

The MAP TRIAL: PHASE III STUDY OF MUSCADINE PLUS (MPX) IN MEN WITH PROSTATE CANCER: A RANDOMIZED DOUBLE-BLIND, PLACEBO-CONTROLLED STUDY OF THE EFFECTS OF MPX CAPSULES ON RISING PROSTATE-SPECIFIC ANTIGEN LEVELS IN ALANINE/ALANINE SOD2 GENOTYPE MEN FOLLOWING INITIAL THERAPY FOR PROSTATE CANCER

Coordinating Center: The Sidney Kimmel Comprehensive Cancer Center (SKCCC) at Johns Hopkins

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Principal Investigator and IND Sponsor:

Channing Paller, MD
The Sidney Kimmel Comprehensive Cancer David H. Koch
Cancer Research Building, 1550 Orleans Street, Room 155
Baltimore, MD 21287 Phone: 410-955-8239,
Fax: 410-614-1860, Email: (cpaller1@jhmi.edu)

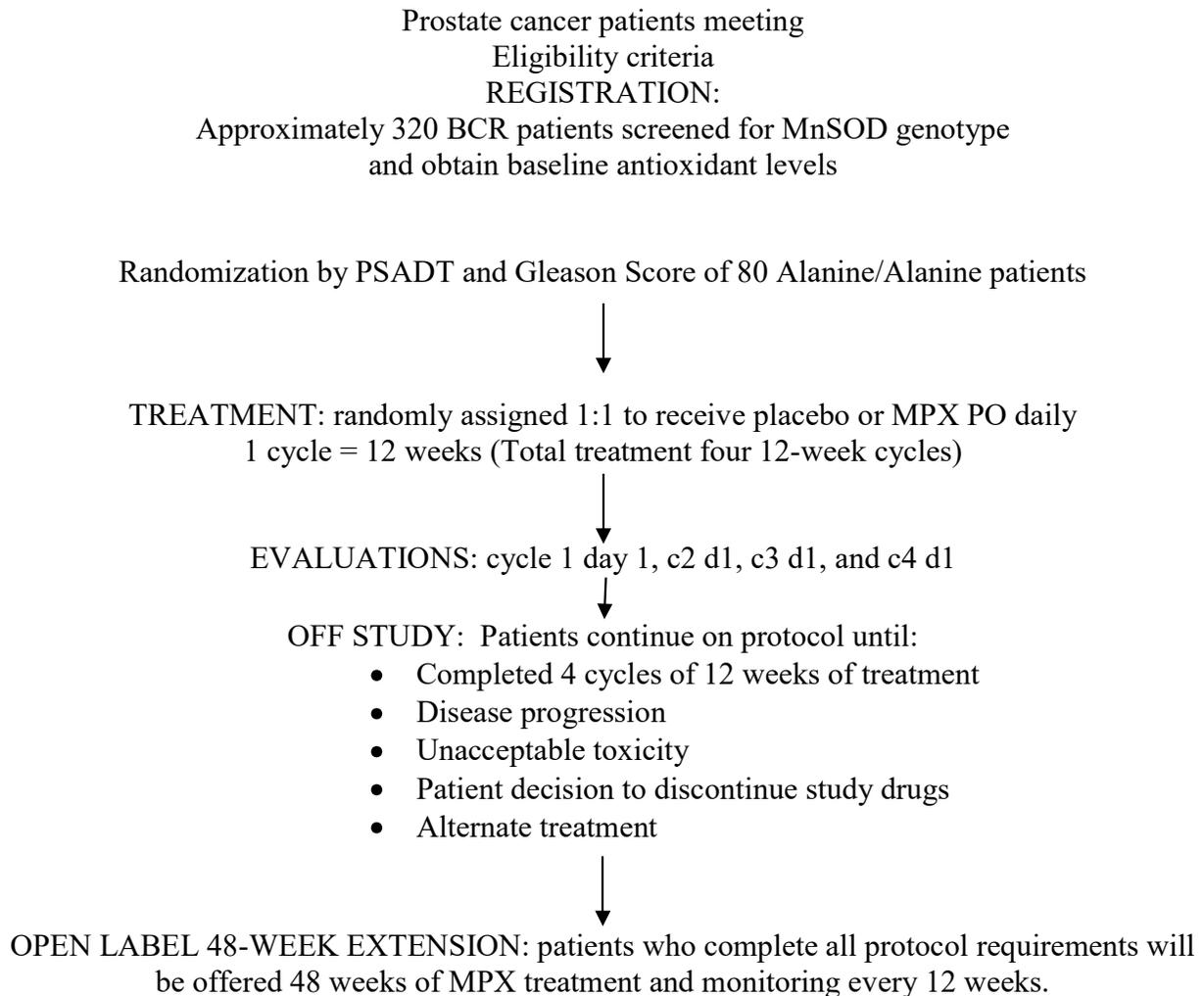
Biostatistics: Marianna Zahurak (Zahurma@jhmi.edu)

Research Nurse: Donna Dowling, RN (ddowlin1@jhmi.edu)

Pharmacist: PCCTC coordinating

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SCHEMA



Drug Names/Abbreviations

Generic name: Muscadine Plus (MPX)

Brand name(s): N/A

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1. OBJECTIVES

1.1 Primary Objectives

1. To determine if men with rising PSA after initial therapy for localized prostate cancer who display the AA genotype of MnSOD and supplement their diet with MPX have greater decrease in PSA slope following treatment compared to men that do not supplement with MPX.

1.2 Secondary Objectives

1. To estimate the prostate specific antigen doubling time (PSADT) of the two treatment groups
2. To estimate the fraction of patients with a decrease in PSA of $\geq 50\%$ from the start of treatment (PSA objective response rate) at 24 and 48 weeks.
3. To estimate the time to disease progression for both treatment groups by PSA progression as well as by radiologic disease progression (i.e. development of metastatic disease)

1.3 Tertiary Objectives (correlative exploratory markers)

1. To correlate baseline and change of levels of antioxidants (selenium, lycopene, and α -tocopheral) with changes in PSA.
2. To correlate measures of oxidative stress (8-OHdG, Malondialdehyde and F2-Isoprostanes) with PSA changes.
3. To store blood for future evaluation of biomarkers associated with MPX effect if the study is positive.

2. BACKGROUND

2.1 Prostate Cancer

The American Cancer Society reports that prostate cancer is the most common cancer in men other than skin cancer and that more than 26,000 men will die of prostate cancer in 2017.¹ Improving techniques in radical surgery and radiation therapy, combined with early detection, allow many prostate cancer patients to enjoy permanent disease control. However, of those patients who undergo radical prostatectomies for clinically localized prostate cancer, a significant fraction (15%) experience biochemical recurrence (PSA >0.2 ng/mL) and one third of those with biochemical recurrence develop metastatic disease within 15 years after surgery. Median time to metastatic disease was 8 years after the recurrence of PSA elevation, and median time to death after metastatic disease was 5 years.²

2.2 Muscadine Plus (MPX) and Placebo

Muscadine Plus (MP), manufactured and sold by Muscadine Naturals, Inc., is an encapsulated blend of 500 mg skin powder of the Noble cultivar of *Vitis rotundifolia*. The study agent uses the same dried grape skins as MP, although isolated from only one farm and standardized in composition, and the same production and encapsulation facilities as the generally available commercial product, but using opaque encapsulation instead of transparent encapsulation used in Muscadine Plus (MP), so as to distinguish the study agent from the commercial product. Because of these differences, our study agent is designated MPX.

The investigational product, MPX is formulated as a brown powder composed of the pulverized skin of *Vitis rotundifolia* of the Noble cultivar. It is encapsulated in clear capsules for oral administration for phase I and white opaque capsules for phase II. Each size 0 capsule contains 500 mg of dried grape skin powder. Size 0 capsules are 7.65 mm in diameter and 21.7 mm long. The capsules are bottled in a 150 cc white HDPE (high-density polyethylene) bottle with 38 mm white ribbed cap, and a neck band seal for consumer protection. Cotton and desiccant are added. Bottles will be labeled at the time of bottling with drug name, date, and lot number. The placebo capsules are rice flour that will be placed in white opaque capsules identical to the ones used for MPX.

In this phase III study, patients will be randomized 1:1 to receive placebo or high (4000 mg) dose MPX. Each patient will receive a 3 month supply of opaque capsules in coded bottles.

2.2.1 Safety profile for Muscadine Plus (MPX)

The *Vitis rotundifolia* grape grows wild in the southeastern United States and is also grown in vineyards and used in winemaking. The Physicians' Desk Reference for Herbal Medicine lists the related *Vitis vinifera* and details many health benefits, concluding, "No health hazards or side effects are known in conjunction with the proper administration of designated therapeutic dosages." The safety of the compound has been demonstrated through the production and sale of muscadine skin powder by Muscadine Naturals and other suppliers. Several companies have sold muscadine grape seed powder and extracts as dietary supplements and muscadine wine commercially for many years with no ill side effects reported. MP has been legally marketed in the United States and surrounding territories since 2005. In the last three years, records from Muscadine Naturals, Inc show that more than 19,000 stock keeping units (SKUs), including bottles and packets, have been delivered without any reported significant adverse effects. The product has been consumed by the public without complaint and without reports of toxicity to the company or to government agencies.

The skin of *Vitis rotundifolia* of the Noble cultivar has been characterized in a number of studies and shown to contain polyphenols that are potent antioxidants.^{3,4} An analysis of the constituent polyphenols of MPX, provided by Muscadine Naturals, shows the following polyphenol content:

Polyphenol Content, 9/10/2010, Analytical Laboratories, Anaheim, CA:

Ellagic acid	2.36 mg/g
Quercetin	18.4 mcg/g

Trans resveratrol 8.77 mcg/g

These polyphenols offer health benefits including slowing prostate specific antigen doubling time in post-prostatectomy prostate cancer patients with biochemical re-occurrence,⁵ reducing markers associated with progression of diabetic complications,^{6,7} inhibiting platelet function as a protective role for the cardiovascular system,⁸ and reducing inflammatory responses.⁹ The polyphenol content has remained stable over several years.

