Title: PHASE II RANDOMIZED PLACEBO-CONTROLLED CLINICAL TRIAL OF GENISTEIN IN REDUCING THE TOXICITY AND IMPROVING THE EFFICACY OF INTRAVESICAL THERAPY

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Study Supporter: DSM Nutritional Products

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Schema:

<table>
<tr>
<td rowspan="3">Enrollment</td>
<td rowspan="3">44 TaT1 Tis superficial bladder cancer patients scheduled to receive BCG intravesical therapy will be consented

Estimated Timeline: until 44 subjects are enrolled (approximately 2 years)</td>
<td rowspan="3">Randomization</td>
<td>Arm 1: BCG intravesical therapy with placebo pills for 10 weeks (22 subjects)</td>
<td rowspan="3">Follow-up</td>
<td>Arm 1: Followed-up according to standard after-treatment care: one-month post-treatment appointment and subsequent doctor visits.</td>
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<tr>
<td>Arm 2: BCG intravesical therapy with 30 mg genistein supplement PO TID for 10 weeks (22 subjects)</td>
<td>Arm 2: Followed-up according to standard after-treatment care: one-month post-treatment appointment and subsequent doctor visits.</td>
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Patients will take either 30 mg tablets of placebo or genistein supplement PO TID, for 6 weeks during BCG intravesical therapy and will continue for 4 weeks following therapy.

1. Abstract:

Patients who are treated with BCG intravesical therapy for TaT1 Tis superficial bladder cancer often develop adverse effects (urinary tract symptoms) which limit the dose (and therefore the efficacy) of therapy and result in poor quality of life. Genistein is a nutritional supplement with anti-inflammatory properties which might help alleviate adverse effects of intravesical therapy that are due to inflammation. Additionally, genistein also has anti-tumor and immunopotentiating properties and has been shown to have no known side effects. Our hypothesis is that genistein given together with BCG intravesical therapy will reduce its adverse effects and improve the efficacy of therapy. Patients (N=44) scheduled for intravesical therapy will be randomly assigned to take 30 mg tablets of genistein supplement (N=22) or placebo (N=22) PO TID during therapy and one month post therapy.

2. Introduction and Background:

BCG intravesical therapy is a form of immunotherapy commonly used to prevent the recurrence of noninvasive bladder cancer, which occurs in approximately 50-80% of bladder cancer patients within 5 years. 10-20% of all superficial bladder cancer patients will progress beyond superficial recurrence within 5 years. Smoking, poor diets, and large body weights are all potential risk factors for recurrence, but there is also a high propensity for bladder cancer recurrence in general due to its aggressive nature. As treatment, BCG intravesical therapy is instilled in the bladder using a urinary catheter. The intravesical therapy remains in the bladder for about two hours until the patient urinates. The treatment is usually once a week for six weeks and then repeated every 3-6 months for two years. However, intravesical therapy typically causes urinary tract symptoms, such as pain, frequency, burning, inflammation, and bleeding in patients. Symptoms may occur after the very first treatment or become worse over time as therapy continues. As a result, the dose of therapy is often reduced so that symptoms can be treated symptomatically with antibiotics or pain medication. Dose reduction is generally done in 50% increments, and the decision to reduce the treatment dosage is often subjective based on each patient's perception of symptoms.

The isoflavone genistein (4,7,4'-trihydroxyisoflavone) is a phytoestrogen found in high levels in soy products and can be taken as a nutritional supplement for its antioxidant benefits and its estrogenic properties. The genistein supplement is sold over the counter and has less soy isoflavone than commonly found in many healthy diets. For comparison, an 8 ounce glass of soy milk has 15 mg of genistein. Studies have used up to 300 mg of genistein supplement daily, and it has been shown as safe, well accepted, and well tolerated with no known significant toxicities.

Recent epidemiologic reports show that some Asian countries with high soy consumption have a smaller incidence of prostate carcinoma and studies suggest it might be due to the anticancer effects of soy. A study of 41 subjects in 2003 showed that there was a significant slow in the rate of PSA rise in prostate cancer patients after taking 100 mg of genistein supplement daily for 6 months[i] A recent study of 52 subjects in 2010 showed that the short-

term, neoadjuvant synthetic aglycone genistein supplement was comparable to high soybean consumption and indicated a possible therapeutic benefit in early CaP, a borderline significant serum PSA reduction, and normalization of PSA in malignant prostate tumors.[ii] Genistein has been reported to have both anticancer and anti-inflammatory effects that may inhibit prostate cancer cell growth and metastasis, as well as an effect on decreased incidence of urinary, gastrointestinal, and erectile dysfunctions when 42 patients were given 100 mg genistein supplement daily for 6 months in conjunction to radiation.[iii] Additionally, a small pilot study of 8 children receiving chemotherapy and/or radiation were given 8 mg of the genistein supplement daily and findings suggest that the genistein supplement reduced the adverse effects of treatment while causing no side effects.[iv] The results from these studies suggest that further clinical investigation should be warranted to apply soy phytochemicals, as a potential prevention regimen for cancer progression .

