Phase 1b/2 study of Chitosan for Pharmacologic Manipulation of AGE (Advanced Glycation Endproducts) Levels in Prostate Cancer Patients

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DEFINITION OF TERMS USED

ADT	androgen deprivation therapy
AE	adverse event
AGE	advanced glycation endproducts
AGER	receptor for AGE
ALT	alanine transaminase
ANC	absolute neutrophil count
AST	aspartate aminotransferase
BID	two times per day
BMI	body mass index
BUN	blood urea nitrogen
CBCD	complete blood count with differential
CDC	Center for Disease Control and Prevention
CML	carboxymethyllysine
CMP	comprehensive metabolic panel
CRP	c-reactive protein
CTCAE	Common Toxicity Criteria for Adverse Events
DSMC	Data Safety Monitoring Committee
ECOG	Eastern Cooperative Oncology Group
eCRF	electronic case report form
FDA	Food and Drug Administration
GCP	Good Clinical Practice
GI	gastrointestinal
GRAS	generally recognized as safe
GSE	grape seed extract
HCC	Hollings Cancer Center
Hgb	hemoglobin
HMS	hypogonadal metabolic syndrome
ICH	International Code of Harmonization
ID	identifier
IDS	Investigational Drug Services
IIT	Investigator Initiated Trial
IRB	Institutional Review Board
LDL	low density lipoprotein
MG	methylglyoxyl
MTD	maximum tolerated dose
MUSC	Medical University of South Carolina
NADHP oxidase	nicotinamide adenine dinucleotide phosphase-oxidase
NYHA	New York Heart Association
OTC	over the counter
PCa	prostate cancer
PI	Principal Investigator
PO	by mouth
PSA	prostate specific antigen
RAGE	receptor for AGE
ROS	reactive oxygen species

SAE	serious adverse event
SIS	Sponsor-Investigator Support Unit
sRAGE	soluble form of RAGE
USP	United States Pharmacopeia
VCAM-1	vascular cell adhesion molecule-1
VLDL	very low density lipoprotein

1 BACKGROUND

1.1 AGEs are Lifestyle-Associated Mediators of Degenerative Diseases.

Despite great progress in the treatment of many cancers, specific populations across the world still suffer disproportionately high levels of cancer incidence and mortality. Cancer disparity is most evident in our African American populations who bear the highest cancer burden for many tumor types. Poor diet, low income, obesity, and a lack of exercise are established lifestyle factors that are known to increase cancer burden and are often more prevalent in African American communities (1-3). As our understanding of tumor biology advances, it is becoming increasingly clear that these interrelated lifestyle factors have distinct molecular consequences on the biologic make up of tumors, altering cell signaling events and gene expression profiles to contribute to cancer disparity outcomes such as its earlier development or its progression to more aggressive disease. Sparse information exists about the genetic and biologic factors that contribute to differential cancer survival and mortality rates observed in minority populations. A greater understanding of the interplay between risk factors and the molecular mechanisms associated with cancer disparity will significantly affect minority health.

We recently reported a potential mechanistic link between sugar-derived metabolites (collectively AGEs or advanced glycation endproducts) and cancer, which may provide a molecular mechanism to account for the consequence of our lifestyle choices that can directly affect tumor biology and contribute to cancer (and generalized health) disparity (4).

1.2 AGE Accumulation Is Inherently Linked to Lifestyle

Systematic reviews and meta-analysis studies support the view that eating unhealthily, being overweight or obese and/or sustaining a sedentary lifestyle can increase risk of cancer, risk of cancer recurrence, and decrease overall survival rates (5,6). A recent statement from Cancer Research UK estimated that lifestyle accounts for around 40% of cancer cases, second only to smoking. This is racially significant as the highest rates of being overweight and obese (defined as a BMI >25) and the lowest adherence to CDC-recommended physical activity guidelines (defined as a minimum of 150 minutes per week) occur among the African American populations at highest risk of developing and dying of cancer (7). A family tradition of high-calorie “soul foods” with a heavy use of fat and sugar exists for many African American families. In addition, low income promotes the use of cheap, unhealthy, and highly processed foods, which can lead to weight gain, obesity, and increased cancer risk. Poverty rates within African Americans communities are among the highest in the country and they are also more likely to live in

designated "food deserts" where people have limited access to healthy affordable food (8). All of these lifestyle factors not only contribute to health disparity and increase cancer risk but significantly contribute to the exogenous AGE accumulation pool in our bodies.

The typical Western diet comprising red meat, refined grains, and high fat/high sugar foods are associated with systemic disease and are particularly AGE-laden, contributing as much as 30% of the AGEs accumulated within our bodies (9). The consumption of AGE-rich diets by mice increases circulating and tissue AGE content to promote conditions such as atherosclerosis, diabetes, and kidney disease, all of which are inhibited by dietary AGE restriction (9). Although human studies are limited, associations between elevated AGE and serum biomarkers of oxidative stress, endothelial dysfunction, inflammation, hyperlipidemia, and hyperglycemia have been identified in patients with impaired renal function and diabetes (9). Evidence supports dietary AGE restriction for the reduction of 8-isoprostanes and TNFα in healthy adults and reduced glucose and insulin resistance and AGE-modified low-density lipoprotein in type II diabetes patients (10).

AGE content in foods is not only dependent on nutritional content but also on how the food is prepared. Cooking methods involving dry heat such as grilling, broiling, and searing, used to improve food flavor, aroma and appearance, accelerate the glycation reaction between sugars and proteins to significantly increase overall AGE content (9). Frying meats, for example, can increase AGE content by as much as 10 fold. Thermal processing and/or irradiation by food manufacturers, used to improve food safety, preservation, and taste also rapidly accelerates the AGE-forming reaction (9). Due to beneficial effects on flavor, synthetic AGEs are now directly added into the manufacturing process for several food items. Processed foods are now one of the most common food items in groceries baskets across the country and due to their relatively low cost are often most heavily used by low-income families.

Recent data from the European Prospective Investigation into Cancer and Nutrition Study conclude that a sedentary lifestyle poses twice the risk of premature death as being overweight or obese (11). Studies of the effects of physical activity on AGE levels are limited and are mainly carried out using animal models but indicate that regular physical activity can help maintain or even reduce AGE levels in our bodies. In obese rats, regular moderate exercise reduced advanced glycation early diabetic nephropathy, lowered plasma AGE-associated fluorescence as well as overall renal AGE content (12). Similarly, increased physical activity in middle-aged senescent rats reduced both cardiac fibrosis and circulating AGE levels (13). In nondiabetic middle-aged women, a 12-week lifestyle modification consisting of an initial educational session followed by encouragement showed that the number of daily walking steps significantly correlated with AGE levels. Decreases in AGEs correlated with reduced body weight and body fat content (14).

In summary, AGEs are inherently linked with poor lifestyle and play a pathogenic role in multiple diseases associated with growing older. Approaches to define the molecular consequences of AGE accumulation may define novel therapeutic targets and potential biomarkers with which to reduce cancer incidence and mortality, particularly in minority populations.