Ellagitannins are the most prevalent polyphenol in MP.⁴ The principal metabolite of ellagitannins, urolithin-A, is preferentially absorbed by the prostate¹⁰ and inhibits the protein complex NF- κ B, potentially leading to increased rates of apoptosis and decreases in cancer cell proliferation.¹¹ Ellagic acid was also found to be a potent DNA methyltransferase modulator in MCF-7 human breast cancer cells.¹²

Other polyphenols in MP, including quercetin,¹³ myricetin,¹⁴ kaempferol,¹⁵ and resveratrol,¹⁶ all have demonstrated anti proliferative activity individually, and two of them, quercetin and resveratrol, along with ellagic acid, have demonstrated synergistic effects greater than what would be expected by adding individual effects.¹⁷

2.2.2 Preclinical Studies of Muscadine Grape Skin Extract

In research conducted while at the National Institutes of Health, co-investigator Dr. Tamaro Hudson showed that *Vitis rotundifolia* skin powder of the Ison cultivar significantly inhibited tumor cell growth in prostate cancer cell lines but did not inhibit growth in normal prostate epithelial cells (PrEC). Prostate tumor cell lines exhibited high rates of apoptosis in response to the extract through targeting of the phosphatidylinositol 3-kinase–Akt and mitogen-activated protein kinase survival pathways. The reduction in Akt activity by *Vitis rotundifolia* skin extract of the Ison cultivar is mediated through a reduction in Akt transcription, enhanced proteasome degradation of Akt, and altered levels of DJ-1, a known regulator of phosphatase tensin homolog (PTEN).³

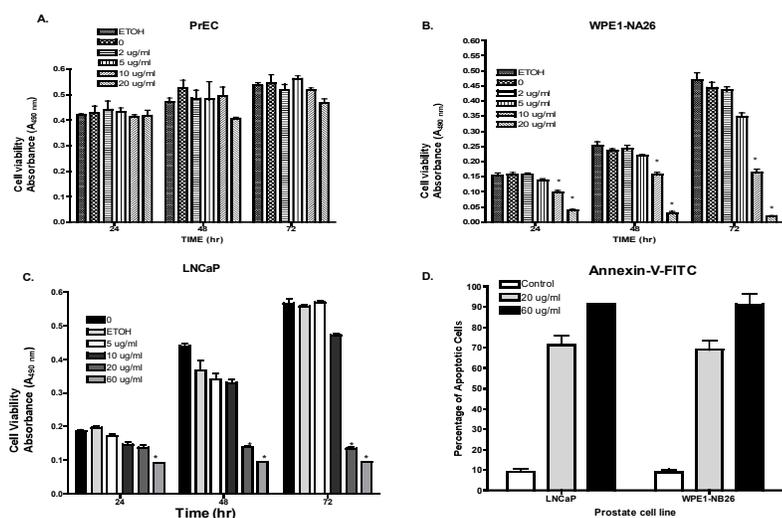


Figure 1. *Vitis rotundifolia* of the Ison cultivar inhibits cell growth in prostate cancer cells but not normal cells and induces apoptosis. The effect of *Vitis rotundifolia* skin extract on the growth rate by MTS assay in: A) PrEC, normal, B) WPE1-NB26 metastatic cells, C) LNCaP. D) *Vitis rotundifolia* of the Ison cultivar induces apoptosis in WPE1-NB26, and LNCaP prostate cells.

An additional *in vitro* study in C4-2 human prostate cells similarly showed that cells treated with muscadine grape skin extract (MSKE) confirmed these findings that it induces apoptosis and also upregulates the stress response of the endoplasmic reticulum and autophagy. Two of the stress response protein markers, IRE-1 alpha and GRP78 were upregulated in the MSKE treated cells compared to the control cells as were the proteins PARP and Caspase 12, involved in apoptosis (Figure 2). It was also shown that MSKE induces autophagy, which was able to be blocked with co-treatment with chloroquine, a known autophagy inhibitor, and that this was also correlated with increases in apoptosis with upregulation of pro-apoptotic proteins BAX, cleaved caspase-3, cleaved caspase-7 and downregulation of the anti-apoptotic protein BCL2.¹⁸

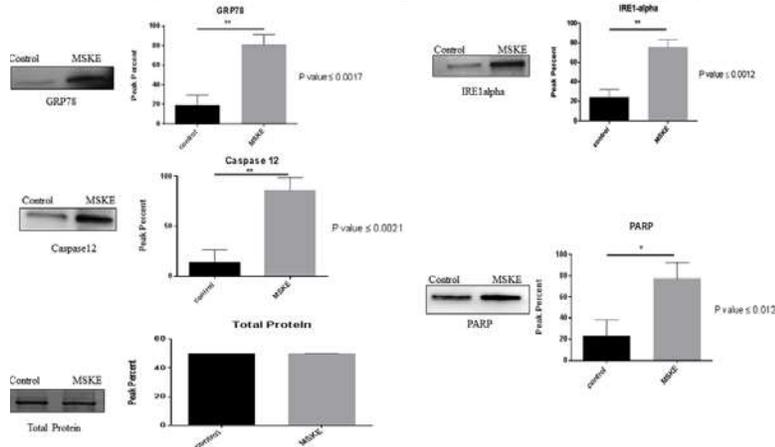


Figure 2 – Western blot analysis of endoplasmic reticulum stress as indicated by markers IRE1-alpha and GRP78 (top) and apoptosis as indicated by PARP, Caspase 12 (bottom)¹⁸.

Although Dr. Hudson had demonstrated *in vitro* that *Vitis rotundifolia* skin extract of the Ison cultivar is effective in causing reduction in cell growth through Akt there was no data that supported its effects *in vivo*. As a result he constructed studies that examined the toxic as well as the preventive effects of the extract. Initial toxicity studies involved relatively healthy athymic nude mice. Three groups of 5 nude mice, 4 to 6 weeks old, were administered *Vitis rotundifolia* skin extract of the Ison cultivar in saline orally at 100, 200, and 400 mg/kg bodyweight 5 days a week for seven weeks, respectively. The remaining control group of mice received saline. The mice were evaluated over the seven weeks for weight loss and moribund appearance. At the end of seven weeks blood and tissue samples were collected. The results revealed that *Vitis rotundifolia* skin extract of the Ison cultivar did not produce any tumors, and had no significant impact on body weight. In addition, histopathological examination of liver, kidney, heart, and lung tissue from treated animals revealed no observable tissue toxicity. Moreover, the hematology profile revealed that leukocytes, erythrocytes, and thrombocytes were within relatively normal range in treated groups compared to control. (Unpublished data from Dr. Hudson’s Laboratory at Howard University.)

A second study performed by Dr. Hudson involved a PC-3 prostate cancer xenograft model of five additional groups of athymic nude mice, 4 to 6 weeks old, were administered 50-600 mg/kg *Vitis rotundifolia* skin extract of the Ison cultivar by oral gavage before, during, and after tumor cell-injection in 0.1 ml saline, 5 days a week for seven weeks. Five animals were in the 600

mg/kg group; the other groups had 10 animals. Four-to-six week old athymic nude male mice were injected in the right flank with 1 million cells. Body weight and diet consumption was recorded weekly throughout the study. After tumors began to grow, their sizes were measured twice a week. No significant differences in mean body weight or food consumption were found between control and treatment groups during the bioassay. Histopathological examination of sections of the liver, lung, and kidney tissues from treated animals indicated that the extract did not elicit toxic effects in any of these organs. *Vitis rotundifolia* skin extract of the Ison cultivar significantly ($p < 0.05$) inhibited PC-3 prostate tumor growth in all five treatment groups compared to the control. (Unpublished data from Dr. Hudson's laboratory at Howard University.)

2.2.3 Phase I and Phase II Clinical Studies of Muscadine Grape Skin Extract

Early clinical studies have now also been completed. A phase I trial was done in 14 men with biochemical recurrent prostate cancer. The phase I study was designed as a dose finding study, showing that the maximal dose, 4,000mg daily, was well tolerated. That dose was chosen as the maximum since it consists of 8 capsules and taking more than that a day raised concerns for adherence to therapy. Six of the 14 patients receive the maximum dose. The most common side effect was flatulence (4 of 14 patients reported). Soft stools, abdominal distention and eructation were also reported and all were grade 1 toxicities. There were no grade 4 adverse events and no study drug related grade 3 adverse events. In this early phase trial, 64% of patients had increases in PSADT with 44% of those who had an increase having an increase of more than 6 months¹⁹.

Given the safety and possible signal of benefit, a phase II trial was completed²⁰. This was a randomized, double-blind trial of 112 men with biochemical recurrent prostate cancer who were assigned to receive either placebo, 500mg or 4,000mg of MPX in a 1:2:2 design. The primary endpoint was change in PSADT. There was no significant difference found between the three groups ($p=0.81$) with a change of 0.9months seen in the placebo group, 1.5 month change in the 500mg group and 0.9 month change seen in the high dose group. There was only one patient in the 4,000mg dose group who had a decrease in PSA of $\geq 50\%$. There were no new safety signals noted in this trial.

MPX was shown to be a safe therapy. However, the overall results have been disappointing but there have been a few people with responses to therapy. This led to the hypothesis that there may be a subset of patients, who can be identified early on, who would benefit from therapy. A previous study showed that in patients with a variant of the manganese superoxide dismutase gene (SOD2), baseline exposure to antioxidants significantly influences the risk of developing prostate cancer.²¹ Manganese superoxide dismutase (MnSOD) is encoded by the SOD2 gene and is one of the major enzymes responsible for detoxification of reactive oxygen species in mitochondria. A mutation at the -9 position results in a change from valine to alanine and alters the structure of the protein.²² This mutation (AA), present in about 25% of prostate cancers, was shown in an observational study that men who had the alanine/alanine genotype and high antioxidant levels had much lower rates of all prostate cancer and clinical aggressive prostate cancer.²¹

Another antioxidant and natural product, pomegranate, has also been studied in the setting of biochemical recurrent prostate cancer. With pomegranate, there were similar findings as above in the MPX trials of no significant change in PSADT in those taking the supplement compared to those on placebo. However, those patients with AA genotype of SOD2 gene did have a significant benefit, with a 12 month increase in PSADT ($p=0.03$, from 13.6 months at baseline to 25.6 months at follow up) compared to only a 1.8 month change in the placebo group (10.9 months at baseline to 12.7 months at baseline).²³

Based on these data, in the phase II trial we completed using MPX, there was a preplanned exploratory analysis to determine if those with the AA genotype had a different response to treatment versus placebo. In that trial, 91 men had SOD2 SNP genotyping done and found that 4 men in the control group, 8 in the 500mg dose group and 11 men in the 4,000mg dose group had the AA genotype. When comparing the treatment effect in those with the AA genotype, there was a signal of benefit. Those in the high dose treatment group had a 10.3 month median increase in PSADT compared to 1.8 months in the control arm. Those in the low dose group had a 2.8 month median increase in PSADT. These results were not statistically significant however are limited due to sample size and are supportive of the larger trial planned.²⁰

2.2.4 Metabolism and Elimination Activity

Metabolism and elimination of these polyphenols in MPX have not been explored in combination. However, ellagic acid, which is also found in pomegranate juice, has been explored in humans. Specifically Seeram et al²⁴ measured ellagic acid in plasma following consumption of pomegranate juice containing 25 mg of ellagic acid and found the peak plasma ellagic acid was at 1 hour, 31.9 \pm ng/ml (0.106 mmol). Cerdá et al.²⁵ investigated blood and urinary metabolites in 6 human subjects on a controlled diet (restricted intake of dietary ellagitannins and polyphenols) after ingestion of pomegranate juice containing 4.37 g/L punicalagin isomers, 0.61 g/L free ellagic acid, 0.6 g/L ellagic acid glucosides and 0.49 g/L anthocyanins. In a preliminary experiment, no juice polyphenols or metabolites were detected in plasma during the first 4 hours or at 24 hours after juice consumption. At 24 hr, 3 main ellagitannin metabolites were found in the urine of treated subjects but not controls. The major metabolite was a glucuronide derivative of 3,8-dihydroxy- 6H-dibenzo[b,d]pyran-6-one, previously detected in rats after ingestion of pomegranate husk ellagitannins; the other metabolites were related conjugates. There was a large variability among the subjects, with the plasma metabolites appearing in some on the first day of juice consumption but remaining nondetectable in others through the experiment. When detected, the concentration range in plasma was 0.5-18.6 μ M. Three of the plasma metabolites were also detected in urine, along with 3 others; the plasma and urine concentrations were unrelated. Percent of ingested ellagic acid equivalents excreted ranged from 1%-50% across individuals. The urinary metabolites were first detected 24hr after ingestion, with maximum excretion occurring between days 3-4.²⁵

A preclinical study of Ison cultivar of *Vitis rotundifolia* skin powder at the Laboratory of Cellular Regulation and Carcinogenesis at the National Cancer Institute, found that the compound significantly inhibited tumor cell growth in prostate cancer cell lines but did not inhibit growth in normal prostate epithelial cells.³ The study is being repeated for the Noble cultivar being proposed for use in this application. Preliminary results from the new study show

that the Noble cultivar used in MP inhibits prostate tumor cell growth in a similar manner to the Ison cultivar grapes (unpublished data from Dr. Hudson's laboratory, Howard University).

