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Evaluating the effect of ADRB2 blockers on PKA/BAD/CREB signaling in the prostate gland
Wake Forest Baptist Comprehensive Cancer Center
CCCWFU # 85716

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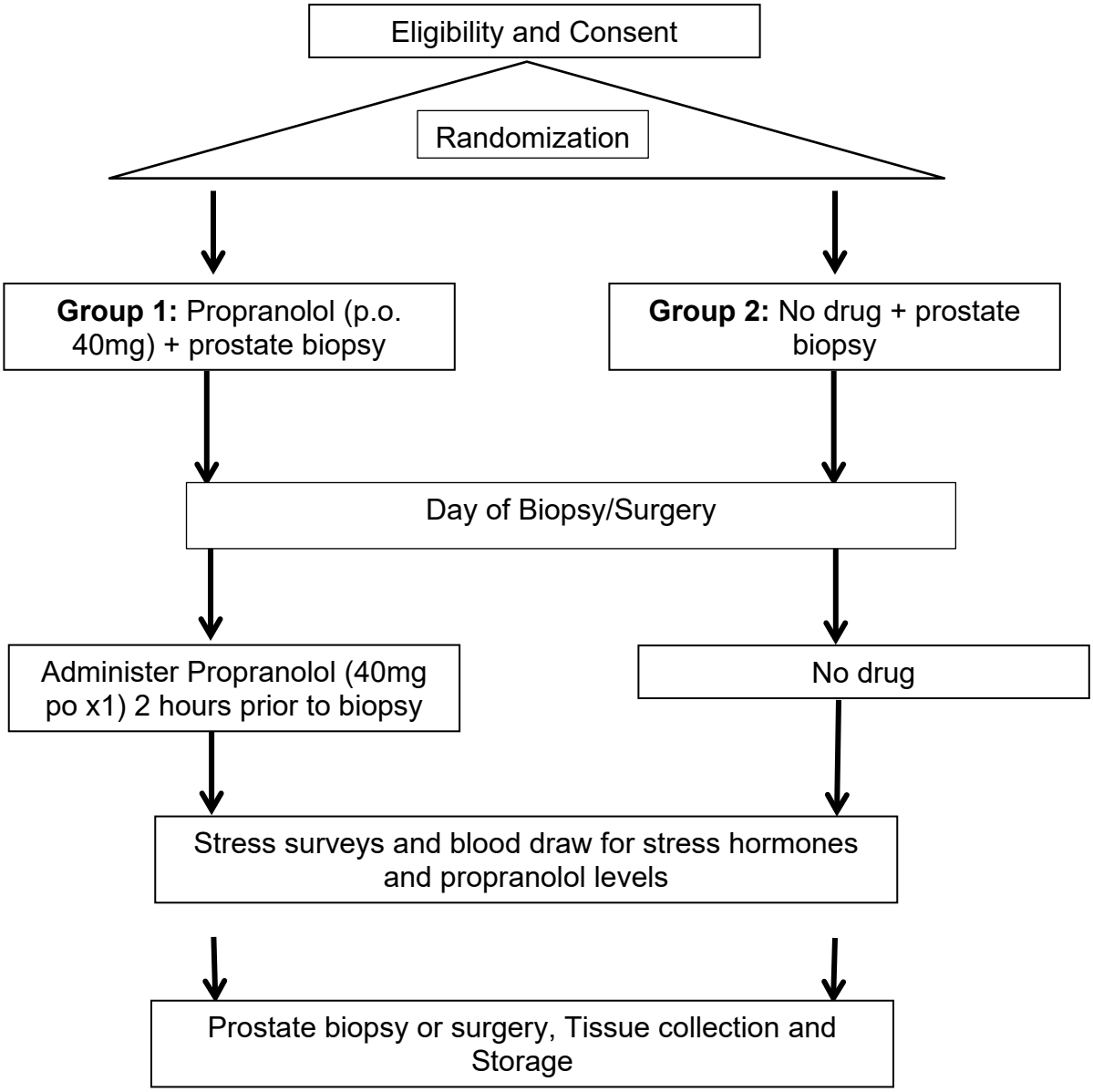

Eligibility and Consent
Randomization
Group 1: Propranolol (p.o. 40mg) + prostate biopsy
Group 2: No drug + prostate biopsy
Day of Biopsy/Surgery
Administer Propranolol (40mg po x1) 2 hours prior to biopsy
No drug
Stress surveys and blood draw for stress hormones and propranolol levels
Prostate biopsy or surgery, Tissue collection and Storage

1.0 Introduction and Background

Increased levels of epinephrine induced by stress inhibit apoptosis and accelerate tumor progression in mouse models of prostate cancer via β2-adrenergic receptor (ADRB2)/ cAMP-dependent protein kinase (PKA)/ BCL2-associated death promoter (BAD) signaling pathway [1]. Furthermore, analysis of 20 prostate biopsies showed significant positive correlation between increased blood epinephrine levels and activation of the ADRB2/PKA/BAD signaling pathway in the prostate gland of men [2]. Because PKA can phosphorylate several substrates in addition to BAD, it is beneficial to analyze phosphorylation of both S75 BAD and S133 CREB to increase assay specificity. Further, antibodies to pS133CREB may be more sensitive compared to pS75 BAD-specific antibodies.

To further improve the specificity and sensitivity of measuring activation of ADRB2 signaling pathway, we have compared mRNA profiles in prostate cancer C42 cells exposed to vehicle or epinephrine using Affimetrix arrays. This comparison identified several transcripts induced by activation of ADRB2 pathway. Should the specificity of these induced transcripts be confirmed, the analysis of these transcripts in collected prostate tissues will also be conducted.

Recently, in a breast cancer mouse model, it has been shown that chronic stress promotes metastasis and that treatment with a beta-blocker can prevent metastasis [3]. Similarly, an association between dysregulation of stress-related signaling pathways, especially the adrenergic pathway and lethal prostate cancer has been reported [4]. These data suggest that stress and ADRB2/PKA/BAD signaling may play an important role in prostate cancer pathophysiology.

In patients with advanced prostate cancer who experience increased stress, elevated epinephrine levels may contribute to inhibition of apoptosis and, hence, therapeutic resistance. Conversely, inhibiting the ADRB2/PKA/BAD signaling pathway by ADRB2-selective beta blockers could be a therapeutic option for patients with increased epinephrine levels [5]. Several epidemiological studies have addressed a potential correlation between the use of beta blockers and prostate cancer incidence and mortality, but they reached contradictory conclusions [6-15]. The results of the most recent studies suggest that activation of ADRB2 is likely to contribute to prostate cancer progression but does not increase prostate cancer incidence. Likewise improvement in overall survival was recently reported for ovarian cancer patients who took beta-blockers [16]. Most patients in these studies took β1-selective blockers, which are poor inhibitors of ADRB2 signaling. A recent study that focused on patients who took propranolol that inhibits ADRB2 signaling reported significant decrease in incidence of several cancers including prostate cancer [15]. Furthermore, our data from preclinical models of prostate cancer suggest that not all patients will respond to therapy with β2-selective blockers due to compensatory signaling pathways that inhibit apoptosis despite of the inactivation of ADRB2/PKA axis. Thus, patients in whom ADRB2/PKA is the major pathway that controls BAD phosphorylation and apoptosis are likely to benefit from ADRB2-selective blockers. In contrast, for patients with multiple redundant

pathways that converge on BAD, the ADRB2-selective blockers will likely need to be given in combination with inhibitors of other signaling pathways.

Beta blockers are commonly administered in the perioperative period. Perioperative beta blocker use has been associated with lower 30-day mortality rates, although the benefit is stronger for those undergoing cardiovascular surgery, and the benefit in the setting of non-cardiac surgery remains controversial [17]. In a 1991 study of women in the perioperative period, a one-time preoperative dose of oral propranolol of 80 mg was well-tolerated and resulted in less anesthetic use [18]. In a more recent randomized study, perioperative doses of 20mg or 40mg were used to reduce anxiety among surgery patients[19]. With the administration of 20mg of oral propranolol, there was a significant decrease in anxiety with fewer cardiovascular effects than the 40mg dose. In the current study, the use of propranolol will be continuously monitored for safety events. Any safety concerns that arise as defined in the protocol will trigger a dose reduction for subsequent patients. Additionally, an interim analysis will assess safety.

2.0 Objectives

2.1 Primary Objective

2.1.1 To compare activation of ADRB2/PKA/BAD signaling pathway in the prostate glands of men two hours after taking or not taking propranolol prior to prostate biopsy or prostatectomy, as indicated by phosphorylated CREB.

2.2 Secondary Objectives

2.2.1 To compare activation of ADRB2/PKA/BAD signaling pathway in the prostate glands of men two hours after taking or not taking propranolol prior to prostate biopsy or prostatectomy as indicated by phosphorylated BAD.

2.2.2 To determine the difference in candidate transcript levels associated with ADRB2/PKA activation between individuals two hours after taking propranolol or not taking propranolol prior to prostate biopsy or prostatectomy.

2.2.3 To determine plasma propranolol levels in individuals taking propranolol two hours after administration prior to prostate biopsy or prostatectomy.

2.2.4 To determine if plasma catecholamine levels in men with prostate cancer can be used as a biomarker to identify patients who show activation of ADRB2 signaling pathway in prostate tumors.

2.2.5 To determine perceived stress level differences in men with prostate cancer prior to prostate biopsy or prostatectomy to examine possible association between perceived stress level and catecholamine levels in blood and activation of ADRB2 pathway in tumors

2.2.6 To determine perceived distress level differences in men with prostate cancer prior to prostate biopsy or prostatectomy to examine possible association between distress level and catecholamine levels in blood and activation of ADRB2 pathway in tumors.

3.0 Study Population

Patients recruited will be individuals scheduled for prostate biopsy or prostatectomy. For this study, 60 individuals will be recruited. Patients will be randomized to receive the treatment (or not) for a total of 30 patients receiving propranolol prior to biopsy or prostatectomy and 30 patients receiving no treatment. It is expected that approximately 10 (5 patients in each group) may have non-evaluable tumor tissue. In case the number of evaluable tumors will be less than 25 for each group, more patients will be recruited to accumulate 25 evaluable samples for each group.

