

AUA Office of Research Early Career Investigators Workshop

Mock Study Section Grant Application

***“A Patient-Centered Approach to Integration of Life Expectancy
into Treatment Decision-Making for Patients with Genitourinary Malignancy”***

A funded K08 application and its review (PI redacted)

PROJECT NARRATIVE

Candidate Overview. I am a Urologic Oncologist and Health Services Researcher who is an Assistant Professor in the [REDACTED] Urology and Director of Health Services Research for the Department of Surgery at [REDACTED] at [REDACTED] Medical Center. During my residency and fellowship, I developed research interests in prostate cancer (PC), comorbidity assessment, life expectancy (LE) and cancer treatment decision making, and digital health. With this award, I hope to combine my health services research training with new expertise in digital health, using mixed methods and online crowdsourcing techniques to create a patient-centered approach to integration of LE into PC treatment decision making.

Candidate's Background. I was formally introduced to urologic health services research, outcomes assessment, and epidemiology during my mid-residency research year in 2009-10. I spent that year working with Dr. [REDACTED], one of the founders of health services research in the field of Urology. The central thesis of my research was that men with even a moderate comorbid disease burden and low- to intermediate-risk PC had such a high likelihood of dying of other causes that treatment was at best inadvisable and at worst harmful. The three most impactful manuscripts from my work at this time: (1) delineated the long-term risks of mortality associated with comorbidity,¹ (2) quantified the rates of overtreatment of men with significant comorbidity and low-risk PC,² and (3) created a PC-specific comorbidity index to improve prediction of other-cause mortality at the point-of-care.^{3,4} Following residency, I completed a three-year combined health services/urologic oncology fellowship through the [REDACTED] Program (earning a Masters of Health Policy and Management) and the [REDACTED] Urologic Oncology. As a fellow, I continued to develop my research portfolio around comorbidity assessment and PC treatment decision making. Among other projects, I led a study with Dr. [REDACTED] defining the impact of age and comorbidity on non-cancer mortality in the Prostate Cancer Outcomes Study, resulting in first-author publications in *Annals of Internal Medicine* and *Urology*.^{5,6}

Current Position. I joined the [REDACTED] faculty as an Assistant Professor in the Division of Urology and Director of Health Services Research for the Department of Surgery in [REDACTED]. My clinical practice primarily consists of comprehensive urologic oncology with a focus on PC. I am one of the four urologic oncologists on the core teaching faculty for our residency program. I oversee a small research team in the [REDACTED] Center for Outcomes Research, which is led by Dr. [REDACTED], the Director of Health Services Research. My research team consists of a masters-level biostatistician and a clinical research coordinator, which I share part time with the biostatistics core and Dr. [REDACTED] team, respectively. I also work closely with Dr. [REDACTED], a national expert in PC outcomes research; we currently have a grant-funded project assessing variation in care around use of ADT in men with advanced PC. I currently have 50% of my time protected for research efforts.

Career Goals and Objectives. My overall career goal is to become a national leader in Urologic Oncology and Health Services Research. My proposed career development award will provide experience in areas in which I need substantial training and mentorship: qualitative analysis (**Aim 1**); conducting structured interviews (**Aim 2**); use of crowdsourcing and conjoint analysis tools (**Aim 3**); and trial design and execution (**Aim 4**). During the period of my award, I will take didactic coursework through [REDACTED] School of Public Health to complement my research. This project will position me well for future work in developing patient-centered approaches to shared decision making. These skills will continue to be in high demand, since "big data" will allow for rapid creation of risk prediction models, but without a robust, patient-centered approach for translation to the clinical setting, the utility of these tools will be diminished.

Mentorship Team

[REDACTED], MD will be the primary mentor for this award. He is [REDACTED] Urology in the Division of Urology [REDACTED] and Associate Director for [REDACTED] at the [REDACTED] Institute. Dr. [REDACTED] is a nationally renowned scientist in PC outcomes research who has a special interest in risk stratification. His research routinely appears in high-impact journals and he has received funding from the National Institutes of Health and Department of Defense. He is editor-in-chief for [REDACTED], one of the major journals in this topic area. He also has a longstanding track record of mentoring numerous junior faculty in PC research. Dr. [REDACTED] has served as my research mentor since I joined the faculty, and we have weekly meetings to discuss research and career development. We have written three papers together and are co-PIs on a Department of Defense grant analyzing the impact of comorbidity on timing of androgen deprivation therapy. Dr. [REDACTED] experience in building a focused research portfolio, managing a research team, and posing key research questions will continue to be critical to my development as a young academician.

Dr. [REDACTED], MD, MSHS will serve as secondary mentor for this award. Dr. [REDACTED] is Professor of Medicine and Director of Health Services Research at [REDACTED]. Clinically, Dr. [REDACTED] is a gastroenterologist, but his research spans numerous disciplines across the field of medicine. Dr. [REDACTED] studies how digital health technologies can be used to improve process and value of healthcare. His group recently received two grants to analyze how conjoint analysis and crowdsourcing can be used to improve case selection in digestive diseases. His research team receives funding from the National Institutes of Health, PCORI, [REDACTED] Foundation, U.S. Department of Veterans Affairs, and from industry sources. My office is within his suite in the [REDACTED] for Outcomes Research and Education, and we have co-hired a study coordinator and app programmer to work on studies of mutual interest. We have weekly meetings to discuss research and career development. We have already published two articles on physician ratings tools and have another publication currently in peer review. We are co-leading a study evaluating wearable biosensors in monitoring post-operative ambulation across six divisions in the Department of Surgery. [REDACTED] mentorship in leadership, asking cross-cutting questions, and high-yield translational research will be seminal to shaping my career.

[REDACTED], MD will serve as co-investigator on this project. He is [REDACTED] Medicine and Executive Co-Director of the [REDACTED] Institute at the University of [REDACTED]. Dr. [REDACTED] is a world-renowned, federally funded health services researcher and a member of the Institute of Medicine. [REDACTED] has done pioneering work on comorbidity assessment and risk stratification and his work has been cited over 22,000 times. [REDACTED] has been a formal mentor to me over the last 6 years, and we have published seven papers together. We recently received a grant from the [REDACTED] to analyze the utility of linking biomarker, tumor-risk, and patient-level variables to predict efficacy of treatment in men with PC. [REDACTED] expertise in comorbidity assessment and his career mentorship will continue to be central to my development as a scientist.

[REDACTED], MD, MPH will serve as co-investigator on this project. [REDACTED] is [REDACTED] Urology at [REDACTED] and is an accomplished health services researcher. In [REDACTED] received [REDACTED] mentored career development award to develop a set of quality-of-care indicators for women with pelvic floor disorders. [REDACTED] is currently PI on two U01 grants funded by the NIDDK and the CDC, and has been PI on three NIH-funded R level grants. [REDACTED] brings expertise in qualitative health services research methodology, including conducting stakeholder interviews, focus groups, and qualitative data analysis.