2.2.5 Absorption, Distribution, Metabolism, and Excretion

Scalbert et al reviewed the metabolism of polyphenols including flavonoids and ellagitannins. Low molecular weight polyphenols are easily absorbed through the gut barrier, whereas large polyphenols are poorly absorbed and require extensive metabolism by the microflora prior to absorption in the colon. Once absorbed, polyphenols are conjugated to glucuronide, sulphate and methyl groups in the gut mucosa and inner tissues, thereby facilitating excretion in the bile and urine, and limiting their toxicity. At the gut barrier, polyphenols are conjugated to O-glucuronides; in the liver, to sulfate esters, and O-methyl esters. Free circulating polyphenol aglycones are only found in plasma if glucuronidation pathways are saturated. De-glucuronidation and demethylation of some polyphenol conjugates, if it does occur at the cellular level, may be restricted to inflammatory states.²⁶

2.2.6 Dosage Rationale

A range of 1500-3000mg daily is most commonly recommended. The range in dosing completed by Dr. Hudson for the mice experiments included 50 mg/kg up to 600 mg/kg daily dosing and demonstrated inhibition of PC3 xenograft model throughout the dosing range, independent of dose. Using the NOAEL/Human Equivalent Dose calculation for a 70 kg male, the dose range would be from 285 mg up to 3415 mg daily. A phase I dose-finding study was done in 14 men with biochemical recurrent prostate cancer, which tested both 500 mg and 4000 mg doses¹⁹. The maximum dose used in this dose-finding study was rounded up to 4000 mg (8 capsules) to account for patients over 70 kgs. The maximum dose of 4000 mg was received by 6 of the 14 men and was well tolerated. The most common side effect was flatulence (4 of 14 patients reported). Soft stools, abdominal distention and eructation were also reported and all were grade 1 toxicities. There were no grade 4 adverse events and no study drug related grade 3 adverse events. In a subsequent Phase II study, the overall rate of AEs was similar between the MPX and placebo control arms²⁰. Of 102 patients that received MPX, only 6 experienced AEs possibly related to study drug. AEs thought to be related included grade 1 flatulence, diarrhea, and fatigue, and grade 2 dyspepsia and reflux disease. There were no serious adverse events or grade 3 or higher toxicities related to study drug. Therefore, we will use a dose of 4,000mg daily. The phase II trial by Paller et al, showed a larger change in PSADT in the 4000 mg AA *SOD2* group.²⁰

2.2.7 Potential for Drug Interactions

Many polyphenols, including quercetin, kaempferol, and gallic acid have been shown to inhibit CYP3A activity.²⁷⁻³¹ CYP3A is a human gene involved in drug metabolism and synthesis of cholesterol. Ellagic acid inhibits CYP2A2, 3A1, 2C11, 2B1, 2B2 and 2C6 in rat liver microsomes.³² However, clinical trials of midazolam³³ and simvastatin³⁴ with pomegranate juice suggest that these polyphenols, at least when combined in pomegranate juice, do not inhibit the activity of CYP3A in healthy human volunteers.

2.3. Rationale for Studying Muscadine Plus (MPX) in Prostate Cancer Patients

PSA is a single-chain glycoprotein produced by the epithelial cells of the prostate. PSA has been used for early detection and monitoring of patients with prostate cancer who receive a variety of treatments. Due to the widespread use of serum PSA to monitor for prostate cancer recurrence following primary treatment, there exists a group of men with a rising PSA as their only evidence of recurrence. These patients may not demonstrate clinical or radiographic evidence of disease progression for an average 8 years from the time of detectable PSA to detectable metastatic disease by standard imaging.² Currently there are limited treatment options for these patients that may delay disease progression or improve survival, including salvage radiation for prior surgical patients, hormonal therapy, and active surveillance.

Although some surgical patients are candidates for salvage radiation, not all patients will want salvage radiation. Even the early initiation of hormonal therapy (e.g., LHRH analogs) has not demonstrated a survival benefit, although Schroder et al suggests an advantage for early hormone therapy in the setting of metastatic regional lymph nodes.³⁵ Furthermore, early initiation of androgen ablation is associated with significant morbidity and impact on quality of life, including fatigue, hot flashes, loss of libido, decreased muscle mass, and osteoporosis with long term use. This group of relatively well men with biochemical recurrence are currently offered androgen ablation therapy or active surveillance (regular PSA monitoring and annual scans) until there is evidence of metastatic disease, because other options have not been available. These patients are excellent candidates for innovative treatments hypothesized to slow the progression of clinical prostate cancer and delay the development metastatic disease.

As the previous section documents, preclinical studies of muscadine grape skin offer evidence that it may extend the time between biochemical recurrence and development of metastatic disease. While the Phase II study described above found no significant difference in PSA doubling time between placebo and either dose of MPX, there was a signal of benefit in the subgroup analysis of men with the AA genotype that received high dose MPX. It is therefore proposed to test the benefits of high dose MPX in capsule formulation in a randomized, controlled study of men who have failed primary therapy, either radiation, surgery or cryotherapy, as primary treatment for prostate cancer. Eligible subjects will have a rising PSA and will have 3 PSA values at least 7 days apart with a recovered testosterone to be able to calculate a baseline PSA doubling time. The primary endpoint of this study will be mean PSA slope during the study period.

2.4 Correlative Studies

2.4.1 Anti-oxidant Status

There are strong correlations between *SOD2* genotype, baseline exposure to anti-oxidants and response to treatment with antioxidants.²¹ Therefore, we think it is important to measure the baseline anti-oxidant status prior to MPX treatment. The most commonly studied antioxidants in prostate cancer include blood levels of selenium, lycopene, and vitamin E isoforms α -tocopherol¹. Levels of vitamin A, C and zinc have also been shown to be altered in prostate cancer patients compared to controls.^{36,37} It is unknown at this time what effect MPX will have

on these levels so they will be measured at baseline and after 12 and 24 weeks on the trial in those with AA genotype who are randomized to MPX or placebo.

2.4.2 Oxidative stress lab correlates

There is evidence that the generation of reactive oxygen species, which result in lipid and protein peroxidation, contribute to the progression of prostate cancer.³⁸ A number of clinical trials, including the SELECT trial of selenium and vitamin E supplementation, studied whether supplementation with anti-oxidants would therefore prevent prostate cancer.^{39,40} While these trials were negative and did not show a benefit in the overall population, there was a subset of patients identified with variations of the SOD2 gene, who did seem to derive clinical benefit from anti-oxidant therapy.^{21,41} Unfortunately, there is no “gold standard” for in vivo measures of oxidation status. The below studies, will be measured at baseline, 12 and 24 weeks.

F2-Isoprostanes are prostaglandin like compounds that are produced by the peroxidation of arachidonic acid which is catalyzed by free radicals and is independent of cyclooxygenase.⁴² Urinary measurement gives an accurate measure of in vivo oxidative stress and is considered to be one of the most reliable biomarker of oxidative stress.⁴³ Within men with prostate cancer, there have been cross-sectional associations with lower levels of 8-isoprostane with higher antioxidant intake.⁴⁴ The data is mixed on association of isoprostanes and incident prostate cancer with some studies showing a positive association with F2-isoprostane level and risk of incident prostate cancer^{45,46} and others showing no association with urinary F2 isoprostanes and incident prostate cancer.^{47,48} However, the literature suggests this is the most reliable biomarker⁴³ and warrants further study in our prostate cancer patients.

8-OHdG – 8-hydroxy-2’deoxyguanosine is a form of damaged DNA caused by excess free radicals and is a marker of DNA oxidation which can be measured in the urine.⁴⁹ At baseline, men with prostate cancer have an intermediate level of 8-OHdG detected in urine which was shown to be transiently increased after radiation therapy in a small study of seven men.⁵⁰ Urinary levels were shown to be decreased in men who were treated in a trial of antioxidants with selenium-enriched yeast.⁵¹ Serum levels were shown in one study of 32 men with localized prostate cancer to be decreased following dietary intervention with lycopene.⁵² Prostatic tissue levels of 8-OHdG were not significantly decreased in a neo-adjuvant trial of 70 men randomized to pomegranate extract or placebo.⁵³ In our population, we hypothesize 8-OHdG will be decreased in those being treated with MPX compared to placebo, and will correlate with response to treatment.

Malondialdehyde (MDA) – Malondialdehyde is a byproduct of lipid oxidative degradation and is the most commonly used correlative study to measure oxidative stress in prostate cancer patients.⁵⁴ There have been mostly consistent results within the published literature with one study showing no difference between levels in patients with metastatic castration resistant prostate cancer compared to controls⁵⁵ but most cross sectional studies showed higher levels of MDA in those with prostate cancer compared to controls.⁵⁶⁻⁶⁵ One study showed a correlation between PSA and levels of MDA but not with vitamin antioxidants.⁶⁶ Another study also showed increased risk of disease progression with higher levels of MDA.⁶⁷ Due to the frequency and consistency of the results, we think it is reasonable to include this in our correlative analysis.

However, there are pitfalls to the measurement some variability based on the method used to measure MDA and it is highly bound in serum.⁶⁸

Additional oxidative correlates that will be store and analyzed if sufficient funding allows.

Superoxide dismutase activity - Superoxide dismutase is a ubiquitous enzyme that converts oxygen free radicals to hydrogen peroxide and oxygen.⁶⁹ In humans there are 3 forms, SOD1 found in cytoplasm, SOD2 found in mitochondria and SOD3 found extracellularly.⁷⁰ SOD1 and SOD2 activity, defined as the amount of enzyme needed to exhibit 50% dismutation of the superoxide radical, can be generated separately from a serum sample. Baseline levels of SOD activity in the study population (AA genotype) will be compared to men in the screened population to determine if genotype influences phenotype of activity levels. It has been previously shown polymorphisms in MnSOD (SOD2) gene are not associated with increased risk of prostate cancer.^{21, 71} However, there are strong correlations between genotype, baseline exposure to anti-oxidant status and response to treatment with antioxidants.²¹ Additionally, higher levels of SOD have been found in prostate cancer patients compared to controls.^{56, 58, 59, 61-63, 72}

2.4.3 Microbiome

Microbiome – An emerging area of interest within oncology is the microbiome and its impact on the effectiveness of therapies.^{73, 74} Even with the prostate and the urine, once considered a “sterile” site, there are bacterial organisms that are theorized to contribute to the pathobiology of prostate cancer.^{75, 76} There are also differences described between the gut microbiome in men with prostate cancer compared to those without prostate cancer.⁷⁷ A natural next question is if the microbiome can be altered in a way to diminish disease progression. There has been some early evidence that intake of another antioxidant, pomegranate, alters the stool microbiome^{78, 79} and stimulates the growth of *Akkermansia muciniphila*,^{80, 81} a potentially beneficial bacterium that has been associated with increased responsiveness to immunotherapy.⁷⁴ In our study, we will characterize the stool microbiome via rectal swab at baseline and 12 weeks in the randomized patient population to determine whether MPX influences the composition of the gut microbiome.