1.3 AGE Accumulation, RAGE, and Stress Response

Persistent, unchecked inflammation and a related increase in oxidative stress are major biologic consequences of poor lifestyle and an underlying factor behind most, if not all, systemic diseases. A healthy diet and regular exercise has been shown to reduce chronic inflammation associated with diabetes and cardiovascular disease in the absence of weight loss, and studies indicate that increased physical activity is associated with lower inflammatory marker levels (19, 20).
A major pathogenic consequence of AGE accumulation is the perpetual activation of immune-mediated chronic inflammation and the generation of ROS, which results in a perpetual inflammatory microenvironment susceptible to disease development. CRP is a marker of inflammation that is linked with increased risk of heart disease, diabetes, and some cancers. In diabetes, serum AGE levels are an independent determinant of CRP levels due to a chronic inflammatory response (15). African Americans have an increased burden of chronic inflammation, which is independent of BMI and other potential confounding factors (16). African Americans have higher CRP levels than Caucasian American, which correlates with obesity and other metabolic and disease risk factors (24). Clinical and epidemiologic evidence also identifies African American race as an independent risk factor for elevated oxidative stress (17) and the increased expression and/or activity of critical oxidative stress markers (18) .

AGEs contribute to immune-mediated chronic inflammation by functioning as ligand for the transmembrane receptor for AGE known as RAGE (or AGER; Fig 1). Mechanistically, AGE-mediated activation of RAGE results in the increased activation of proinflammatory transcriptional regulators, including nuclear factor-kappa B (NF-κB), STAT3, and hypoxia inducible factor 1 (HIF1). In diabetes, RAGE activation perpetuates NF-κB activation in a feedback loop involving *de novo* synthesis of NFκB-p65, which functions to maintain a persistent pool of this key proinflammatory regulator. Increased activation of these critical transcription factors increases the secretion of cytokines/chemokines such as IL1β, IL6, and TNFα, leading to the increased recruitment of lymphoid and myeloid immune cells into tissues, elevated ROS production, and an inflammatory response (19). To perpetuate the cycle and add further fuel to the fire, reactive intermediates generated during AGE formation (i.e., Schiff's Bases and Amadori products) can directly increase ROS production and increased ROS presence can in turn further promote the formation of AGE precursors such as methylglyoxal to create a cyclic and persistent inflammatory response (20) . Significantly, antioxidants can inhibit AGE-induced changes in glucose consumption and lower ROS levels. AGE activation of RAGE increases heme oxygenase-1, nuclear translocation of NF-κB, and increased endothelial expression of vascular cell adhesion molecule-1 (VCAM-1), all of which function to increase oxidative stress and elevated levels of ROS (21). RAGE loss of function inhibits all of these AGE-mediated effects.

1.4 AGEs and Cancer

It is now generally accepted that chronic inflammation, oxidative stress, and cancer are intrinsically linked, and an inflammatory microenvironment is an essential element for both the onset and growth of tumors. In precancerous lesions, a constant state of inflammatory response and increased ROS production can cause both genetic and epigenetic alterations to alter gene

expression and increase cancer risk (22). In established tumors, inflammation is thought to mediate cross-talk between cancers cells and the stroma, resulting in the active recruitment of immune cells to the tumor microenvironment and increased oxidative stress (22). The inflammatory milieu created contributes to tumor onset and progression by promoting genetic instability, cell survival, growth, and metastatic potential.

Although the mechanistic links between AGEs and lifestyle have been identified in diseases such as diabetes and cardiovascular disease (9), a potential contribution to the development and progression of cancer is relatively understudied. AGE presence in human tumors was first demonstrated in larynx, breast, and colon tumors by immune-histochemical staining. Exogenous AGE treatment of breast (23) and prostate (24) immortalized cancer cell lines promotes cell growth, migration, and invasion. In prostate cancer, AGE-modified basement membrane promotes the invasive properties of prostate epithelial cells and correlates with decreased survival (24). A recent article found that the dietary-derived AGE carboxymethyl-lysine was associated with modestly increased risk of pancreatic cancer and may partially explain the positive association between red meat and pancreatic cancer (25). Increases in the exogenous AGE pool mediated by poor diet and a sedentary lifestyle may contribute to tumor development and growth through the perpetual activation of immune response. This would be particularly significant in population groups with the highest prevalence of poor lifestyle and cancer risk, such as our African American communities, which evidence suggests may have higher AGE accumulation levels (4).
The receptor for AGEs, RAGE, is also overexpressed in a number of tumors and evidence suggests a direct link between RAGE activation with the proliferation, survival, migration, and invasion of tumor cells. Loss of RAGE in inflammatory mouse models confers resistance to skin carcinogenesis and suppresses tumor growth (19). In prostate cancer, RAGE preferentially interacts with AGE over other potential RAGE ligands and AGE treatment of prostate cancer cells induces both cell growth and invasion (26).

Our group examined circulating and tumor AGE levels in clinical specimens of prostate cancer and identified a race specific, tumor-dependent pattern of accumulation (4) . AGE levels were significantly elevated in both serum and tumor, with highest accumulation occurring in more aggressive tumors. When examined in a matched cohort of patients, high AGE levels in the serum correlated with high AGE accumulation in cancer tissue (4). Significantly, when the data were stratified by race, AGE metabolite levels were significantly higher in serum from African American cancer patients compared with Caucasian. These initial data indicate that AGEs may represent a potential mechanistic link between cell metabolism and cancer, which may also provide a biologic consequence of the lifestyle risk factors that drive cancer health disparity.

Based on associations between active metabolism, lifestyle, and immune response, increases in exogenous AGE accumulation may represent a biologic mechanism contributing to cancer disparity and may represent a novel paradigm to explaining the increased cancer incidence and mortality figures observed within minority populations. A series of recent articles has highlighted the tumor-associated immune response as a critical pathway contributing to cancer disparity in

African Americans. An examination of expression differences based upon tumor composition shows that cytokine signaling associated with an increased immune response was found to be a predominant pathway increased in African American prostate cancer patients (2). Upon closer analysis, the majority of race-specific differential gene expression was found in the stromal compartment of the tumor (2). A similar race-specific increase in immune response gene copy number and gene expression was seen in matched radical prostatectomy tissues (27) and in Gleason 6 prostate tumors (1).

An analysis of more than 500 genes previously associated with prostate cancer shows that African American prostate tumors have significant upregulation of NF-κB and inflammatory cytokine factors (IL6, IL8, IL1B, C-X-C chemokine receptor type 4, and fatty acid synthase) compared with European Americans (28). In breast cancer, race is an independent predictor of elevated IL6 levels (29). Clinical and epidemiologic evidence also identifies African American race as an independent risk factor for elevated oxidative stress and ROS levels. For example, NADPH oxidase catalyzes the reduction of superoxide ($O_2\cdot$) radicals to ROS. Significantly, HUVEC cells from African Americans show higher levels of nitric oxide, lower superoxide dismutase activity, and increased expression of the NADPH oxidase subunit p47phox protein than their Caucasian counterparts (18). These combined data further indicate that the immune-mediated inflammatory response may be elevated in African American cancer patients and therefore may be more susceptible to the pathogenic effects of AGE accumulation. Such a heightened inflammatory response may be a major contributor to the development and progression of cancer and contribute to the dire cancer incidence and survival rates observed in this population.