In a large prospective cohort of 2000 Chinese men, the association between dietary isoflavone and lower urinary tract symptoms (LUTS) were studied using standardized structured questionnaires. A total of 96.2 % of subjects reported some consumption of genistein, glycitein, or daidzein. In ordinal multinominal logistic regression, subjects with dietary total isoflavone of more than 5.1 mg were significantly less likely to suffer from more severe LUTS.[v] The aforementioned studies suggest that the use of genistein in combination with intravesical therapy will allow for more effective treatment of bladder cancer.

3. Investigational Product Information:

This study will use the synthetic non-soy genistein supplement, Bonistein™ . Bonistein™ has been compounded into tablets named I-Cool ®. Each I-Cool tablet contains 30 mg of genistein (Bonistein™). I-Cool ® and the corresponding placebo tablets are supplied by DSM Nutritional Products. DSM Nutritional Products as the study product supplier, provides Certificate of Analysis to Study Sponsor. Patients enrolled in the study will be receiving I-Cool ® tablets or the corresponding placebo tablets.

4. Objectives:

4.1 Primary objective

The primary study endpoint is the change in severity of urinary symptoms over time as determined by the IPSS questionnaire score, specifically comparing the symptoms at the 1st BCG treatment (baseline) to the symptoms at the 6th week of treatment. The difference in the degree of urinary symptoms between the placebo and genistein supplement arms will help reflect whether genistein supplement can improve the quality of life in BCG intravesical therapy patients.

4.2 Secondary objective

a) A secondary study endpoint will be the presence of cancer and the rate of recurrence as determined by the 10-week biopsy or subsequent standard follow-up visits.

b) A second secondary study endpoint will be the total dose of intravesical therapy administered over the 6 weeks of treatment. If subjects develop fewer urinary tract symptoms as a result of the genistein supplement, then hypothetically, intravesical therapy dosing would not have to be reduced as much. This will be measured by looking at the level of dose reduction for each treatment week and comparing it across the two arms.

5. Study Design and Methods:

At the pre-operative office visit before intravesical therapy, eligible patients will be screened and enrolled into the study by going through the consent process and signing the consent and HIPAA forms. Each patient will need to report all other medications so that the study team can assess whether certain medications might affect trial outcome.

Patients will be randomized into Arm 1 (placebo) or Arm 2 (genistein supplement) at a ratio of 1:1 stratified by one dichotomized prognostic factor, age. The dichotomization will take place based on whether they are age 60 and above or whether they are age 59 and below. This will allow for similar age groups to be placed in each arm, so that the degree of urinary symptoms while receiving treatment is not skewed depending on the patient's age.

Patients are treated with intravesical therapy once a week for 6 weeks and then return to the office approximately four weeks later for a post-operative visit and biopsy, as is standard care. Study subjects will take their genistein or placebo tablets for a total of 10 weeks, which includes the length of their treatment, as well as the 4 weeks before the post-op visit. Each patient will fill out an International Prostate Symptom Score (IPSS) questionnaire at their baseline office visit when consented. Patients will fill out an IPSS questionnaire at each subsequent intravesical therapy session and their final one-month follow-up appointment (at 10 weeks) either in person or via phone call with a study team member. The IPSS questionnaire will gauge their urinary symptoms throughout their therapy and provide measurable symptom scores for the two arms. The biopsy at 10 weeks, as well as standard 3 months follow-up appointments, is standard care for superficial bladder patients and will help determine cancer rate of recurrence.

	Baseline	BCG Week 1-6[b]	10 Week [c]
Placebo Arm	• Screening (Physical Exam,[a] Vitals, [a] complete blood count and serum chemistry analyses, [a] Urine Dipstick, IPSS Questionnaire) • Enrollment • Randomization	• Urine Dipstick, Interval History, Vitals, IPSS Questionnaire*, • BCG Intravesical Therapy • 6 weeks of placebo (30 mg, PO TID) • Continue taking placebo for remaining 4 weeks before biopsy	• Bladder Cystoscopy (Biopsy recommended, but not mandatory) • Urinary cytology (if inidicated) • Interval History • IPSS Questionnaire* • Physical Exam • Vital signs • Complete blood count and serum chemistry panel is recommended, but not mandatory
Genistein Supplement Arm	• Screening (Physical Exam, [a] Vitals, [a] complete blood count and serum chemistry analyses, [a] Urine Dipstick, IPSS Questionnaire) , • Enrollment, • Randomization	• Urine Dipstick, Interval History, Vitals, IPSS Questionnaire*, • BCG Intravesical Therapy • 6 weeks of genistein Supplement (30 mg, PO TID) • Continue taking genistein Supplement for remaining 4 weeks before biopsy	• Bladder Biopsy (pathology) • Urinary cytology • Interval History • IPSS Questionnaire* • Physical Exam • Vital signs • Complete blood count and serum chemistry panel is recommended, but not mandatory

[a] Within 2 months before the first dose of study treatment.

