strategies for treatment of fatigue.[14,15] Biologic mechanisms associated with fatigue are multi-factorial. Common biologic pathways implicated in fatigue symptomatology for both cancer-related and non-cancer related fatigue include systemic inflammation, impairment of mitochondrial function, and cardiac dysfunction.[6,16,17] Interventions targeting these pathways may be of particular relevance to older adults given the association between aging and dysregulation of systemic inflammation, mitochondrial function and cardiac health and is in line with the principles of geroscience.[18-23]

Muscadine grape extract (MGE) is a rich dietary source of bioactive phenols, including ellagic acid, gallic acid, anthocyanins, and flavan-3-ols, which have been studied with particular interest for their potential anti-inflammatory and anti-cancer properties.[24-39] Preclinical and clinical data from the investigative team show effects of MGE on inflammation, mitochondrial bioenergetics and the symptom of fatigue. This pilot study proposes translation of these observations to a high need clinical setting to test the effect of MGE supplementation on older adults with self-reported fatigue. This study will provide data on the effects of MGE on fatigue, function and biologic correlates to evaluate mechanisms.

1.1 Preclinical Studies with Proprietary MGE

Data from the Investigative Team Gallagher/Tallant Lab:
The MGE administered in the following preclinical studies and Phase 1 clinical trial is a proprietary compound that was prepared from the skins and seeds of muscadine grapes by Piedmont Research and Development Corporation (PRDC). The skins and seeds have high levels of polyphenols, micronutrients found in plants that provide health benefits. The MGE produced by PRDC has a higher concentration of total polyphenols as compared to other muscadine grape nutritional supplements on the market (Figure 1). Mass spectrometry analysis showed that the MGE from PRDC has a higher content of specific polyphenols as compared to other muscadine grape supplements (Figure 2). It should be noted that the proprietary MGE from PRDC is prepared from an aqueous solution of ground seeds and skins; therefore, resveratrol, which has limited solubility in water, was not detected.

1.2 Rationale for testing MGE to improve fatigue

Several mechanisms to support a potential MGE effect on fatigue have been suggested by pre-clinical studies. Data are highlighted below.

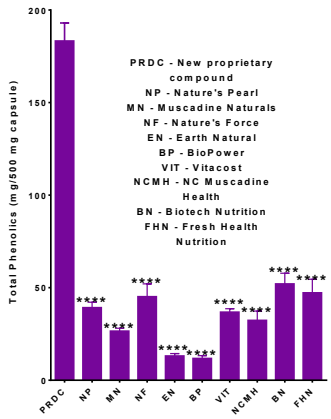


Figure 1. Total phenolic content of MGEs determined by Folin-Ciocalteu assay using gallic acid as a standard. n=3 separate lots, ****p<0.001 compared to PRDC.

A significant effect of MGE on exercise tolerance, an established fatigue surrogate, was observed in transgenic (mRen2)27, hypertensive female rats administered MGE (0.2 mg total phenolics/mL; in the drinking water starting at 14 weeks of age after the onset of high blood pressure. At 40 weeks of age (26 weeks of treatment), exercise tolerance was assessed on a treadmill (10.2 m x min^{-1}, 5% inclination). Time to fatigue (TTF: min) was increased 2.3-fold in MGE-treated animals compared to controls (**Figure 3**). Workload (*W: g X m*) was 2.4-fold higher in MGE-treated animals compared to untreated rats (**Figure 4**). At the end of the study, there were no significant differences in systolic blood pressure (166 ± 6 vs 162 ± 3 mm Hg) or body weight (365 ± 10 and 385 ± 8 g) between the control rats and the MGE-treated rats. This improvement in exercise tolerance was sustained following 56 weeks of treatment in 70 week-old rats (data not shown), suggesting that MGE consumption improves exercise capacity in aging adult female hypertensive rats independent of changes in blood pressure or body weight. MGE also

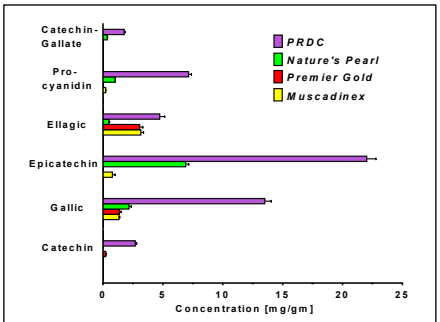


Figure 2. Concentration of specific phenolic compounds in MGEs were determined by ultra-high pressure liquid chromatography/mass spectrometry. n=3 separate lots.

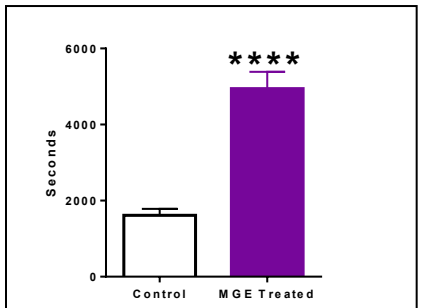


Figure 3. MGE enhanced exercise capacity (time to fatigue) in female hypertensive rats. n=5; ****<0.0001

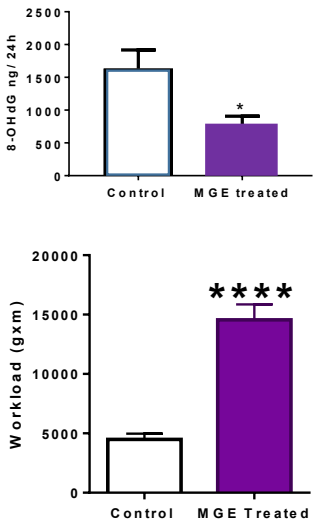


Figure 4. MGE enhanced exercise workload in female hypertensive rats. n=5; ****<0.0001

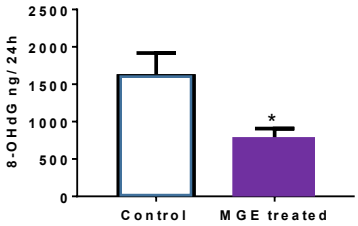


Figure 5. MGE treated rats had a decreased concentration of urinary 8-OHdG, indicating reduced oxidative stress. n=5; *p<0.05

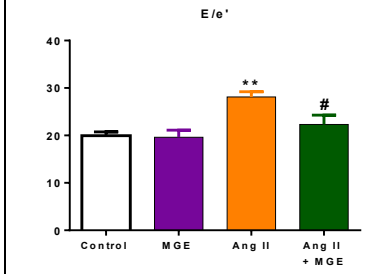


Figure 6. Ang II increased left ventricular filling pressure (E/e'), a measure of diastolic function, while MGE co-administration prevented these effects. E/e' was measured by echocardiography at week 4. **p<0.01 compared with control, # p<0.05 compared to Ang II alone.

reduced urinary 8-OHdG (**Figure 5**), a marker of oxidation, suggesting that oxidative stress may be involved in the improvement in exercise capacity.