3. Eligibility Criteria

3.1 Inclusion Criteria

Patients meeting the following conditions are eligible for registration and participation in the study:

1. Subject has histologically or cytologically confirmed adenocarcinoma of the prostate
2. Subject has undergone definitive treatment (surgery, surgery with radiation therapy, cryotherapy, radiation therapy or brachytherapy) for the primary prostate tumor.
 - a. A subject with a rising PSA post-prostatectomy should consider radiation as a potentially curative alternative. If subject declines radiation or is not a candidate for radiation, he may be considered eligible in this setting.

3. Subject has a rising PSA on a minimum of 3 time points (2 rises) within the 12 months prior to study initiation (this will include the PSA measurement taken at the screening visit, but not at the baseline day 0 study visit). Note: Fluctuations in PSA are allowed per Bubley et al 1999 working group criteria. ⁸²

For purposes of calculating PSADT:

- a) All PSA values used in the calculation should be ≥ 0.20 ng/ml and overall should follow a rising trend;
- b) Record every available PSA drawn within the last 12 months of the most recent local PSA;
- c) The minimum requirement is 3 PSA values obtained over 3 months with a minimum of 4 weeks between measurements;
- d) If there are 4 or more PSAs available, the time interval between the first and last PSA measurements must be at least 3 months, and, there is no minimum time interval requirement between any two PSA measurements;
- e) For radiotherapy only patients, record PSA nadir value and collection date.

PSADT must be positive according to Memorial Sloan Kettering Cancer Center Prostate Cancer Nomograms under this link:

<http://www.mskcc.org/applications/nomograms/prostate/PsaDoublingTime.aspx>

4. One of the following criteria must be met.
 - a. Absolute level of PSA >0.4 ng/mL following surgery. (surgery only)
 - b. Absolute level of PSA >0.4 ng/mL for subjects treated with multiple treatment modalities (e.g., surgery + radiation, surgery + cryotherapy, etc.).
 - c. A rise by 2 ng/mL or more above the nadir PSA will be considered the standard definition for biochemical failure after radiation therapy with or without hormonal therapy. (radiation only)
5. Subject is >18 years of age.
6. Subject has life expectancy of greater than 12 months.
7. Subject has ECOG performance status 0, 1 or 2
8. Subject has testosterone level of ≥ 1.5 ng/mL at screening.
9. Subject has normal organ and marrow function as defined below:
 - a. Leukocytes $>3,000/\text{mcL}$
 - b. absolute neutrophil count $>1,500/\text{mcL}$
 - c. platelets $>100,000/\text{mcL}$
 - d. total bilirubin ≤ 1.5 x upper limit of normal except for Gilberts
 ≤ 2.5 x upper limit of normal
 - e. AST(SGOT)/ALT(SGPT) ≤ 2.5 X upper limit of normal
 - f. creatinine ≤ 2.5 upper limit of normal
10. Subject agrees to abstain from other commercially available MuscadinePlus (MP) products (Vinetra, MPlus or MP capsules) while participating in this study.

11. Subject's use of other dietary/herbal supplements (e.g. saw palmetto, selenium, pomegranate juice or pills, acai concentrated extract, etc) has been stable for at least 2 months prior to screening and the subject agrees not to stop or change the dose(s) while participating in the study.
12. Subject has signed a written informed consent document and agrees to comply with requirements of the study.
13. CT or MRI chest/abdomen/pelvis and bone scan without evidence of metastatic disease as an inclusion.
14. Subject agrees to genotyping of *MnSOD2* gene and any genetic counseling. Only those with AA genotype will be randomized.

3.2 Exclusion Criteria

Subjects meeting the following conditions are not eligible for participation in the study:

1. Subject has known radiographic evidence of metastatic disease, except for presence of positive lymph nodes from the surgical pathology. Pelvic/intraperitoneal lymph nodes less than 2.0 cm maybe considered nonspecific and the patient would be eligible. If there is any clinical suspicion for metastatic disease, CT and Bone Scan must be performed to rule out metastatic disease, within the last four months, per standard of care.
2. Subject has received any therapies that modulate testosterone levels (e.g., androgen ablative/anti-androgen therapy, 5 alpha reductase inhibitors) for a minimum of 12 months prior to study.
3. Subject has had prior or concomitant treatment with experimental drugs, high dose steroids, or any other cancer treatment within 4 weeks prior to the first dose of the study product.
4. Subject has consumed any Muscadine Plus over the past 2 months.
5. Subject has a known allergy to muscadine grapes, ellagic acid or rice
6. Subject has uncontrolled concurrent illness including, but not limited to, ongoing or active infection, symptomatic congestive heart failure, unstable angina pectoris, cardiac arrhythmia, or psychiatric illness/social situations that would limit compliance with study requirements.
7. Subject has negative PSA doubling time (negative doubling time corresponds with decreasing PSA) Doubling time may be computed using the Sloan Kettering prediction tools posted at <http://www.mskcc.org/applications/nomograms/prostate/PsaDoublingTime.aspx>

3.3 Inclusion of Minorities

Men of all races and ethnic groups are eligible for this trial.

4. REGISTRATION PROCEDURES

4.1 General Guidelines

Eligible patients will be registered on study centrally at the Sidney Kimmel Comprehensive Cancer Center (SKCCC) by the SKCCC Coordinating Center Study Manager.

To register a patient, the following documents must be completed emailed to SKCCC Coordinating Center Study Manager.

- Signed patient consent form
- Research authorization/HIPAA form
- Registration Form
- Copies of the following records required for assessment of eligibility:
 - prostate cancer pathology report
 - baseline laboratory studies including CBC with differential, liver and kidney function tests, testosterone, and PSA values
 - Other materials considered pertinent for confirming patient eligibility

Following registration, patients should begin protocol treatment within two weeks. Issues that would cause treatment delays should be discussed with the Principal Investigator. The Study Coordinator should be notified of cancellations as soon as possible.

Each participating institution will order agents directly from Johns Hopkins. Agents may be ordered by a participating site only after the initial IRB approval for the site has been forwarded to the Johns Hopkins Study Coordinator.

Randomization

Once eligibility is confirmed, patients will be randomized to a treatment group according to the randomization schedule. Patients will be randomized to receive either placebo or MPX in a 1:1 ratio. Following registration, patients should begin protocol treatment within two weeks.

The investigators, study site personnel and the subject will remain blinded to each subject's treatment throughout the course of the study. Dr. Sam Denmeade (medical monitor) and Dr. Bill Wagner (Distributor) will provide access to blinded subject treatment information in the case of a medical emergency.

Stratification

Stratification will be by pretreatment PSADT (≤ 9 or > 9 months) and Grade Groups (Grade Groups 1 and 2, equivalent to Gleason $\leq 3 + 4$, and Grade Groups 3, 4 and 5, equivalent to Gleason $\geq 4 + 3$).

4.3 Data Reporting and Regulatory Requirements

All information will be collected on study-specific CRFs by study staff. These data will be reviewed for completeness and accuracy by the Principal Investigator at each site.

CRFs will be used to capture study results and data. The study coordinator or other authorized study personnel will transcribe data from source documents onto paper or eCRFs. Before or between visits, the Protocol Chair, IND Sponsor, or designee may request copies of the CRFs for preliminary medical review. Once the CRFs are complete and source-verified, the investigator may sign and verify the accuracy of all data contained within the CRF.

The Protocol Chair

The Protocol Chair, Channing Paller, MD, 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
- Reviewing and ensuring reporting of Serious Adverse Events (SAEs)
- Reviewing data from all sites

Lead Center

The Lead Center (SKCCC) 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 AE's and SAE's to the Protocol Chair and all other applicable parties.
- Facilitating audits by securing selected source documents and research records from participating sites for audit, or by auditing at participating sites.

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 to the Lead Center (SKCCC)
- Registering all patients with the Lead Center (SKCCC) by submitting registration form, signed informed consent, and eligibility documentation 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 Center (SKCCC)
- Collecting and submitting data according to the schedule specified by the protocol

4.4 Data Entry

Data collected during this study will be entered into a secure database. Staff at the lead coordinating center will be responsible for the initial study configuration and setup of the database and for any future changes.

4.4.1 Case report forms completion

Electronic case report forms (CRFs) will be generated by the coordinating center for the collection of all study data. Investigators will be responsible for ensuring that data entry into the CRFs are kept up-to-date.

The paper Eligibility Checklist CRF must be completed using black ink. Any errors must be crossed out so that the original entry is still visible, the correction clearly indicated and then initialed and dated by the individual making the correction.

eCRFs will be completed within 2 weeks of the patient coming to the clinic and all relevant supporting documentation such as scans, progress notes, nursing notes, blood work, pathology reports, etc., will be submitted via email to the SKCCC Coordinating Center Study Manager. All patient names or other identifying information will be removed prior to being sent to the Coordinating Center or non-redacted source documents can be sent via a password -protected/ secured document transfer based on each institution's guidelines.

Authorized representatives of the Coordinating Center may visit the satellite sites to perform audits or inspections, including source data verification.

The purpose of these audits or inspections is to systematically and independently examine all trial-related activities and documents to determine whether these activities were conducted and data were recorded, analyzed, and accurately reported according to the protocol, Good Clinical Practice (GCP), and any applicable regulatory requirements.

4.4.2 Source documents

Study personnel will transcribe clinical data from each patient's source documents (ie, the patient's medical record) into the database. Source documentation will be made available to support the patient research record. Study monitors will review entries on the CRFs at regular intervals, comparing the content with source documents.

4.4.3 Record retention

The investigator will maintain adequate and accurate records to enable the conduct of the study to be fully documented and the study data to be subsequently verified. After study closure, the investigator will maintain all source documents, study-related documents, and the CRFs. Because the length of time required for retaining records depends upon a number of regulatory and legal factors, documents should be stored until the investigator is notified that the documents may be destroyed. In this study, records are to be retained and securely stored for a minimum of 7 years after the completion of all study activities.

4.5 Data Management

4.5.1 Lead research program coordinators

A Lead research program coordinator at the coordinating center will be assigned to the study. A Lead Research Program Coordinator will manage the study activities at each of the participating sites. The responsibilities of the Lead Research Program Coordinator include project compliance, data collection, data entry, data reporting, regulatory monitoring, problem resolution and prioritization, and coordination of the activities of the protocol team.

4.6 Study Monitoring and Quality Assurance

Regularly scheduled registration reports will be generated to monitor patient accruals and the completeness of registration data. Routine data quality reports will be generated to assess missing data and inconsistencies. Accrual rates and the extent and accuracy of evaluations and follow-up will be monitored periodically throughout the study period, and potential problems will be brought to the attention of the principal investigator for discussion and action.

Random-sample data quality and protocol compliance audits will be conducted by the study team at least once a year, more frequently if indicated. Audits by the coordinating center may entail (1) shipping source documents and research records for selected patients from participating sites to the coordinating center for audit, or (2) on-site auditing of selected patient records at participating sites.

All clinical work conducted under this protocol is subject to Good Clinical Practice (GCP) guidelines. This includes inspection of study-related records by the lead site, sponsor, its designee, or health authority representatives at any time.

4.6.1 Data and Safety Monitoring

This is a JHU-investigator-sponsored IND or IDE trial, DSMP Level 2 per the SKCCC DSMP. The PI shall internally monitor the progress of the trial, including review and confirmation of all safety/treatment-related outcomes, response assessments, safety reports and/or any related source documentation. PI shall also establish additional external data/safety monitoring oversight (DSMB, etc), if required by IND-sponsor and detailed within protocol. IND-sponsor is ultimately responsible for external monitoring of JHU site, and also all subsites if JHU is coordinating center. Authorized representatives of the Coordinating Center may visit the satellite sites to perform audits or inspections, including source data verification. The purpose of these audits or inspections is to systematically and independently examine all trial-related activities and documents to determine whether these activities were conducted and data were recorded, analyzed, and accurately reported according to the protocol, Good Clinical Practice (GCP), and any applicable regulatory requirements

Regular registration reports will be generated to monitor patient accruals and the completeness of registration data. Routine data quality reports will be generated to assess missing data and inconsistencies. Accrual rates and extent and accuracy of evaluations and follow-up will be monitored periodically throughout the study period, and potential

problems will be brought to the attention of the lead site principal investigator for discussion and action.