3.1 Inclusion criteria:

1) Men >18 years of age
2) Patients undergoing prostate biopsy or prostatectomy
3) Individuals able to understand and willing to sign an IRB-approved informed consent document

3.2 Exclusion criteria:

1) Men taking propranolol daily for any reason are excluded
2) Men with baseline SBP <120 or HR <63
3) Men unable to swallow pills
4) History of current or past medical or psychiatric illness that would make participation difficult or not feasible at the discretion of the principal investigator or co-investigators.

3.3 Inclusion of Women and Minorities

Men of all races and ethnicities who meet the above-described eligibility criteria are eligible to participate. Due to the sex specific nature of prostate cancer, women will not be included in this study.

Based on WFBCCC population estimates and based on the sample size of 60 patients total for this study, we plan to enroll approximately 15% Black

or African American (N=9). Given the low rates and/or population statistics in our catchment area we expect no or very low accruals within the Asian, American Indian, or Pacific Islander populations. Additionally, we may expect approximately 5% of the sample to be Hispanic, which may account for an N = 3 Hispanic individuals. Should we not meet or exceed these estimates, the PI will engage the Cancer Center Health Equity Advisory Group to discuss strategies to enhance recruitment in these target populations.

3.4 Accrual

Five (5) individuals per month for one year

4.0 Methods

4.1 Registration Procedures

All patients entered on any WFBCCC trial, whether treatment, companion, or cancer control trial, **must** be registered, within 24 hours of Informed Consent, into WISER. Patients **must** be registered prior to the initiation of treatment.

You must perform the following steps in order to ensure prompt registration of your patient:

1. Complete the Eligibility Checklist (Appendix A)
2. Complete the Protocol Registration Form (Appendix B)
3. Alert the Cancer Center registrar by phone, *and then* send the signed Informed Consent Form, Eligibility Checklist and Protocol Registration Form to the registrar, either by fax or e-mail.

   Contact Information:
   Protocol Registrar PHONE (336) 713-6767
   Protocol Registrar FAX (336) 713-6772
   Protocol Registrar E-MAIL (registra@wakehealth.edu)

   *Protocol Registration is open from 8:30 AM - 4:00 PM, Monday-Friday.

4. Fax/e-mail ALL eligibility source documents with registration. Patients **will not** be registered without all required supporting documents.

Note: If labs were performed at an outside institution, provide a printout of the results. Ensure that the most recent lab values are sent.

To complete the registration process, the Registrar will:

- assign a patient study number
- Randomized Group Assignment
- register the patient on the study

4.2 Methods

Compare activation of ADRB2/PKA/BAD signaling pathway in the prostate glands of men who take or do not take ADRB2-selective beta blockers

Patients scheduled for prostate biopsy.

The research staff screens the OR and clinic schedules for potential patients and provides a list to the investigator. Dr. Hemal or co-Investigator will discuss the study with the patient and inform the research nurse or coordinator (research staff) about the patients who agreed to participate in the study. Research staff will inform Tissue Procurement Lab (Director Dr. Kucera; Libby McWilliams, Tumor Procurement Coordinator) and courier about the scheduled date and time when prostate samples will be collected.

On the day of sample collection Dr. Kucera's lab will prepare box with dry ice for prostate biopsy samples, a box with wet ice for blood samples as well as 2 ml cryovials (Corning, cat#430488) to collect biopsies and tubes to collect blood (Na heparin, green-capped tube, 10 ml total blood volume).

Courier will pick up boxes from Tissue Procurement Lab (Director Dr. Kucera; Hanes Building Rm 4032, tel 336-716-3721) and deliver to research staff at Charlois clinic (140 Charlois Boulevard, Winston-Salem, 27103).

Recruited patients scheduled for prostate biopsy will be randomized into two groups. Group one will receive 40 mg propranolol given by research nurse 2 hours prior to prostate biopsy. The research nurse should withhold drug and contact treating physician if SBP <120 or HR < 63. Group two will receive no treatment.

All patients will fill out stress surveys on the biopsy day.

All patients will give approximately 10 mL of blood approximately 20 min prior to prostate biopsy to test plasma catecholamine levels (collected in 10 mL green top tube (sodium heparin) and sent on ice within 1 hour to clinical lab for processing)and propranolol level (collected in 10 mL green top tube (sodium heparin) and sent on ice to The Tissue Procurement lab for processing and storage as described below). For group one that takes propranolol, this blood sample will be drawn at 1.5 -2 hours after taking the propranolol tablet.

For the biopsy, at least 1 core of the prostate specimen is to be flash frozen (on dry ice -80C) for laboratory analysis. Each biopsy sample will be collected into separate cryovials. The samples will be picked up by courier at Charlois clinic and delivered to Tissue Procurement laboratory.. The tissue samples will be frozen at -80 C.

At Tissue Procurement Lab, the blood sample will be centrifuged to prepare plasma. Plasma will be transferred into cryovials and stored at -80 C.

Patients scheduled for prostatectomy.

Research staff will inform Tissue Procurement Lab (Director Dr. Kucera; Libby McWilliams, Tumor Procurement Coordinator) about the scheduled date and time when samples from prostate cancer patients will be collected at OR. Prior to the day of sample collection at specified time Dr. Kucera's lab will provide vials and freezing media to collect prostatectomy samples and plasma samples (Na heparin, green cup).

Recruited patients scheduled for prostatectomy will be randomized into two groups. Group one will receive 40 mg propranolol given by research staff 2 hours prior to prostatectomy. The research staff should withhold drug and contact the study physician performing the procedure and/or the anesthesiologist if SBP <120 or HR < 63. Group two will receive no treatment. All patients will fill out stress surveys prior to prostatectomy.

Research staff will collect stress questionnaire and will collect blood into the vials with Na heparin that will be stored on ice (+4C). All patients will give approximately 10 ml of blood approximately 20 min prior to prostatectomy to test plasma catecholamine levels and propranolol level. For group one that takes propranolol, this blood sample will be drawn at 1.5 -2 hours after taking the propranolol tablet. Blood will be collected into two 10 mL green top (sodium heparin) tubes. One tube will sent on ice within 1 hour to the clinical lab for processing of fractionated catecholamines. The other tube will be sent on ice to The Tissue Procurement lab for processing and storage. The plasma will be separated, transferred to cryovials, and stored in -80C at the Tissue Procurement Lab

Prior to anesthesia patient will be evaluated by anesthesiologist for hypotension and bradycardia. Prostatectomy samples will be collected by Tissue Procurement Coordinator and placed into cryovials and flash-frozen. At least 3 vials with the prostate specimens will be flash frozen (on dry ice -80C) for laboratory analysis and stored at the Tissue Procurement Lab.

Prostate samples will be analyzed for the overall status of prostate tissue (by pathologist) and for activation of ADRB2/PKA signaling pathway [2,20-22].

Analysis of ADRB2/PKA signaling pathway will be conducted by two methods: 1) western blotting to measure phosphorylation of CREB and BAD; 2) analysis of mRNA expression signature that reflects activation of ADRB2/PKA signaling pathway. Methods related to western blotting of material and RNA analysis will be per manufacturer's instructions with modifications to adapt the protocol to the particular conditions of the experiments and will be kept in laboratory notebooks in the analyzing laboratory. Results will be kept in laboratory notebooks, but will also be recorded on the associated data collection forms (Appendices J-M) where appropriate and will ultimately be entered into a REDCap database.

Experimental plan (Kulik laboratory):

1. Test whether LCM can be used to excise epithelial cells from frozen sections of prostate tissue for analysis of CREB and BAD phosphorylation by Western blotting, and for the analysis of mRNA by qPCR or RNA seq.

2. Recruit patients prior to prostate biopsy or prostatectomy. We plan to recruit 60 patients to this study and expect that 10 patients may not have evaluable tumor tissue. Patients will provide medical history and pre-study blood samples. On the day of prostate biopsy, the patients will fill out two stress surveys, being given propranolol or no treatment approximately 2 hours prior to biopsy or surgery, give blood, have prostate biopsy/prostatectomy, at which point prostate sample will be collected.

3. Compare CREB phosphorylation, BAD phosphorylation in prostates of men who took and who did not take propranolol. Based on our recent study of activation of ADRB2/PKA pathway in prostate biopsies we anticipate that in ~ 20% patients (10 of 50) ADRB2 pathway will be activated [2]. If the selective beta blocker propranolol inhibits ADRB2/PKA pathway, then we will not see CREB and BAD phosphorylation in prostates of patients who take propranolol. In the no treatment group, patients are expected to show elevated BAD and CREB phosphorylation in their prostates. The levels of CREB and BAD phosphorylations will be determined by probing Western blots with antibodies to pS133CREB and total CREB; pS112BAD and total BAD. Ratios of signals from pS133CREB/total CREB and pS112BAD/total BAD will be used to compare CREB and BAD phosphorylations in prostate tissue samples.

4. Free plasma propranolol (pharmacologically active) will be measured at The Proteomics and Metabolomics Shared Resource (Dir. Dr. Cristina Furdui) using chromatographic separation and fluorometric detection (linear detection range 5-400 µg/L) as described in [20-22].

5. Using prostate cancer cell models and tissues from the tumor bank, we will test if there is an mRNA signature of activation of ADRB2/PKA signaling pathway.

6. Compare mRNA signature of activation of ADRB2/PKA signaling pathway in prostates samples of men who take propranolol versus those who take nothing. Analysis of forskolin-induced genes using Affimetrix platform has been reported for HEK293T, MIN6 cell lines and primary hepatocytes and identified respectively 6, 14 and 17 mRNA transcripts induced over 3 fold [23]. These results suggest that it would be possible to identify transcripts induced by ADRB2/PKA signaling in prostate cells as well. Our pilot studies identified 8 transcripts induced in prostate cells by epinephrine. Experiments to confirm the specificity of these transcripts for ADRB2/PKA signaling pathway are currently ongoing.

   Results of these experiments will inform whether blood epinephrine levels can be determined above which ADRB2/PKA pathway is activated in prostate cells; whether beta blockers inhibit activation of ADRB2/PKA pathway in prostates and which method of monitoring activation and inhibition of ADRB2/PKA pathway is most adequate for analysis of clinical samples.