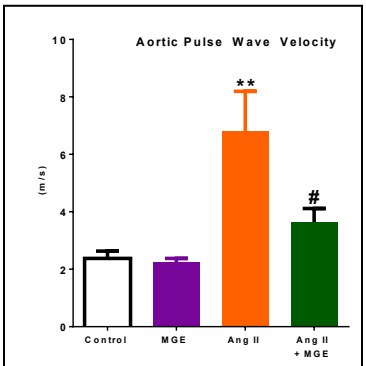


Figure 7. Ang II increased pulse wave velocity, a measure of arterial stiffness, while co-administration of MGE prevented these effects. **p<0.01 compared with control, # P<0.05 compared to Ang II alone.

To assess the effect of MGE on cardiac damage, a potential underlying mechanism for fatigue, male Sprague-Dawley rats were pretreated for 1 week with MGE in their drinking water (0.2 mg/mL total phenolics) followed by a 4-week infusion with Ang II (24 µg/kg/h) which increased blood pressure from 114 mmHg ± 1.9 to 211 mmHg ± 8.5. MGE administration had no effect on blood pressure or systolic function in normotensive or hypertensive rats but ameliorated the Ang II-induced decrease in diastolic function **(Figure 6**) and increase in interstitial cardiac fibrosis by reducing myocardial collagen III and prevented hypertension-induced aortic stiffness (**Figure 7**). These MGE effects were mediated by attenuating Ang II-induced dysregulation of inflammation (NF--κβ, IL-6), oxidative stress (Nox, NADPH oxidase, catalase), proliferation (MAP kinase), and fibrotic signaling pathways (TGF--β/Smad, CTGF) (data not shown due to page limitations). These results suggest that MGE may improve patient fatigue by preventing cardiac and vascular damage.

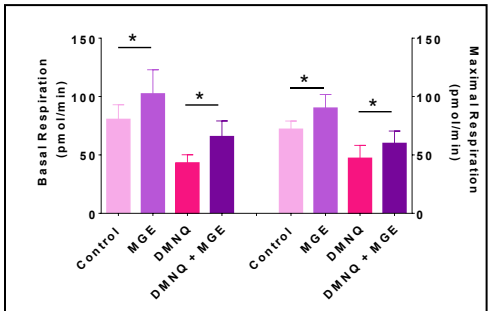


Figure 8. Mitochondrial metabolism was measured as the Oxygen Consumption Rate (OCR) by Seahorse analysis. MGE prevented the DMNQ-mediated reduction in basal and maximal respiration rates. *p < 0.05; n=3.

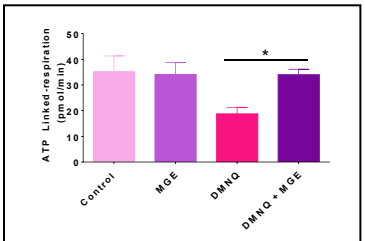


Figure 9. Mitochondrial metabolism was measured as the Oxygen Consumption Rate (OCR). MGE prevented the DMNQ-mediated reduction in ATP linked to respiration. *p < 0.05; n=3.

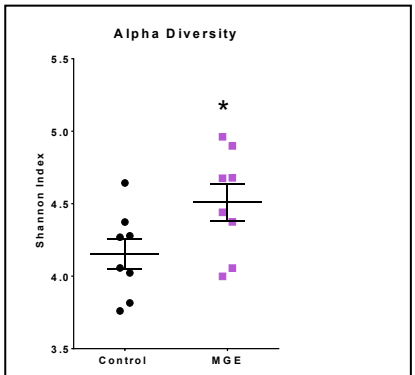


Figure 10. MGE increased microbial diversity in mouse fecal samples.

<u>Agents that preserve mitochondrial integrity and maintain or increase mitochondrial respiratory capacity may prevent cardiac tissue damage due to oxidative stress and thereby reduce fatigue.</u> Cardiac cells were treated with the redox cycling agent, 2,3 dimethoxynaphthoquinone (DMNQ; 15 µM) which generates both superoxide and hydrogen peroxide intracellularly and thus mimics pathologies of elevated reactive oxygen species. DMNQ increased cardiac cell cytotoxicity as measured by LDH release; this effect was prevented by MGE (30 µg total phenolics/mL). DMNQ increased the potential of the mitochondrial membrane, which was also prevented by MGE, suggesting protection of mitochondrial membrane integrity. Cell respirometry measurements showed that DMNQ reduced the oxygen consumption rate (OCR) by 50% (both basal and maximal respiration, **Figure 8**), while co-treatment with MGE prevented this reduction, suggesting that the protective effect may be mediated by preservation of mitochondrial function. MGE prevented the decrease in ATP-linked respiration (**Figure 9**). The basal extracellular acidification rate (ECAR) was increased 2-fold with MGE pretreatment when compared to DMNQ alone (13.6 ± 2.3 DMNQ+MGE vs. 6.8 ±.0.88 DMNQ, n=3, p<0.01), suggesting that MGE may also increase glycolysis as a response to stress. These data suggest that treatment with MGE enhances mitochondrial function and cellular bioenergetics during