[REDACTED], MD, MSHS will serve as co-investigator on this project. [REDACTED] is Professor of Medicine and Director of Division of General [REDACTED] of the [REDACTED] School. [REDACTED] is a noted health services researcher who has extensive experience in physician stakeholder engagement in the context of developing appropriateness measures. [REDACTED] is widely published in high-impact journals such as *NEJM* and *JAMA*. Dr. [REDACTED]' expertise in study design, and stakeholder engagement will substantially add to the proposed research, and her experience in bridging the gap between research and policy will be essential to translating our research findings to the policy level.

Research Background and Significance

Relevance of Life Expectancy to PC. LE has become an increasingly important factor in risk stratification and treatment decision making for men with PC, since limited LE predicts lower likelihood of sufficient longevity to benefit from treatment,^{1,5-8} higher morbidity,⁹ and decreased treatment effectiveness.^{10,11} For early-stage PC, men with limited LE are unlikely to benefit from definitive local therapy, since randomized controlled trial evidence has shown that significant survival benefits do not accrue until 8–10 years after treatment.^{12,13} We recently showed that older men with significant comorbidity also have worse quality of life after surgery or radiation,⁹ and even have decreased effectiveness of treatment¹¹ when compared with healthier patients. Regarding the latter, among 140,553 men >65 years with low- and intermediate-risk PC from SEER-Medicare, we found that 10-year absolute risk reduction in cancer mortality due to PC

| Charlson Group | Treatment Type | Cumulative Incidence of PC Mortality at 15 years after Diagnosis (%) | 15-Year Absolute Risk Reduction: Aggressive vs. Nonaggressive Treatment (%) |
|----------------|----------------|--|---|
| Charlson 0 | Aggressive | 6.5 | 6.1 |
| | Nonaggressive | 12.6 | |
| Charlson 1 | Aggressive | 5 | 4.3 |
| | Nonaggressive | 9.3 | |
| Charlson 2 | Aggressive | 4.9 | 3.9 |
| | Nonaggressive | 8.8 | |
| Charlson 3+ | Aggressive | 5.6 | 0.9 |
| | Nonaggressive | 6.5 | |

Table 1. 15-year Absolute Risk Reduction in PC Mortality for Aggressive vs. Nonaggressive Treatment of Early-Stage PC Across Comorbidity Subgroups ([REDACTED]).

treatment decreased with increasing comorbidity at diagnosis: 6.1%, 4.3%, 3.9%, and 0.9% for those with Charlson 0, 1, 2, and 3+, respectively (Table 1).¹¹ Others have found a similar relationship with advancing age.¹⁰ As a result, over the last decade LE has been formally incorporated into guidelines, which recommend against aggressive therapy for men with LE <10 years and very low-, low-, and some intermediate-risk PC.¹⁴

Overtreatment and Undertreatment of PC by Life Expectancy. Despite the strong role of LE in treatment guidelines for PC, patients with limited LE are often overtreated for indolent cancer and undertreated for high-risk cancer. There is strong evidence for overtreatment of indolent tumors in this setting. Among 96,032 men in SEER-Medicare with low- and intermediate-risk PC diagnosed from 1992 to 2007, we found that over half had LE <10 years, and over half of men with LE <10 years were treated aggressively with surgery or radiation (Figure 1).¹⁵ Overtreatment of low-risk PC among men with limited LE has also been confirmed by us and others in the VA and other population-based registries.^{2,16,17} Although not all patients with limited LE should necessarily pursue non-aggressive treatment, we feel that these overwhelming trends suggest that LE is poorly incorporated into treatment decision making.

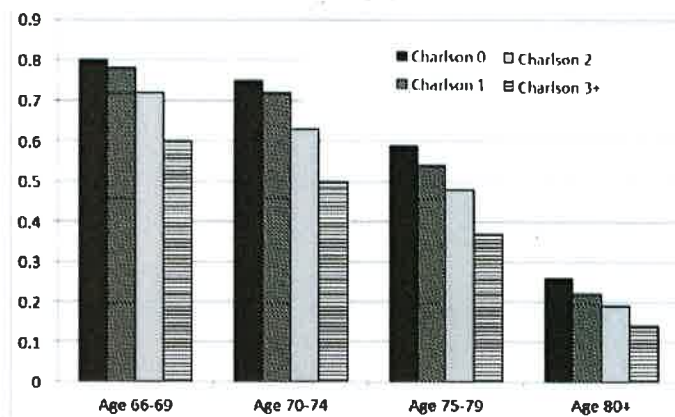


Figure 1. Probability of aggressive treatment by age and comorbidity among men with low/intermediate-risk prostate cancer

Conversely, we and others have also shown that patients with limited LE are often undertreated for high-risk PC. One reason for this is underestimation of LE. In a study of 19,190 men with high-risk non-metastatic PC from the National Prostate Cancer Register of Sweden, men in their seventies with minimal comorbidity were found to receive aggressive treatment only 10% of the time despite a greater than 10-year LE.¹⁸ Another reason for undertreatment is underestimation of mortality associated with high-risk tumors. In contrast to our previous findings in low/intermediate-risk disease, we recently showed that older men with moderate comorbidity derive substantial reductions in cancer mortality from aggressive treatment of high-risk PC as early as 5 years after treatment, suggesting that aggressive treatment of high-risk tumors may be beneficial.¹⁹ In all, these studies suggest that LE is being inappropriately utilized in situations where patients may still stand to benefit from aggressive treatment.

Life Expectancy Prediction Tools. Because LE is a critical and often overlooked consideration in treatment decision making, we and others have created tools for predicting LE that can be used at the point of care. We recently developed an age-adjusted, PC-specific comorbidity index that provides 2-, 5- and 10-year probabilities of death from causes other than PC according to the patient's age and comorbidity (Figure 2).^{3,4} This scale has been internally validated, and work is underway to externally validate it across a nationwide sample of 200,000 men in the VA. We have also operationalized survival data from SEER-Medicare to provide cutoffs defining 10-year LE for men with early-stage PC using claims-based comorbidity assessment.^{15,20} For example, among 96,032 men with low- and intermediate-risk PC, we showed that LE was <10 years for men aged 66–69 with Charlson scores ≥2, for men aged 70–74 with Charlson scores ≥1, and for all men >age 75 regardless of Charlson score. These studies suggest that prediction of other-cause mortality is feasible with data that are available to physicians at the point-of-care.

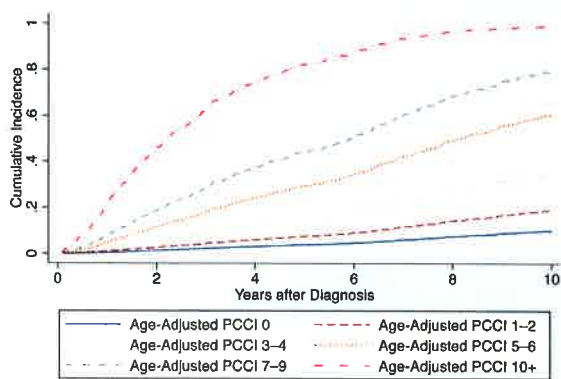


Figure 2. Risk of non-prostate cancer mortality by age-adjusted PCCI Score

Results of Trials of Incorporating Life Expectancy into Treatment Decisions. Despite availability of LE prediction tools, studies of these tools in clinical practice have shown surprisingly little impact on decisional conflict and overtreatment. In a study of 105 men with PC receiving a LE estimate and 123 receiving standard care, 18% of the standard of care group reported significant decisional conflict (>37.5 points on the decisional conflict scale) compared with 10.5% of in the LE counseling group (p=0.08).²¹ However, there was no difference in treatment choice (active surveillance vs. active treatment) and the average difference in decisional conflict was modest and nonsignificant (–2.7 points (95% CI –6.8–1.3)). This study suggests that merely

providing LE estimates to patients does not substantially reduce conflict associated with treatment choice. One potential reason for this is lack of targeting of the intervention to relevant situations: LE information is undoubtedly more impactful if it is targeted to patients with low- and intermediate-risk cancers and limited LE, rather than patients who are healthy and/or have higher-risk disease. Another reason for the relatively low impact of this study is the lack of a patient-centered implementation strategy, which would optimize communication strategies to ensure comprehension by patients.