The existence of AGE metabolites, their connections with diet and lifestyle, and their contribution to systemic disease are relatively unfamiliar to the general public as well as the cancer research community. Although emerging research has identified increased levels of AGEs in the circulation and tumor of cancer patients and has identified a significant role in carcinogenesis for their cognate receptor RAGE, it is not known if the same AGE–RAGE–mediated biologic pathways established in other systemic diseases are at play in the tumor microenvironment and to what extent AGEs derived from poor diet and a sedentary lifestyle contribute. Overall, supporting evidence for dietary restriction and/or physical activity interventions to reduce AGE levels in humans is hampered by the need for long-term high-quality randomized control trials with larger cohorts and defined disease outcomes. The difficulties of effecting these changes over long periods of time in a wide spectrum of patients are well known. The development of pharmacologic methods to manipulate AGE levels could be a significant aid to defining the role of AGEs in cancer development and progression.

1.5 Prostate Cancer and the Hypogonadal Metabolic Syndrome: Role for AGEs?

The primary treatment for advanced prostate cancer is ADT. While highly effective at producing regression of prostate cancer, ADT is not curative. Furthermore it has significant side effects due to the lack of testosterone. Adverse effects of ADT include decreases in bone mineral density; metabolic changes such as weight gain, decreased muscle mass, and increased insulin resistance;

decreased libido and sexual dysfunction; hot flashes; gynecomastia; reduced testicle size; anemia; and fatigue (30). Several observational studies suggest an increased risk of diabetes (31,32) and cardiovascular events (33), although most published studies report that ADT is not linked to greater cardiovascular mortality. Randomized trials have found value in treatments for some adverse effects including bone loss (bisphosphonates, denosumab, selective estrogen receptor modulators), markers of metabolic syndrome (exercise, diet, metformin), gynecomastia (tamoxifen, prophylactic radiation), muscle loss (resistance and aerobic exercise), and hot flashes (venlafaxine, medroxyprogesterone, cyproterone acetate, gabapentin).

A subset of ADT toxicities is referred to as the HMS because of its resemblance to the metabolic syndrome in the diabetes and cardiovascular literature. The HMS consists of increased body fat (particularly subcutaneous fat), obesity, insulin resistance, sarcopenia, elevated triglycerides and VLDLs, and increased adiponectin. Additional significant metabolic toxicities include loss of bone mass, and increased risk of an acute cardiac event. The HMS differs from the standard metabolic syndrome by the absence of hypertension (present in standard metabolic syndrome), and the elevated levels of adiponectin (reduced in standard metabolic syndrome; (34)).

Components of the metabolic syndrome (whether pre-existing or associated with ADT) are associated with poor outcomes from prostate cancer treatment (35). Obesity is associated with shortened progression-free survival after active surveillance (36), prostatectomy (37) and radiation (38,39), and with shortened time to castration-resistant disease in subjects receiving androgen deprivation therapy (40). Poor glycemic control is associated with biochemical recurrence after radical prostatectomy (44) and in more advanced patients (41), and with impaired overall survival (42).

Since AGEs are implicated in the metabolic syndrome described from diabetic and cardiac subjects, it is likely that they will play a role in the metabolic syndrome associated with prostate cancer and androgen deprivation. Thus, development of methods to modulate AGE levels may provide additional tools to improve cancer-specific outcomes in prostate cancer patients.

1.6 Pharmacologic Manipulation of AGE levels

AGEs accumulate in our tissues and organs over time and contribute to the development and complications associated with diseases of advancing age, including diabetes, cardiovascular disease, renal failure, arthritis, and neurodegenerative disorders (43). The rate of AGE accumulation in our bodies results from a balance between (i) their endogenous accumulation during the breakdown of sugar via the non-enzymatic, spontaneous glycosylation of proteins, lipids, and DNA; (ii) their exogenous intake through the foods we consume and other lifestyle factors such as drinking alcohol, smoking, and a sedentary lifestyle; and (iii) their inefficient removal via renal and/or enzymatic clearance, around 10% to 30% of exogenous AGEs are absorbed intestinally but only a third of those are excreted in urine and feces (9). AGEs bind to several receptors proteins, most clearly the RAGE. Receptor engagement then activates a variety of signaling pathways, including NFkB. These documented features of AGE production,

accumulation, elimination, and signaling provide guidance for the development of pharmacologic approaches to AGE modification. Agents with at least some activity in diabetic or chronic kidney disease populations (animal models or patients) include glucose regulators, antioxidants, RAGE regulators, and oral AGE-binding resins.

1.6.1 AGE-binding resins.

Sevelamer consists of polyallylamine that is crosslinked with epichlorohydrin. In overall structure it is a polymer with free amino groups. The marketed form sevelamer hydrochloride is a partial hydrochloride salt being present as approximately 40% amine hydrochloride and 60% sevelamer base. Sevelamer is normally used to lower phosphorus levels, and is given chronically to patients on dialysis. The amine groups of sevelamer become partially protonated in the intestine and interact with phosphate ions through ionic and hydrogen bonding. Sevelamer has also been shown to bind AGEs in vitro, and to lower AGE levels in the plasma of patients with chronic kidney disease (44). While having little toxicity, sevalamer is extremely expensive, with current wholesale costs of $1200/month.

1.6.2 Chitosan as an AGE sequestering agent.

Chitosan is a linear polysaccharide composed of randomly distributed β(1→4)-linked D-glucosamine (deacetylated unit) and *N*-acetyl-D-glucosamine (acetylated unit). It is made by treating the chitin shells of shrimp and other crustaceans with an alkaline substance such as sodium hydroxide. Chitosan can also be recovered commercially from the stems of mushrooms. Like sevelamer, chitosan is also a polymer decorated with free amino groups that become partially protonated at the intestinal pH. It can bind phosphorus and other anions, as well as AGEs. In contrast to sevelamer, chitosan is derived from food sources and is safe and inexpensive. These parallels suggest that chitosan could be a clinically useful AGE-lowering agent.

Several studies in mice and humans implicate carbonyl stress from methylgloxal (MG)-derived AGEs in diabetic nephropathy (45,46). Chitosan directly binds MG-derived AGEs *in vitro* and lowers serum and tissue levels of AGEs in mouse models (46). Furthermore chitosan treatment reduced AGE-related nephropathy in mice, both histologically and clinically. A small clinical trial of chitosan administration to chronic dialysis patients demonstrated an improvement in several biochemical parameters of nephropathy with the treatment (47). However no studies to date have demonstrated that chitosan can reduce either serum or tissue levels of AGEs in humans with AGE-related diseases. Similarly there are no clear data as to the *in vivo* mechanism by which chitosan reduces AGEs in animal models.