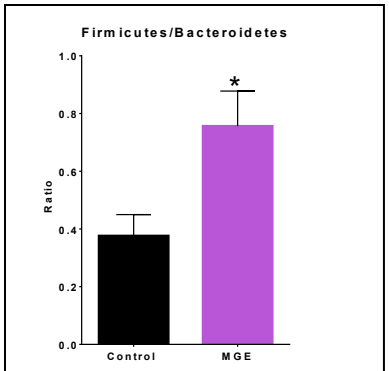


Figure 11. MGE altered the ratio of the relative abundance of Firmicutes to Bacteroidetes

oxidative stress and thus may effectively prevent tissue damage from oxidative stress to reduce fatigue 4T1 Triple negative breast cancer cells were injected into the mammary fat pad of 6-week-old female Balb/c mice. After 2 weeks, tumors were surgically removed and mice received either drinking water or 0.1 mg phenolics/mL of MGE in the drinking water. Mice were sacrificed after 4 weeks; tissues and fecal samples were collected for analysis. Alpha diversity (**Figure 10**) and the Firmicutes to Bacteroidetes ratio (**Figure 11**) were significantly increased in MGE-treated mice compared to the control, indicating enhanced microbial richness and increased energy harvest by the gut microbiome could improve symptoms of fatigue. Butyrate-producing bacteria, such as *Ruminococcus*, *Butyricicoccus* and *Lachnospiraceae*, were increased with MGE ($p<0.05$) as well as the anti-inflammatory compound butyrate compared to other short-chain fatty acids (25.0 ± 2.7% vs 75.3 ± 15.5%; $p<0.01$). Of note, the MGE supplement is a complex mixture of polyphenols that are further metabolized by the host cells or the gut microbiome to secondary and tertiary metabolites, including short chain fatty acids. **These metabolites may serve as additional energy sources, feeding directly into the TCA cycle and leading to enhanced energy production by the mitochondria, to reduce fatigue.**

1.3 Clinical Studies with MGE

The investigative team completed a Phase 1 trial (N=23) (ClinicalTrials.gov NCT02583269, IND 128937) in patients with advanced stage solid tumors who progressed on prior therapies (mean age 72 years) to assess the safety and tolerability of proprietary MGE. Five dose levels of the extract from one to five pills twice daily (320 to 1600 mg total phenolics/day) were tested in a standard 3 + 3 design. No concerning safety events were identified; maximum tolerated dose was not reached.[40]

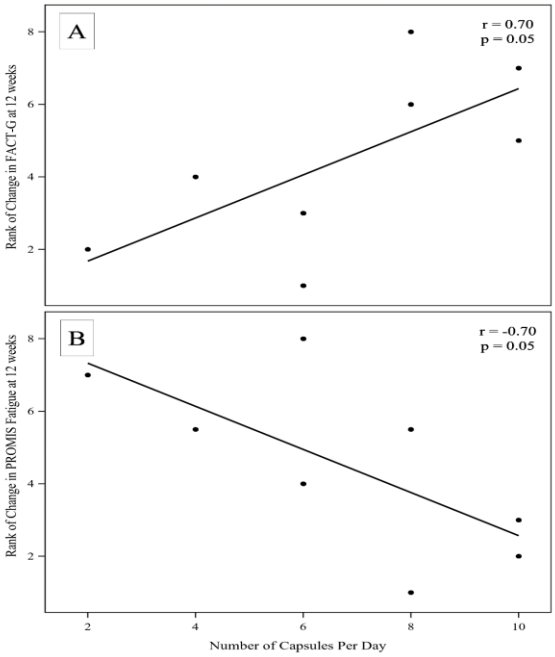


Figure 12

Median time on therapy was 8 weeks, with 29% of patients treated beyond 16 weeks and a median overall survival of 7.2 months. MGE adherence was high (98%) and the most common side effects were flatulence, diarrhea, and constipation. In this heavily pre-treated population, among patients on study at 12 weeks (n=8), **higher dose level of MGE was correlated with increases in the FACT-G score (r=0.70, p=0.05) and decrease in fatigue (r=-0.70, p=0.05)** (**Figure 12**). In the blood, the mean total phenolic level increased from baseline of 2.12 mg/mL (n = 18, sd = 304) to 2.33 mg/mL at week 8 (n = 16, sd =245; p = 0.01). An MGE breakdown component urolithin A glucouronidate, a marker for the ingestion/metabolism of MGE, was measurable in the urine. At baseline, the mean UAG levels were low with a mean of 0.4 µg/mL (n=18, sd=1.5) and increased after 8 weeks of MGE treatment to a mean of 17.4 µg/mL (n= 16, sd=44.4; p <0.01).

Additional clinical studies:

The investigative team is currently conducting a double blind randomized trial comparing MGE and placebo among men with prostate cancer (N=160) receiving anti-androgen therapy to evaluate effect on fatigue. Recruitment is on-going (IND 139288, NCT03496805).

1.4 Rationale for MGE dosing:

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 including those reported in 1.2 providing preliminary data for this application. This dose is roughly equivalent to 8 capsules of MGE daily (each capsule contains 500mg MGE which corresponds to approximately 165mg of total phenolics). 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 the phase 2 prostate study and for this proposed pilot, in which effects on fatigue will be assessed in subjects treated for or surviving cancer.*

2.0 Objectives

2.1 Primary Objective(s)

2.1.1 To evaluate whether administration of MGE supplementation (4 tablets twice daily, approximately 1320mg total phenolics) decreases PROMIS Fatigue score from baseline to 12 weeks compared to placebo.

Hypothesis: Participants randomized to daily oral MGE administration will experience greater decline in self-reported fatigue (PROMIS Fatigue) from baseline to 12 weeks (primary outcome) compared to those receiving placebo.

2.2 Secondary Objective(s)

2.2.1 To evaluate whether administration of MGE supplementation (4 tablets twice daily) causes changes in physical function (Pepper Assessment Tool for Disability [PAT-D], Short Physical Performance Battery), physical fitness (6-minute walk), physical activity (Minnesota Leisure Questionnaire), and sedentary behavior (Sedentary Behavior Questionnaire) from baseline to 12 weeks compared to placebo.

Hypothesis: Participants on the intervention will have improved physical function, fitness and activity while sedentary behavior will be lower at 12 weeks compared to the placebo arm.

2.2.2 To compare changes in health related quality of life (PROMIS Global Health) at 12 weeks in participants randomized to MGE group vs. placebo.

Hypothesis: Participants on the intervention will report improved quality of life at 12 weeks compared to the control arm.

2.2.3 To compare changes in the Fried frailty index at 12 weeks in participants randomized to MGE vs. placebo.

Hypothesis: Participants on the intervention arm will demonstrate improvement in frailty status compared to the placebo arm.

2.3 Exploratory Objectives

2.3.1 To explore whether MGE administration alters 8-hydroxy-2 deoxyguanosine, peripheral blood mitochondrial function, IL-6, TNF-alpha, C-reactive protein, hepatocyte growth factor from baseline to 12 weeks compared to the placebo arm.

2.3.2 To explore whether MGE administration alters the microbiome from baseline to 12 weeks compared to the placebo arm.

2.3.3 To explore whether 8-hydroxy-2 deoxyguanosine, peripheral blood mitochondrial function, IL-6, TNF-alpha, C-reactive protein, hepatocyte growth factor and the microbiome may mediate changes in self-reported fatigue and physical function, fitness and activity from baseline to 12 weeks.