[b] A range of 7 days before or after the scheduled visit is allowed or per standard of care. If BCG treatment is interrupted or discontinued Urine Dipstick, Interval History, and Vitals may not be collected per standard of care.

[c] A range of 2-3 weeks before or after the 10 week is allowed or per standard of care.

*IPSS Questionnaire may be collected via phone call.

6. Participant Selection:

44 patients will be enrolled in the trial, with 22 subjects on each arm. Potential subjects will be superficial bladder cancer patients in the Department of Urology who are scheduled for their standard-of-care BCG intravesical therapy.

Inclusion Criteria:

1. Male or female gender
2. 18 years or older
3. Diagnosis of superficial bladder cancer
4. Scheduled for induction BCG intravesical therapy
5. Willing and able to give blood sample
6. Willing and able to fill out a pill diary to ensure compliance
7. Willing and able to sign informed consent

***Birth Control is not required for this study!

Exclusion Criteria:

1. Patients who are pregnant
2. Diagnosis of muscle-invasive bladder cancer
3. Unwillingness to follow study protocol and compliance procedures
4. HIV positive or immunocompromised
5. Receiving concurrent immunotherapy or chemotherapy
6. Presence of concurrent second cancer (active, not history)

7. Quality of Life:

This study follows standard of care for all superficial bladder cancer subjects. All subjects will receive standard BCG intravesical therapy for six weeks and return for a one-month post-operative visit and biopsy. Both the control and intervention arms will receive the same care, other than taking the genistein supplement or placebo. While genistein supplement is not a standard part of treatment, it has been shown to not have any significant toxicities. It is expected that the subjects taking genistein supplement will have a better quality of life due to a possible reduction in urinary symptoms, such as hematuria, infection, frequency, urgency, and retention, when compared to those subjects receiving standard treatment without the genistein supplement. Each patient's perception of quality of life will be seen indirectly throughout the BCG treatment, as it is a supplemental question on the IPSS Questionnaire.

Quality of life will be assessed for both arms during an interim analysis and stopping criteria will be determined on the degree of individual AEs as well as an interim analysis of both arms. Subjects with toxicities of Grade 3 or higher according the NCI CTCAE Version 4.0 will be immediately discontinued from the study pill. Additionally, the interim analysis will take place after 22 patients (11 on each arm) have been enrolled, treated, and biopsied. If the genistein supplement arm has statistically significant toxicities reported or greater degree of bladder cancer biopsies then we will immediately discontinue any future study enrollment. If the genistein supplement arm has significant Grade 3 toxicities, we will reduce the genistein supplement given by 1 tablet for 10 more patients, and if the toxicities persist after that, the study will be stopped.

8. Statistical Consideration:

8.1 Power analysis

We hypothesize that, at 6 weeks, the symptom score will increase by 6 (SD = 3.5) relative to the baseline in the placebo arm, while it will only increase by 3 (SD = 3.5) in the synthetic genistein supplement arm. A sample size of 20 patients in each arm can reach 83% power to detect the hypothesized difference at the significance level of 0.05 by one-sided Mann-Whitney test.

In an effort to reach a sample size of 20 patients on each arm, we also hypothesize that there will be a 10% drop-out or non-compliance rate. We will accrue 44 patients, 22 subjects on each arm, for this study with the expectation that 10% will drop out. With a 10% drop out rate included, this would result in a total of 40 compliant patients.

8.2 Statistical analysis plan

8.2.1 Primary analysis

The IPSS score change at 6-week from baseline will be calculated with 95% confidence interval and compared between two experiment groups through Mann-Whitney test. The baseline, minimum three treatment period IPSS scores, and the 10-week biopsy IPSS scores are required in order for the patient to be evaluable.

8.2.2 Secondary analysis

a) For the secondary end point of recurrence rate defined by 10-week biopsy, the Chi-squares test is used to test the rate difference between the two arms, and a logistic regression is needed to further adjust potential covariates, e.g. total BCG therapy dose.
b) For the secondary end point of the total dose of intravesical therapy administered over 6 weeks, we consider the two-sample t test and/or sum rank test to test the difference between two arms. If genistein supplement works by reducing symptoms and maintaining the therapy dose, we expect to see a higher total therapy dose in its arm than in the placebo arm.
c) We will also explore the change of IPSS score over time by the GEE model in which the repeated measurements of IPSS score are treated as outcome. Treatment, time, interaction between treatment and time, and intravesical therapy dose enter the model as predictors and time-dependent covariate.