Barriers to Applying Life Expectancy to Cancer Decision Making. Substantial barriers exist to applying LE data to clinical decision making at the both physician and patient levels, though there is surprisingly little information on this in the setting of cancer decision making. A recent study investigated physician-level barriers to incorporation of LE in non-cancer care of older adults by conducting semi-structured interviews of 28 primary care practitioners (PCPs).²² This study showed that PCPs: often use clinical experience rather than validated tools to measure LE; often consider LE without discussing it with patients; and disagree on whether LE needs to be specifically addressed in discussions. The authors concluded that these barriers need to be addressed in order to improve uptake of LE in clinical decision making. Interestingly, there is little to no information known about what *physician-level* barriers exist to applying LE to cancer care, though cancer diagnoses are uniquely emotional, complicated, and often managed by subspecialists.²³ There is also little data on how *patients with cancer* consider LE in medical decision making.²⁴ Furthermore, beyond the reflections of patients and physicians on past experiences, it is currently unknown how LE is actually discussed in the context of cancer decision making: In which situations is it specifically mentioned? Is it described numerically or in general terms? What is the emotive context? Identifying and addressing barriers to communication of LE from the perspectives of both subspecialists and patients is the goal of **Aims 1 & 2** of this award.

Conjoint Analysis in PC Decision Making. Conjoint analysis (CA), a form of tradeoff analysis that helps consumers make complex decisions, may allow for new insights into how patients value LE in the context of other tradeoffs in PC treatment decision making.²⁵ CA is a computer-based exercise that requires the patient to iteratively choose between competing “treatment profiles” consisting of tradeoffs relevant to the decision at hand, in order to help patients identify how much they value certain attributes of the decision. For example, in clinically localized PC, the competing profiles may list the likelihood of side effects, likelihood of sufficient longevity to benefit from treatment, and projected reduction in cancer morbidity and mortality with treatment (“the attributes”). Using data generated by the choices a patient makes, the tool can determine the relative worth of the different attributes to the patient, which can then be used in shared decision making. CA has been successfully applied in clinically localized PC. Among 48 men with newly diagnosed PC using a PC-specific CA, decisional conflict decreased by 38% ($p < 0.0001$), including significant improvements in value clarity, feeling effective, feeling supported, and reducing uncertainty.²⁶

Crowdsourcing Conjoint Analysis Tools to Understand How Individuals and Populations Value LE. In addition to being an interventional tool, CA also is an excellent platform to study how groups of individuals value certain attributes, namely LE, in the context of competing tradeoffs. By adapting the data from a patient’s CA, one can model the LE at which he or she would choose non-aggressive treatment, based on decisions made in the context of scenarios with differing LE, risk of cancer mortality, and treatment-related side effects. As an extension of this concept, obtaining CA data from diverse *population*—patients and non-patients, physicians and lay community, different sociodemographic groups—allows for assessment of how individual-level factors (sociodemographics, physician vs. non-physician, patient vs. lay community) affect the valuation of LE in cancer decision making. Online crowdsourcing of CA tools—soliciting a large group of participants in the lay community to complete CA exercises via the internet—provides an ideal method for composing such a dataset. This information would enable identification of LE cutoffs in which the majority of individuals prefer a “non-aggressive” treatment profile in the context of typically encountered side effect and treatment benefit tradeoffs. This approach would provide a patient-centered perspective to expert panels creating guidelines around LE, since currently these cutoffs are determined solely by expert opinion. This is the intent of **Aim 3** of our proposal.

Using CA to Target Life Expectancy Interventions. Analysis of crowdsourced CA datasets will also allow us to identify situations where most individuals believe LE should affect decision making, thereby optimizing the utility of the intervention. A small subgroup obviously has limited LE and clearly need to be counseled about their LE. Yet most PC patients are younger or healthier and do not need to be counseled about LE, and there are numerous borderline situations where LE may or may not need to be explicitly discussed. Regression models generated from the CA can determine LE cutoffs where individuals begin to favor non-aggressive treatment, in the context of side effect and treatment benefit profiles typically encountered in PC. These are the situations where LE interventions are most likely to be helpful in shared decision making. Testing whether providing

patient-specific LE data in these situations improves decisional conflict, knowledge, shared decision making between doctors and patients, and reduces overtreatment is the goal of **Aim 4** of our proposal.

Overview: Plan for Research. We will conduct a series of incremental studies with an overarching goal of building a patient-centered approach to integrating LE into PC treatment decision making. **Aim 1** will delineate how LE is currently communicated to patients with PCs through qualitative analysis of treatment consultation transcripts. **Aim 2** is to engage patients with limited LE and urologic oncology specialists through structured interviews to identify opportunities to improve communication information regarding LE. **Aim 3** will use online crowdsourcing of CA to study how individual characteristics affect valuation of LE in the context of other tradeoffs and to identify areas of consensus where LE appears to drive “non-aggressive” treatment choices. **Aim 4** will test whether patient-specific LE estimates—communicated in a patient-centered approach as determined in **Aims 1 & 2** and targeted to specific high-yield situations as identified in **Aim 3**—improve decisional conflict, quality of LE data discussed, and reduce overtreatment in men with newly diagnosed PC.

Approach

Aim 1: To characterize how LE is currently communicated to patients with newly diagnosed prostate cancer through qualitative analysis of treatment consultation discussions

Hypothesis. Current practice in communication of LE will be highly variable in terms of incidence of discussion of LE, mode of communication, emotive context, positioning within discussion, and time devoted.

Approach. We will audio record and transcribe treatment counseling discussions for men with newly diagnosed early-stage PC. We will analyze content of the clinical encounters around LE using qualitative content analysis as well as semi-quantitative metrics such as incidence of discussion; mode of description (numeric, probabilistic, generalization); emotive content (positive, negative, neutral); adjacent contextualizing information (risks of cancer mortality, treatment side effects); position within consultation (beginning, middle, end); and length of time devoted to the topic. This approach has been previously used to study content of healthcare encounters.²⁷

Participants and Inclusion/Exclusion Criteria. We will recruit 30 patients from the [REDACTED] Urologic Oncology clinic with newly diagnosed early-stage prostate cancer. At least one quarter of patients will have LE <10 years. LE will be estimated by claims-based age and comorbidity cutoffs as established in our previous work.^{15,20} In order to identify patients who have dealt with treatment decisions where LE is relevant, we will include men with Gleason scores ≤7. We will exclude men <18 years of age, those with difficulty communicating or dementia, and non-English speakers. In order to obtain diversity in terms of specialists, we will include at least 1 genitourinary oncologist, 1 urologist, and 1 radiation oncologist, with each counseling <5 patients.