2.3.4 To explore whether MGE administration causes changes in cognitive speed (Digital Symbol Substitution Test) from baseline to 12 weeks compared to placebo.

3.0 Patient Selection

3.1 Inclusion Criteria

3.1.1 Self-reported history of cancer diagnosed > 12 months prior to enrollment excluding non-melanoma skin cancer with no evidence of disease at enrollment.

3.1.2 Eligible solid tumor cancer types include Stage 1-3 breast, lung, head and neck, colorectal, anal, prostate, melanoma, bladder/ureteral, esophageal, gastric, pancreatic, kidney, liver/biliary, uterine, cervical, ovarian, sarcoma. (superficial disease and in situ disease only is excluded)

3.1.3 Eligible hematologic malignancies include lymphoma any subtype any stage in remission, multiple myeloma in remission, leukemia any subtype in remission.

3.1.4 Eligible prior cancer treatment modalities include surgery, radiation, chemotherapy, hormonal therapies, immunotherapy, biologic therapies.

3.1.5 All anti-cancer therapy completed > 12 months prior to enrollment

3.1.6 Age 65 years and older

3.1.7 Presence of self-reported fatigue defined by a response of “somewhat, quite a bit or very much” to the screening question “During the past seven days, did you feel fatigued: Not at all, a little bit, somewhat, quite a bit, very much?”

3.1.8 Ability to walk without requiring assistance from another individual (use of cane or walker acceptable)

3.1.9 Normal organ and marrow function as defined below:

- leukocytes ≥3,000/mcL
- absolute neutrophil count ≥1,500/mcL
- platelets ≥100,000/mcL
- total bilirubinwithin normal institutional limits
- AST(SGOT)/ALT(SGPT) ≤2.5 X institutional upper limit of normal
- creatinine clearance ≥30 mL/min

3.1.10 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 active malignancy or on-going cancer treatment including oral anti-estrogen therapy, immunotherapy, biologic therapy.

3.2.2 men receiving androgen deprivation therapy

3.2.3 use of Coumadin or Warfarin

3.2.4 symptomatic congestive heart failure

3.2.5 lung disease requiring oxygen

3.2.6 end stage renal disease requiring dialysis

3.2.7 inability to swallow capsules

3.2.8 chronic nausea or diarrhea defined by a frequency of ≥ once per week

3.2.9 hemoglobin <10 g/dl

3.2.10 diagnosis of dementia

3.2.11 uncontrolled intercurrent illness including, but not limited to ongoing or active infection, unstable angina pectoris, cardiac arrhythmia, or psychiatric illness/social situations that would limit compliance with study requirements.

3.2.12 known untreated hypothyroidism

3.2.13 allergy to muscadine grapes or muscadine grape preparations

3.3 Inclusion of Women and Minorities

Recruitment to this study is expected to be similar to national data on cancer survivors population and the demographics of the WFBCCC. We expect >50% of enrolled participants to be women survivors. We will not enroll children (≤ 18 years) as the research pertains to older adult survivors. Based on our cancer center data, we expect approximately 3% of participants to be Hispanic/Latino (n=2). The breakdown of racial categories is expected to be as follows: 14% Black or African American (n=9), 84% White (n=54) and 2% more than 1 race (n=1). Should we

not meet or exceed these estimates, the PI will engage the Office of Cancer Health Equity to discuss strategies to enhance recruitment in these target populations. Our cancer center has policies in place to enhance recruitment of minority participants and will provide translation of the protocol and informed consent into Spanish.

4.0 Recruitment and Registration Procedures

4.1 Recruitment

Participants will be recruited from the community via advertisement in the Center for Health Aging and Alzheimer's Prevention Newsletter, newspaper advertisement, postcard mailing, and through sharing information with interested community organizations. Subjects will also be recruited from the Wake Forest Baptist Cancer Center (WFBCCC) using flyer advertisement including but not limited to Survivorship Clinics, mailings to participants, and messages via MyWakeHealth, listing the trial in the WFBCCC clinical trial website used by providers and monitoring trial recruitment through the Cancer Control and Survivorship Disease Oriented Team. Interested participants will contact the MGE Team Clinical Coordinators who will conduct prescreening by phone and coordinate in person visits for eligible patients in the Clinical Research Unit, or at the High Point, Wilkes, or Statesville clinic sites.

4.2 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 or encountered at a clinic visit. 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 re-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.

4.3 Registration

All participants entered on any WFBCCC trial, whether treatment, companion, or cancer control trial, **must** be linked to the study in EPIC within 24 hours of Informed Consent. Participant **must** be registered prior to the initiation of treatment.

1. Complete the Eligibility Checklist (Appendix A)
2. Complete the Protocol Registration Form (Appendix B)
3. Register the participant in OnCORE – this will alert the cancer center registrar, who will then review the signed informed consent and confirm the eligibility.
   Contact Information:
   Protocol Registrar PHONE (336) 713-6767

Protocol Registrar FAX (336) 713-6772
Protocol Registrar E-MAIL (registra@wakehealth.edu)

4. Participant **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

To complete the registration process, the study team will:
1. Register the participant on the study
2. Assign a participant study number
3. Randomize the participant

4.4 Blinding

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

5.0 Study Outcomes and Study Measures

5.1 Primary Outcome

5.1.1 Patient Reported Outcomes Measurement System (PROMIS) Fatigue 7a will be used to assess fatigue. 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.[41-44]

5.2 Secondary Outcomes

5.2.1 The **Pepper Assessment Tool for Disability (PAT-D)** will used to assess self-reported physical function. The (PAT-D) is a 19-item survey designed by Rejeski et al. to assess domains of physical function in older adults which contains subscales on mobility, instrumental (IADLs), and basic activities of daily living (ADLs).[45,46]

5.2.2 The **Short Form Minnesota Leisure Time Activity Questionnaire (MLTA)** will assess self-reported physical activity.[7]

5.2.3 The **Short Physical Performance Battery (SPPB)** will be used to objectively assess lower extremity physical function. This validated measure comprises a short walk, repeated chair stands, and balance test. Lower scores on the SPPB have been associated with increased risk of disability, hospitalization and worse survival among older adults with and without cancer[47-49].

5.2.4 The **6-minute walk** will be measured to assess physical fitness. The 6-minute walk is easy to administer in a clinical setting, accurately assesses submaximal exercise

capacity, is an independent predictor of mortality and is correlated with peak V02 testing.[50]

5.2.5 The **Longitudinal Aging Study Amsterdam** (LASA) **Sedentary Behavior Questionnaire** will be used measure sedentary behavior[51].

