A phase II double-blind, randomized placebo-controlled study to determine the efficacy of Coenzyme-Q10 in killing prostate cancer cells *in vivo.*

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A. Study Purpose And Rationale

In recent years, there has been a growing interest in using herbal and nutritional supplements for the prevention of prostate cancer. Products such as selenium, vitamin E, lycopene and more recently Coenzyme-Q10 (CoQ10) have all been implicated as possibly conferring protection against the disease. The main purpose of this study is to determine if CoQ10 has any physiologic effects in human prostate cancer. We will endeavor to determine if ingestion of CoQ10 prior to robotic radical prostatectomy can induce apoptosis in prostate cancer cells and reduce vascularity within the cancerous portions of the gland. The outcomes will be determined pathologically.

1. Background Information of Coenzyme Q10:

Coenzyme-Q10 or ubiquinone [2,3-dimethoxy, 5-methyl, 6-decaprenyl benzoquinone] is distributed throughout human tissues, and it is most well-recognized as a crucial component of oxidative phosphorylation in mitochondria.[1] Through this process CoQ 10 converts carbohydrates and fatty acids into ATP to drive cellular processes. Similarly, CoQ10 participates in oxidation/reduction reactions in many other cellular membranes and organelles. In its reduced form CoQ10 is a potent antioxidant and is hypothesized to be involved in free-radical scavenging.

CoQ10 was first isolated in 1957 by Dr. Frederick Crane in Wisconsin, who extracted the compound from beef heart mitochondria.[2] It was first studied in the treatment of congestive heart failure and the majority of its notoriety is from cardiovascular studies over the past 14 years. Heart tissue has an extremely high energy consumption rate, thus CoQ10 is highly concentrated in myocardium. Severity of heart failure has been correlated with low levels of CoQ10 in a number of studies.[3,4] The safety and side effect profile of CoQ10 has been established in these studies and many others conducted around the world. An interesting aside is that in eight symposia from 1976 through 1993, over 300 papers presented by over 200 physicians and scientists from 18 different countries have thoroughly elicited the clinical and biomedical characteristics of CoQ10.

2. Prior Safety and Efficacy Data:

Safety and efficacy of CoQ10 has been established in a number of these studies.[5.6,7] A study done at Temple University established clinical response in the treatment for cardiomyopathy at blood levels of 2.5pg/mL and higher during therapy without any significant side effects reported.[6] Similarly studies by Mortensen *et al.* from Denmark reported no adverse reactions to CoQ10 supplementation.[7]

CoQ10 is normally present in blood at 1pg/mL. It is believed that CoQ10 in cells is highly functional but that in plasma CoQ10 is nonfunctional, existing in equilibrium between absorption and metabolism.[3] These statements ate derived from studies involving cardiovascular treatment and rely on CoQ10 functioning in the mitochondria and contributing to sub-cellular processes occurring throughout a variety of tissues. However, its free-radical scavenging capability is believed to occur in a non-specific 'tanner and therefore be related to higher serum levels and supposed tissue penetration. The goal for CoQ10 therapy in prostate cancer is therefore to create a biomolecular environment that challenges the growth and progression of prostate cancer cells.

3. Q-Gel® Softsules and rationale for use:

In previous studies, to increase the levels to 2pg/mL required at least 100mg/day of supplementation.[8] The desire for serum levels of at least 2pg/mL or higher served as the impetus for initiating research to find a form of CoQ10 that could be readily absorbed and create high plasma levels. A new process called Bio-Solv® improved the dissolution and absoption of

CoQ10 and was recent employed Tischon Corp. in the making of their product, Q-GeI™ Softsules®. This producet demonstrated a greater increase in plasma concentration of CoQ10 when compared with previously tested softgel, tablet and powder-filled capsules.[9] In one study, at 21 days patients taking Q-Gel™ Softsule® had a mean plasma concentration of greater than 3pg/mL while the other versions of supplemental CoQ10 did not reach levels of 2pg/mL. In a second study, in which QGeI™ Softsule® was compared with a softgel, the prior reached mean plasma levels of 3pg/mL at 28 days while the latter reached a mean plasma level of 1.25pg/mL.

Because prior studies indicate a rather large dose necessary to raise plasma levels it is believed that high blood levels of CoQ10 are needed to reach deficient tissues. The use of Q-Gel™ Softsule® —because of its ability to reach higher plasma levels in a more rapid time than previously studied formulations of the drug — is a desirable medical choice for this study because of the relatively short time period (six weeks) allotted between diagnosis, medical treatment and prostatectomy.