1.6.3 Biodistribution of oral chitosan.

The systemic absorption and distribution of chitosan following oral exposure are likely influenced by the molecular weight of the polymer. The effect of molecular weight on chitosan absorption has been evaluated in male Sprague Dawley rats (48). Oral gavage administration of chitosan with molecular weights of 3.8, 7.5, 13, 22, or 230kDa resulted in maximum plasma

chitosan concentrations (Cmax) of 20.23, 9.30, 5.86, 4.32, or less than 0.5µg/mL, respectively. The results of this study suggest that the absorption of chitosan from the gastrointestinal tract following oral exposure is inversely related to chitosan molecular weight, as there is likely low bioavalability associated with the higher molecular weight chitosan polymers.

The biodegradation of chitosan influences absorption and distribution because both are dependent on molecular weight. The biodegradation of chitosan invivo is dependent on the degree of deacetylation (49). Enzymatic degradation of chitosan depends on the ability to hydrolyze glucosamine -glucosamine, glucosamine-N-acetyl-glucosamine and N-acetyl-glucosamine-N-acetyl-glucosamine linkages (50). Degradation of chitosan in vertebrates is thought to occur predominantly by lysozymes and bacterial . While eight human chitinases have been identified with three showing enzymatic activity, their capacity to degrade chitosan has not been investigated (50,51).

1.6.4 Toxicity of oral chitosan.

Under the auspices of the National Toxicology Program, chronic oral dosing of chitosan (Mw 82KD, 86% deacetylated) for 6 months, was studied in rats (52). Serum levels of cholesterol, triglycerides, and phosphorous were significantly decreased in rats exposed to 9% chitosan (equivalent to 5.2gm/kg/day), as were serum levels of vitamin A and vitamin E; serum levels of 1,25(OH) vitamin D were increased. Vitamin E concentrations in the livers of exposed rats were significantly lower than those in control rats. Exposure to chitosan also had digestive effects, including increases in fecal weight and moisture, and decreases in percent fat digested. There was a decrease in thymus and liver weights, along with decreased fatty tissue in the livers of exposed rats. There was no effect of any dose of chitosan on longevity.

Chitosan is widely regarded as being a non-toxic, biologically compatible polymer (53). It is approved for dietary applications in Japan, Italy and Finland (54). In the United States it is widely marketed (without FDA approval) as a weight loss agent that binds fats and prevents their absorption (55)(56)(57). In a study on fat chelation, 4.5 g/day chitosan (Mw and DD not noted) in humans was not reported toxic, although no significant reduction in fat was found (58). Additional, no significant effects were reported following oral administration of chitosan at up to 6.75 g per day for 8 weeks in male and female volunteers (59). It has been approved by the FDA for use in wound dressings (60). Wound contraction and increased healing were found using chitosan (61).

Chitosan has been extensively tested in human for indications related to obesity, glucose control, hypertension, renal failure, and the metabolic syndrome. Table 1 is a partial selection of trials that can be found in the published literature. These studies include 371 patients treated with oral chitosan, in doses up to 5,250mg daily, and for periods of up to 1 year. These doses include the range of proposed dosing in our study. A variety of benefits were claimed. No study reported SAEs or significant (grade 3,4) AEs. When reported, compliance was generally high, and in randomized studies, was similar between the chitosan and placebo arms. Described AEs

predominately included GI complaints (constipation, bloating, full feeling, gas) but these were also described in the placebo arms. Where attribution was made, constipation was described as "not related" to the study drug. Three studies made no comment on chitosan toxicity. While the toxicity in these studies may be unclear, it is unlikely that the chitosan intervention was highly toxic. (58,62-72)

Table 1. Toxicity of Chitosan in Humans

disease	study type	chitosan	placebo	chitosan dose	duration	effects	toxicity	ref
renal Dz on dialysis	randomized	40	40	1350mg/d vs placebo	12 wks	lower creatinine, cholesterol; improved Hb, wt	"no clinically problematic symptoms"	1
elderly	single arm	18	18	5100mg/d	8 wks	immune cytokine increase	"no safety problems"	2
high cholesterol	crossover	65	65	2400mg/d vs placebo	3 months	no reduced chol, ApoE	no SAEs. Reported AEs were mild (GI complaints, pains, swelling, rash, heart palpitation, insomnia). 7/65 (chito) vs 5/65 (placebo) dropouts	3
high cholesterol women	randomized	46	44	1200mg/d vs placebo	56 days	lowered chol	few; no SAE; 4/46 AE in chitosan (thirst x 3, apthous ulcer x 1), 2/44 AE in placebo (abd fullness, HA)	4
healthy males	single arm	7		5,250mg/d	12 days	no reduced fat absorption	no comment	5
healthy adult	randomized (chitosan vs orlinostat)	6	6	2670mg/d	21 days	no increase in fat excretion	"well-tolerated, with no subjects reporting SAEs or discontinuing study. Majority of AEs were gastrointestinal in nature, reported by 8% of subjects on chitosan"	6
adult	randomized	44	44	1000mg/d vs placebo	112 days	no reduced wt or body fat	72.3% completed study	7
overweight adults	randomized	15	15	not stated	28 days	no wt loss; nl fat-soluble vits	no SAEs; compliance 91.5% (chitosan) vs 96.0% (placebo); constipation in 6/15 chitosan and 2/15 placebo; 2/15 chito and 2/15 placebo withdrew	8
normal volunteers	single arm	10		540mg/d	28 days	reduced glucose, ox stress	no comment	9
high BMI	randomized (chitosan vs placebo)	50	50	1,600mg/d	1 year	reduced BMI, waist circumference	no aAE except "few cases" of constipation equally distributed between grps.	10
high BMI	randomized (chitosan vs placebo)	64	ew	2,500mg/d	90 days	reduced A1c; reduced BMI, improved QOL	AEs mild, unrelated to Rx; 6/64 in chitosan (URI, constipation, body ache, HTN, 4/32 in placebo (high triglycerides, fracture)	11
obese adults	randomized	6	6	2,250mg/d	3 months	decreased weight, BMI, improved insulin sensitivity	no comment	12

Because of the broad use of chitosan for many different studies, plus its use as a pharmacologic excipient and a topical agent, the FDA has had many occasions to review chitosan. The FDA has not made any proscription for use of this agent. It is freely available in every health food store in the country, as well as multiple on-line locations. The manufacturer of our proposed chitosan preparation (Primex EHF, Iceland) reports that they have sold over 400,000,000 doses of chitosan in the past few years. This broad use of chitosan, in the community and in peer-reviewed clinical trials, gives confidence that the agent is safe.

2 STUDY OBJECTIVES

2.1 Study Overview

The overall goal of this Phase Ib/2 study is to identify a safe dose of chitosan for pharmacologic reduction of AGE levels in PCa subjects.

2.2 Primary Objective

The primary objective is to determine the maximum tolerated dose (MTD) of chitosan in subjects with PCa on ADT. The MTD will be defined as the dose that produces no more than 1 dose-limiting toxicity (DLT) in 6 subjects.

2.3 Secondary Objectives

The mechanistic studies under this section are to document any systemic effects from the chitosan treatment, related magnitude and timing of AGE depletion to chitosan effects, and determine if chitosan can reduce tissue, as well as serum, AGE levels.