5.2.6 The **PROMIS Global Health Short Form (SF)** will assess global QOL This is a 10-item instrument representing multiple domains inclusive of physical, mental and social health in adults[52].

5.2.7 **Adherence** will be measured by pill count at study completion.

5.2.8 The following measures of interest will be collected to allow characterization of frailty (Fried Frailty Phenotype), **Center for Epidemiologic Studies Depression Scale (CES-D), MLTA questionnaire, Grip strength, self-reported weight loss**.[7,53-55]

5.3. Exploratory outcomes

5.3.1 Peripheral blood will be collected at the baseline and week 12 study visit, and will be processed to obtain serum for the measurement of cytokines including interleukin 6 (Il-6), tumor necrosis factor-alpha (TNF-α), C reactive protein (CRP) and HGF and polymorphonuclear neutrophils (PMNs) to measure mitochondrial function. Cytokines and 8-OHdG will be measured using human ELISA kits (Abcam) and mitochondrial function will be assessed using a Seahorse XF Cell Mito Stress Test (Agilent).

5.3.2 Urine will be collected at baseline and 12 weeks to measure the oxidative stress marker 8-OHdG and urolithin A glucouronidate (UAG), a marker for the ingestion/metabolism of MGE. UAG, which is generated from the ingestion of MGE polyphenolic ellagitannins, will be measured using an LCMS-8050 triple quadrupole mass spectrometer in the Wake Forest School of Medicine Proteomics and Metabolomics Shared Resource.

5.3.4 Fecal specimens will be collected at baseline and at ~12 weeks using an at-home fecal sampling kit. Samples will be collected by the patient prior to the initiation of intervention and again near the completion of intervention. Patients will be asked to mail samples in a pre-paid package to the laboratory. DNA will be isolated from fecal samples using the Qiagen DNeasy PowerSoil Pro kit protocol. Metagenomic sequencing will be performed by CosmosID Inc. (Rockville, MD). In brief, DNA libraries will be prepared using the Illumina Nextera XT library preparation kit, with a modified protocol. Library quantity will be assessed with Qubit (ThermoFisher). Libraries will be sequenced on an Illumina HiSeq platform 2x150bp. Fecal samples will be read at 12M read-depth. Unassembled sequencing reads will be directly analyzed by CosmosID bioinformatics platform for multikingdom microbiome analysis and quantification of organism's relative abundance. Fecal microbiomes will be assessed by metagenomic sequencing and bioinformatics. A list of all the microbiologic species will be recorded, along with their relative abundance recorded as a % relative abundance of the total microbiome.

5.3.5 Digit Symbol Substitution Test (DSST)[56,57] is a measure of attention and perceptual speed, amenable to change with exercise or fatigue in which participants are given a

series of numbered symbols and then asked to draw the appropriate symbols below a list of random numbers. The score is the number of correctly made matches in 2 minutes (120 seconds).

5.4. Adverse events (AEs)

AEs will be assessed by the study team at each visit (by phone or in person). AEs of interest are flatulence, diarrhea, nausea, dyspepsia, constipation, abdominal pain (see Appendix P). Other possibly/probably attributable adverse events will be recorded in addition to any serious adverse event.

6.0 Intervention Plan

6.1 Study-Related Activities

Measures / Timepoints	Pre-Study[a]	Baseline	Wk 2	Wk 4	Wk 8	Wk 12	End of Trtmt Visit[h]
Informed consent	X						
Medical and oncologic history	X						
Concomitant medications assessed by self-report[j]	X	X	X	X	X	X[g, j]	
Karnofsky Self-Reported Performance Rating Scale		X					
Vital signs		X				X[j]	
Adverse event assessment		X	X	X	X	X[g, j]	X
Drug dispensing[b]		X					
Pill count						X[g, j]	
Telephone follow-up[c]			X	X	X		
Cognitive/Physical function & fitness (DSST, SPPB, 6-min walk, grip strength)		X				X[j]	
CBC with differential[d]	X					X[j]	
Serum chemistries[d]	X					X[j]	
(Partially fasting) labs for phenolic levels (blood and urine)[e]		X				X[j]	
(Partially fasting) labs for correlative studies[e] (blood and urine)		X				X[j]	
Stool sample collection[f]		X				X[j]	
PROMIS Fatigue survey		X	X	X	X	X[g, j]	X
Other patient reported outcomes (PAT-D, LASA, PROMIS Global Health, CES-D, MLTA, Self-reported weight loss)[j]		X				X[g, j]	
Participant Feedback						X[i, j]	

[a] Labs to assess eligibility must be completed within 30 days prior to the first dose of MGE/placebo.
[b] Study drug (MGE or placebo) will be dispensed as a 90-day supply at the baseline visit.
[c] Adherence and symptom review will be assessed at each study visit. In addition, participants will be contacted by phone as follows, ±3 days: Wk2, Wk4, Wk8.
[d] 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) with differential: WBC count with differential, platelet count, hemoglobin/hematocrit.
Serum chemistries (complete metabolic panel, CMP): sodium, potassium, chloride, bicarbonate, blood urea nitrogen, creatinine with creatinine clearance, glucose, aspartate aminotransferase (AST), alanine aminotransferase (ALT), total bilirubin, alkaline phosphatase.
[e] Phenolic levels and other research labs will be obtained at baseline, and 12 weeks (±14 days). Patients are asked to avoid coffee, tea, fruit juice, fruit, chocolate or wine after midnight prior to blood draw.
[f] Two kits will be provided to participant at baseline, with detailed instructions for how and when to collect stool at home.
[g] If a participant is unable to attend an in person study visit for any reason including COVID-19 related reasons, all patient reported measures will be collected in a phone study visit.

[h] End of Treatment Visit will be conducted by phone 30 days (+14 day window) after last dose of study medication. For those who end treatment prior to week 12, the End of Treatment Visit can be combined with another existing study visit, if that visit occurs 30-44 days after the last dose of study medication.

[l] Participant feedback will be collected in person at Wk12 visit (+30 day window), or by phone at Wk16.

[j] Follow-up study visits will occur at 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.

6.2 Intervention Administration

Treatment will be administered on an outpatient basis. 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.