Experimental plan (clinical):
Prior to prostate biopsy or prostatectomy, a complete history and physical will be performed per standard of care; this can be completed during the initial consult. The necessary clinical information, including most recent PSA and testosterone if available (per standard-of-care) will be retrospectively obtained from what is charted and recorded per Appendix G.

Study related activities for the day of prostate biopsy or prostatectomy should be recorded on the Surgery Day Worksheet (Appendix N). Patients will fill out both the perceived stress[24] form (Appendix C) and the Distress Thermometer (Appendix D)[25]. Patients will receive either propranolol 40mg po x1 or no treatment 2 hours prior to prostate biopsy or prostatectomy. Approximately 20 minutes prior to prostate biopsy or prostatectomy, Group 1 will have approximately 10 mL of blood collected into two green top (sodium heparin) tubes. Group 2 will have the same amount of blood collected into two green tops. There will be no time limit prior to the biopsy or prostatectomy. Plasma will be separated and stored for future analysis of propranolol levels.

Epinephrine (fractionated catecholamines) levels will be performed per standard clinical laboratory procedures on all patients.

Information regarding tissue and plasma collection will be recorded on the Tissue Collection Form (Appendix H) or the Blood Collection Form (Appendix I).
All stored plasma or tissue will be stored in the Tumor Tissue Core.

Tumor Tissue Core
Wake Forest Baptist Comprehensive Cancer Center
Tumor Tissue Core Facility
Care of Dr. Greg Kucera
Hanes Building Rm 4049

5.0 Treatments

Study treatment is defined as any investigational treatment(s), marketed product(s), placebo, or medical device(s) intended to be administered to a study participant according to the study protocol.

Clinical supplies [study treatment(s) provided by the Sponsor] will be stored as a stock bottle at the Urology clinic in a locked area, accessible to only study staff. Supplies at the main facility will be obtained from the IDS pharmacy.

5.1 Treatments Administered

5.1.1 The study treatment(s) to be used in this trial are outlined in section 4.2.

6.0 Dose Modifications

Modifications to the dose will be made if Safety Concerns as outlined in 5.1.1 arise in the propranolol/surgery group.

6.1 Definition of a Safety Concern

6.1.1 Safety Concerns in this study will include bradycardia or hypotension requiring intervention by the anesthesiologist beyond standard interventions to mitigate the condition (CTCAE grade 3 or greater), or any adverse event attributable to propranolol that results in a prolonged stay in post anesthesia care unit. If any event of this nature occurs during the study, the dose will be reduced as described in Section 5.2.1 for subsequent patients.

6.2 Dose Reduction

6.2.1 If a subject has CTCAE grade 3 hypotension or bradycardia (as per section 5.1.1), the dose of propranolol will be reduced to 20 mg for subsequent patients. The study will be terminated if there is an additional CTCAE grade 3 event at the lowest dose of 20mg.

6.2.2 For any CTCAE grade 4 or 5 hypotension or bradycardia attributable to pre-operative propranolol dosing, the study will be terminated.

7.0 Outcome Measures

7.1 Primary Outcome

7.1.1 CREB phosphorylation determination by western blot in prostate tissue from men two hours after taking or not taking propranolol prior to prostate biopsy.

7.2 Secondary Outcomes

7.2.1 BAD phosphorylation by western blot in prostate tissue from men two hours after taking or not taking propranolol prior to prostate biopsy

7.2.2 Difference in levels of transcripts that reflect ADRB2/PKA activation as measured by real-time PCR in prostate tissue from men two hours after taking or not taking propranolol prior to prostate biopsy or prostatectomy.

7.2.3 Plasma propranolol levels as measured by fluorometric detection from men two hours after taking or not taking propranolol prior to prostate biopsy or prostatectomy.

7.2.4 Plasma catecholamine levels (including epinephrine) measured by ELISA from men two hours after taking or not taking propranolol prior to prostate biopsy or prostatectomy.

7.2.5 Self-perceived stress as measured by The Perceived Stress Questionnaire (Appendix C) from men on the day of surgery before taking propranolol, prior to prostate biopsy or prostatectomy.

7.2.6 Distress score as measured by The Distress Thermometer (Appendix D) from men on the day of surgery before taking propranolol, prior to prostate biopsy or prostatectomy.

8.0 Analytic Plan

8.1 Power Calculation/Sample size

Based on the preliminary data, the baseline level of CREB phosphorylation was 0.73 with a standard deviation of 0.26 [2]. Using these values, we determined that there is 80% power to detect a difference of 0.21 between groups using a 2-sample t-test (alpha=0.05, 2-sided). Thus, if the control group has a value of 0.73 (based on the preliminary data) then we would be able to detect a difference if the value of CREB phosphorylation is below 0.52. Based on the preliminary data, this effect size is 0.81, and would correspond to a 29% relative change in CREB phosphorylation. This calculation is based on have 25 patients in each group being evaluable (i.e., having tumor tissue available to be evaluated).

8.2 Primary Objective

Since this is a randomized trial, the two groups can be compared for CREB phosphorylation levels using a two-sample t-test. In addition to the 2-sample t-

test, descriptive statistics will be calculated for this primary outcome within each group. These statistics include n, mean, standard deviations and 95% confidence intervals.

Based on the preliminary data, the baseline level of CREB phosphorylation was 0.73 with a standard deviation of 0.26 [2]. Using these values, we determined that there is 80% power to detect a difference of 0.21 between groups using a 2-sample t-test (alpha=0.05, 2-sided). Thus, if the control group has a value of 0.73 (based on the preliminary data) then we would be able to detect a difference if the value of CREB phosphorylation is below 0.52. Based on the preliminary data, this effect size is 0.81, and would correspond to a 29% relative change in CREB phosphorylation. This calculation is based on have 25 patients in each group being evaluable (i.e., having tumor tissue available to be evaluated). Sixty patients are to be enrolled to allow up to 10 patients (5 per group) to possibly not have evaluable tissue available at the time of analyses.

8.3 Secondary Objective

There are 6 secondary objectives; each of these is focused on comparing the levels of a potential biomarker in patients. These measures are activation of ADRB2/PKA/BAD signaling pathway in the prostate glands, transcripts levels associated with ADRB2/PKA activation, plasma propranolol levels, plasma catecholamine levels, perceived stress levels, and distress levels. For each of these measures 2-sample t-tests will be calculated to compare the groups who were randomized to receive propranolol (yes/no). In addition to the 2-sample t-tests, descriptive statistics will be calculated for each measure within each group. These statistics include n, mean, standard deviations and 95% confidence intervals.

8.4 Interim Analysis

An interim analysis will occur after 10 patients (5 in each group) are recruited to determine if dosing or administration route of propranolol needs to be adjusted. This interim analysis will not include any statistical testing, but rather will be used to assess the safety of the trial.

If CREB phosphorylation will be observed in patients with epinephrine blood levels over 1nM who took propranolol, the dose of propranolol will be increased to 80mg. Exclusion criteria for this dose will be reviewed based on interim analysis.

Safety Review

Safety will be addressed on a per patient basis as described in Section 5.0 and as reiterated below. Because this pilot study involves only 1 dose of medication (initially 40mg; if 40mg is not effective in inhibiting CREB phosphorylation dose will be increased to 80mg) with a peak effect expected in 1-4 hours and a half-life of 3-6 hours, the safety assessment window will be in the perioperative period for up to 4 hours after dosing. Blood pressure will be monitored at 3h and 4h after the propranolol is taken. Patients will be allowed to leave if SBP >90. If blood pressure not acceptable after 4 hours, the patient will continue to be monitored and may receive IV fluids if SBP/DBP <90/60 after 6h post dose.

If there is CTCAE grade 3 bradycardia or hypotension, then the dose for subsequent patients will be modified per section 5.2. The study will be terminated if grade 4 or 5 cardiac events attributable to propranolol dosing occur. While safety will be addressed promptly on a per patient basis, a review of all AEs will occur at the safety interim analysis after 10 patients have been accrued (5 in each group). If other safety events are identified by the investigators at this interval analysis, then the propranolol dosing may be modified for subsequent subjects as directed by Section 5.2 or in a manner designated by the PI to ensure safety of the patients.

Finally, if a safety event as described in Section 5.1.1 occurs, the study will be locked and all activities suspended for 24 hours to further evaluate patient recovery and to monitor for other potential events. If patient recovery is deemed acceptable by the PI, the trial will be unlocked with subsequent patients following the modified dose reduction plan as described in Section 5.2.

Occurrence of an SAE other than those listed above will be addressed following the SAE protocol (Appendix F).

8.5 Accrual Rate

The accrual rate is anticipated to be 5 per month, thus recruitment should be complete within 12 months. Since analysis will occur on the samples that are taken during prostate biopsy, the overall length of this study is anticipated to be less than 18 months (allowing for the possibility that accrual goes slower than predicted or that time for lab analyses takes some time to be performed).

9.0 Data Management

Informed consent document	
Subject Eligibility Checklist (Appendix A)	
Protocol registration form (Appendix B)	WISER
Perceived Stress Form (Appendix C)	REDCap
Distress Thermometer (Appendix D)	REDCap
Adverse Events Log (Appendix E)	WISER

Baseline Medical History Form (Appendix G)	WISER
Tissue Collection Form (Appendix H)	
Blood Collection Form (Appendix I)	
Data Collection Form (prostate pCREB and pBAD) (Appendix J and K)	REDCap
Plasma Outcomes Form (Appendix L)	REDCap
RNA Data Collection Form (Appendix M)	REDCap
Surgery Day Worksheet (Appendix N)	

10.0 Confidentiality and Privacy

Confidentiality will be protected by collecting only information needed to assess study outcomes, minimizing to the fullest extent possible the collection of any information that could directly identify subjects, and maintaining all study information in a secure manner. To help ensure subject privacy and confidentiality, only a unique study identifier will appear on the data collection form. Any collected patient identifying information corresponding to the unique study identifier will be maintained on a linkage file, store separately from the data. The linkage file will be kept secure, with access limited to designated study personnel. Following data collection subject identifying information will be destroyed (state the anticipated time the data will be destroyed, e.g. three years after closure of the study, and the method of destruction), consistent with data validation and study design, producing an anonymous analytical data set. Data access will be limited to study staff. Data and records will be kept locked and secured, with any computer data password protected. No reference to any individual participant will appear in reports, presentations, or publications that may arise from the study.