(Include evaluation of Tishcon Meds for consistency across lot)

4. Coenzyme QI0 and Prostate Cancer:

Prostate cancer is the most common type of cancer found in American men, other than skin cancer. Although men of any age can get prostate cancer, it is found most often in men over 50. In fact, more than eight of ten men with prostate cancer are over the age of 65. The American Cancer Society estimates that there will be about 179,300 new cases of prostate cancer in the United States this year, and about 37,000 men will die of this disease.[10]

The wide prevalence of prostate cancer continuously fuels a search for both traditional and alternative therapies for the prevention, control and cure of prostate cancer. The use of CoQ 10 in the treatment of prostate cancer is based on a number of relatively contemporary studies demonstrating CoQ10 as an effective anti-tumor factor in prostate cancer cells grown in tissue. Most recently a study from Spain showed a markedly different response to CoQ10 in malignant prostate cancer cells in reference to phospholipids hydroperoxide glutathione peroxidase (PHGPx) gene expression and the creation of reactive oxygen species (ROS) as compared to non-malignant cells. In addition, CoQ10 lowered cell growth without adversely affecting cell growth in the nonmalignant cell line.[11]

5. Discussion *of the Study Population:*

Before asserting that CoQ10 is an effective nutritional supplement in the prevention of prostate cancer, it should first be established to hinder the growth of prostate cancer cells *in vivo* to demonstrate that the coenzyme can penetrate prostate tissue and have an effect on prostate cancer cells. The ideal population for this study is patients first diagnosed with prostate cancer that could be started and maintained on CoQ10 for a period of time and then evaluated in comparison to a group not being treated with CoQ10. For obvious reasons the length of time in which patients with a diagnosis of prostate cancer is limited. Studies concerning the *watchful waiting* of prostate cancer patients employ strict guidelines for those who can be "watched" and have been controversial and inconclusive to date. For patients with relatively low-risk prostate cancer, defined by a PSA less than 10, Gleason of six or lower, clinical stage of Tic or T2a[12] and age under 65, typically wait six weeks following their initial biopsy and diagnosis before undergoing radical prostatectomy. This allows for the prostate tissue to recover from inflammation and disruption of the anatomy associated with biopsy, allowing the surgeon a more clear and definable operative field. For patients with higher-risk cancer diagnosed by biopsy, it is often inappropriate to wait six weeks or to proceed directly to surgery without another intervention (radiotherapy, hormone therapy). "High-risk" patients are defined as intermediate or high-risk patients by the same criteria cited above: Clinical Stage T2b or greater, PSA > 10, Gleason 7 or greater. For the reasons described above, low-risk prostate cancer patients are the ideal study group for this study.

Lastly, there are many approaches to radical prostatectomy employed by a number of surgeons. Despite all types of surgery having the capability of providing adequate tissue samples, controlling for type of surgery and surgeon would control for any bias or confounding factors. Robotic prostatectomy is an emerging and more popular surgical technique with five to ten cases being performed weekly here at

Columbia Presbyterian by Dr. David Samadi. This further refines our study population to low-risk prostate cancer patients designated for radical robotic prostatectomy.

6. Laboratory Evaluation:

Each patient enrolled in the study will have their initial biopsy and surgical specimen processed by the Experimental Pathology Lab and evaluated by members of the

Department of Pathology here at Columbia Presbyterian Medical Center. The pathology evaluation will be overseen by Mahesh Mansukhani, MD.

Both pre-operative and operative tissue samples will be stained and evaluated for a number of biochemical markers including Bcl-2, CD3I, p27, Ki-67 and

Cleaved Caspase 3. Bcl-2 is a marker of cell death (apoptosis), CD31 is a marker of angiogenesis, p27 is a general marker expressed by many malignant cells and is therefore a general marker of tumor burden, Ki-67 is a marker of proliferating cells, and the Cleaved Caspase 3 is a marker of cell death.

B. Study Design

This will be a phase II trial to determine efficacy of ingested CoQ10 in inducing a physiologic effect in cancer-containing human prostate gland. This will be a randomized masked clinical trial in which patients will be designated to receive CoQ10 or placebo for 6 weeks prior to robotic radical prostatectomy. The study will enroll 40 patients, 20 of which will be randomized to placebo and 20 of which will be randomized to CoQ10 supplementation. The patients will take the prescribed dose of CoQ10 (or placebo) every day for 6 weeks prior to their scheduled prostatectomy. At the time of surgery, tissue samples of the excised prostate gland will be sent for immunohistochemical staining and will be evaluated based on evidence of apoptosis, vascularity and cell growth.

C. Study Procedure

At the time of enrollment, all patients will meet both principal investigators and sign an informed consent. They will have the following blood tests drawn: PSA (prostate specific antigen), Liver Function Tests (LFTs), Complete Blood Count (CBC), Basic Metabolic Panel (BMP), serum CoQ10 levels, Testosterone. These will establish baseline levels by which lab values at six weeks can be compared to monitor for toxicity and/or possible benefit.