- Identify systemic effects of chitosan on redox status (RedoxSys, serum oxidized glutathione), inflammation (plasma cytokines, Toll-like receptor signaling), and insulin resistance (HOMA-IR).
- Determine if chitosan modifies bowel permeability (plasma endotoxin) and microbiome diversity (16s rDNA sequencing).
- Correlate changes in serum AGE levels (pan-AGE, carboxymethyllysine, methylglyoxal, pentosidine adducts) with changes in tissue AGE levels as determine by skin autofluorescence.
- Define the frequency of a $\geq$ 30% reduction in total AGE levels from the pretreatment level. A frequency of 50% will be considered "interesting" for further study.

3 SUBJECT SELECTION

3.1 Inclusion Criteria

1. Confirmation of adenocarcinoma of the prostate that is documented by one of the following: pathology report or clinic note with documented history of prostate cancer.
2. Subjects must be receiving ADT with a GnRH agonist or antagonist, with or without an anti-androgen or testosterone sythesis inhibitor. The current testosterone level must be documented to be <50ng/dL at enrollment. Subjects whose ADT is interrupted may enroll or continue on study as long as the testosterone is documented to remain <50ng/dL for the entire duration of study participation. Subjects who have undergone orchiectomy are also eligible.
3. Subjects must have adequate hematologic, renal, and hepatic function at baseline, as follows:
    - Hematology parameters: ANC >1000/mcL, platelets > 100,000/mcL, Hgb >8.0gm/dL

- Renal Function: eGFR of ≥ 45mls/min using Cockkroft and Gault formula
- Liver Function: Total bilirubin ≤ULN, AST and ALT <1.5x ULN

4. Able to swallow and retain oral medication
5. ECOG performance status of 0 – 2
6. Ability to sign written informed consent
7. Testosterone level <50ng/dL at time of enrollment.
8. Age 18 or older.
9. May have had prior radiation therapy, surgery, or cryoablation for primary prostate cancer
10. May have had prior cytotoxic chemotherapy for metastatic prostate cancer, prior treatment with genomically-targeted agents, or Provenge

3.2 Exclusion Criteria

1. Known allergy to chitosan or shellfish.
2. History of receiving more than 2 classes of ADT.
3. Chronic constipation (BM < 3x weekly), history of malabsorption or history of daily laxative use.
4. Patients requiring medication administration with lunch or dinner or at a frequency of three or more times per day are not eligible.
5. Current use of chitosan, sevelamer, and/or glucosamine.
6. Patients currently on diclofenac or any of the restricted therapies listed in section 4.5

3.3 Study Registration

The SIS Unit will provide patient registration services for this study. The SIS unit will conduct a patient eligibility audit review of all redacted eligibility source documents prior to patient registration. After obtaining signed informed consent and completion of required baseline assessments, eligible subjects will be registered. A unique subject number will be assigned to each patient. The SIS Unit will issue a patient registration confirmation email to the enrolling study team at the time of registration. This confirmation will include the patient's assigned cohort dose level and study ID number.

4 TREATMENT ADMINISTRATION

4.1 Androgen deprivation therapy

During the course of the study, subjects will continue to receive ADT therapy per subject's primary oncologist. Changes to ADT administration will be made at their discretion, but the testosterone level should remain <50ng/dL during the entire study..

4.2 Chitosan administration

Subjects will be enrolled sequentially into four cohorts; the starting dose level is cohort 1. These cohorts will take chitosan as described in Table 2. Three patients will be enrolled into cohort 1, and no patients will be enrolled into the next cohort until the final patient in cohort 1 will have completed 28 days of treatment.

Chitosan will be taken up to 30 minutes before, or during, the middle and evening meals (BID dosing) or the largest meal of the day (Q day dosing). Chitosan will not be taken with breakfast, and the subjects should endeavor to take any chronic medicines before or with breakfast to minimize the chance of absorption of pharmaceuticals to chitosan. Any pharmaceuticals should be taken at least 1 hour before or 2 hours after chitosan. Any chitosan doses lost through vomiting will not be made up.

Table 2. Dose escalation cohorts

Cohort	Chitosan
-1	500mg Q day
1	500mg BID
2	1000mg BID
3	1500mg BID
4	2000mg BID

4.3 Medication Compliance Assessment

Patients will be required to track daily doses of study drugs by maintaining a daily medication diary. The subject will be asked to bring his drug diary and pill bottle back for each clinic visit to assess drug compliance.

4.4 Discontinuation of Therapy

Participation in this study should be discontinued for any of the following reasons:

- Change in anti-cancer therapy
- Procedures requiring general anesthesia.
- Development of any medical condition that, in the opinion of the treating physician, requires discontinuation of the study intervention
- Withdrawal of consent by the subject
- Evidence of allergic reaction to any component of the treatment regimen
- Any grade 3-4 hematologic or non-hematologic toxicity (other than in 4.5) that does not resolve, with drug discontinuation, to grade 1 or less in 14 days.

4.5 Restricted Therapies During Study Therapy

Current use of chitosan, sevelamer, and/or glucosamine.

Drugs Eliminated by Biliary Excretion (defined as greater than 4% excretion in bile):

- Chemotherapeutic Agents
    - Irinotecan
    - Paclitaxel
    - Vincristine
- Anti-Hyperlipidemics
    - Ezetimibe
    - Fluvastatin
    - Pravastatin
    - Rousvastatin
    - Simvastatin
- Anti-Hypertensives
    - Ramipril
    - Spironolactone
    - Valsartan
- Analgesics
    - Acetaminophen
- NSAIDS
    - Diclofenac
    - Indomethacin
- Anti-histamines
    - Desloratadine
    - Fexofenadine
    - Levocetrizine
- Anti-diabetics
    - Rosiglitazone
- Immunosuppressants
    - Mycophenolic acid
- Anti-Psychotics
    - Quetiapine
- Anti-retrovirals
    - Lopinavir
    - Ritonavir
- Antibiotics
    - Ceftriaxone
    - Trovafloxacin
- Others
    - Cyclobenzaprine
    - Ketoconazole
    - Montelukast

4.6 Supportive Care during Therapy

It is unlikely that specific supportive care interventions will be needed for the study interventions. General supportive care for the subjects will be at the discretion of the treating physician.

5 SCHEDULE OF INTERVENTIONS AND ASSESSMENTS

5.1 General Considerations

- Informed consent must be signed prior to any study-specific assessments being done
- Screening assessments may be done up to 28 days prior to registration. Screening assessments completed within 28 days of day 1 do not have to be repeated.
- Day 85 or End of Study Assessments may be done +/- 3 days.
- All research and clinical blood samples must be collected while the subject is fasting.

5.2 Assessments and Procedures

The following clinical assessments are made at the time subjects are screened for the trial. Some of these may need to be repeated prior to day 1; see Appendix A for more details regarding the schedule of procedures and assessments.

- A full medical history and physical examination to review patient medical history, medication list, overall health and any side effects they may be experiencing. Medical history will include:
    - Extent of cancer to include metastatic disease
    - Concomitant medications
    - current ADT therapy
- Height, weight
- CBCD
- CMP (sodium, potassium, chloride, bicarbonate, BUN, creatinine, glucose, albumin, alkaline phosphatase, AST, ALT, calcium)
- PSA, testosterone
- Research blood, urine, and stool samples as outlined (see Appendix A)
- 24hr food intake recall questionnaire
- Quality of life questionnaires (FACT-P and AUA; see Appendices B, C)
- Measurement of tissue AGE level by skin autofluorescence using the AGE Reader device.