The Data Safety Monitoring Board (DSMB) for this clinical study will contain a minimum of five members, including at least one oncologist, as statistician, and a lay representative. Members may not have a conflict of interest with any aspect of the clinical trial. The DSMB will provide a written recommendation to the IND Sponsor and Protocol Chair after each meeting. In turn, the study team will forward these summaries to the JHU IRB, and JHU SKCCC SMC. The operating plan of the DSMB will be as follows:

- Meetings will be held at least semi-annually, and potentially more frequently if needed.
- Meetings will be conducted in-person or via video/teleconference, with a participant sign-in sheet collected at each meeting. The PI and study staff may attend the open portion of this meeting to review data and answer any question the DSMB might have).
- Approximately one week prior to each DSMB meeting, the study team will submit the following items to DSMB members for review and discussion at the meeting:
 - A summary of the clinical trial's progress to date;
 - The latest IRB-approved protocol and informed consent;
 - A summary of all adverse events, serious adverse events, deaths, and withdrawals to date;

Note that the DSMB may recommend halting trial accrual or all study activity if, after review, serious safety concerns warrant this action. If the DSMB recommends halting study accrual or all study activity, then the study team must notify the JHU SKCCC SMC, JHU IRB, and the FDA immediately.

4.7 Clinical Trial Agreement

This trial is being conducted under one or more clinical trial agreements that contain, among other terms, the publication policy, indemnity agreements, and financial arrangements for the study.

5. TREATMENT PLAN

This is a randomized, double-blind, placebo controlled multicenter study to compare the change in slope and PSADT from baseline between placebo and MDX treatment groups. Approximately 320 subjects will be registered and genotyped to find 80 men with AA genotype for enrollment. Those with AA genotype will be enrolled and randomized 1:1 (40 to placebo and 40 to MPX). Treatment on study will last 48 weeks and will be administered on an outpatient basis.

Appropriate dose delays and discontinuation recommendations for MPX are described in Section 6. Adverse event reporting requirements and potential risks are described in Section 7. No investigational or commercial agents or therapies other than those described below may be administered with the intent to treat the patient's malignancy.

5.1 MPX Administration

Each treatment cycle consists of once daily oral dosing of 4000 mg MPX, every day throughout each 12 week (84 day) cycle. Patients may continue to receive additional cycles of study drug and will be followed every three months with standard visits with their physician until completion of 48 weeks of study treatment, disease progression, or until they wish to discontinue the drug.

Patients will self-administer the assigned number of capsules on a daily schedule. Patients will return capsule containers and any unused study drug to the study coordinator or research nurse at each scheduled follow-up visits. Returns will be documented on the drug accountability log by the pharmacist or pharmacist designee.

Compliance with study product consumption will be evaluated by subject interview and by patient pill diaries (Appendix B) that will be collected at each visit. Compliance will be recorded as a percent of scheduled intakes of study product consumed. Non-compliance will be defined as consumption of <80% of the scheduled intakes of study product.

Patients will be asked to refrain from adding other vitamin or herbal supplements during the study period and follow-up. If a patient does not comply, he will first be counseled that he may be withdrawn from the study. If he persists in taking vitamins or herbal supplements, he will be withdrawn from the study.

Participants will be encouraged to administer the MPX capsules as follows:

- 1) MPX Capsules must be swallowed whole
- 2) MPX Capsules should be taken with water
- 3) MPX capsules should be taken at least 60 minutes prior to eating or 2 hours after eating
- 4) Ingestion of capsules may be spread out over 2 hours if necessary
- 5) Vomited doses (or missed doses for any given day) will not be made-up. If a dose is missed at the usually administered time, it may be made up later that same day.
- 6) If the vomiting occurs after 3 sequential doses and is thought to be related to study drug, the patient should refrain from taking additional MPX capsules and report this to study team.

5.2 Blinding

The double-blind portion of the study will end when every subject has either completed the study, been treated for 48 weeks, or discontinued the study. A subject is considered to have completed the study if he experiences PSA progression as defined in section 5.4. A subject will also be considered to have completed the study if they have not experienced PSA progression but remain on-study at the time the study ends at 48 weeks. Patients will be informed if they are on the MPX or placebo arm. At protocol defined progression or completion of 48 weeks of treatment, patients will be offered an additional four 12-week cycles of MPX treatment, and will come for a visit every 12 weeks, without correlates but with PSA drawn every 12 weeks. Patients who come off study for any other reason will not be offered open label, extension MPX.

Subjects will have an end of study visit and are required to have CT and bone scans prior to beginning the open label extension, and at discharge.

Blind Breaking Procedures

In the event of a medical emergency in which the investigator believes that knowledge of study drug treatment is required, the investigator should contact the Principal Investigator, Channing Paller, and the Distributer, Dr. Bill Wagner and/or Karen Wagner: bill@muscadinenaturals.com or karen@muscadinenaturals.com. For emergencies or complications: Please call Karen Wagner's cell phone at 404-323- 0003.

Every effort must be made to contact the Principal Investigator prior to breaking the blind. The date and reason that the blind was broken must be conveyed to the lead site and recorded on the appropriate CRF if the blind is broken for a participant. In the event the team should break the blind, the reason will be documented in a note for the study file and on the appropriate CRF.

When the predetermined timing of the primary analysis has been reached, the double-blind treatment period of this study will end and the study blind will be broken.

Patients will not be informed to which arm they were assigned. Patients who complete all protocol requirements will be offered 48 additional weeks of MPX treatment and monitoring every 12 weeks.

5.3 General Concomitant Medication and Supportive Care Guidelines

Patients may not be taking any other medications for the goal of treating their prostate cancer. If they are taking medications for other purposes, they may continue per the guidance of the doctor.

Patients who have an on-going study drug-related serious adverse event upon study completion or at discontinuation from the study will be contacted by the investigator or his/her designee periodically until the event is resolved or determined to be irreversible.

5.4 Definition of Treatment Response and Disease Progression

Objective Response (OR): Defined as a decrease of 30% or more in the PSA compared with the baseline level, confirmed by a second PSA at least 3-4 weeks later.

Progressive Disease (PD): Defined as:

- Radiographic Progression
Radiographic progression for soft tissue lesions will be determined using RECIST 1.1. Radiographic progression for bone lesions will be determined by radionuclide bone scan using the consensus guidelines of the PCWG2 criteria, Or
- Unequivocal clinical progression: physical symptoms felt to be cancer related (in the opinion of the investigator), Or
- PSA Progression: Clinically significant increase in PSA as defined below.

- *For subjects whose PSA declined from baseline:* record time from start of therapy to first PSA increase that is $\geq 50\%$ and ≥ 5 ng/mL above the nadir, and which is confirmed by a second value ≥ 3 weeks later.
- *For subjects whose PSA has not declined from baseline:* PSA progression $\geq 50\%$ and ≥ 5 ng/mL after 12 weeks

Stable Disease (SD): Disease Assessment that does not qualify as either objective response or progressive disease.

5.5 Duration of Therapy

Patients may continue on therapy for up to 48 weeks.

Patients may be removed from the study if any of the following occurs.

- Completion of 48 weeks of study therapy
- Disease progression (see Section 5.4)
- A new illness that prevents further administration of treatment
- Unacceptable adverse event(s)
- Patient decides to withdraw from the study
- General or specific changes in the patient's condition that renders the patient unacceptable for further treatment in the judgment of the investigator
- Noncompliance: Subjects who do not meet 80% dosing compliance will be counseled, if they are deemed non-compliant at their next visit they will be withdrawn from the study

Patients who complete all protocol requirements will be offered an additional 48 weeks of MPX treatment and monitoring every 12 weeks.

5.6 Duration of Follow-Up

A single follow-up visit will be scheduled 4 weeks following last dose of study drug.

6. DOSING DELAYS

Criteria for Treatment Delays and Discontinuation Due to Toxicity

Grade or Description of Toxicity	Action
Grade 1 treatment-related toxicity	No dose modification
Grade 2 treatment-related toxicity unresponsive to therapy	Per investigator discretion, continue treatment with careful monitoring or withhold treatment until toxicity returns to \leq grade 1.
Grade 3 treatment-related toxicity unresponsive to therapy	Withhold treatment until toxicity returns to \leq grade 2
Repeated grade 3 treatment-related toxicity within 3 months, or multiple grade 3 treatment-related toxicities	Discontinue Treatment

Grade 4 treatment-related toxicity	Discontinue Treatment
Treatment delays > 2 weeks due to treatment-related toxicity	Discontinue Treatment

As this is a blinded study, there will be no dose reductions. All treatment discontinuations due to toxicity will be documented in the patient’s medical record and case report form.

7. ADVERSE EVENTS: List and Reporting Requirements

This study will use the descriptions and grading scales found in the revised CTCAE version 4.03 for AE reporting that can be found at http://ctep.cancer.gov/protocolDevelopment/electronic_applications/ctc.htm.

Information about all AEs, whether volunteered by the subject, discovered by investigator questioning, or detected through physical examination, laboratory test or other means, will be collected, recorded, and followed as appropriate.

7.1 Definitions

7.1.1 Adverse Event

An AE is defined as any undesirable sign, symptom or medical condition occurring after starting the study drug (or therapy) even if the event is not considered to be related to the study. An undesirable medical condition can be symptoms (e.g., nausea, chest pain), signs (e.g., tachycardia, enlarged liver) or the abnormal results of an investigation (e.g., laboratory findings, electrocardiogram). Medical conditions/diseases present before starting the study treatment are only considered AEs if they worsen after starting the study treatment (any procedures specified in the protocol). New medical conditions / diseases occurring before starting the study treatment but after signing the informed consent form will not be recorded as AEs. Additionally, expected progression of the disease being studied will not be recorded as an adverse event.

Laboratory abnormalities: Laboratory abnormalities present at the screening visit will be recorded as pre-treatment signs and symptoms. After study treatment administration, all grade 3 and 4 clinical laboratory results that represent an increase in severity from baseline will be reported as AEs. A grade 1 or 2 clinical laboratory abnormality should be reported as an AE only if it is considered clinically significant by the investigator (induce clinical signs or symptoms or require therapy).

7.1.2 Serious Adverse Events

A Serious Adverse Event (SAE) is an AE that meets one of the following criteria:

- Results in death
- Is immediately life threatening
- Results in persistent or significant disability or incapacity

- Requires inpatient hospitalization or prolongation of existing hospitalization for >24 hours
- Is suspected of leading to a congenital anomaly or birth defect
- Is an important medical event that may jeopardize the subject or may require medical intervention to prevent one of the outcomes listed above

Events **not** considered to be SAEs are hospitalizations for:

- Routine health assessment requiring admission for baseline/trending of health status (e.g., routine colonoscopy)
- Medical/surgical admission for purpose other than remedying ill health state and was planned prior to entry into the study. Appropriate documentation is required in these cases.
- Admission encountered for another life circumstance that carries no bearing on health status and requires no medical/surgical intervention (e.g., lack of housing, economic inadequacy, care-giver respite, family circumstances, administrative).