Primary Outcomes. We will use ATLAS.ti (Berlin, Scientific Software Development) to extract segments of discussion related to LE using keywords (e.g. “life expectancy,” “survival,” “death,” “dying,” “death from [something else, other causes, cancer]”). Content analysis will be conducted using the constant comparative method.²⁸ We will use an open coding approach to qualitatively assess and label segments of discussion related to LE within each interview, then compare codes: between patients within each disease group, then between patients of different disease groups. This approach maximizes the ability to capture common themes within and between groups. Two investigators will independently code all transcripts; consensus themes and subthemes agreed on by the coders will constitute the final data.

We will then code each segment of discussion for semi-quantitative outcomes, including incidence of discussion of LE; mode of description (e.g. numeric, probabilistic, generalization); emotive content (positive, negative, neutral); adjacent contextualizing information within 100 words (risks of cancer mortality, side effects); position within visit (before history taking, during diagnosis, during counseling, during side effects discussion, at closing); length of time devoted to topic.

Plans for Use of Information. A comparison of current standard practice with best practices as ascertained in **Aim 2** will be crucial to optimizing communication of LE data in our patient-centered intervention in **Aim 4**.

Aim 2: To identify opportunities on how to optimize communication of LE to patients with prostate cancer through structured interviews of patients and specialty physician stakeholders

Hypothesis. Physicians and patients will have different knowledge, beliefs, and attitudes regarding how LE should be integrated into treatment decision making, in particular how numeric LE data should be optimally communicated (e.g. number of years, probability of survival) and if/when it should be routinely discussed.

Approach. We will conduct one-on-one structured interviews of (1) patients with PC and limited LE and (2) specialty physicians (Urologists, Radiation Oncologists, Medical Oncologists) who typically counsel these patients. Specific questions will address anxieties in giving and receiving information on LE, typical sources of information used to estimate LE, opinions on the best mode of communication of LE data, if/when LE should be routinely discussed, and how it should be contextualized (See interview questions in **Appendices 1 and 2**). Structured interviews will be audiotaped and transcribed. Qualitative content analysis using the constant comparative method²⁸ will be used to derive themes and emergent concepts from transcribed interviews. The methods will be informed by prior work by Dr. [REDACTED] who has content expertise with structured interviews.

Participants and Inclusion/Exclusion Criteria. For patient structured interviews: We will recruit 15 patients from the [REDACTED] Oncology clinic with established diagnoses of early-stage PC and LE less than 10 years at the time of diagnosis of their disease, using criteria described in **Aim 1**. Since we intend for patients to reflect on their experiences in the decision making process, we will conduct interviews with patients after they have made treatment decisions. In order to identify patients who have dealt with decisions where LE has been relevant, we will include patients with clinically localized prostate adenocarcinoma with Gleason scores of 7 or less at the time of original diagnosis. We will exclude patients under 18 years of age, those with difficulty communicating or dementia, and non-English speakers.

For physician structured interviews: We will recruit 15 physicians from [REDACTED] who typically counsel and treat patients with PC, including Urologists (5 physicians), Radiation Oncologists (5 physicians), and Medical Oncologists (5 physicians). We will recruit physicians from a variety of practice settings (academic/private). We will limit recruitment to physicians whose practice at least partly consists of genitourinary oncology. The topical guide to be used for the interviews will be based on our team's previous work in benign urology.

Primary Outcomes. We will analyze transcribed audiotapes of interviews using the constant comparative method,²⁸ which is rooted in the grounded theory approach to qualitative analysis. This involves first using an open coding approach to qualitatively assess and label segments of discussion within each interview, then comparing codes: between patient interviews within each disease group, between physician interviews within each specialty, between patient disease groups, between physician specialty groups, then finally between physician and patient groups. This approach maximizes the ability to capture common themes within and between groups. We will have two investigators independently code all transcripts; consensus themes and subthemes agreed on by the coders will constitute the final data.

Plans for Use of Information. Information from the structured interviews will inform development of the implementation approach in **Aim 4**. For example, if patients prefer LE information to be communicated as an average longevity (e.g. "LE of 4 years") or above/below a cutoff value (e.g. "LE below 10 years"), then this is the way that this data will be expressed. Additionally, if there are barriers identified in the structured interviews (e.g. assurance of accuracy of estimates), these issues will be addressed (i.e. provide data on accuracy).

Aim 3: To identify community preferences regarding which LE cutoffs are best suited to nonaggressive treatment in early stage prostate cancer using online crowdsourcing of conjoint analysis tools

Hypotheses. (1) While the impact of LE information on treatment decisions is highly influenced by individual preferences, there may be situations where the vast majority of patients, physicians, and laypeople agree that risks outweigh rewards in the setting of limited LE. Identification of these areas of consensus using crowdsourcing methods will provide a reference for developing guidelines that may differ from expert opinion. (2) Individual-level factors (e.g. sociodemographics, physician vs. patient vs. lay community) may impact how persons value LE in cancer treatment decision making. (3) Crowdsourced data can define the cutoffs at which LE begins to drive non-aggressive treatment decisions, which can help to target LE interventions in our patient-centered implementation approach (**Aim 4**).

Approach. We will use an adaptive choice-based conjoint analysis platform (Sawtooth, North Orem, Utah) to create a conjoint analysis tool specific to tradeoffs encountered in PC. Conjoint tools will test the key attributes comprising a cancer treatment choice: (1) Risk of non-cancer mortality (LE); (2) Risk of cancer mortality; (3) Risk of cancer metastasis; and (4) Risks of side effects of treatment. CA tools will be administered to patients, physicians, and a large group of individuals in the lay community using online crowdsourcing. The CA will require participants to iteratively choose between scenarios that represent "aggressive" treatment (i.e. lower cancer mortality but treatment-related side effects) and "non-aggressive" treatment (i.e. higher risk of cancer spread and mortality but no side effects) in the context of varying LEs. We will use multilevel random

coefficient logistic regression analysis to evaluate how LE is valued (i.e. “part-worth utility”) by patients, physicians, and the community in the context of varying tradeoffs. Models will also identify LE cutoffs where some (>25%) or most (>50%) patients, physicians, and community members choose “non-aggressive” treatment in the context of commonly encountered tradeoffs. This information will help target scenarios where LE is relevant for implementation (**Aim 4**).

Participants and Inclusion/Exclusion Criteria. We will enroll 50 patients from [REDACTED] with early-stage PC to complete the CA exercise. We will also recruit 50 physicians (of any specialty) from [REDACTED] to complete the CA. We will then partner with Cint®, a survey research firm with access to multiple research survey panels across the US comprising 1 million individuals, to capture 30,000 community members to complete the CA exercise.