On day 29 of the study, a phone call will be made to the patient to assess any possible adverse events (AEs).

The following clinical assessments are made on day 85/End of study

- Interim medical history and focused physical exam to review:
    - Changes to the patient's medical history,
    - Changes to ADT

  - Changes to familial history of diabetes
  - Change to medication list
  - Overall health and any side effects they may be experiencing.
- Height, weight
- CBCD
- CMP (sodium, potassium, chloride, bicarbonate, BUN, creatinine, glucose, albumin, alkaline phosphatase, AST, ALT, calcium)
- PSA
- Research blood and urine samples as outlined (see Appendix A)
- 24hr food intake recall questionnaire
- Measurement of tissue AGE level by skin autofluorescence using the AGE Reader device.
- Day 85: Quality of life questionnaires (FACT-P and AUA; see Appendices B, C)

5.3 Subject Recruitment

This study will take place at the MUSC and one other cancer center (to be determined).

- Subjects will be identified by the designated IRB approved study team or will be referred by colleagues who care for the patient populations under study.
- Designated IRB approved study research team personnel may screen records to identify potential subjects. Records and/or areas that may be reviewed include pathology/surgical reports, and pharmacy records.
- Potential subjects will only be approached for the study after they have been informed of the study by someone involved with their clinical care.
- Potential subjects who have previously agreed to be contacted for research may be approached by the study team.

5.4 Informed Consent Process

- Subjects who are interested in participating will be interviewed in clinic. A designated IRB approved member of the study will explain the nature of the study, the benefits and risks. Informed consent will be obtained by designated IRB approved research members in clinic. Consent will be documented by obtaining the subject's signature on the approved consent form, and documenting the consent process through a progress note in the participant's clinical or research record.

- Consent may be obtained the same day the patient is presented with the trial.

5.5 Disease Assessment

Disease response will be obtained by the assessment of PSA at enrollment and at the time the patient comes off study. PSA will not be repeated at these times if it has been done within 2 weeks for clinical care. Imaging studies will be done only if clinically indicated and/or at the discretion of the subject's primary oncologist.

5.6 Toxicity Assessment

See Section 7.

5.7 Schedule of Assessments

See Appendix A.

5.8 Correlative Studies

Samples will be processed and stored locally in Dr. Lilly's lab in HO334. The trial will be carried out at MUSC and one other cancer center (to be determined). Correlative studies will be done and will be exchanged between participating institutions. Samples may be stored for future use.

6 STUDY DRUG INFORMATION - CHITOSAN

6.1 Agent Description.

Chitosan is a natural polycationic linear polysaccharide derived from partial deacetylation of chitin. It is the structural element in the shell of insects, certain shellfish, and the cell walls of fungi. After cellulose, it is the most abundant natural polysaccharide. Chistosan is made up of B-(1-4)-lined D-glucosamine and N-acetyl-D-glucosamine randomly distributed within the polymer.

This study will use LipoSan Ultra® brand of chitosan, to reduce blood and tissue levels of advanced glycation endproducts, as an adjunct to prostate cancer treatment. LipoSan Ultra® is a proprietary combination of chitosan and succinic acid. Its composition and manufacture is covered by US Patent No. 6,130,321; it is manufactured in the US by Swanson Health. LipoSan Ultra® has also been awarded a certificate of compliance with the Tún Standards for Certified Natural Products.

6.1.1 Supply

Chitosan will be provided in 500mg capsules. Chitosan will be stored at the investigational pharmacy located at each participating center. Chitosan will be distributed by the investigational pharmacy at each participating center. Due to COVID-19 restrictions Chitosan can also be mailed to the subject directly. It will be purchased from an external supplier and repackaged by the investigational pharmacy at each participating center. The packaging will include the label information required per 21 CFR part 312.

6.1.2 Storage.

The capsules should be stored at a controlled room temperature, with excursions to 15-30°C (59-86°F) permitted. All capsules should be kept in a secured storage area.

6.1.3 Administration.

See section 4.2 for chitosan administration instructions.

6.1.4 Known Potential Toxicities.

Patients with allergies to any shellfish should not take Chitosan as it can cause anaphylactic shock. Known toxicities of chitosan include included flatulence, increased stool bulkiness, bloating, mild nausea, and heartburn. Chitosan may interfere with how your body absorbs some medications, including certain beta blockers (propranolol), blood thinners (warfarin), diuretics (thiazides), cholesterol medications (zetia), diabetes medications (rosiglitazone), and antibiotics (ciprofloxacin). It is important that you take the study drugs as instructed by the study doctor to avoid any possible drug interactions.

7 RISKS AND BENEFITS

7.1 Potential Risks

See Section 6.1.4 for the potential risks of Chitosan.

- The physical risk to participants is minimal. Venipuncture may produce momentary discomfort from needle stick, bruising or infection. Fainting could occur. The volume of blood removed is insignificant.
- The risk of distress from inappropriate release of PHI is low, due to the plan for de-identification of clinical data, as well as the likelihood that the clinical collaborators will already be involved in the subjects' health care.

7.2 Protection Against Risk

- The proposed procedures are inherently of low physical risk. We believe the psychological risks are also extremely low, since the investigators will already be treating the patients.
- We do not anticipate the occurrence of adverse events requiring treatment, or necessitating a data and safety monitoring board.
- Samples and results will be coded through the assignment of a unique study number to subject. Study labs will not have access to the ID log that contains the subject identifier. It will be stored separately from the research data.

7.3 Potential benefits of the proposed research to the subjects and others

There is no direct benefit to the participating subjects. Society may benefit if the new assays provide greater certainty about the diagnosis and behavior cancer and its response to therapy. In view of the extremely low risk of this study, the risk:benefit ratio seems favorable.

7.4 Withdrawal of subjects

Subjects can withdrawal from the study at any time without if affecting their regular care. The subject can be removed from study at any time if it is determined it is not in the subject's best interest to remain on the trial, the subject is non-compliant, or if the study is closed.

If the subject withdraws or is withdrawn from the study, the study team will stop collecting new information and specimen from the subject, but information and specimens already collected may still be used in the data analysis.

8 ADVERSE EVENTS

8.1 Definitions

Adverse Events (AE). An AE is defined as any untoward medical occurrence in a patient or clinical investigation subject administered a pharmaceutical product and that does not necessarily have to have a causal relationship with this treatment. An AE can therefore be any unfavorable and unintended sign (including an abnormal laboratory finding), symptom, or disease temporally associated with the use of the study drugs, whether or not is it causally related. During clinical trials, adverse events can be spontaneously reported or elicited during open-ended questioning, examination, or evaluation of a subject. (In order to prevent reporting bias, subjects should not be questioned regarding the specific occurrence of one or more adverse events.)

Adverse events will be named and graded using CTCAE v 5.0 and will be recorded prospectively during the study and entered into the REDCap database. Adverse events will be reported from the first day of study intervention until the last day of study intervention.