11.0 Data Safety and Monitoring

The principal investigator will be responsible for the overall monitoring of the data and safety of study participants. The principal investigator will be assisted by other members of the study staff.

12.0 Reporting of Unanticipated Problems, Adverse Events or Deviations

Any unanticipated problems, serious and unexpected adverse events, deviations or protocol changes will be promptly reported by the principal investigator or designated member of the research team to the IRB and sponsor or appropriate government agency if appropriate.

13.0 Adverse Events List and Reporting Requirements

13.1 Adverse Event List for Propranolol

Cardiovascular: Bradycardia; congestive heart failure; intensification of AV block; hypotension; paresthesia of hands; thrombocytopenic purpura; arterial insufficiency, usually of the Raynaud type.

Central Nervous System: Light-headedness, mental depression manifested by insomnia, lassitude, weakness, fatigue; catatonia; visual disturbances; hallucinations; vivid dreams; an acute reversible syndrome characterized by disorientation for time and place, short-term memory loss, emotional lability, slightly clouded sensorium, and decreased performance on neuropsychometrics. For immediate-release formulations, fatigue, lethargy, and vivid dreams appear dose-related.

Gastrointestinal: Nausea, vomiting, epigastric distress, abdominal cramping, diarrhea, constipation, mesenteric arterial thrombosis, ischemic colitis.

Allergic: Hypersensitivity reactions, including anaphylactic/anaphylactoid reactions, pharyngitis and agranulocytosis; erythematous rash, fever combined with aching and sore throat; laryngospasm, and respiratory distress.

Respiratory: Bronchospasm.

Hematologic: Agranulocytosis, nonthrombocytopenic purpura, thrombocytopenic purpura.

Autoimmune: Systemic lupus erythematosus (SLE).
Skin and mucous membranes: Stevens-Johnson Syndrome, toxic epidermal necrolysis, dry eyes, exfoliative dermatitis, erythema multiforme, urticaria, alopecia, SLE-like reactions, and psoriasiform rashes. Oculomucocutaneous syndrome involving the skin, serous membranes and conjunctivae reported for a beta blocker (practolol) have not been associated with propranolol.

Genitourinary: Male impotence; Peyronie's disease.

13.2 Adverse Event Characteristics

- **CTCAE term (AE description) and grade:** The descriptions and grading scales found in the revised NCI Common Terminology Criteria for Adverse Events (CTCAE) version 4.0 will be utilized for AE reporting. All appropriate treatment areas should have access to a copy of the CTCAE version 4.0. A copy of the CTCAE version 4.0 can be downloaded from the CTEP web site (http://ctep.cancer.gov).

- **'Expectedness'**: AEs can be 'Unexpected' or 'Expected' (see Section 7.1 above) for expedited reporting purposes only.

- **Attribution** of the AE:

- Definite – The AE **is clearly related** to the study treatment.
- Probable – The AE **is likely related** to the study treatment.
- Possible – The AE **may be related** to the study treatment.
- Unlikely – The AE **is doubtfully related** to the study treatment.
- Unrelated – The AE **is clearly NOT related** to the study treatment.

13.3 DSMC SAE Reporting Requirements

The Data and Safety Monitoring Committee (DSMC) is responsible for reviewing SAEs for WFBCCC Institutional studies as outlined in Appendix F. DSMC currently requires that all unexpected 4 and all grade 5 SAEs on these trials be reported to them for review. All WFBCCC Clinical Research Management (CRM) staff members assisting a Principal Investigator in investigating, documenting and reporting an SAE qualifying for DSMC reporting are responsible for informing a clinical member of the DSMC as well as the entire committee via the email notification procedure of the occurrence of an SAE.

13.4 WFBH IRB AE Reporting Requirements

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

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

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

Any unanticipated problems involving risks to subjects or others occurring at another site conducting the same study that has been approved by the WFBH IRB (external events) must be reported to the WFBH IRB within 7 calendar days of the investigator or other members of the study team becoming aware of the event.

Any event, incident, experience, or outcome that alters the risk versus potential benefit of the research and as a result warrants a substantive change in the research protocol or informed consent process/document in order to insure the safety, rights or welfare of research subjects.

14.0 Pharmaceutical Information

A list of the adverse events and potential risks associated with the investigational or commercial agents administered in this study can be found in Section 12.1.

14.1 Pharmaceutical Accountability

Propranolol will be provided by the study as per section 5.0.

14.2 Propranolol

Product description: Propranolol hydrochloride is a stable, white, crystalline solid available in 10, 20, 40, 60 and 80 mg tablets. For this study, 40 mg tablets will be used.

Solution preparation: NA
Storage requirements: Store at room temperature. Protect from light
Stability: Propranolol is stable.
Route of administration: Take by mouth 2 hours before prostate biopsy
Disposal: Dispose of any unused tablets in line with local and institutional regulations.

References

1. Hassan S, Karpova Y, Baiz D, et al. Behavioral stress accelerates prostate cancer development in mice. *The Journal of clinical investigation.* 2013;123(2):874-886.
2. Hassan S, Karpova Y, Flores A, et al. A pilot study of blood epinephrine levels and CREB phosphorylation in men undergoing prostate biopsies. *International urology and nephrology.* 2014;46(3):505-510.
3. Le CP, Nowell CJ, Kim-Fuchs C, et al. Chronic stress in mice remodels lymph vasculature to promote tumour cell dissemination. *Nature communications.* 2016;7:10634.
4. Lu D, Sinnott JA, Valdimarsdottir U, et al. Stress-Related Signaling Pathways in Lethal and Nonlethal Prostate Cancer. *Clinical cancer research : an official journal of the American Association for Cancer Research.* 2016;22(3):765-772.
5. Kulik G. Targeting psychoemotional stress to treat prostate cancer. *Asian journal of andrology.* 2013;15(3):362-363.
6. Armaiz-Pena GN, Allen JK, Cruz A, et al. Src activation by beta-adrenoreceptors is a key switch for tumour metastasis. *Nature communications.* 2013;4:1403.
7. Braadland PR, Ramberg H, Grytli HH, Tasken KA. beta-Adrenergic Receptor Signaling in Prostate Cancer. *Frontiers in oncology.* 2014;4:375.
8. Cardwell CR, Coleman HG, Murray LJ, O'Sullivan JM, Powe DG. Beta-blocker usage and prostate cancer survival: a nested case-control study in the UK Clinical Practice Research Datalink cohort. *Cancer epidemiology.* 2014;38(3):279-285.
9. Grytli HH, Fagerland MW, Fossa SD, Tasken KA. Association between use of beta-blockers and prostate cancer-specific survival: a cohort study of 3561 prostate cancer patients with high-risk or metastatic disease. *Eur Urol.* 2014;65(3):635-641.
10. Grytli HH, Fagerland MW, Fossa SD, Tasken KA, Haheim LL. Use of beta-blockers is associated with prostate cancer-specific survival in prostate cancer patients on androgen deprivation therapy. *Prostate.* 2013;73(3):250-260.
11. Perron L, Bairati I, Harel F, Meyer F. Antihypertensive drug use and the risk of prostate cancer (Canada). *Cancer causes & control : CCC.* 2004;15(6):535-541.
12. Rodriguez C, Jacobs EJ, Deka A, et al. Use of blood-pressure-lowering medication and risk of prostate cancer in the Cancer Prevention Study II Nutrition Cohort. *Cancer causes & control : CCC.* 2009;20(5):671-679.
13. Shah SM, Carey IM, Owen CG, Harris T, Dewilde S, Cook DG. Does beta-adrenoceptor blocker therapy improve cancer survival? Findings from a population-based retrospective cohort study. *British journal of clinical pharmacology.* 2011;72(1):157-161.
14. Wang HM, Liao ZX, Komaki R, et al. Improved survival outcomes with the incidental use of beta-blockers among patients with non-small-cell lung cancer treated with definitive radiation therapy. *Annals of oncology : official journal of the European Society for Medical Oncology / ESMO.* 2013;24(5):1312-1319.
15. Chang PY, Huang WY, Lin CL, et al. Propranolol Reduces Cancer Risk: A Population-Based Cohort Study. *Medicine.* 2015;94(27):e1097.

16. Watkins JL, Thaker PH, Nick AM, et al. Clinical impact of selective and nonselective beta-blockers on survival in patients with ovarian cancer. *Cancer.* 2015;121(19):3444-3451.
17. London MJ, Hur K, Schwartz GG, Henderson WG. Association of perioperative beta-blockade with mortality and cardiovascular morbidity following major noncardiac surgery. *Jama.* 2013;309(16):1704-1713.
18. Dyck JB, Chung F. A comparison of propranolol and diazepam for preoperative anxiolysis. *Canadian journal of anaesthesia = Journal canadien d'anesthesie.* 1991;38(6):704-709.
19. Khadke VV, Khadke SV, Khare A. Oral propranolol--efficacy and comparison of two doses for peri-operative anxiolysis. *Journal of the Indian Medical Association.* 2012;110(7):457-460.
20. Ray K, Trawick WG, Mullins RE. Assay of total and free propranolol in plasma by liquid chromatography with fluorometric detection. *Clinical chemistry.* 1985;31(1):131-134.
21. Salman SA, Sulaiman SA, Ismail Z, Gan SH. Quantitative determination of propranolol by ultraviolet HPLC in human plasma. *Toxicology mechanisms and methods.* 2010;20(3):137-142.
22. Sood SP, Green VI, Mason RP. Routine methods in toxicology and therapeutic drug monitoring by high-performance liquid chromatography. IV. A rapid microscale method for determination of propranolol and 4-hydroxypropranolol in plasma. *Therapeutic drug monitoring.* 1988;10(2):224-230.
23. Zhang X, Odom DT, Koo SH, et al. Genome-wide analysis of cAMP-response element binding protein occupancy, phosphorylation, and target gene activation in human tissues. *Proceedings of the National Academy of Sciences of the United States of America.* 2005;102(12):4459-4464.
24. Cohen S, Kamarck T, Mermelstein R. A global measure of perceived stress. *Journal of health and social behavior.* 1983;24(4):385-396.
25. Donovan KA, Grassi L, McGinty HL, Jacobsen PB. Validation of the distress thermometer worldwide: state of the science. *Psycho-oncology.* 2014;23(3):241-250.