Primary Outcome. Predicted probability of “aggressive” vs. “non-aggressive treatment” choice associated with various life expectancies across tradeoffs (e.g. risk of cancer death, risk of treatment side effects) that are commonly encountered in early-stage PC. **Secondary Outcomes.** (1) Interactions of sociodemographic/tumor risk factors with LE in predicting treatment choice. (2) Predicted probability of aggressive treatment associated with LE among patient/physician/community subgroups.

Statistical Analysis. Regression Model. We will create a multilevel random coefficients model (accounting for non-independence of repeated testing of subject) predicting aggressive vs. non-aggressive treatment choice using data from the conjoint exercises. The primary predictor in our models will be LE. Covariates will include: sociodemographic characteristics; absolute risk reduction in cancer mortality (difference in cancer mortality from selected conjoint option to unselected conjoint option); absolute risk reduction in metastasis (difference in metastasis from selected conjoint option to unselected option); absolute risk reduction in side effects (difference in individual side effect from selected conjoint option to unselected option), the baseline value for side effects, cancer mortality, and metastatic risk. Similar methods have been used previously by Dr. [REDACTED]’s team.

Analysis of Primary Outcome. We will conduct this analysis in parallel for patient, physician, and crowdsourced datasets. (1) We will determine how much a given LE is “worth” by describing the equivalent tradeoff patients, physicians, and laypeople are willing to make in terms of absolute risk reduction in cancer mortality, absolute risk reduction in metastasis, or absolute difference in side effects of treatment. (2) We will determine the LE cutoffs in which patients, physicians, and laypeople choose “non-aggressive treatment” with probabilities of >25%, >50%, and >75% while holding other tradeoffs within the range typically expected for PC. (3) We will use regression models to identify LE cutoffs where probability of non-aggressive treatment among patients, physicians, and laypeople is >25%. These “LE-relevant” situations will be targeted in **Aim 4**.

Analysis of Secondary Outcomes. We will compare model coefficients for LE across patient, physician, and crowdsourced datasets using postestimation Wald tests to determine if the impact of LE on treatment choice varies between groups. In order to characterize the impact of sociodemographics and tumor variables on how LE is valued in decision making, we will determine the interactions between LE and sociodemographic and tumor risk factors in predicting treatment choice.

Plans for Use of Information. Defining the LE cutoffs at which patient/physician/community stakeholders begin to choose non-aggressive treatment options in the context of commonly encountered tradeoffs will allow for targeting of our LE intervention to situations where it is likely to have the greatest impact in **Aim 4**.

Aim 4: To determine if a patient-centered, targeted approach to communication of LE improves decisional conflict, quality of LE discussion, and reduces rates of overtreatment of patients with prostate cancer and limited LE

Hypothesis. Patient-centered communication of LE in situations in which crowdsourcing data suggests that LE is relevant will reduce decisional conflict, improve quality of LE data discussed, and reduce overtreatment.

Approach. We will conduct a pilot randomized trial of provision of patient-specific LE estimates to men with newly diagnosed early stage PC in situations identified to be “LE-relevant” by crowdsourcing from **Aim 3**. In addition to patient-specific LE estimates, we will provide “talking points” to physicians on how to communicate LE data based on identification of best practices from **Aims 1 & 2**. We compare decisional conflict, quality of LE discussion, and rates of overtreatment between the intervention and standard-of-care arms.

Participants and Inclusion/Exclusion Criteria. We will recruit 15 [REDACTED] patients with newly diagnosed early-stage PC identified as having LE-relevant scenarios by crowdsourcing in **Aim 3**. LE estimates will be

determined using claims-based definitions. We will exclude men <18 years, those with difficulty communicating, and non-English speakers.

Randomization Strategy. Our intervention will be randomized at the level of the patient, among those determined to have LE-relevant scenarios by crowdsourcing. Randomization of patients will help to balance study arms in terms of measured and unmeasured patient characteristics. However, this strategy does not account for possible imbalance in the numbers of subjects in each study arm assigned to each counseling physician. This bias will be mitigated by use of a multilevel statistical model adjusting for physician identity.

Primary Outcomes. Decisional conflict as measured by decisional conflict score (DCS). **Secondary Outcomes.** (1) Qualitative content of outpatient treatment consultation. (2) Odds of aggressive vs. non-aggressive treatment.

Statistical Analysis. Decisional Conflict. We will employ a validated decisional conflict scale to estimate uncertainty associated with treatment choice (See Appendices 3 & 4). Effect sizes of 0.3 to 0.4 are considered meaningful. To determine difference in decisional conflict score between those exposed to the intervention vs. those not exposed within physician clusters, we will use a multilevel random coefficients model. We will use multivariate linear regressions predicting DCS score within physician clusters as our fixed (level I) equations. The primary predictor will be the intervention. The level II equations will predict β estimates for our primary predictor (the LE intervention) from our level I equation, using physician cluster as the primary predictor.

Quality of LE Discussion. We will audiotape and transcribe outpatient treatment counseling visits for both subjects receiving the intervention and those randomized to no intervention. We will analyze the transcripts to determine: if LE is mentioned; the proportion of time it is discussed; how LE is characterized by the physician (probability, numeric, generalization); how many questions patients ask about LE. We will also use the ATLAS.ti co-occurrence tool to examine emotional context surrounding LE. We will use multilevel random coefficients models as detailed above to compare these semi-quantitative outcomes between intervention arms.

Treatment Choice. For the treatment choice outcome, we will again use a multilevel random coefficients model (with physician identity as the level II variable) to determine the average impact of the intervention on aggressive versus non-aggressive treatment choice within physician clusters. Random effects for both intercept and slope for our primary regressor within physician clusters will again be calculated. A secondary analysis will investigate congruence of treatment choice with NCCN guidelines, using the same model structure.

Power Calculation. Since this is a pilot study, we will initially aim to enroll 15 patients in order to provide proof of concept. Pilot data from this study will provide the foundation an R01 grant application, which would permit a multicenter trial with adequate funding and recruitment pool to robustly test hypotheses. As a reference, in order to detect a clinically meaningful difference (0.5) in DCS between patients in the intervention arm vs. control, we would require 68 patients per group to be powered at 80% with a two-tailed type I error of 0.05.

Anticipated Limitations. One expected limitation will be length of time needed to accrue an adequate number of subjects, since our target population of older, sicker patients is underrepresented. However, based on our clinic characteristics (total annual volume ~200 patients, with approximately 15% eligible), we believe that accrual period for a 15-patient study would be approximately 1 year, assuming a 50% screen failure rate.

PCRP Overarching Challenges. This proposal directly aligns with the PCRP overarching challenge to develop strategies to optimize the physical health of men with prostate cancer. In developing a pragmatic approach to improve the integration of LE into decision making, we hope to reduce the overtreatment of men with limited LE for low- and intermediate-risk PC. We have previously shown that treatment of men with limited LE with surgery and radiation therapy is not only unnecessary but also results in worse treatment-related morbidity compared with healthier men. Therefore efforts to reduce overtreatment are critical to reducing harm and improving overall health of these men.