6.3 Investigational Agent

This study uses a specific formulation of 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 and the on-going Phase 2 study in prostate cancer patients (FDA IND 128937; FDA IND 139288). The muscadine grapes are pressed and processed on site per good manufacturing practice (FDA Code of Federal Regulations 21). The extract is dissolved in water, 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 and phenolic levels occurs numerous times during the production process and will continue at regular intervals until study completion. The MGE will be tested biannually for total phenolics using the Folin-Ciocalteu method, with gallic acid as the standard, and microbes will be assessed annually by American Testing Lab (San Diego, CA). MGE will be dispensed by the WFBCCC Investigational Drug Studies Pharmacy which managed the Phase 1 and on-going Phase 2 study.

6.4 Placebo Description

Placebos will be supplied by Clinical Encapsulation Services (CES) (Schenectady, NY), an FDA-registered facility, registration number 16691092818. The capsules contain microcellulose tinted to match MGE and are encapsulated with a vegetable capsule of hypromellose of the same size as the MGE capsules. Placebo were 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.

Storage requirements: Capsules should be stored at room temperature.
Route of administration: Placebo is available for oral administration.

6.5 General Concomitant Medication and Supportive Care Guidelines

If participants start Coumadin/Warfarin while on study, we will advise them of the potential interaction with MGE. Participants who start Coumadin/Warfarin while on study will be asked to stop dosing study drug (see section 6.7 regarding indications for discontinuation of intervention). Participants will be instructed to inform their prescribing providers of the recommendation to recheck within 7 days of starting medication and again within 7 days of completing our study drug.

6.6 Study Assessments

All in-person study-related activities will occur at the Wake Forest Baptist Medical Center Clinical Research Unit, or at the High Point, Wilkes, or Statesville clinic sites and do not need to be coupled with standard-of-care clinic visits.

6.6.1 Vitals signs, performance status

Vital sign assessment consists of heart rate, blood pressure, respiration rate, temperature. Height and weight will be collected at baseline with weight repeated at follow-up.

Performance status will be assessed at baseline using the Karnofsky Self-Reported Performance Rating Scale (KPS).

6.6.2 Laboratory assessments (safety)

The following laboratory studies will be obtained at follow-up:

- Complete blood count (CBC) with differential: Total white blood cell count (WBC) with differential, hemoglobin/hematocrit, and platelet count.
- Blood chemistry (CMP): Sodium, Potassium, Chloride, Bicarbonate, Blood Urea Nitrogen, Creatinine, Glucose, total bilirubin, alkaline phosphatase, AST (SGOT), ALT (SGPT).
- Two 10mL K2 EDTA tubes of blood will be collected for banking at baseline and follow-up, which is optional and determined at the time of consent
- Critical laboratory values will be reported to the PI who will communicate with the participant.

6.6.3 Laboratory assessments (other)

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

Correlative research labs (refer to **section 10.0**)

6.6.4 Physical function assessments

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. The 6-minute walk will be performed to assess fitness. Grip strength will be performed to calculate a Fried Frailty Index. These measures will be performed by trained research nurses per protocol in the CRU and at participating sites.

6.6.5 Cognitive assessment

Attention and perceptual speed will be measured with the Digital Symbol Substitution Test (DSST). Participants are presented a series of numbered symbols and then asked to draw the appropriate symbols below a list of random numbers. This will be conducted by trained study coordinators.

6.6.6 Patient reported outcomes

The PROMIS fatigue survey will be administered at baseline, 2, 4, 8, 12 weeks and the End of Treatment Visit (@ week 16). Other patient-reported outcomes will be administered at baseline and 12 weeks as outlined in the study calendar. If unable to come in person for follow-up study visit due to COVID-19 or other intercurrent event, patient reported data will be collected by phone.

6.7 Duration of Study Intervention

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

- Intercurrent illness that prevents further administration of treatment;
- Unacceptable adverse event(s) attributable to MGE;
- Patient misses more than 28 consecutive days of treatment for any reason;
- Participant takes Coumadin or Warfarin while on study
- 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

6.8 Duration of Follow Up

Patients will be followed for toxicity/adverse events for 30 days after the last dose of the study drug.

Patients will be instructed to inform the study staff of any medical concerns that arise during the first 30 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, manuscript under review). Participants will also be called by the study team for the End of Treatment Assessment tele-visit 30 days after last study dose to review toxicity and assess resolution/stabilization of reported AEs.

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 6.5) will be asked to continue study visits per protocol. If they are unable or unwilling to come to the CRU or participating site as planned, study staff will attempt to collect

fatigue and other QOL data by phone 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.

6.9 Criteria for Removal from Study Intervention

All patients will be expected to complete their 12-week study visit, unless they withdraw consent or are medically unable, even if they have stopped MGE. If they are unable or unwilling, they will be contacted by phone and asked to complete the survey-based data.

Patients will be removed from study intervention when any of the criteria listed in **section 6.7** apply.

7.0 Dosing Delays/Dose Modifications

7.1 Missed doses

If a dose of MGE/placebo is missed, the patient will be instructed to take his/her 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 patient will be removed from the study intervention. Unused pills will be collected at the follow-up study visit.

7.2 Dose modification

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

Subjects who notify the study team of potential grade 2 or higher study drug-related toxicity concerns will be contacted by the study PI for further information. Subjects will permanently discontinue the study drug for any attributable grade 4 toxicity. If participants experience grade 2 or 3 toxicity potentially attributable to study drug, dosing will be held and appropriate supportive care recommendations made. Subjects will be re-assessed weekly until toxicity has resolved to grade 0-1. If the subject continues to experience grade 2 or 3 toxicity, he/she will hold the study drug for up to 27 days, and the study team will again follow up with the patient 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 within 27 days, the subject will restart the study drug. If the same grade 2 or higher possibly-attributable toxicity returns despite appropriate supportive care measures (ie diarrhea despite use of anti-diarrheal medication), the subject will permanently discontinue the study drug.

Guidelines for study drug discontinuation include 1) any attributable grade 4 toxicity, 2) any attributable grade 2 or 3 toxicity that does not resolve within 27 days or recurs upon re-initiation of study drug, 3) study drug not taken for >28 consecutive days for any reason.

Guidelines for holding MGE/placebo: 1) any attributable grade 2 or 3 toxicity; 2) participant experiences medical or personal concern that is not attributable to study medication but prohibits study drug administration (i.e. inability to swallow, hospitalization).

8.0 Adverse Events List and Reporting Requirements

8.1 Adverse Event List for MGE

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

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 7.1 above) for expedited reporting purposes only.
- **Attribution** of the AE:
  - Definite – The AE **is clearly related** to the study intervention.
  - Probable – The AE **is likely related** to the study intervention.
  - Possible – The AE **may be related** to the study intervention.
  - Unlikely – The AE **is doubtfully related** to the study intervention.
  - Unrelated – The AE **is clearly NOT related** to the study intervention.