Prior to being randomized, all patients will have prostate biopsy tissue tested for Bcl-2, CD31, p27, Ki-67 and Cleaved Caspase 3.

Following enrollment, patients will be randomized to either treatment or placebo arm of the trial. Randomization and preparation of the medicine will be handled by Tara McCann, a separate researcher responsible for randomization and distribution of medicines. The study coordinator will be responsible for all of the patient contact prior to and following randomization. Patients will take either the treatment CoQ10 or placebo every day for six weeks prior to prostatectomy.

During the treatment period, the study coordinator will contact the patients on a minimum of two separate occasions separated by a two week time period. During these interviews patients will be asked about side effects, compliance issues and follow-up will be scheduled.

Upon pathologic diagnosis status post radical prostatectomy, prostate tissue will be evaluated once again for Bcl-2, CD31, p27, Ki-67 and Cleaved Caspase 3. The investigating pathologist is to be blinded and will not have access to which patients were in the treatment or placebo arms.

At baseline and at 6 weeks time, each patient will have the following blood tests drawn: PSA (prostate specific antigen), Liver Function Tests (LFTs), Complete Blood Count (CBC), Basic Metabolic Panel (BMP), serum CoQ10 levels, Testosterone. LFTs, CBC and BMP will be drawn to monitor for any toxicity related to CoQ10.

At six weeks, each patient will have a formal side effect profile questionnaire to complete.

D. Stastical Analysis

Using a two-sided t-test we will be able to detect differences in the outcome variables of up to 1.25 standard deviations with 80% power at a significance at 0.05.

E. Study Drugs

Coenzyme-Q10

F. Study Devices

None

G. Study Questionnaires

SWOG Quality of Life Questionnaire. The SWOG questionnaire is used because it is a validated questionnaire that asks specific questions regarding common side effects with supplement use. Though no side effects have been previously reported, we are interested in identifying any that might exist.

H. Study Subjects

All eligible patients referred to or being treated at the Department of Urology will be offered participation in this study.

Inclusion Criteria:

- All patients must be male.
- All patients must be diagnosed with prostate cancer.
- All patients must be designated "low-risk" and able to tolerate the six week preoperative treatment regimen without adverse effect to prognosis. The designation of "low-risk" is defined by the following criteria:
    - Age <65
    - Clinical Stage Tlc or T2a
    - PSA < 15
    - Gleason Score 7
- All patients must undergo radical robotic prostatectomy.
- Patients must be willing to refrain from taking any other supplements or vitamins during the six weeks they are taking CoQ10.

Exclusion Criteria:

- Patient is unable to take an oral supplement.
- Neoadjuvant therapy including radiation, chemo- or hormone therapy.
- Patient is "high-risk" and is unable to wait six weeks for prostatectomy as urgent surgery is indicated or other therapies (radio- hormone therapy) will be initiated first. The "high-risk" designation is defined by the following criteria:
    - Age > 65
    - Clinical Stage > T2b
    - PSA > 20
    - Gleason score > **7**

I. Recruitment Of Subjects

All eligible patients referred to or being treated at the Department of Urology at the New York Presbyterian Hospital will be offered study participation.

J. Confidentiality Of Study Data

Any information obtained during this study will remain confidential. A record of each subject's progress will be kept in a designated research protocol room under lock and key at the Department of Urology in the New York Presbyterian Hospital.

Information obtained from this study may be used for other research purposes and publication. Subjects will not be identified by name in any publication.

No information that can identify a subject in any way will be released without the subject's consent, except as specifically required by law.

K. Potential Conflict Of Interest

None

L. Location Of Study

The study will be conducted at the Department of Urology offices in the Atchley Pavilion at the New York Presbyterian Hospital or at Eastside facility at 16 East 60 St. in Manhattan.

M. Potential Risks

There are no anticipated risks for participating in this study. The six week time period necessary for taking the medicine prior to surgery is an accepted period of time to wait prior to surgery for low-risk prostate cancer patients. Those patients that could potentially have a worse outcome by delaying the date of surgery will not be enrolled.

N. Potential Benefits

Enrolled patients will most likely not benefit from this trial. CoQ10 may induce apoptosis and reduce the vascularity of their prostate cancer decreasing the viability and virulence of their cancer. Most likely, this study will provide information regarding the relationship between prostate cancer cell viability and CoQ10. This may help future prostate cancer patients and generations to better prevent and treat prostate cancer. Patients will be provided with the results of all laboratory data examined during this study.

O. Alternative Therapies

There are no alternatives to CoQ10 prior to prostatectomy. Patients may choose not to participate in this study.

P. Compensation To Subjects

None

Q. Costs To Subjects

None

R. Minors As Research Subjects

None

S. Radiation

None

T. References

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