PCRP Focus Areas. Our proposal addresses two of the PCRP focus areas. We will address population science by using crowdsourced, population-based conjoint analysis data to determine how the community values LE in the context of other tradeoffs typically encountered in PC decision making. Crowdsourced data will also be used to target our LE intervention to clinical scenarios where patients believe this information is relevant. We will also address survivorship, including psychosocial impact on the patient and family by identifying ways to optimize communication of LE to patients and their families during treatment counseling discussions, with the ultimate goal of reducing decisional conflict and stress around treatment choice.

Appendix 1: Physician Structured Interview

Can you recount a situation when you incorporated life expectancy into a treatment decision making discussion for a patient with prostate cancer? Kidney cancer? Bladder cancer? Can you tell me about that experience? What went well? What could have been improved?

Beliefs

1. How do you feel about communicating information regarding life expectancy to patients with newly diagnosed genitourinary malignancy?
2. What are your greatest anxieties about communicating this information?
3. Are there other barriers to communicating this information?
4. Do barriers to communication vary with regard to certain disease types or patient types?
5. Should life expectancy always be discussed with patients? In what situations should it be discussed?

Knowledge

1. What is life expectancy?
2. What sources of information do you use to estimate life expectancy?
3. If you don't use life tables or nomograms to estimate life expectancy, what criteria do you generally use?
4. What form of life expectancy data to prefer to use – average longevity in years, probability that patient will live to a certain timepoint, or another method?
5. How do you account for variability in life expectancy estimates for the individual patient?
6. What other data would you like to characterize life expectancy, if any?

Communication

1. How do you typically communicate information on life expectancy to your patients with newly diagnosed cancer (percentage, average longevity, generalization)?
2. Do any patient factors affect how you communicate this information?
3. Do any disease-related factors affect how you communicate this information?
4. Do you routinely pair information on life expectancy with any other information? If so, what information?
5. When do you typically discuss life expectancy during the visit?

Decision Making

1. How does life expectancy affect your decision making for low-risk prostate cancer? Intermediate-risk prostate cancer? High-risk prostate cancer? Advanced prostate cancer?
2. How does life expectancy affect your decision making for non-muscle-invasive bladder cancer? Muscle-invasive bladder cancer? Advanced bladder cancer?
3. How does life expectancy affect your decision making for small renal masses? Larger renal masses? Advanced kidney cancer?
4. Patients often ask what the physician would do if he or she were in the same situation. What do you tell patients?
5. Patients often ask what other patients would do in a similar situation. What do you tell patients?
6. If you had information on what other patients or physicians would do in a similar situation, would you give it to patients?

Appendix 2: Patient Structured Interview

When you were diagnosed with cancer, did your physician discuss your life expectancy? Can you tell me about that experience?

Beliefs

1. How do you feel about hearing information regarding your own life expectancy when considering treatment of your cancer?
2. What are your greatest anxieties about hearing this information?
3. Are there other barriers to you hearing this information?
4. Have any other doctors discussed your life expectancy with you with regard to medical decision making? Is it a similar experience in all situations or different when it's cancer?
5. Do you believe that life expectancy should be discussed with cancer patients? Why or why not?

Knowledge

1. What is life expectancy?
2. Have you ever sought information on your own life expectancy? What information source did you use?
3. What form of life expectancy data do you prefer to use when you seek it on your own – average longevity in years, probability that you will live to a certain timepoint, or another method?
4. What other data would you like to characterize life expectancy, if any?

Communication

1. How have doctors typically communicated information on life expectancy to you (percentage, average longevity, generalization)?
2. How did your cancer doctors communicate this information to you?
3. How would you prefer for your cancer doctors to communicate life expectancy information to you?
4. Was other data on your cancer prognosis presented to you? Any information on side effects of treatment? How was this information presented?
5. How did your doctor explain the expected benefit of treatment?

Decision Making

1. Did your life expectancy affect the way you made decisions regarding your cancer? How so?
2. Regarding which treatment to choose, did you seek out information on what other patients have done in similar situations?
3. Regarding which treatment to choose, did you seek information on what your doctor would do if it were he or she in the same situation? How did you use that information?
4. If you had information on what other patients or physicians would do in a similar situation, would you use it? How?

Appendix 3: Decisional Conflict Scale

When considering whether or not to pursue aggressive treatment [will list relevant aggressive treatment options for each cancer type] vs. non-aggressive treatment [will list relevant non-aggressive treatment options for each cancer type]:

| | Strongly Agree | Agree | Neither Agree Or Disagree | Disagree | Strongly Disagree |
|---|--------------------------|--------------------------|----------------------------------|--------------------------|--------------------------|
| | [0] | [1] | [2] | [3] | [4] |
| 1. I know which options are available to me. | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> |
| 2. I know the benefits of each option. | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> |
| 3. I know the risks and side effects of each option. | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> |
| 4. I am clear about which benefits matter most to me. | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> |
| 5. I am clear about which risks and side effects matter most. | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> |
| 6. I am clear about which is more important to me (the benefits or the risks and side effects). | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> |
| 7. I have enough support from others to make a choice. | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> |
| 8. I am choosing without pressure from others. | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> |
| 9. I have enough advice to make a choice. | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> |
| 10. I am clear about the best choice for me. | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> |
| 11. I feel sure about what to choose. | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> |
| 12. This decision is easy for me to make. | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> |
| 13. I feel I have made an informed choice. | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> |
| 14. My decision shows what is important to me. | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> |
| 15. I expect to stick with my decision. | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> |
| 16. I am satisfied with my decision. | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> |

Decisional Conflict Scale

Appendix 4: Decisional Conflict Scale Scoring Manual

Items are given a score value of:

0 = 'strongly agree'; 1 = 'agree'; 2 = 'neither agree nor disagree'; 3 = 'disagree'; 4 = 'strongly disagree'.

TOTAL SCORE

16 items [items 1-16 inclusive] are: a) summed; b) divided by 16; and c) multiplied by 25.

Scores range from 0 [no decisional conflict] to 100 [extremely high decisional conflict].

UNCERTAINTY SUBSCORE

3 items [10, 11, 12] are: a) summed; b) divided by 3; and c) multiplied by 25.

Scores range from 0 [feels extremely certain about best choice] to 100 [feels extremely uncertain about best choice].

INFORMED SUBSCORE

3 items [1, 2, 3] are: a) summed; b) divided by 3; and c) multiplied by 25.

Scores range from 0 [feels extremely informed] to 100 [feels extremely uninformed].

VALUES CLARITY SUBSCORE

3 items [4, 5, 6] are: a) summed; b) divided by 3; and c) multiplied by 25.

Scores range from 0 [feels extremely clear about personal values for benefits & risks/side effects] to 100 [feels extremely unclear about personal values]

SUPPORT SUBSCORE

3 items [7, 8, 9] are: a) summed; b) divided by 3; and c) multiplied by 25.

Scores range from 0 [feels extremely supported in decision making] to 100 [feels extremely unsupported in decision making].

EFFECTIVE DECISION SUBSCORE

4 items [13, 14, 15, 16] are: a) summed; b) divided by 4; and c) multiplied by 25.

Scores range from 0 [good decision] to 100 [bad decision].

NOTE: The Cochrane systematic review of trials of patient decision aids [3] uses this scoring method; other papers may present scores ranging from 1 [low decisional conflict] to 5 [high decisional conflict]. If the SCORE or SUBSCORE is reported as a percentage or on a 0-100 scale no conversion is required.