Appendix A – Subject Eligibility Checklist

IRB Protocol No. 00043227	WFBCCC Protocol No. 8 5 7 1 6
Study Title: Evaluating the effect of the ADRB2 blockers on PKA/BAD/CREB signaling in the prostate gland	
Principal Investigator: Ashok Hemal, M.D., George Kulik, D.V.M, Ph.D.	

Inclusion Criteria (as outlined in study protocol)	Criteria is met	Criteria is NOT met	Source Used to Confirm * (Please document dates and lab results)
Men > 18 years of age	☐	☐	
Patients undergoing prostate biopsy or prostatectomy	☐	☐	
Individuals able to understand and willing to sign an IRB-approved informed consent document	☐	☐	
Exclusion Criteria (as outlined in study protocol)	**Criteria NOT present**	**Criteria is present**	**Source Used to Confirm * (Please document dates and lab results)**
Men taking propranolol daily for any reason are excluded	☐	☐	
Men with baseline SBP <120 or HR <63	☐	☐	
Men unable to swallow pills	☐	☐	
History of current or past medical or psychiatric illness that would make participation difficult or not feasible at the discretion of the principal investigator or co-investigators	☐	☐	

This subject is ☐ eligible / ☐ ineligible for participation in this study.

WISER Assigned PID: _____

Signature of research professional confirming eligibility:_____
Date: _____

Signature of Treating Physician**: _____
Date: _____

* Examples of source documents include clinic note, pathology report, laboratory results, etc. When listing the source, specifically state which document in the medical record was used to assess eligibility. Also include the date on the document. Example: “Pathology report, 01/01/14” or “Clinic note, 01/01/14”

**Principal Investigator signature can be obtained following registration if needed

Appendix B – Protocol Registration Form

DEMOGRAPHICS

Patient: Last Name: _____ First Name: _____

MRN: ___ ___ ___ ___ ___ ___ ___ ___ DOB (mm/dd/yy): ___ ___ / ___ ___ / ___ ___

ZIPCODE: ___ ___ ___ ___ ___

Ethnicity (choose one): ☐ Hispanic
☐Non-Hispanic

Race (choose all that apply): ☐ WHITE ☐BLACK ☐ ASIAN
☐ PACIFIC ISLANDER ☐ NATIVE AMERICAN

Primary Diagnosis: _____

Date of Diagnosis: ___ ___ /___ ___/___ ___

Randomization

This patient has been randomly assigned to the following treatment arm (Check one):

_____ Propranolol and Prostate biopsy or prostatectomy (Group 1)

_____ Prostate biopsy or prostatectomy only (Group 2)

Randomization Recorded by: _____ Date___ ___ / ___ ___ / ___ ___Time_____

PROTOCOL INFORMATION

Date of Registration: ___ ___ / ___ ___ / ___ ___

MD Name (last) : _____

Date protocol treatment started: ___ ___ / ___ ___ / ___ ___

Informed written consent: ☐ YES ☐ NO
(consent must be signed prior to registration)

Date Consent Signed: ___ ___ / ___ ___ / ___ ___

PID # (to be assigned by WISER): ___ ___ ___ ___ ___ ___ ___ ___

Protocol Registrar can be contact by calling 336-713-6767 between 8:30 AM and 4:00 PM, Monday – Friday. Completed Eligibility Checklist and Protocol Registration Form must be hand delivered, faxed or e-mailed to the registrar at 336-7136772 or registra@wakehealth.edu.

Appendix C – Perceived Stress

Perceived Stress Scale – 4*

To be filled out by clinical staff

WISER PID: __ __ __ __ __ __ __ __ **Date Completed:** __ __ / __ __ / __ __

PI: Ashok Hemal, M.D., George Kulik, D.V.M., Ph.D. **Study Number:** 8 5 7 1 6

To be filled out by the patient:

Instructions: This set of questions asks about your feelings over the **LAST 2 WEEKS**. Please indicate how often you have felt this way.

Questions

1. In the last 2 weeks, how often have you felt that you were unable to control the important things in your life?
   - ☐0 = Never
   - ☐1 = Almost Never
   - ☐2 = Sometimes
   - ☐3 = Fairly Often
   - ☐4 = Very Often

2. In the last 2 weeks, how often have you felt confident about your ability to handle your personal problems?
   - ☐0 = Never
   - ☐1 = Almost Never
   - ☐2 = Sometimes
   - ☐3 = Fairly Often
   - ☐4 = Very Often

3. In the last 2 weeks, how often have you felt that things were going your way?
   - ☐0 = Never
   - ☐1 = Almost Never
   - ☐2 = Sometimes
   - ☐3 = Fairly Often
   - ☐4 = Very Often

4. In the last 2 weeks, how often have you felt difficulties were piling up so high that you could not overcome them?
   - ☐0 = Never
   - ☐1 = Almost Never
   - ☐2 = Sometimes
   - ☐3 = Fairly Often
   - ☐4 = Very Often

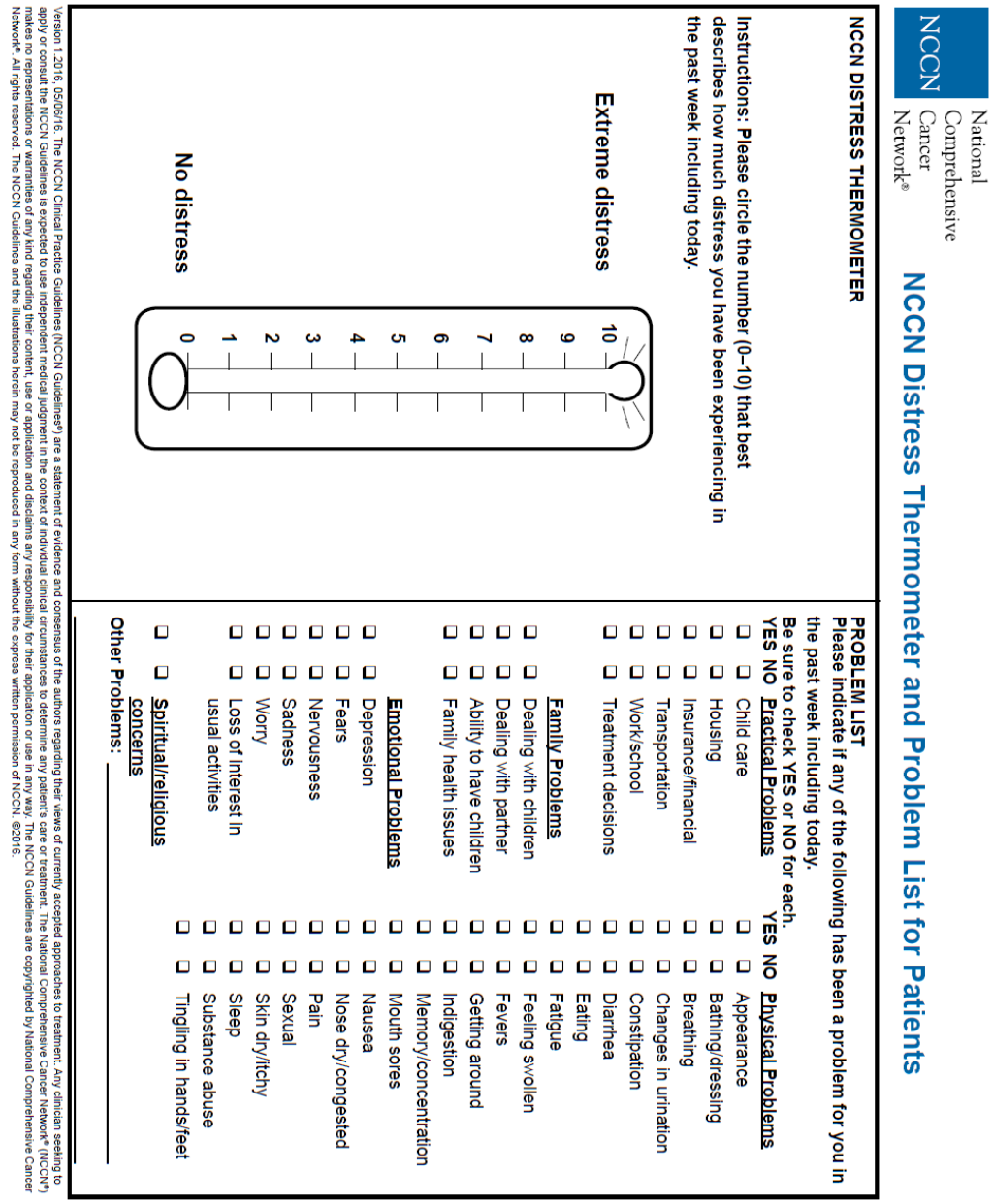
*Modified from:
Cohen, S., Kamarck, T, and Mermelstein, R. "A Global Measure of Perceived Stress." Journal of Healthand Social Behavior, Vo. 24, No. 4 (Dec.,1983)

Appendix D – Distress Thermometer

WISER PID: __ __ __ __ __ __ __ __ Date Completed: __ __ / __ __ / __ __

PI: Ashok Hemal, M.D., George Kulik, D.V.M., Ph.D.

Study Number: 8 5 7 1 6



NCCN National Comprehensive Cancer Network®

NCCN Distress Thermometer and Problem List for Patients

NCCN DISTRESS THERMOMETER

Instructions: Please circle the number (0–10) that best describes how much distress you have been experiencing in the past week including today.

Extreme distress 10 9 8 7 6 5 4 3 2 1 0 No distress

PROBLEM LIST

Please indicate if any of the following has been a problem for you in the past week including today.

Be sure to check YES or NO for each.