7.2 Assessment of Adverse Events

7.2.1 Severity / Grade

The investigator will make an assessment of grade for each AE and SAE reported during the study, which will be recorded in the appropriate CRF. The assessment will be based on the National Cancer Institute's CTCAE (Version 4.03) which can be found at http://ctep.cancer.gov/protocolDevelopment/electronic_applications/ctc.htm

When an AE becomes more severe, the worsening is considered a new AE, and must be documented and recorded according to section 7.2, Documentation and Reporting, below.

7.2.2 Relatedness / Causality

The relationship of an AE to the administration of the study drug is to be assessed by the investigator according to the following definitions:

- No (unrelated, not related, no relation): The time course between the administration of study drug and the occurrence or worsening of the adverse event rules out a causal relationship and another cause (concomitant drugs, therapies, complications, etc.) is suspected.
- Yes (related): The time course between the administration of study drug and the occurrence or worsening of the adverse event is consistent with a causal relationship and no other cause (concomitant drugs, therapies, complications, etc.) can be identified.

The following factors should also be considered:

- The temporal sequence from study drug administration - The event should occur after the study drug is given. The length of time from study drug exposure to event should be evaluated in the clinical context of the event.

- Underlying, concomitant, intercurrent diseases - Each report should be evaluated in the context of the natural history and course of the disease being treated and any other disease the subject may have.
- Concomitant medication - The other medications the subject is taking or the treatment the subject receives should be examined to determine whether any of them might be recognized to cause the event in question.
- Known response pattern for this class of study drug - Clinical and/or preclinical data may indicate whether a particular response is likely to be a class effect.
- Exposure to physical and/or mental stresses - The exposure to stress might induce adverse changes in the recipient and provide a logical and better explanation for the event.
- The pharmacology and pharmacokinetics of the study drug - The known pharmacologic properties (absorption, distribution, metabolism, and excretion) of the study drug should be considered.

7.2.3 Expectedness

Unexpected AE: An AE, which varies in nature, intensity or frequency from information on the investigational drug/agent provided in the product IB, package insert or safety reports. Any AE that is not included in the IB, package insert, safety reports or informed consent is considered “unexpected”.

Expected (known) AE: An AE, which has been reported in the IB, package insert or safety reports. An AE is considered “expected”, only if it is included in the IB document as a risk. Potential risks associated with the study product are nausea, vomiting, and allergic reactions.

7.3 Documentation and Reporting

7.2.1 Prompting Subjects

An AE may be detected by a physical examination or may be reported by a subject without having been prompted. The investigator will also periodically prompt subjects with the following non-leading question: “How have you felt since your last visit?” To avoid bias, it is important that the prompt be non-leading.

7.2.2 All Adverse Events

AEs will be defined with the NCI-CTCAE v4.0. All AEs, both related and unrelated, will be recorded on the appropriate study-specific CRFs, including the following information:

- Specific type of reaction in standard medical terminology
- Severity
- Relatedness
- Duration, including onset, of the event
- Description of treatment action or change in study drug administration or dose
- Resolution

Adverse events should be recorded from the start of treatment with MPX through one week following the last dose of study drug. Details of changes in AEs should be recorded even if they occur after the protocol prescribed follow-up. Investigators should follow-up subjects having serious or related AEs until the resolution of the event. Details of the resolution will be recorded in the CRF.

7.2.3 Serious Adverse Events

All SAEs (including deaths) occurring from the first dose of the study drug through 1 week after the last dose of study drug will be collected and reported.

Subjects who have an ongoing SAE related to the study procedures and/or medication(s) may continue to be periodically contacted by a member of the study staff until the event is resolved or determined to be irreversible by the investigator.

SAEs will be reported promptly to the IND Sponsor and Lead Study Coordinator within 24 hours of initial discovery of the SAE. If this falls on a weekend or holiday, an email notification is acceptable but must be followed by an SAE reporting form on the next business day.

SAE reports and any other relevant safety information are to be sent to:

Channing Paller, MD

Email: cpaller1@jhmi.edu

Fax: 410-614-8160

If Dr. Paller cannot be reached, please contact

Dr. Michael Carducci at carducci@jhmi.edu

After the initial AE or SAE report, the investigator is required to proactively follow each subject and provide further information to the safety department in regards to the subject's condition.

All AE(s) and SAE(s) will be followed until:

- Resolution
- The condition stabilizes
- The event is otherwise explained
- The subject is lost to follow-up
- Death

As soon as relevant information is available, a follow-up SAE report will be submitted to the IND Sponsor and Lead Study Coordinator.

7.2.4 Institutional Review Board Reporting

Investigators at each participating site will be responsible for reporting SAEs to their local IRB in a timely manner per institutional standards. Follow-up information will also be

provided to the local IRB per institutional standards as soon as relevant information becomes available.

7.2.5 Food and Drug Administration (FDA)

All reporting to the FDA will be completed by the IND Sponsor.

7.5.7.1 Expedited IND Safety Reports

7 Calendar-Day Telephone or Fax Report:

The IND Sponsor is required to notify the FDA of any fatal or life-threatening adverse event that is unexpected and assessed by the investigator to be possibly related to the investigational agent. Such reports are to be telephoned or faxed (301-827-9796) to the FDA within 7 calendar days of first learning of the event. Follow-up information will be submitted to the FDA as soon as relevant information is available.

15 Calendar-Day Written Report:

The IND Sponsor is required to notify the FDA of any SAE that is unexpected and related to the investigational agent in a written IND Safety Report.

Written IND Safety Reports should include an Analysis of Similar Events in accordance with regulation 21 CFR § 312.32. All safety reports previously filed with the IND concerning similar events should be analyzed. The new report should contain comments on the significance of the new event in light of the previous, similar reports.

Written IND safety reports with Analysis of Similar Events are to be submitted to the FDA within 15 calendar days of first learning of the event. Follow-up information will be submitted to the FDA as soon as relevant information is available.

7.5.7.2 IND Annual Reports

In accordance with the regulation 21 CFR § 312.33, the IND Sponsor shall within 60 days of the anniversary date that the IND went into effect submit a brief report of the adverse events and progress of the investigation. Please refer to Code of Federal Regulations, 21 CFR § 312.33 for a list of the elements required for the annual report. All IND annual reports will be submitted to the FDA by the IND Sponsor.

8. PHARMACEUTICAL INFORMATION

8.1 Muscadine Plus

Chemical Names:

Ellagic acid (2,3,7,8-Tetrahydroxy-chromeno[5,4,3-cde]chromene-5,10-dione), gallic acid (3,4,5-trihydroxybenzoic acid), trans resveratrol (trans-3,5,4'-Trihydroxystilbene), epicatechin ((2R,3S)-2-(3,4-dihydroxyphenyl)-3,4-dihydro-2H-chromene-3,5,7-triol), and other polyphenols including quercetin (2-(3,4-dihydroxyphenyl)-3,5,7-trihydroxy-4H-

chromen- 4-one), myricetin (3,5,7-Trihydroxy-2-(3,4,5-trihydroxyphenyl)- 4-chromenone), and kaempferol (3,5,7-trihydroxy-2-(4-hydroxyphenyl)-4H-1-benzopyran-4-one)

Other Names: MPX

Classification: Antineoplastic

CAS Registry Number:N/A

Molecular Formula (Ellagic Acid): C₁₄H₆O₈

M.W.: 302.197g/mol

Description: Investigation ongoing. Probable mechanisms listed under modes of action.

Mode of Action: The principal compounds in MPX have been found to use the following mechanisms:

Ellagic acid inhibits DNA Methyltransferase. DNA Methyltransferases (DNMTs) are a family of enzymes that regulate chromatin methylation and use S-adenosyl methionine (SAM) as the methyl donor. Ellagic acid's metabolite, urolithin-A inhibits the protein complex NF-κB, potentially leading to increased rates of apoptosis and decreases in cancer cell proliferation. Extracts from *Vitis rotundifolia* have shown inhibition of the phosphatidylinositol 3-kinase-Akt pathway.

How Supplied:

Patients will take 8 MPX pills or 8 placebo pills daily. MPX is supplied by Muscadine Naturals Inc. MPX/Placebo (the form of Muscadine Plus to be used in this study) is supplied by Muscadine Naturals as a white, opaque gelatin capsule, size 0, containing 500mg of brown fine powdered muscadine grape skins or matching placebo of rice powder.

All study personnel will be blinded. Drug will be packaged as follows:

MPX/Placebo 500 mg capsules #280

Drug Ordering: No starter supply will be sent. Once a patient is randomized, a PCCTC contractor will ship a 12 week patient specific supply of MPX/placebo. Pharmacies may reorder for more supply. Please allow up to 10 days for delivery.

Storage: Store MPX capsules at room temperature, 15 to 30 °C (59 to 86 °F). Do not store above 30°C. Avoid exposure to excessive moisture.

Stability: Shelf life stability studies of the intact bottles are ongoing.

Route of Administration: Orally

Administration: MPX capsules must be administered whole. It should be taken with water, 1 hr prior or 2 hrs after eating. Patients may consume all capsules over a 2 hour period if needed. If vomiting occurs during dosing then hold the remainder of the capsules and resume with the next scheduled dose. If vomiting occurs after taking 3 sequential doses thought to be related to the study drug, then patient should stop from taking any additional capsules.

Dispensing: Patient should take 8 capsules from the 280 count bottle once a day.
(Total 8 capsules/day)

Potential Drug Interactions: No drug interactions have been documented.

Special Handling: N/A

Patient Care Implications: N/A

Drug Destruction: Patient returns may be destroyed per hospital policy, following documentation of returns per your IDS policy.

8.2 Study Medication Accountability and Destruction (MPX)

All study drug required for completion of this study will be provided by Johns Hopkins Hospital (JHH) Investigational Drug Services (IDS). Complete drug request form and fax to 410-502-1036. The IDS service can be reached by phone at 410-502-1036 or emailed at oncpharmacy@jhmi.edu.

The recipient will acknowledge receipt of the drug by returning the appropriate documentation form indicating shipment content and condition. Damaged supplies will be replaced.

Accurate records of all study drug received at, dispensed from, returned to and disposed of by the study site should be recorded by using the Drug Inventory Log. Inspections of the study drug supply for inventory purposes and assurance of proper storage will be conducted as necessary. Any significant discrepancy will be recorded and reported to JHH IDS or their designee and a plan for resolution will be documented.

Study drugs will not be loaned or dispensed by the Investigator to another Investigator or site, unless specifically requested by JHH IDS in writing.

Temperature records for MPX must be made available to the JHH IDS or other Sponsor nominated monitoring teams for verification of proper study drug storage.

Only completely unused study drug vials (MPX) should be retained by the site until a representative from JHH IDS or other JHH IDS-designated personnel have completed an inventory. Partially used and completely used vials should be destroyed according to the site's guidelines, and their disposition should be recorded on the Investigational Drug

Accountability Record Form. All returned medication is to be documented on the Drug Accountability Record Form per institutional policy.

Study drug will either be disposed of at the study site according to the study site's institutional standard operating procedure or returned to JHH IDS with the appropriate documentation. JHH IDS or someone else designated by the JHH IDS will personally inspect the study drug inventories before returning or destroying supplies of either medication. If the study site chooses to destroy study drug, the method of destruction must be documented and JHH IDS must evaluate and approve the study site's drug destruction standard operating procedure prior to the initiation of drug destruction by the study site.

9. CORRELATIVE/SPECIAL STUDIES

Complete instructions for the collection processing, and shipment of samples will be provided in a separate laboratory manual. All specimens should be correctly labeled with patient initials, study-specific subject ID number, protocol number, and date of collection.