If the average SCORE or SUBSCORE is reported on the 1-5 scale then to convert these scores to the equivalent 0-100 scale: a) subtract 1 from the score; b) then multiply by 25.

If the SCORE or SUBSCORE is reported as a sum of items that used the 1-5 scale then to convert these scores to the equivalent 0-100 scale: a) divide the score by the number of items summed; b) then subtract 1; c) then multiple by 25.

~~Source: <http://www.jco.org/jco/article/doi/10.1200/JCO.2007.11.1111>~~

FACILITIES AND OTHER RESOURCES

Overview. [REDACTED] is a full-service, acute tertiary care hospital and the largest nonprofit hospital in the western United States. [REDACTED] is located on a 24-acre site, which includes a 1.6 million sq. ft. main complex and 12 other structures, for a total of more than 4.1 million sq. ft. This tertiary care facility contains over 800 beds for Internal Medicine, Obstetrics, Gynecology, Pediatrics and Surgery; a 28-bed rehabilitation and outpatient surgery unit; a 29-bed Skilled Nursing and Assessment Unit; and the 150-bed [REDACTED] Critical Care [REDACTED]. [REDACTED] is a major teaching facility of the [REDACTED] School of Medicine, with over 240 full-time faculty members in ten different departments. [REDACTED] has its own professorial series, and most attending physicians at [REDACTED] participate in the training of medical students, interns, residents and fellows. Translational and clinical research at [REDACTED] falls under the purview and oversight of the [REDACTED] Institute, ranked among the top ten non-university biomedical research institutions in the nation in terms of funding from the NIH. It includes over 270,000 square feet of laboratory and laboratory support space. Core facilities are shared resources servicing the entire [REDACTED] community.

Scientific Environment. As the largest non-profit academic medical center in the western United States and the largest teaching hospital affiliated with the [REDACTED] School of Medicine at [REDACTED], boasts a world-renowned faculty and over 60 competitive graduate medical education programs for more than 350 residents and fellows. A major expansion of research infrastructure has increased wet lab space to 400,000 sq. ft. with the completion of the new [REDACTED] housing clinical programs, research laboratories, and expanded vivarium space. In addition, intensive recruitment efforts during the last five years have added over two dozen outstanding faculty investigators, whose research spans from molecular genetics to outcomes research. *Over 1370 projects involve more than 310 principal investigators. Federal funding for research at [REDACTED] has increased by more than 14% per year over the last decade. As of early January 2012 there were 1034 active IRB-approved clinical studies conducted by more than 286 PIs. Since 2006, clinical research has shown robust growth with a 9% average annual increase in clinical trials.*

The PI has recently been accepted as a candidate member of [REDACTED] in the Cancer Prevention and Genetics group. [REDACTED] a rapidly growing part of the [REDACTED] and supports much of the research infrastructure for cancer related studies. [REDACTED] is a 72,000 sq. ft. state-of-the-art clinical facility that pioneered the concept of complete cancer services within one location over 25 years ago. Services are provided to over 9,000 patients per year and include diagnostic evaluation, multidisciplinary treatment teams, genetic cancer risk assessment, cancer prevention, and hospice care.

Department of Surgery. Highly-trained surgeons perform approximately 32,000 surgeries in 42 operating rooms at [REDACTED] each year. More than one-third of all surgeries are now performed as minimally invasive procedures, in which physicians use the most advanced technology available for laparoscopic and robotic surgery. Over the last 15 years, we have added a larger nucleus of full-time academic surgeons and surgical scientists to *augment cutting-edge translational research*. This combination has enriched our surgical residency and fellowship programs and brought the most complex surgical services to our campus.

Division of Urology. The [REDACTED] Division of Urology is comprised of 45 urologists, of which 12 are faculty members affiliated with the urology residency program. The Division conducts more than 300 major urologic oncology cases per year. The urology faculty has representation in all major urological subspecialties, including pediatrics, oncology, female/reconstructive urology, infertility, endourology, and transgender medicine. The urology faculty holds \$3.6M in NIH funded research. The urology residency program is a 5-year program including 10 categorical residents in urology.

Outcomes Research and Education [REDACTED]. The [REDACTED] is a group of academicians (3 faculty members, 1 PhD epidemiologist, 5 masters-level research staff, 3 research assistants, and 2 administrators) that supports high quality health services research. The [REDACTED] is housed in an office suite occupying 2400 square feet on the [REDACTED] Medical Campus. Members of the center have published extensively in healthcare quality, patient-reported outcome (PRO) development, healthcare decision making, health economics and digital health sciences. Members of [REDACTED] routinely interact with social scientists, epidemiologists, health economists, psychometricians, biostatisticians, computer scientists, engineers and physicians focused in multiple medical specialties. [REDACTED] scientists work closely across [REDACTED] and partner with a vibrant external community of university, government and healthcare institutions. This model positions [REDACTED] to carry out high-level research in an efficient and effective manner across a broad

spectrum of topics. Most recently, faculty members of the [REDACTED] have obtained grant funding from PCORI and two grants from the statewide [REDACTED] Precision Medicine.

Office And Laboratory At [REDACTED] Dr. [REDACTED] office is located in the [REDACTED] Outcomes Research and Education [REDACTED], a suite of office space that houses health services researchers within the institution. [REDACTED] occupies 2400 square feet in the [REDACTED] Building at [REDACTED] is hard-wired to the [REDACTED] T1 internet connection and includes a video conference room, shared work spaces for staff and students, and private offices for the Director, Deputy Director, and Key Collaborators. Dr. [REDACTED] private office contains a high speed iMac equipped with STATA statistical software, color printer, and access to scanner and high-volume copier. Dr. [REDACTED] clinical research coordinator, [REDACTED] MPH, also has an office within the suite, which is equipped with a high speed Dell computer loaded with STATA statistical software, ATLAS.ti, Microsoft Office, and web-based access to RedCap database management software. [REDACTED] video conferencing room includes a plasma screen monitor, whiteboards and boardroom table and chairs. [REDACTED] uses 10 computers as well as 10 executive-style desks and chairs, five large file cabinets, five large bookshelves, several tables and chairs, and two high-capacity multi-function printers/scanners/fax machines. [REDACTED] also maintains multiple active licenses for research software programs, including SAS, Stata, Sawtooth, TreeAge, Microsoft Office Suite, EndNote, ATLAS.ti, and several graphic design programs.

[REDACTED] Biostatistics And Bioinformatics [REDACTED] The Biostatistics and Bioinformatics [REDACTED] is a shared institutional resource for biostatistical consulting and related methodological research. It is recognized as a critical component of the research infrastructure at [REDACTED] and serves as a focal point from which institute investigators may draw statistical expertise for planning, management and analysis of their studies. Services are provided free of charge for faculty members actively working on grant-related research and working on research that will build towards grant funding. Types of research supported include clinical trials, translational research, epidemiology, and behavioral and basic science. In addition to this core institutional support, Dr. [REDACTED] employs a part-time biostatistician (0.4FTE), [REDACTED] MPH, for dedicated time to his research projects, who is housed within the biostatistics core.