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. All Adverse Events that occur during protocol intervention and are coded as either 1) **unexpected grade 4,** 2) **unplanned inpatient hospitalization ≥ 24 hours (regardless of grade)**, or **grade 5 (death)** must be reported to the STRC using the using the SAE console in WISER. All WFBCCC Clinical Protocol and Data Management (CPDM) staff members assisting a Principal Investigator in investigating, documenting and reporting an SAE qualifying for STRC reporting are responsible for informing a clinical member of the STRC as well as the entire committee via the email notification procedure of the occurrence of an SAE.

8.4 WFUHS IRB AE Reporting Requirements

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

devices, procedures or other interventions, or as a result of questionnaires, surveys, observations or other interactions with research subjects.

All members of the research team are responsible for the appropriate reporting to the IRB and other applicable parties of unanticipated problems involving risk to subjects or others. The Principal Investigator, however, is ultimately responsible for ensuring the prompt reporting of unanticipated problems involving risk to subjects or others to the IRB. The Principal Investigator is also responsible for ensuring that all reported unanticipated risks to subjects and others which they receive are reviewed to determine whether the report represents a change in the risks and/or benefits to study participants, and whether any changes in the informed consent, protocol or other study-related documents are required.

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

Any 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.

8.5 Sponsor Reporting Requirements

This study has no third part sponsor. This study is funded by a CTSI pilot grant.

9.0 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.

9.1 Pharmaceutical/Device 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) used under this protocol. These logs shall record quantities of study drug received and quantities dispensed to patients, including lot number, date dispensed, patient identifier number, protocol number, dose, quantity returned, balance remaining, and the initials of the person dispensing the medication.

9.2 Muscadine Grape Extract (MGE)

Product description: This pilot 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 participants with advanced cancer (FDA IND 128937; clinicaltrials.gov NCT02583269) and a phase 2 study in participants with prostate cancer (FDA IND 139288; clinicaltrials.gov NCT03496805).

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.

10.0 Correlative/Special Studies

Blood: To be collected at baseline, and 12 weeks. Approximately 20 mL of blood will be collected into an EDTA tube to measure cytokines and growth factors, including interleukin 6 (Il-6), tumor necrosis factor-alpha (TNF-α), C reactive protein (CRP), HGF, and polymorphonuclear neutrophils (PMNs) to measure mitochondrial function. Samples will be collected by CRU or participating site nursing staff according to a protocol they establish with the study team. Samples will then be transported on ice to the Cell & Molecular Biology Core in the Hypertension Center at BioTech Place (575 Patterson Ave, Winston-Salem, NC 27101), using a commercial courier service. Samples will be stored at -80°C in the Hypertension Center (3rd Floor, BioTech Place) indefinitely until batched analysis. Analysis will take place in the Cell & Molecular Biology Core.

Two 10mL K2 EDTA tubes of blood will be collected for banking at baseline and follow-up, which is optional and determined at the time of consent.

Urine: At least 20 mL of urine will also be collected at baseline and follow-up for 8-OH deoxyguanosine levels and urolithin A glucouronidate (UAG), stored at -80°C indefinitely until batched analysis. Samples will be transported on ice using commercial courier service to the Cell & Molecular Biology Core in the Hypertension Center at BioTech Place (575 Patterson Ave, Winston-Salem, NC 27101).

Stool samples: Fecal specimens will be collected at baseline and at ~12 weeks using an at-home fecal sampling kit. Two kits will be provided to participant at baseline, with detailed instructions for how and when to collect stool at home. Participants will be asked to mail samples in a pre-paid package to the laboratory. DNA will be isolated from fecal samples using the Qiagen DNeasy PowerSoil Pro kit protocol. Metagenomic sequencing will be performed by CosmosID Inc. (Rockville, MD). In brief, DNA libraries will be prepared using the Illumina Nextera XT library preparation kit, with a modified protocol. Library quantity will be assessed with Qubit (ThermoFisher). Libraries will be sequenced on an Illumina HiSeq platform 2x150bp. Fecal samples will be read at 12M read-depth. Unassembled sequencing

reads will be directly analyzed by CosmosID bioinformatics platform for multikingdom microbiome analysis and quantification of organism's relative abundance. Fecal microbiomes will be assessed by metagenomic sequencing and bioinformatics. A list of all the microbiologic species will be recorded, along with their relative abundance recorded as a % relative abundance of the total microbiome.

11.0 Data Management

Item/Form	Location
Informed consent document	WakeOne
Protocol registration & race/ethnicity verification	OnCore
Study visit data collection forms: Baseline, week 12	REDCap*
Medications and supplements	OnCore
Labs	REDCap*
Correlative labs	REDCap*
Adverse event ascertainment	REDCap*
Pre-toxicity and adverse events log	REDCap*
Patient surveys	REDCap*
Cognitive/Physical function & fitness forms	REDCap*
Telephone surveys	REDCap*
Off-treatment/Off-study form	REDCap*/OnCore

*This project will utilize REDCap Clinical Data Interoperability Services. This is a special feature for importing data into REDCap from WakeOne. It provides an adjudication process whereby REDCap users can approve all incoming data from WakeOne before it is officially saved in their REDCap project. REDCap Clinical Data Interoperability Services can only be enabled by a REDCap administrator who serves as an honest broker to PHI. REDCap's Clinical Data Interoperability Services can only be accessed by users with valid WakeOne credentials. Using the Clinical Data Interoperability Service requires using the Medical Record Number (MRN) as a key to automatically gather demographics and laboratory data and reduces data entry errors.

12.0 Statistical Considerations

12.1 Analysis of Primary Objective

To address the primary objective, we will use a mixed effects model with a constraint of a common baseline mean across treatment groups and an unstructured covariance matrix to model all fatigue measures over time, and use linear contrasts to estimate the difference in change and corresponding 90% confidence interval between the two groups from baseline to follow-up.[58] All outcomes will be measured even if the participants discontinue the intervention, to provide an estimate of effectiveness.

12.2 Analysis of Secondary Objectives

For secondary outcomes listed in Objective 2.2.1, 2.2.2, and 2.2.3, which are based on baseline and follow-up measures, we will estimate 95% confidence intervals for the differences between the two

groups for the secondary outcomes at 12 weeks. We will also fit a ANCOVA model adjusted for the baseline value of the outcome to each secondary outcome.