9.1 Blood and saliva

Blood and saliva will be collected at baseline for *SOD2* genotyping. (see Study Calendar).

Complete instructions for the collection processing, and shipment of samples will be provided in a separate laboratory manual.

9.2 Serum

Blood for measuring serum levels of antioxidants (selenium, lycopene, alpha-tocopherol) and oxidation marker MDA will be collected for 80 patients at baseline, 12 and 24 weeks (see Study Calendar).

Instructions for the collection, processing, and shipment of samples will be provided in a separate laboratory manual.

9.3 Urine

Urine for measuring F2-Isoprostanes and 8-OHdG (markers of oxidative stress) will be collected at baseline, 12 weeks, and 24 weeks on study (see Study Calendar) for randomized patients.

Instructions for the collection, processing, and shipment of samples will be provided in a separate laboratory manual.

9.4. Microbiome analysis

Rectal swabs will be collected at baseline, and at 12 weeks on study (see Study Calendar).

10. STUDY CALENDAR

Treatment Period	Screen for AA ¹	Screen for Elig.	On Study Visits -Blinded					Extension ² Open Label		Follow Up ¹³
			Weeks	Day -28 to day -1	0	12 W ± 7 days	24 W ± 7 days	36 W ± 7 days	48 W or DC ³ ± 7 days	
Informed Consent	√									
Inclusion/Exclusion Criteria ¹¹	√	√								
Medical History	√	√ ⁶								
Pathology Review		√								
Complete physical examination		√					√		√	
Interval History and Brief physical examination ¹⁰		√		√	√	√		√		√
Vital Signs (temperature, BP, heart rate)		√	√	√	√	√	√	√	√	√
Weight		√	√	√	√	√	√	√	√	√
Height		√								
ECOG Performance status		√	√	√	√	√	√	√	√	√
CMP ⁹		√		√	√	√	√	√	√	√
CBC w/diff.		√		√	√	√	√	√	√	√
PSA		√ ⁴	√ ⁴	√	√ ³	√	√	√	√	√
CT/Bone Scan ¹		√					√ ⁷		√	√ ¹⁴
Testosterone		√								
Blood for MnSOD genotyping & antioxidant ⁸	√									
Serum Collection for Correlative Studies ⁵	√		√	√	√ ³					
Rectal Swab for Correlative Studies			√	√						
Urine Collection for Correlative Studies			√	√	√ ³					
Adverse Event Assessment			√	√	√	√	√	√	√	√
Concomitant Medication		√	√	√	√	√	√	√	√	√
Dispense Study Drug and Consumption Diary			√	√	√	√		√		
Collect Returned Study Product and Pill Diary ¹²				√	√	√	√	√	√	

¹ Baseline evaluations are to be conducted within 4 weeks prior to start of protocol therapy. Scans are allowed within 90 days. CT of the abdomen and pelvis. With contrast preferred; but without contrast allowed.

² Patients on study drug who complete protocol requirements may choose to start MPX after completing 48 weeks on the blinded portion of the study or after discharge with documentation of protocol defined progression (per Section 5.4). In order to remain a double blinded trial, patients and investigators will not know to which arm they were assigned and thus it is patient and investigator judgement if they want to try unblinded MPX for up to an additional 48 weeks.

³ If patient leaves the study for any reason prior to 24 weeks but > 12 weeks on trial, please record whether DC reason was patient meeting protocol defined progression (defined in Sec. 5.4 of this protocol) or DC reason was investigator judgement. If reason was protocol-defined progression, record whether there was pain, metastases on bone or CT scan and/or PSA progression. Also collect serum and urine for correlative studies, and perform CT and bone scans unless scans and patient samples have been collected in the past 8 weeks.

⁴ It is important to have ≥ 3 pre-trial PSA values prior to day 0 and to collect a day 0 PSA value for on-study calculations.

⁵ One tube at original screening of 320 patients; two tubes at baseline, 12 weeks, and 24 weeks for patients on MPX trial.

⁶ With medical history record prior prostatectomy, prior radiation (brachytherapy, SBRT, etc.) plus start and end dates of any prior hormonal therapy including name of drug.

⁷ For patients discharged prior to 48 weeks, open label MPX drug will be offered only to patients who meet protocol defined progression per Section 5.4.

⁸ All 320 patients should get genotype (5ml blood or blood and saliva), one 10ml tube of blood for serum tests for antioxidants

⁹ Comprehensive metabolic panel to include measurement of sodium, potassium, chloride, bicarbonate, BUN, creatinine, glucose, total bilirubin, calcium, total protein, albumin, AST, ALT, and alkaline phosphatase

¹⁰ Brief physical examination includes cardiovascular, pulmonary, abdominal and Symptom-directed; includes investigating any new abnormalities and weight. Interval history includes any hospitalizations, illnesses, changes in medication

¹¹ All inclusion/exclusion criteria should be assessed at screening and confirmed prior to randomization

¹² Compliance will be assessed by reviewing both returned # of pills and pill diary

¹³ The last visit will be 28 days (± 3 days) following last dose of study drug.

¹⁴ CT/Bone Scan is performed at off study visit only if it was not performed within the previous 4 weeks.

¹⁵ Assess systems per standard of care at the study site and as clinically indicated by symptoms.

11. EVALUATION AND STATISTICAL METHODS

Study design: This is a multicenter, double-blind, randomized study to evaluate the benefit of MPX supplementation in the subset of men who display the AA genotype of MnSOD. Based on a previous randomized phase II trial of MPX at Hopkins¹, PSA doubling time (PSA-DT) was prolonged in this subgroup of men.

The primary objective of this trial will be to determine how the rate of change in PSA is affected by MPX supplementation and compare this treatment effect to a control arm given a placebo.

The rate of change in PSA, before and during treatment, for each patient will be quantified using the log slope which is calculated from a regression model of the log of PSA values on time. This quantity is the estimate directly used in the standardized method for the calculation of PSA-DT² (PSA-DT is calculated as the natural log of 2 divided by the log slope).

Different PSA kinetic measurements have previously performed differently in different settings³.

In a randomized clinical trial, it is convenient if the distribution of the outcome to be compared between groups is suitable for analysis with a parametric test. The distribution of log slope, or the change in log slope, can reasonably be assumed to be approximately normal. By comparison, PSA-DTs are problematic when PSA values are not increasing exponentially, as in the setting where treatment may result in a PSA-DT less than zero. In this case the DT for the patient is assigned an arbitrary large value and any calculated changes in DT will necessarily involve this imputed value. As a result, assumptions of normality for the distributions of DTs, and changes in DTs, are not be reasonable, and would preclude parametric analyses. The log slope as a continuous quantity is therefore the chosen kinetic for this randomized clinical trial.

We expect the average DTs of patients enrolling on this study to be in the range of 6 to 15 months, which correspond to average log slopes of 0.1155 ng/mL/month and 0.0462 ng/mL/month respectively. The historical post-treatment average slope estimates from the previous Hopkins trial were 0.033 ng/mL/month (average PSA-DT of 21 months) on the MPX arm and 0.083 ng/mL/month (average PSA-DT of 8.4 months) on the control arm. We have powered this study using the difference in these historical estimates as the clinically relevant post-treatment difference to be detected in this study: 0.05 ng/mL/month (or a PSA-DT

difference of 12.6 months). It should be noted that for the same log slope difference between treatment arms, if the average post-treatment PSA rates of change are greater (steeper slopes), the detectable difference in DTs would be smaller and if the rates of change are less (slopes are less steep) the detectable DT difference will be larger.

Randomization: Stratified randomization will be performed to maintain balance between the arms as much as possible. Stratification will be by pretreatment PSADT (≤ 9 or >9 months) and Grade Groups (Grade Groups 1 and 2, equivalent to Gleason $\leq 3 +4$, and Grade Groups 3, 4 and 5, equivalent to Gleason $\geq 4 +3$). Block randomization, with varying block sizes, will be used to ensure reasonable balance between the arms. Randomization assignments will be generated by the study statistician prior to initiation of the study using an R (version 3.4), and made available to the study team through the Biostatistics Shared Resources website.

End of Study: The date the study ends for the purpose of the final analysis is the date at which all randomized subjects have either completed the study or prematurely discontinued treatment and withdrew. This date will be designated as the primary analysis date for data cutoff and for all statistical analyses of efficacy and safety. Following the collection and adjudication of all data for all randomized subjects (follow-up up to and including the primary analysis date for data cutoff, the study blind will be broken (Section 5.2, Blinding).

Primary Objective: Determine if men who display the AA genotype of MnSOD and supplement their diet with MPX have greater changes in PSA slope following treatment compared to men that do not supplement with MPX.

Sample size and accrual: This two arm randomized study will determine if there are differences in the change in PSA slope (post supplementation minus pre supplementation) between patients supplementing with MPX (Arm A) and patients that are not (Arm B). The accrual rate for each site is expected to be 1 patient per month. With 16 to 17 sites, is expected that we could accrue 320 patients over 4 years, for which we will have baseline MnSOD genotype and baseline antioxidant status, of which approximately 80 would be of the required AA genotype and eligible for randomization to mpX or placebo. Overall the study would take 4 years of accrual and another year for follow-up. PSA slope will be defined by the linear regression line of the natural log of PSA (in ng/mL) against time (in months). The primary statistical analysis will be an analysis of covariance (ANCOVA) to compare the two arms at the final analysis, adjusting for baseline PSA slope. Implications of the sample size are given based on simulations which include a single interim analysis for futility midway through the trial. The predictive probability approach for the futility analysis is described in the interim analysis section below. Based on the previous study of MPX at Hopkins, we conservatively estimate that the difference in the post-treatment PSA slope (MPX minus placebo), adjusting for pre-treatment PSA, in this group of patients with the AA genotype will be -0.05 with the MPX arm having lower post-treatment slopes (MPX slope 0.033 ng/mL/month versus untreated 0.083 ng/mL/month). Patients with the AA genotype will be randomized to MPX (Arm A) and placebo (Arm B) in a 1:1 ratio. Using a two sided 5% type I error allowance, a sample size of 78 (39 per arm), and minimal correlation between the pre and post treatment PSA slopes, this study will have 83% power to detect a difference in post-study PSA slope of -0.05. Use of the ANCOVA design with covariate adjustment for pre-treatment PSA slope will tend to increase the power depending on the level of the correlation between paired PSA slope measurements (i.e., pre- and post-treatment). As an example, for a correlation (ρ) of 0.30,

the power would increase to 87%.

Interim analysis for primary objective: We plan to monitor the primary efficacy objective for futility once, after treating half the planned number of patients (i.e., 39), using a predictive probability approach. The ANCOVA analysis for the primary objective is a linear regression using the post treatment PSA slope as the outcome and study arm and baseline PSA slope as covariates. The monitoring plan is based on calculations of the likelihood of a significant result if the trial were to continue to the end. At the time the trial is monitored, 100 simulations of the future results of the trial will be generated and analyzed. If the probability that the trial would end with a significant result falls below a cut off value, the study will be stopped for futility. In Bayesian linear regression, all the parameters are given a prior distribution. A common objective prior for linear regression is the g -prior of Zellner². This prior uses the covariate information in the data to specify the prior variance for the regression coefficients. An inverse-gamma prior distribution is typically used for the residual variance, σ^2 . We will use information obtained at the time of the interim analysis to describe the distributions of these parameters.