Serious Adverse Event (SAE). An SAE is any untoward medical occurrence that at any dose**:**

- results in death,
- is life-threatening (defined as an event in which the patient was at risk of death at the time of the event; it does not refer to an event which hypothetically might have caused death if it were more severe),
- requires inpatient hospitalization or causes prolongation of existing hospitalization,
- results in persistent or significant disability/incapacity,
- is a congenital anomaly/birth defect,
- results in the development of drug dependency or drug abuse,
- is an important medical event (defined as a medical event(s) that may not be immediately life-threatening or result in death or hospitalization but, based upon appropriate medical and scientific judgment, may jeopardize the patient or may require intervention (e.g., medical, surgical) to prevent one of the other serious outcomes listed in the definition above.) Examples of such events include, but are not limited to, intensive treatment in an emergency room or at home for allergic bronchospasm; blood dyscrasias or convulsions that do not result in hospitalization.

8.2 Reporting of SAEs

Following the subject's registration, all SAEs should be collected and reported, including those thought to be associated with clinical trial procedures. SAE terminology and severity grading will

be based on CTCAEv4. The following categories and definitions of causal relationship to study drug should be used:

Definitely related:	An adverse event occurring in a plausible time relationship to drug administration, and which cannot be explained by concurrent disease or other drugs or chemicals. The response to withdrawal of the drug should be clinically plausible. The event must be definite pharmacologically or phenomenologically, using a satisfactory rechallenge procedure if necessary and feasible.
Possibly related:	An adverse event with a reasonable time sequence to administration of the drug, but which could also be explained by concurrent disease or other drugs or chemicals. Information on drug withdrawal may be lacking or unclear.
Not related:	An adverse event with a temporal relationship to drug administration which makes a causal relationship improbable, and in which other drugs, chemicals or underlying diseases provide plausible explanations.

Adverse events classified as "serious" require expeditious handling and reporting to the coordinating center and Sponsor-Investigator. SAEs must be reported to all of these entities within 24hrs of becoming aware of the event, *whether or not the event is related to the study drugs or procedures*. If only limited information is initially available, follow-up reports are required. The original SAE form must be kept on file at the study site.

SAEs should be reported on the 102898 SAE eCRF via REDCap at redcap.musc.edu. The coordinating center will be responsible for submission to the Sponsor-Investigator, HCC-DSMC and FDA as applicable. SAEs should be reported to the site's IRB per institutional standards.

The site should receive confirmation from MUSC that the SAE report was received. If not, please contact the SIS Unit immediately.

Collection of complete information concerning SAEs is extremely important. Thus, follow-up information which becomes available as the SAE evolves, as well as supporting documentation (e.g., hospital discharge summaries and autopsy reports), should be collected subsequently, if not available at the time of the initial report, and immediately sent using the same procedure as the initial SAE report.

AEs should be followed to resolution or stabilization, and reported as SAEs if they become serious. This also applies to subjects experiencing AEs that cause interruption or discontinuation of study

drugs, or those experiencing AEs that are present at the end of their participation in the study; such subjects should receive post-treatment follow-up as appropriate. All SAEs must be collected which occur within 30 days of discontinuation of dosing or completion of the patient's participation in the study if the last scheduled visit occurs at a later time.

8.3 Unanticipated Adverse Device Effects

An unanticipated adverse device effect (UADE) is any serious adverse effect on health or safety, any life-threatening problem or death caused by, or associated with a device, if that effect, problem, or death was not previously identified in nature, severity, or degree of incidence in the application; or any other unanticipated serious problem associated with a device that relates to the rights, safety, or welfare of subjects.

UADEs will be reported to the FDA by the PI within 10 working days of being notified of the event.

9 DATA SAFETY MONITORING

9.1 Responsible Individuals and Organizations.

The Principal Investigator will be responsible for monitoring the safety and efficacy of the trial, executing the DSM plan, and complying with all reporting requirements. This will be accomplished under the oversight of the HCC DSMC.

The HCC DSMC is responsible for monitoring data quality and patient safety for all interventional IITs at HCC. The HCC DSMC will have oversight of this protocol. The HCC DSMC will meet at a minimum on an annual basis to discuss the investigator-initiated trial. Also, the IIT will be audited by the DSMC auditor or external agency at least once a year.

The DSMC reviews all IRB reportable serious adverse events, monitoring/ auditing reports, and protocol deviations and has the authority to recommend closure and/or suspension for trials on which there are safety or trial conduct issues and may submit recommendations for corrective actions. The DSMC recommendations for modifications to the trial (if requested) are forwarded to the principal investigator. The principal investigator is notified of this recommendation in order that he/she may alert all investigators involved in the trial with regard to the potential action. At this time the principal investigators may submit to the DSMC additional information that could affect the Committee's decision.

All IRB reportable serious adverse events, monitoring/ auditing reports will be reviewed by the HCC DSMC for review during the DSMC monthly meetings. The SIS Unit will forward the event report to the HCC DSMC so that the information can be reviewed at the next available DSMB meeting. During the DSMB review, the DSMB can make recommendations for any further study action. The SIS Unit will maintain a copy of the DSMB approval letters for each event review within this study's central file.

9.2 Data Collection

Electronic CRF's will be provided for the recording of data and reporting that requires expedited review, such as SAE submissions. All data should be substantiated by clinical source documents organized within a patient research record. ICH Good Clinical Practices are to be followed. Data submission guidelines are outlined in the 102508 Operations Manual.

Electronic data for on study and follow-up patient data is submitted via the electronic system called REDCap. REDCap is managed from MUSC as a consortium partner under their CTSA. REDCap CRF is a secure, Web-based application designed to capture and manage research study data.

The system has been reviewed for 21CFR Part 11 compliance and has been deemed "21CFR 11 Capable." Users of the REDCap system are limited to members of the IRB approved research team who are delegated data management responsibilities, typically the study coordinator and data manager.

9.3 Food and Drug Administration Review

An IND for chitosan will be submitted to the FDA in accordance with 21 CFR 312.

Review of the AGE Reader by the Food and Drug Administration is not indicated as this study does not meet the significant risk device criteria under 21 CFR 812.3(m). This study will follow the abbreviated reporting requirements found under 21CFR 812.2(b), which includes that the device will be labeled in accordance with 21CFR 812.5 (Caution – investigational device. Limited by Federal lab to investigational use). The label or other labeling shall describe all relevant contraindications, hazards, adverse effects, interfering substances or devices, warnings and precautions.

10 STATISTICAL METHODS AND POWER ANALYSIS

10.1 Escalation Design

The investigators will use a 3+3 design for assignment to cohorts. The cohorts are shown in Table 3. An initial 3 subjects will be enrolled at dose level 1. Dose-limiting toxicities (DLT) will be defined only in the first 28 days of therapy. If there are no DLTs after the final patient has been treated for 28 days, then the next cohort will be enrolled in dose level 2, and so forth. If a DLT occurs, then 3 more subjects will be enrolled into that cohort. If two DLTs occur in the first 3 patients in a dose level, then dose will be de-escalated and 3 additional patients will be enrolled in the lower dose

Table 3. Clinical Trial Cohorts

Dose Level	Chitosan	number
-1	500mg QD	3 (+3)
1	500mg BID	3 (+3)
2	1000mg BID	3 (+3)
3	1500mg BID	3 (+3)
4	2000mg BID	3 (+3) +30

level unless 6 have already been treated at that dose level. The MTD will be defined as the dose at which no more than 1 out of 6 subjects experiences a DLT.