12.3 Analysis of Exploratory Objectives

To analyze the biomarker outcomes in Objective 2.3.1, which are measured continuously, we will also use the ANCOVA approach described for the secondary objectives, and will construct 95% for the differences in the change between the two groups at 12 weeks.

To meet Objective 2.3.2, we will examine the proportion of different bacteria taxa at each time point, and will use a marginalized two-part beta regression model to account for the compositional nature of the data.[59] If we are unable to fit this model, we will examine changes using McNemar-Bowker tests for within-person changes and for mammary and gut composition at 12 weeks.

To meet Objective 2.3.3, we will include the biomarker data in the models of the primary and secondary outcomes described above, and evaluate if the effects of the intervention on each outcome is attenuated in the presence of the biomarker. Attenuation of 10% or more in the intervention parameter estimate will indicate potential mediation in the presence of a clinically meaningful change of both the primary or secondary outcome and the biomarker of interest.

For Objective 2.3.4, we will compare the change in the DSST score between the 2 groups at 12 weeks, using a mixed model with linear contrasts.

12.4 Power and Sample Size

The minimally important difference (MID) established for the fatigue scale is 3-5, and the SD of change in our pilot study was 6.4 over 8 weeks in 12 participants. Using the upper limit of the one-sided 90% CI for this SD (9.0) to account for potential bias, indicates an effect size of ≥0.33 would be meaningful. For pilot studies, rather than calculating power based on hypothesis testing, use of 80% or 90% one-sided confidence intervals are frequently suggested.61-63 With N=30 participants per group, for a difference of 0 the upper bound of the 90% CI is 0.33; i.e., if we observe no difference between the groups, it would be unlikely that the true effect size is greater than 0.33.To adjust for 5% drop out we will inflate our sample to 32/group. For secondary objectives, which are based on baseline and follow-up measures, we will estimate 90% confidence intervals for the differences between the two groups for the secondary measures of interest. Adherence rates will be estimated using pill counts and examined using a mean and 95% confidence interval. Subgroup analyses of the outcomes will be explored among those that achieved ≥80% adherence vs. those that did not.

12.5 Estimated Accrual Rate

The expected accrual is 64 participants. We anticipate accruing an average of 3 participants per week.

12.6 Estimated Study Length

Participants will be followed for a total of 12 weeks for study procedures. A 30-day phone visit will be conducted after the last dose of the study drug thus the study will be completed 12-16 weeks after the last participant is accrued.

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Appendix L - Frailty Index Calculation Form

Score will be programmed to calculate automatically, after other forms are entered into the database.

Study team to review weight loss collected on baseline form:

1. Have you had more than 10 pounds of unintentional weight loss in the past year? (Baseline form)
   Yes ___ No ___

Study team to record the following:

2. Review the response to question #7 on the CES-D survey which states "everything I did was an effort". Did the participant answer either "3-4 days /week" or "5-7 days per week"? Yes ___ No ___

3. Review the response to question #20 on the CES-D survey which states "I could not get going". Did the participant answer either "3-4 days /week" or "5-7 days per week"? Yes ___ No ___

Study team to review data from 4-meter walk trials from SPPB are recorded

4-meter walk test, faster of 2 trials (seconds, with up to 2 decimal places) _____

Study team to review data from from grip strength testing are recorded:

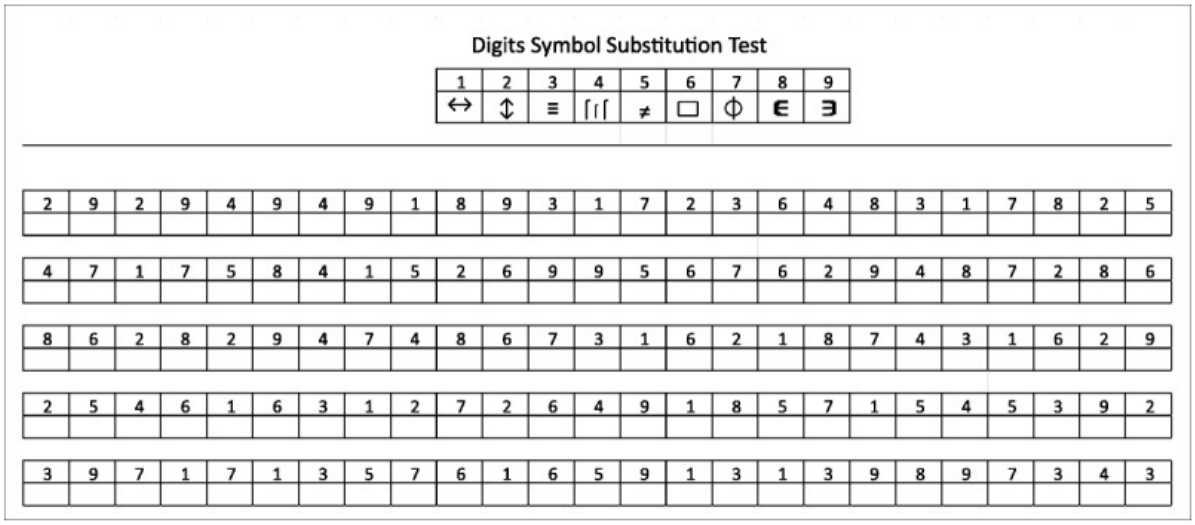
Strongest trial for right hand: (kg) _____

Strongest trial for left hand: (kg) _____

Study team to review MLTQ was collected and recorded.

Appendix R: Digit Symbol Substitution Test (DSST) Script

****This is a sample of the test. Permission was received to obtain and use the full test.****



Next I am going to have you copy some symbols. Take a look at these boxes up top. Notice that they have a number up top and a symbol below. Each number has its own symbol.

Now look at the boxes down here. The boxes have numbers in the top but the bottom is empty. What I am going to have you do is put the symbol in bottom boxes that should go there. Let me demonstrate.

Here is the 6, the 6 has this symbol, so you would put in the box under the 6. Here is the 8, the 8 has this symbol, so you would put in the box under the 8. Here is the 3, the 3 has this symbol, so you would put in the box under the 3.

Now I am going to have you fill in the boxes up to the heavy black line here.

Good! Now that you know how to do them, when I say BEGIN, I am going to have you start here and fill in as many boxes as you can without skipping any. Work as quickly as you can without making any mistakes. Complete each row before going to the next. Keeping working until I tell you to stop.

Ready? BEGIN.