The interim analysis will occur after 50% of the patients ($m=39$) have been treated and assessed for post-treatment PSA slope. At this time the ANCOVA model will be fit and the variance of the residuals, SSR_g , and the posterior distribution of the regression (ANCOVA) coefficients β will be calculated using the g prior with $g = m$. From this information, we predict the results of the trial by simulating the remaining future patients' data from the predictive distribution and performing an ANCOVA analysis on the combined interim data and simulated data. We will base the predicted probability of ultimately finding a significant difference on 100 simulations. The following steps will be used to generate the i^{th} simulated value.

1. Generate $\sigma^{2(i)}$ from its inverse-gamma posterior.
2. Generate $\beta^{(i)}$ from its (conditional) posterior distribution.
3. Generate \tilde{Y}_j , $j=1, \dots, n-m$ from $N(X_j \beta^{(i)}, \sigma^{2(i)})$
4. Perform the final ANCOVA analysis based on the interim data and the predicted data.
5. Repeat for the required number of simulations predicting the final outcome (100).
6. Calculate the predicted probability of rejecting the null hypothesis at the end of the study.

If interim analysis simulations indicate that the probability of a successful trial would be less than 10%, we will consider stopping the trial for futility. The table below gives the operating characteristics of the monitoring plan based on 2000 simulations for different scenarios, each scenario corresponding to different treatment effects and different levels of correlation between the pre-treatment and post-treatment PSA slope. The seventh row shows results using the hypothesized treatment effect of a mean difference of -0.05 with a standard deviation of 0.075, and minimal correlation. We do expect some correlation between pre and post-treatment values, so power will tend to increase with increasing correlation. The significance level for the final analysis is 0.05. Scenarios 1-3 give operating characteristics under the null (no treatment effect). **Table 1.** Operating characteristics of monitoring rule from 2000 simulated studies with one interim analysis based on 100 predicted study conclusions and final analysis based on an ANCOVA model. Simulations use a g -prior with $g=39$ (i.e. the sample size at the time of the interim analysis). All simulations assume the standard deviation of post-treatment PSA slope is 0.075.

Scenario	Treatment Effect	Correlation (pre- and post-treatment measures)	Stop for Futility %	Would have stopped for Futility and would have Rejected %	Overall studies rejecting Ho %
1	0.0	0.1	34.0%	0.10%	4.3%
2	0.0	0.2	33.5%	0.35%	5.6%
3	0.0	0.3	34.5%	0.20%	4.4%
4	-0.025	0.1	21.9%	1.00%	31.8%
5	-0.025	0.2	20.4%	0.80%	30.6%
6	-0.025	0.3	17.5%	1.40%	35.0%
7	-0.050	0.1	4.5%	1.25%	82.7%
8	-0.050	0.2	4.4%	0.75%	81.9%
9	-0.050	0.3	3.0%	0.95%	87.3%
10	-0.075	0.1	0.4%	0.30%	98.8%
11	-0.075	0.2	0.4%	0.20%	99.1%
12	-0.075	0.3	0.2%	0.15%	99.7%

Safety monitoring and early stopping rules for safety: The toxicity in each arm of the study will be monitored after every patient. The prior for this study will assume that 1 of 6 patients may experience grade 3-4 diarrhea. The prior probability that the treatment is too toxic may therefore be taken to be a Beta distribution with parameters 1.5 and 5.5. This distribution corresponds to assuming a 1 in 4 chance that the risk of toxicity is 30% or higher and 90% certainty that the risk is between 3% and 49%. The stopping rule applies this prior distribution to the observed number of patients experiencing toxicity and computes the resulting probability of toxicity. If the posterior certainty that toxicity is 30% or higher, based on Bayes rule and these assumptions, is 75% or higher ($\geq 3:1$ odds in favor of the treatment being too toxic), the study should stop. The following tables show the resulting monitoring rule and operating characteristics. For example, the rule will call for stopping the study if 5 patients out of the first 8 experience toxicities. The next table shows the percent of the time that the stopping rule will stop the study under different hypothetical risks of toxicity, along with the average sample size (based on 5000 simulations).

Stop if DLTs	3	4	5	6	7	8	9
in N patients	3-4	5-7	8-9	10-12	13-15	16-18	19-22

10	11	12	13	14	15
23-25	26-28	29-31	32-34	35-37	38-39

Risk of AE	0.10	0.20	0.25	0.30	0.35	0.40	0.45	0.50
% of Time Study Stops	0.7%	7.7%	19.0%	39.3%	60.9%	79.3%	92.0%	97.8%
Expected Sample Size	38.7	36.9	34.2	29.6	24.3	19.4	14.6	11.2

Analysis plans for primary objective

PSA Slope: The primary objective will be based on the intent-to-treat (ITT) population and assessed with an ANCOVA model, with post-treatment PSA slope as the independent variable and study arm and baseline PSA slope as covariates. A two-sided t test at the 0.05 significance level will be used to determine if, adjusting for baseline, the post-treatment PSA slope has been reduced with MPX supplementation (Arm A). Scatterplots of post treatment PSA slope by baseline slope

will be used to visualize the fitted ANCOVA model. Boxplots of the observed changes will also be used to visualize the results.

Secondary Efficacy Assessment

PSA Doubling Time (PSADT): The PSADT will be calculated as $\ln(2) (=0.693) / \beta$ (=slope of the linear regression fit to \ln PSA vs. time in months). If the patient's slope is less than or equal to zero, we will assign them a PSADT of 100 months, which is consistent with the literature.⁴⁸

A secondary statistical test of the difference between the two groups will be assessed by a nonparametric Wilcoxon Rank Sum test of the percentage change in PSADT (logarithm of the ratio of PSADT on-study divided by the pre-treatment PSADT). We will also analyze the log of the PSADT ratio by the analysis of variance (ANOVA) adjusted for the stratification factors. Confidence intervals (95%) for the change from baseline in PSADT will also be computed for the two dose groups. The paired t-test (or Wilcoxon signed-rank test) will be used to assess the within treatment group differences of PSADT on study compared to the baseline. Patients with negative slopes will be assigned PSADT of 100 months.

PSA Reduction of $\geq 50\%$ / Objective Response: An objective response (OR) is defined as a decrease in PSA by 50% or more after the start of treatment. The objective response rate (ORR) is estimated as the fraction of men for whom an objective response is observed. The time to objective response, defined as the time from start of treatment to an observed objective response, as well as the average duration of response, defined as the time from objective response to disease progression, will also be summarized and compared between groups.

PSA Progression: The distribution of time to PSA progression will be estimated using Kaplan-Meier method for both treatment groups combined and separately. The stratified logrank test will be used to test the hypothesis that the time to PSA progression distributions for the 2 treatment groups are the same. The same stratification factors used for the randomization will be used to stratify the logrank test.

Disease Progression (Metastatic disease): The distribution of time to disease progression will be estimated using Kaplan-Meier method for the treatment groups combined and separately. The stratified logrank test will be used to test the hypothesis that the time to disease progression distributions for the 2 treatment groups are the same. The same stratification factors used for the randomization will be used to stratify the logrank test.

Toxicity Assessments: Safety analyses will be performed on all subjects that received at least one dose of MPX. The proportion of toxicities by type and grade according to the Common Terminology Criteria for Adverse Events (CTCAE) 4.0 will be reported with exact binomial 95% confidence intervals for each arm of the study.

Correlative Studies

Correlative studies seek to improve understanding patient response to MPX, as well as the biological mechanism or mechanisms of action. Correlative studies include: (1) correlating PSA response with antioxidant levels and (2) correlating PSA response with markers of oxidative stress.

We will look at baseline levels of selenium, lycopene, and α -tocopherol. Three measures of oxidative stress (F2-Isoprostanes, 8-OHdG, and Malondialdehyde) taken at three time points (baseline, week 12, and week 24) will be compared by study arm for changes following treatment. Comparisons will be visualized using boxplots of the raw data on a logarithmic scale. Analytic comparisons will be made with multivariable regression analysis using generalized estimating equations to assess oxidative stress outcomes as a function of MPX treatment, time, and other factors such as Gleason score, baseline PSA, and age. A compound symmetric covariance structure will be assumed for these regression models. With sample sizes of 40 per group, a standardized effect size of 40% would be detectable with 79%-88% power assuming within-subject correlations of 0.10-0.30 and a two-sided alpha of 0.10. Although multiple generalized estimating equation models will be explored, one for each measure of oxidative stress, we will not adjust for multiple comparisons.

Additional Analyses

Baseline Characteristic Analyses: Qualitative baseline characteristics will be summarized with the number and percent of subjects in each treatment group with the characteristic. Quantitative characteristics (e.g., age, height, and weight) will be summarized with the median, mean, standard deviation, minimum value, and maximum value. Categorical characteristics will be tabulated.

The number and percent of subjects, who are randomized, treated with randomized study drug, prematurely discontinued study drug, are lost to follow up, and completed four 12 week cycles of follow-up after randomization will be summarized.

The number and percent of subjects with each medical history will be summarized. Fisher's exact test will be used to assess treatment group differences in the proportions of subjects with each medical history. The number and percent of subjects that receive each medication that is not the study drug will be summarized.

There will be no adjustment for multiple statistical comparisons. We recognize that carrying out multiple significant tests increases the risk of false positive results among the collection of all analyses. Therefore, except for the primary outcome, all other analyses are considered as secondary, tertiary, or exploratory.

ECOG Performance Status: ECOG Performance Status will be captured as on a 6-point categorical scale from 0 (normal activity) to 5 (dead) and summarized using shifts from baseline to the 24-week and 48-week evaluations. Baseline is defined as the last ECOG value prior to or on the date of randomization. The percentage of patients with an increase of at least 1 point from baseline will be presented for each treatment group at both the 24-week and 48-week evaluation time points.

Adherence: Participants who completed 4 cycles of study and had at least 80% of intake of the planned consumption will be defined as study compliant. An overall probability of study compliance will be estimated with a 95% confidence interval. The difference in proportion of compliance between two groups will be tested using Fisher's exact test at a two-sided alpha level of 0.05. Summary statistics will also be provided for the time from the first dose to the last dose of study drug.

APPENDIX A: ECOG PERFORMANCE STATUS SCALE

Grade	Descriptions
0	Normal activity. Fully active, able to carry on all pre-disease performance without restriction.
1	Symptoms, but ambulatory. Restricted in physically strenuous activity, but ambulatory and able to carry out work of a light or sedentary nature (e.g., light housework, office work).
2	In bed <50% of the time. Ambulatory and capable of all self-care, but unable to carry out any work activities. Up and about more than 50% of waking hours.
3	In bed >50% of the time. Capable of only limited self-care, confined to bed or chair more than 50% of waking hours.
4	100% bedridden. Completely disabled. Cannot carry on any self-care. Totally confined to bed or chair.
5	Dead

APPENDIX B: Study Drug Compliance - Pill Diary

Patient should be provided with diary pages for 3-4 months, enough to log study drug compliance between study visits.

Cycle Day	Date of dosing	Time of Dose	Number of Tablets capsules	Comments/Side Effects
1				
2				
3				
4				
5				
6				
7				
8				
9				
10				
11				
12				
13				
14				
15				
16				
17				
18				
19				
20				
21				
22				
23				
24				
25				
26				
27				
28				

28 day Pill Diary History # _____ Subject's ID #: _____

- Take 8 capsules from the bottle once every day
- Your pills should be taken **ONE** hour before food or **TWO** hours after food.
- **Bring your diary and pill supply with you to each clinic visit. Return all unused pills and empty bottles.**
- **Tell us the time of your last meal (CLINIC VISIT DAY ONLY):** _____

Subject's signature: _____

Date: _____

Reviewer's signature: _____

Date: _____

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