YES	NO	Practical Problems	YES	NO	Physical Problems
☐	☐	Child care	☐	☐	Appearance
☐	☐	Housing	☐	☐	Bathing/dressing
☐	☐	Insurance/financial	☐	☐	Breathing
☐	☐	Transportation	☐	☐	Changes in urination
☐	☐	Work/school	☐	☐	Constipation
☐	☐	Treatment decisions	☐	☐	Diarrhea
			☐	☐	Eating
		Family Problems	☐	☐	Fatigue
☐	☐	Dealing with children	☐	☐	Feeling swollen
☐	☐	Dealing with partner	☐	☐	Fevers
☐	☐	Ability to have children	☐	☐	Getting around
☐	☐	Family health issues	☐	☐	Indigestion
			☐	☐	Memory/concentration
		Emotional Problems	☐	☐	Mouth sores
☐	☐	Depression	☐	☐	Nausea
☐	☐	Fears	☐	☐	Nose dry/congested
☐	☐	Nervousness	☐	☐	Pain
☐	☐	Sadness	☐	☐	Sexual
☐	☐	Worry	☐	☐	Skin dry/itchy
☐	☐	Loss of interest in usual activities	☐	☐	Sleep
			☐	☐	Substance abuse
☐	☐	**Spiritual/religious concerns**	☐	☐	Tingling in hands/feet

Other Problems: _____

Appendix E – Adverse Events Log

WFBCCC 85716 Adverse Event (AE) Log

PI: Ashok Hemal, MD; George Kulik, DVM, PhD

PID: __ __ __ __ __ __ __ __

MRN: __ __ __ __ __ __ __ __

Date: __ __/__ __/__ __

Adverse Event CTC Term	**Value** (-5 if non-numeric)	**Grade** (0-5) per CTC	**Start Date**	**Attribution** 1=Related 2=Probably 3=Possible 4=Unlikely 5=Unrelated	**Treating MD Initials/Date**	**End Date**	**Expected** 1=Yes 0=No	***Serious Adverse Event (SAE)** 1=Yes 0=No	**Dose Limiting Toxicity (DLT)** 1=Yes 0=No	**Action Taken** 1=None 2=Tx withheld 3=Tx D/C 4=Tx adjusted 5=Other	**Reportable?** 1=IRB 2=DSMC 3=FDA 4=Sponsor

*Serious Adverse Event: Hospitalization; Disability; Birth Defect; Life-threatening; Death.

CTCAE Version 4 - http://evs.nci.nih.gov/ftp1/CTCAE/CTCAE_4.03_2010-06-14_QuickReference_8.5x11.pdf

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Appendix F – Mandatory DSMC SAE Reporting Guidelines

Data and Safety Monitoring Committee (DSMC) Serious Adverse Event (SAE) Notification SOP	**Date: 02/11/2021**

Mandatory DSMC SAE Reporting Requirements in WISER

This document describes reporting requirements of adverse events from **WFBCCC Investigator Initiated interventional trials to the Data and Safety Monitoring Committee (DSMC).** A trial is considered a **WFBCCC Investigator Initiated interventional trial** if the following criteria are met:

1) The Principal Investigator (PI) of the trial is a member of a department at the Wake Forest University Baptist Medical Center.
2) WFBCCC is considered as the primary contributor to the design, implementation and/or monitoring of the trial.
3) The trial is designated as "Interventional" using the Clinical Research Categories definitions provided by the NCI in the Data Table 4 documentation. (https://cancercenters.cancer.gov/GrantsFunding/DataGuide#dt4)

There are two distinct types of WFBCCC Investigator Initiated interventional trials based on where patient enrollment occurs. These include:

1) Local WFBCCC Investigator Initiated interventional trials defined as trials where **all patients are enrolled from one of the WFBCCC sites.** These include the main outpatient Cancer Center clinics (located in Winston-Salem) as well as WFBCCC affiliate sites located in Bermuda Run (Davie Medical Center), Clemmons, Lexington, High Point, or Wilkesboro.
2) Multi-Center WFBCCC Investigator Initiated interventional trials defined as trials where patients are enrolled from other sites in addition to WFBCCC sites. There are three types of trials that are included in this category:
    a. Trials sponsored by the NCI Community Oncology Research Program (NCORP) that are conducted at multiple sites where the PI is a member of a department at the Wake Forest University Baptist Medical Center.
    b. Trials sponsored by Industry that are conducted at multiple sites and the PI is a member of a department at the Wake Forest University Baptist Medical Center.
    c. Trials sponsored by WFBCCC that are conducted at multiple sites and the PI is a member of a department at the Wake Forest University Baptist Medical Center.

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WFBCCC # 85716

All Adverse Events (AEs) and Serious Adverse Events (SAEs) that occur on any patients enrolled on WFBCCC Investigator Initiated Interventional trials must be entered into the WISER system. The only exception to this requirement is for patients enrolled on NCORP trials at non- WFBCCC sites. AEs and SAEs for NCORP patients enrolled at WFBCCC sites must be entered into the WISER system. Once these AEs and SAEs are entered in WISER, certain actions must be taken regarding the reporting of specific Adverse Events to the DSMC.

All Adverse Events that occur during protocol intervention (defined below) and are coded as either 1) **unexpected grade 4,** 2) **unplanned inpatient hospitalization > 24 hours (regardless of grade)**, or **grade 5 (death)** must be reported to the DSMC using the using the SAE console in WISER.

A research nurse or clinical research coordinator when made aware that an adverse event meets one of the above criteria has occurred on a WFBCCC Investigator Initiated interventional trial, is responsible for informing a clinical member of the DSMC by phone (or in-person) about the adverse event. The nurse/coordinator should contact the treating physician prior to calling the DSMC clinical member to obtain all details of the SAE, as well as all associated toxicities to be recorded along with the SAE. In addition, this nurse or coordinator is responsible for entering the adverse event information into the SAE console in WISER. Once the adverse event has been entered into the SAE console an email informing the entire DSMC will be generated.

THESE REPORTING REQUIREMENTS APPLY TO any staff member on the study team for a WFBCCC Institutional Interventional trial. Ultimately, the protocol PI has the primary responsibility for AE identification, documentation, grading and assignment of attribution to the investigational agent/intervention. However, when an AE event as described above is observed, it is the responsibility of the person who observed the event to be sure that it is reported to the DSMC.

What is considered during protocol intervention?

During protocol intervention is considered to be the time period while a patient is on study treatment or during the time period within 30 days of last study treatment (even if patient begins a new (non-study) treatment during the 30 days). This window of 30 days should be the standard window to be used in all protocols unless a specific scientific rationale is presented to suggest that a shorter window can be used to identify events. If it is a trial sponsored by Industry and the sponsor requires a longer window for monitoring of SAEs, then the longer window of time specified by the sponsor should be followed.

What is considered as an Unexpected Grade 4 event?

Any grade 4 event that was not specifically listed as an expected adverse event in the

protocol should be considered as unexpected. A grade 4 adverse event can be considered to be unexpected if it is an event that would not be expected based on the treatment being received or if it is unexpected based on the health of the patient. In either case, if there is any uncertainty about whether a grade 4 adverse event is expected or unexpected it should be reported to DSMC.

DSMC notification responsibilities of the person (e.g., nurse) handling the reporting/documenting of the SAE in WISER:

1. Make a phone call (or speak in person) to the appropriate clinical member of the DSMC according to the schedule as listed below (page if necessary).
2. Enter a new SAE into the SAE module that is located in the Subject>> CRA Console in WISER WITHIN 24 HOURS of first knowledge of the event. Information can be entered and saved, but the DSMC members will not be notified until a date is entered into the DSMC Notification Date Field. This will ensure that all persons that need to be made aware of the event (i.e., PI, study team members and DSMC members) will be notified; remember to file a copy of the confirmation.
3. Document that the appropriate person(s) on the DSMC has been contacted. Indicate the name of the DSMC clinician that was contacted and the date and time contacted in the Event Narrative field in the SAE console of the particular subject.
4. Document whether or not the protocol should be suspended based on the discussion with the DSMC clinician. This is the major function of the email notification. Enter whether the protocol should be suspended in the Event Narrative Field.
5. Follow up/update the clinical member(s) of DSMC regarding any new developments or information obtained during the course of the SAE investigation and reporting process.

Elements needed to complete the SAE form in the Subject Console in WISER (see Screen Shot 3):

1. Event Date
2. Reported Date
3. Reported by
4. If Grade 5, enter Death Date
5. If Grade 5, enter Death occurred: within 30 days
6. Event Narrative: Brief description (include brief clinical history relevant to this event, including therapies believed related to event). Begin narrative with the DSMC clinician who was notified and Date/Time notified. In addition, state attribution by DSMC clinician as either “Unrelated”, “Unlikely”, “Possibly”, “Probably”, or “Definitely”. Always include the following here:
    i. DSMC clinician name, date/time contacted and comments
    ii. Date of last dose before the event

iii. Is suspension of the protocol needed? Y/N

7. Treating Physician comments
8. PI comments, if available
9. Protocol Attribution after discussion with DSMC clinician
10. Outcome (Fatal/Died, Intervention for AE Continues, Migrated AE, Not Recovered/Not Resolved, Recovered/Resolved with Sequelae, Recovered/Resolved without Sequelae, Recovering and Resolving)
11. Consent form Change Required? Y/N
12. SAE Classification ***This is required in order for the email notification to be sent***
13. Adverse Event Details – Enter all details for each AE associated with the SAE.
    a. Course start date
    b. Category
    c. AE Detail
    d. Comments
    e. Grade/Severity
    f. Unexpected Y/N
    g. DLT Y/N
    h. Attributions
    i. Action
    j. Therapy
    k. Click ADD to attach the AE Detail to the SAE.
14. Enter Date Notified DSMC -- ***This is required for the email notification to be sent***
15. Click Submit. The auto-generated notification email will disseminate within 5 minutes. If you do not receive an email within 5 minutes, check that you have entered the "Date Notified DSMC" and the "SAE Classification". If these have been entered and the email still has not been received, take a screen shot of the SAE in WISER and immediately email it out to all of the DSMC members listed in this SOP. In the subject line, indicate that this is a manual transmission of the SAE in lieu of the auto-generated email. It is required that a notification goes to the DSMC members immediately so that their assessment can be obtained within the 24 hour period requirement. Contact the Cancer Center Programmer/Analyst to alert that there is an issue with the auto-generated email.