9. Adverse Event Reporting:

According to 21 CFR 312.32, *Adverse event* means any untoward medical occurrence associated with the use of a drug in humans, whether or not considered drug related. A *Serious adverse event* or *serious suspected adverse reaction* is an adverse event or suspected adverse reaction is considered "serious" if, in the view of either the

investigator or sponsor, it results in any of the following outcomes: Death, a life-threatening adverse event, inpatient hospitalization or prolongation of existing hospitalization, a persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions, or a congenital anomaly/birth defect. Important medical events that may not result in death, be life-threatening, or require hospitalization may be considered serious when, based upon appropriate medical judgment, they may jeopardize the patient or subject and may require medical or surgical intervention to prevent one of the outcomes listed in this definition. Examples of such medical events include allergic bronchospasm requiring intensive treatment in an emergency room or at home, blood dyscrasias or convulsions that do not result in inpatient hospitalization, or the development of drug dependency or drug abuse.

Adverse events will be recorded at each study visit via direct patient communication as well as through patient questionnaires. The severity of the AEs will be graded according to NCI CTCAE Version 4.0. Subjects with toxicities of Grade 3 or higher will be immediately discontinued from the study pill for the remainder of treatment. Any BCG intravesical therapy modifications will be done according to doctor analysis with an effort on alleviating toxicities as needed. In order to be evaluable, patients will need to receive at least three BCG instillations. If BCG treatment is interrupted or discontinued, only IPSS scores will be obtained via phone call. If a SAE occurs, we will promptly report it to our IRB upon discovery and follow the subject until the SAE is resolved. Adverse events will be reported to the IRB according to their requirements**.**

IND safety reports will be sent to the FDA in accordance with 312.32 the FDA's *Guidance for Industry and Investigators: Safety Reporting Requirements for INDs and BA/BE studies*. IND safety reports will document any aggregate adverse events, adverse reactions suspected adverse reactions, or serious adverse events. If the event is considered to be unexpected fatal or life-threatening, the IND safety report will be sent as soon as possible and within 7 business days of the investigator being notified. If necessary, the investigator will report any serious or life-threatening event initially by facsimile or telephone prior to formal submission. IND safety reports will also be submitted to the FDA if any related safety information is released from other sources. Any nonserious adverse events will be reported to the FDA in aggregate on an annual basis.

10. Data and Safety Monitoring Plan (DSMP):

Once the study has reached 50% accrual, investigators will plan to initially evaluate the results. A group of physicians from the Winship Cancer Institute at Emory University will review the data and questionnaires to see how patients are reacting to the genistein supplement arm or whether there are drug compliance issues, such as failure to take the pills per protocol. If the genistein supplement arm has statistically significant toxicities reported or greater degree of bladder cancer biopsies then we will immediately

discontinue any future study enrollment. If the genistein supplement arm has significant Grade 3 toxicities, we will reduce the genistein supplement given by 1 tablet for 10 more patients, and if the toxicities persist after that, the study will be stopped.

Also, per Winship Cancer Institute's Data Safety Monitoring Plan, their Data Safety Monitoring Committee will review the study conduct annually as well as monitor for regulatory compliance.

11. References and Appendices

[i] Soy Isoflavones in the Treatment of Prostate Cancer. Nutrition and Cancer. 2003 Nov;47(2):111-117. Hussain M, Banerjee M, Sarkar FH, Djuric Z, Pollak MN, Doerge D, Fontana J, Chinni S, Davis J, Forman J, Wood DP, Kucuk O.

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[iii] Soy Isoflavones in Conjunction with Radiation Therapy in Patients with Prostate Cancer. Nutrition and Cancer. 2010 Oct;62(7):996-1000. Ahmed IU, Forman JD, Sarkar FH, Hillman GG, Heath E, Vaishampayan U, Cher ML, Andic F, Rossi P, Kucuk O.

[iv] Soy Isoflavones Ameliorate the Adverse Effects of Chemotherapy in Children. Nutrition and Cancer. 2010 Oct; 62(7): 1001-1005. Tacyildiz N, Ozyoruk D, Yavuz G, Unal E, Dincaslan H, Dogu F, Sahin F, Kucuk, O.

[v] The association between isoflavone and lower urinary tract symptoms in elderly men. Br J Nutr. 2007 Dec;98(6):1237-42. Wong SY, Lau WW, Leung PC, Leung JC, Woo J.