Definition of DLT:

- Grade 3 or higher hypophosphatemia
- Grade 3 or higher of any of the following toxicities, that the investigator deems related to chitosan, that do not improve or resolve within 7 days of onset: flatulence, increased stool bulkiness, bloating, nausea, heartburn

10.2 Expansion Cohort Design and Statistical Analysis.

Once the MTD is established, 27 additional subjects will be recruited at that dose level to better define toxicity and changes in AGEs. The maximum number of patients that can be accrued is 54. The primary measure of change in AGE is defined as a binary indicator of a 30% or greater decrease in plasma total AGE level between baseline and day 85 specimens. The proportion of patients with a 30% or greater decrease with be calculated with its exact 95% confidence interval. An exact binomial test will be based on a null hypothesized true proportion of 0.25 (i.e., 25% of patients have a ≥30% reduction in AGE levels) which is too low to be of further interest. With an alternative of 0.50 and 33 patients, assuming a 1-sided alpha of 0.05, the power is 0.88. Graphical displays will be made to demonstrate patterns in AGE between baseline and day 85. The following analyses will be performed, and then data will be stratified by race, and analyses repeated to evaluate differences by race. Summary statistics (mean change between timepoints, standard deviations, medians) will be reported. Scatterplots and paired t-tests will be used to compare the mean change between baseline and day 85 with a 2-sided alpha of 0.05 for changes in AGE levels and changes in other variables of interest (e.g. race, BMI). Linear regression will be used to evaluate associations between continuous variables and changes in AGE levels with changes in AGE levels as the outcome. Transformations will be taken as appropriate and residual plots will be examined to evaluate assumptions of linear regression model. The investigators will add baseline levels of clinical parameters in the linear regression model to account for starting levels. Regression coefficients will be evaluated in terms of clinical effect size and statistical significance. Model results will be reported with 95% confidence intervals.

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APPENDIX A. SCHEDULE OF PROCEDURES AND ASSESSMENTS

Study Activity	Screen[c]	Day 1[c]	Day 14[g]	Day 29	Day 57[g]	Day 85/ End of study[a, d]	Specimen
Demographics	X						
Full Physical Exam,	X						
Focused Physical Exam,		X				X	
Medical History to include: • Duration of ADT • Extent of cancer • Personal history of diabetes mellitus • Current medications	X	X					
Interim Medical History		X				X	
Anthropometric Measurements (height, weight)	X	X				X	
CBCD	X	X				X	
CMP (fasting)[g]	X	X				X	
Phosphorus	X	X				X	
PSA	X	X				X	
Assessment of toxicity		X	X[g]	X[g]	X[g]	X	
testosterone	X	X				X	
Chitosan			X----------X				
Drug diary review[e]						X	
Informed consent	X						
Insulin Resistance (HOMA-IR)	X[f]					X	Plasma[b]
CRP, AGE species and precursors	X[f]					X	Serum[b]
RedOx status (ORP, reduced glutathione)	X[f]					X	Plasma[b]
RAGE, TLR expression, signaling	X[f]					X	Whole blood[b]
Microbiome profiling	X[f]					X	Stool
AGE species	X[f]					X	Urine[i]
Tissue AGE level by autofluorescence	X					X	
Food Recall Dietary AGE content[h]	X[f]					X	
QOL (FACT-P and AUA)	X[f]					X	

a) All days are +/- 5 days
b) 2x7mL EDTA blood (=plasma), 2x7mL clot tube (=serum), 1x7mL sodium heparin tube (=whole blood).
c) Screening assessments to be done within 28 days of registration. Screening assessments may be used for the day 1 assessments if they were completed within 28 days of day 1. The CMP used for eligibility confirmation does not have to be fasting, but a fasting CMP will need to be collected prior to day 1 drug administration. Fasting is defined as nothing containing calories since the previous midnight.
d) If the subject goes off study less than 2 weeks after start of dosing, "end of study" labs and questionnaires will not be done.
e) The drug diary will be given to each subject for completion during the study.
f) Research procedures (excluding informed consent) may be done at any point after informed consent and prior to first dose of drug (on day 1). Research blood, urine and stool samples will be taken to the participating research lab(s) for processing.

g) On day 14,day 29, and day 57 (+/- 5 days), compliance and adverse events will be assessed via phone using the phone contact guide in appendix D.
h) Food recall using the NCI quick food scan and the NIH's "Eating at America's Table Study" Quick food scan.
i) 10mL of urine collected in a sterile cup such as those used for clinical urine specimens

APPENDIX B: FACT-P QUALITY OF LIFE QUESTIONNAIRE

See Next Page

Study ID:
Initials:
Date:

FACT-P (Version 4)

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

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

	SOCIAL/FAMILY WELL-BEING	**Not at all**	**A little bit**	**Some-what**	**Quite a bit**	**Very Much**
GS1	I feel close to my friends……	**0**	**1**	**2**	**3**	**4**
GS2	I get emotional support from my friends…….	**0**	**1**	**2**	**3**	**4**
GS3	I get support from my friends…….	**0**	**1**	**2**	**3**	**4**
GS4	My family has accepted my illness…….	**0**	**1**	**2**	**3**	**4**
GS5	I am satisfied with family communication about my illness……..	**0**	**1**	**2**	**3**	**4**
GS6	I feel close to my partners (or the person who is my main support……	**0**	**1**	**2**	**3**	**4**
Q1	Regardless of your current level of sexual activity, please answer the following question. If you prefer not to answer it, please mark this box ☐ and go to the next section.					
GS7	I am satisfied with my sex life…….	**0**	**1**	**2**	**3**	**4**

Study ID:
Initials:
Date:

FACT-P (Version 4)

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

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

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

Study ID:
Initials:
Date:

FACT-P (Version 4)

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

	ADDITIONAL CONCERNS	Not at all	A little bit	Some-what	Quite a bit	Very Much
C2	I am losing weight……....	0	1	2	3	4
C6	I have a good appetite……..	0	1	2	3	4
P1	I have aches and pains that bother me…….	0	1	2	3	4
P2	I have certain parts of my body where I experience pain	0	1	2	3	4
P3	My pain keeps me from doing things I want to do……..	0	1	2	3	4
P4	I am satisfied with my present comfort level……..	0	1	2	3	4
P5	I am able to feel like a man…….	0	1	2	3	4
P6	I have trouble moving my bowels……...	0	1	2	3	4
P7	I have difficulty urinating……	0	1	2	3	4
BL2	I urinate more frequently than usual……	0	1	2	3	4
P8	My problems with urinating limit my activities……..	0	1	2	3	4
BL5	I am able to have and maintain an erection…….	0	1	2	3	4