The Clinical Members of DSMC to Notify by Phone or Page:

Monday	Tuesday	Wednesday	Thursday	Friday	Saturday	Sunday
Lesser	Hughes	Goodman	Reed	Porosnicu	Seegars	Lesser
Hughes	Goodman	Reed	Porosnicu	Seegars	Lesser	Hughes
Goodman	Reed	Porosnicu	Seegars	Lesser	Hughes	Goodman
Reed	Porosnicu	Seegars	Lesser	Hughes	Goodman	Reed
Porosnicu	Seegars	Lesser	Hughes	Goodman	Reed	Porosnicu
Seegars	Lesser	Hughes	Goodman	Reed	Porosnicu	Seegars

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Glenn Lesser, MD – Hematology Oncology 6-9527 / 6-7972 / Pager 336-806-8397
Mercedes Porosnicu, MD-- Hematology Oncology 6-7980 / 6-0230 / Pager 336-806-9150
Ryan Hughes, MD – Radiation Oncology 3-3600 / Pager 336-806-9865
Michael Goodman, MD -- Hematology Oncology 6-7970 / Pager 336-806-7283
Daniel Reed, MD -- Hematology Oncology 3-3841 / Pager 336-806-0637
Mary Beth Seegars, MD -- Hematology Oncology 6-4815 / Pager 336-806-9948

Definition of Unavailable:

As a general guideline if the first clinician that is contacted does not respond to the phone call or page within 30 minutes, then initiate contact with the next DSMC clinician listed in the table above on the particular day the SAE is being reported. Allow up to 30 minutes for the new DSMC clinician to respond to a phone call or page before contacting the next member in the table. These times (30 minutes) are a general guideline. Best judgment as a clinical research professional should be used giving considerations of the time of day, severity of the SAE, and other circumstances as to when it is appropriate to contact backup clinicians. If the event occurs near the end of day, then leave messages (voice or email) as appropriate and proceed with submitting the DSMC notification form. It is important to take reasonable steps and to document that some type of contact has been initiated to one or more of the clinical members of DSMC.

DSMC CLINICAN RESPONSIBILITY:

It is the responsibility of the DSMC clinician to review all reported events, evaluate the events as they are reported; and communicate a response to the Investigator, event reporter and the members of DSMC. The review will include but not be limited to the information reported; there may be times when additional information is needed in order for an assessment to be made and further communication directly with the investigator may be warranted. DSMC reserves the right to disagree with the Investigator's assessment. If DSMC does not agree with the Investigator, DSMC reserves the right to suspend the trial pending further investigation. If there is any immediate danger or harm that could be present for a future patient based on the information provided in the DSMC report then an immediate suspension of enrollment should be considered.

AMENDMENTS TO PREVIOUS REPORTS

If all pertinent information is unavailable with the initial submission, once the additional information is available **do not submit a new report**. Rather, go to the original email that was sent to the DSMC and using that email "reply to all". Entitle this new email "**Amendment** for (list date of event and patient ID)" this will avoid duplications of the same event. List the additional information being reported. This information needs to be entered into WISER as well. To do this, go to the Subject console and click SAEs on the left column. Click on the appropriate SAE number that needs updating. Then click Update. This will allow

additional information to be added.

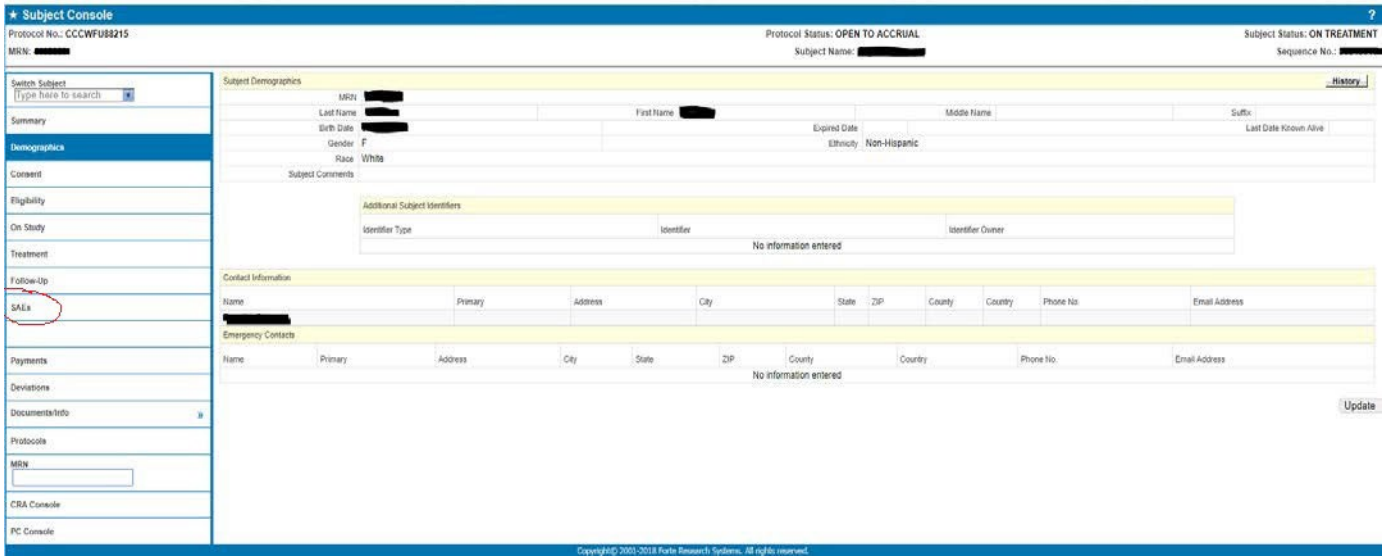
Acronyms

AE – Adverse Event
DSMC-Data and Safety Monitoring Committee
SAE-Serious Adverse Event
WFBCCC – Wake Forest Baptist Comprehensive Cancer Center
NCI-National Cancer Institute
WISER –Wake Integrated Solution for Enterprise Research

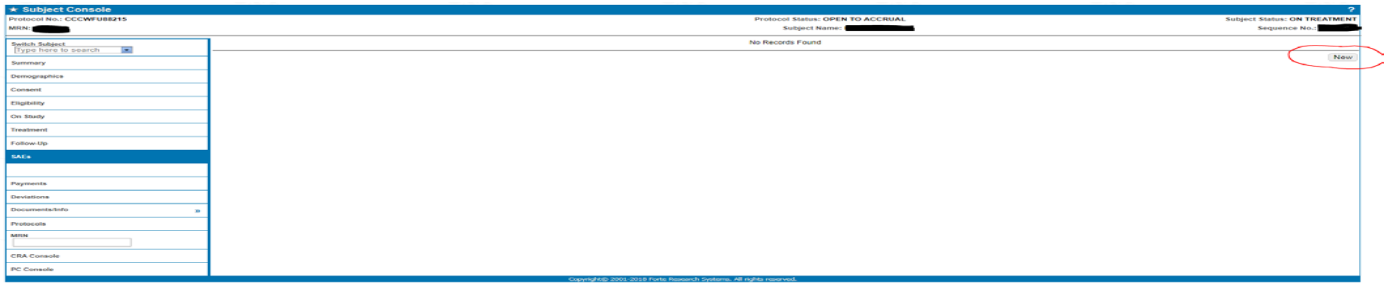
Screen Shots:

The following screen shots come from the SAE Console within the Subject Console in WISER.

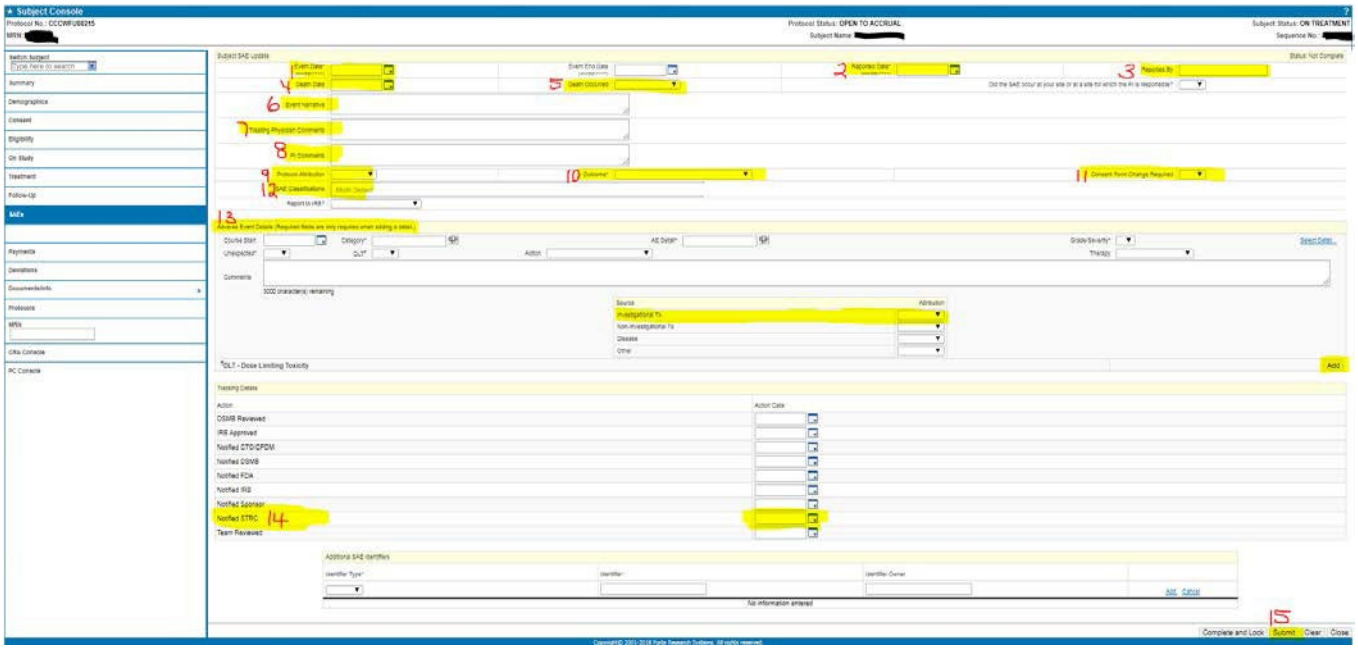
Screen Shot 1:



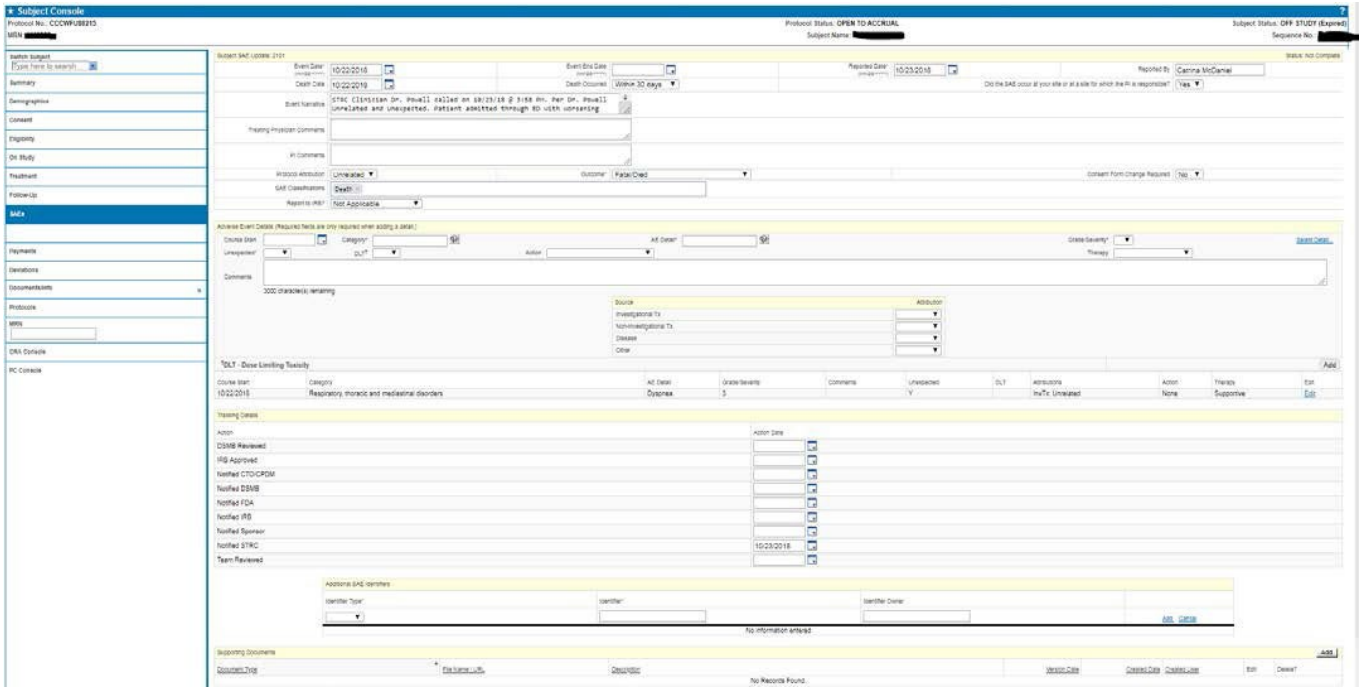
Screen Shot 2:



Screen Shot 3:



Screen Shot 4:



Appendix G - Baseline Medical History

Baseline Medical History and Data Collection Form

WISER PID: _____ **Date Completed:** __ __ / __ __ / __ __

PI: Ashok Hemal, M.D. and, George Kulik, D.V.M., Ph.D.

Study Number: CCCWFU 85716

1. Oncologic history
   1. Prior prostate cancer therapies:

      ☐ chemotherapy ☐ radiation ☐ surgery ☐ hormone ☐ other: _____

      ☐ None

Regimen Name	Total # of Treatments	Date of Last Treatment, or 'ongoing' for certain HT
1.		
2.		
3.		
4.		
5.		
Site of RT	Total Dose in Gy	Date of Last Treatment
1.		
2.		
3.		
Type of surgery	Date of surgery	
1.		
2.		

Hormone Therapy Name	Dose	Date of Last Treatment
1.		
2.		

Other	Dose	Date of Last Treatment
1.		
2.		

2. Concurrent medications and supplements
   a. List all prescription and over-the-counter medications (includes prescription supplements)

Medication Name	Is it PRN?	Medication Name	Is it PRN?
1.	yes no	11.	yes no
2.	yes no	12.	yes no
3.	yes no	13.	yes no
4.	yes no	14.	yes no
5.	yes no	15.	yes no
6.	yes no	16.	yes no
7.	yes no	17.	yes no
8.	yes no	18.	yes no
9.	yes no	19.	yes no
10.	yes no	20.	yes no

3. Vital signs:
   a. HR_____
   b. BP____ /____
   c. RR_____
d. Temp _____(°F)
e. Weight _____(kg)

4. Blood Draws

   a. Cancer-specific tumor marker measurement (if available):

      Prostate cancer:
      Testosterone, Date __ __ /__ __ / __ __ Value: _____
      PSA, Date __ __ /__ __ / __ __ Value: _____

Signature of research professional:_____ Date: __ __ / __ __ / __ __

Appendix H – Tissue Collection

TISSUE COLLECTION FORM

This form is for the collection of core samples of prostate tissue.

Sample Handling: Tubes used for collecting sample should be labelled with the patient study number (Patient ID), initials and date. Samples should be frozen in liquid nitrogen and stored in the Tumor Tissue Core at -80 until use.

Tumor Tissue Core
Wake Forest Baptist Comprehensive Cancer Center
Tumor Tissue Core Facility
Care of Dr. Greg Kucera
Hanes Building Rm 4049
Medical Center Blvd
Winston-Salem NC 27157

WISER PID: __ __ __ __ __ __ __ __ Date Completed: __ __ / __ __ / __ __

PI:, Ashok Hemal, M.D., George Kulik, D.V.M., Ph.D.

Study Number: CCCWFU 85716

Drug Received

Propranolol ☐ Yes ☐ No

Tissue Sample

Sample ID: _____

Tissue pathology:_____

Time of Collection

Time Sample Collected _____am/pm Time in storage _____am/pm

Location of Sample

Freezer ID: _____ Shelf: _____ Box:_____

Person Collecting Tissue_____ Date__ __ / __ __ / __ __

Deviations or notes regarding sample collection

_____

_____

_____

Appendix I – Blood Collection

BLOOD/PLASMA COLLECTION FORM

This form is for the collection of research blood samples to be stored for future use.
Sample Handling: Plasma should be aliquoted in cryotubes or equivalent tubes and labelled with the patient study number (Patient ID), pre or post draw, initials and date. Samples should be frozen and stored in the Tumor Tissue Core at -80 until use.

Tumor Tissue Core
Wake Forest Baptist Comprehensive Cancer Center
Tumor Tissue Core Facility
Care of Dr. Greg Kucera
Hanes Building Rm 4049
Medical Center Blvd
Winston-Salem NC 27157

WISER PID: __ __ __ __ __ __ __ __ Date Completed: __ __ / __ __ / __ __

PI: Ashok Hemal, M.D., George Kulik, D.V.M., Ph.D.

Study Number: CCCWFU 85716

Drug Received

Propranolol ☐ Yes ☐ No

Plasma Sample

Sample ID: _____

Time of Collection

Time Draw Collected _____am/pm

Time 1 tube sent to clinical lab for catecholamine processing _____am/pmTime

plasma was separated _____am/pm

Time plasma placed in storage _____am/pm

Location of Sample

Freezer ID: _____ Shelf: _____ Box:_____

Person Processing plasma _____Date__ __ / __ __ / __ __

Freeze Thaw Cycles (note tube number, date and amount of sample used)

Deviations or notes regarding collections and use

Appendix J: CREB Phosphorylation

DATA COLLECTION FORM

WISER PID: __ __ __ __ __ __ __ __ Date Completed: __ __ / __ __ / __ __

PI: Ashok Hemal, M.D., George Kulik, D.V.M., Ph.D.

Study Number: CCCWFU 85716

Prostate CREB Phosphorylation Worksheet				
Densitometric Values				
Sample ID	Date	pCREB	panCREB	Normalized pCREB

Appendix K: BAD Phosphorylation
DATA COLLECTION FORM

WISER PID: __ __ __ __ __ __ __ __ Date Completed: __ __ / __ __ / __ __

PI: Ashok Hemal, M.D., George Kulik, D.V.M., Ph.D.

Study Number: CCCWFU 85716

Prostate BAD Phosphorylation Worksheet				
Densitometric Values				
Sample ID	Date	pBAD	panBAD	Normalized pBAD

Appendix L: Plasma Outcomes

DATA COLLECTION FORM

WISER PID: __ __ __ __ __ __ __ __ Date Completed: __ __ / __ __ / __ __

PI: Ashok Hemal, M.D., George Kulik, D.V.M., Ph.D.

Study Number: CCCWFU 85716

Sample ID	Date	Propranolol levels (ng/ml)	Epinephrine (ng/ml)	Initials	Notes

Appendix M: RNA Analysis

WISER PID: __ __ __ __ __ __ __ __ Date Completed: __ __ / __ __ / __ __

PI: Ashok Hemal, M.D., George Kulik, D.V.M., Ph.D.

Study Number: CCCWFU 85716

RT-PCR Worksheet				
Sample ID or PID	Date	Transcript	Relative expression	Initials

Appendix N: Surgery Day Worksheet

SURGERY DAY WORKSHEET

WISER PID: __ __ __ __ __ __ __ __ Date Completed: __ __ / __ __ / __ __

PI: Ashok Hemal, M.D., George Kulik, D.V.M., Ph.D.

Study Number: CCCWFU 85716

Propranolol Administration

Group Assignment (Randomization Assignment on Protocol Registration Appendix B)

☐ Group 1 (Propranolol) ☐ Group 2 (no Drug)

☐ Yes ☐ No Time:_____ Dose:_____ Initials_____

Post-randomization Activities (1-2hr Post-Drug if randomized to Group 1)

Blood Draw: ☐ Yes ☐ No Time_____ Initials_____

Perceived Stress Survey: ☐ Yes ☐ No Time:_____ Initials_____
Score_____

Distress Thermometer Survey: ☐ Yes ☐ No Time _____ Initials_____
Score_____