Faculty and Staff at the Biostatistics and Bioinformatics [REDACTED] (1) Coordinate and manage statistical activities at [REDACTED] to ensure that investigators have ready access to statistical consultation and support; (2) Provide statistical expertise in the design of experiments and studies, including research proposal development, sample size determination, randomization procedures and plans for interim reviews and final analysis; (3) Assist with the writing of statistical components of manuscripts; (4) Review the integrity and statistical soundness of all studies involving human subjects; (5) Provide statistical analysis for projects using appropriate statistical and computing methodologies and assist in the interpretation and presentation of results; (6) Interact and collaborate with the Clinical Research Office in the development of protocols and the monitoring and reporting of clinical data; (7) Maintain a computing facility with up-to-date software for statistical analysis to support program project investigators; and (8) Conduct biostatistical and bioinformatics methodology research on practical problems arising in basic science population and clinical studies.

Clinical Research Office (CRO). The Clinical Research Office (CRO) has overall responsibility for the performance of cancer trials and other cancer research studies at the [REDACTED] and provides support and services to faculty and staff conducting oncology research.

The CRO provides the following support for cancer clinical trials: (1) Facilitate, direct and coordinate cancer clinical research within the [REDACTED] (2) Serve as a single point of contact for research-related resources including protocol design, forms development, data coordination, and regulatory compliance; (3) Support clinical research by providing a centralized, specifically trained, and supervised core staff of clinical research nurses, research coordinators, regulatory specialists, and data managers; (4) Develop and maintain an active study portfolio accessible by all [REDACTED] cancer researchers and staff; (5) Assist in protocol review and assess the feasibility of clinical research studies within [REDACTED]; (6) Serve as a central resource for clinical research education, training and staff development for physicians and research staff; (7) Develop and promulgate standards, policies, and procedures for the proper conduct of clinical research in accordance with ethical standards and in compliance with federal, state, and local regulatory requirements; (8) Provide assistance and advice to investigators regarding FDA documentation including submission of IND applications; provide support for IRB submissions and communications; (9) Facilitate clinical research by interfacing with research pharmacy, laboratory, pathology, medical records, budgeting, grants and contracts offices, and with external research sponsors; and (9) Collaborate with research informatics faculty and staff to provide standard databases for research; data management tools; and monitoring of study accrual.

BUDGET JUSTIFICATION

Key Personnel

[REDACTED], M.D., M.S.H.P.M., Principal Investigator (50% effort, 6 calendar months). Dr. [REDACTED] is the Director of Health Services Research for the Department of Surgery and Assistant Professor of Surgery in the Division of Urology. Dr. [REDACTED] is a clinical Urologic Oncologist and his research is focused on the integration of life expectancy into decision making for patients with genitourinary cancer. As the Principal Investigator of this career development award, Dr. [REDACTED] will draft and submit the IRB application, ensure regulatory compliance, conduct stakeholder interviews, obtain consent from participants, direct the research team in collecting key data, develop data collection databases, clean the datasets, direct the research team in development of statistical models and interpretation of data, draft manuscripts, submit manuscripts for publication, present research at scientific meetings, and draft research updates for the funding agency.

[REDACTED], M.D., Primary Mentor. Dr. [REDACTED] is the [REDACTED] Prostate Cancer and a Professor of Urology at [REDACTED]. Dr. [REDACTED] is a clinical Urological Oncologist and an expert in the treatment of prostate cancer. He is also an expert in risk stratification in prostate cancer and has published extensively on the role of PSA kinetics on prostate cancer outcomes. As the co-primary mentor, he will provide mentorship for Dr. [REDACTED] career development as a urologic oncologist and surgeon-scientist, including weekly meetings, guidance for directed readings, collaborative research, and help building national collaborations in the field. In terms of the execution of the proposed research, he will supervise and participate in study design, data analysis, interpretation, and writing and editing of publications.

[REDACTED], M.D., M.S.H.S., Secondary Mentor. Dr. [REDACTED] is the [REDACTED] Health Services Research and Director of the [REDACTED] Outcomes Research and Education [REDACTED]. He is also Professor of Medicine and Public Health [REDACTED]. As the co-primary mentor, [REDACTED] will provide mentorship for Dr. [REDACTED] career development as a health services researcher, including weekly meetings, guidance for directed readings, collaborative research, and further development of speaking and writing skills. He will also provide content expertise in the areas of qualitative analysis, digital health, and conjoint analysis. In terms of the execution of the proposed research, he will supervise and participate in study design, analysis of results, interpretation, and writing and editing of publications.

[REDACTED], M.D., Collaborator. Dr. [REDACTED] is the [REDACTED] Professor of Internal Medicine and co-Director of the Health Policy Research Institute at the [REDACTED]. He is a widely published health services researcher, a member of the Institute of Medicine, and a previous Chair of an IOM subcommittee on Cancer Survivorship. As co-mentor on this grant, he will mentor Dr. [REDACTED] in the content areas of health services research, life expectancy and clinical management of patients with urologic malignancy, and help to develop Dr. [REDACTED]'s national connections within health services research. In terms of the execution of the proposed research, he will participate in study design, analysis of results, interpretation, and editing of publications.

[REDACTED], M.D., M.P.H., Collaborator. [REDACTED] is an NIH-funded urologist who is board-certified in Female Pelvic Medicine and Reconstructive Surgery. [REDACTED] is the Associate Director of Urological Research at [REDACTED], and [REDACTED] Associate Professor of Surgery. In November of 2010 she moved to [REDACTED], where [REDACTED] holds a faculty appointment and close collaborations. For this career development award, [REDACTED] will provide expertise in conducting structured interviews and qualitative data analysis. She will also mentor Dr. [REDACTED] in transitioning to achieving academic independence through independent publications and his future application for R-level NIH funding. In terms of the execution of the proposed research, she will participate in study design, analysis of results, interpretation, and editing of publications.

[REDACTED], M.D., Collaborator. [REDACTED] is an Associate Professor of Medicine and the Director of General Internal Medicine at [REDACTED] who has a research interest in the clinical

and financial implications of improving the quality and safety of healthcare. She is currently PI of an AHRQ R01 that examines the effects of the Medicare Hospital Readmissions Reduction Program on clinical outcomes among seniors hospitalized with heart failure. As co-mentor on the career development award, Dr. [REDACTED] will provide expertise in stakeholder engagement, health services research, and health policy. She will also mentor Dr. [REDACTED] in his transition to becoming an independent federally funded health services researcher. In terms of the execution of the proposed research, she will participate in study design, analysis of results, interpretation, and editing of publications.

Non-Key Personnel

Clinical Research Coordinator (42% effort, 5 calendar months) A Clinical Research Associate working with Dr. [REDACTED] will oversee the project and function as the database manager for the research team. The responsibilities will include overseeing study recruitment, facilitating and maintaining records for informed consent, facilitating transcription of stakeholder interviews and treatment consultation visits, overseeing the biostatistician for database development, maintaining a study database, interfacing with the biostatistician to design data queries. He/she will ensure that the data collected in the prospective patient accrual study is entered properly into a HIPAA-compliant database. As needed, he/she will assist with chart abstraction. A portion of the [REDACTED] salary and fringe will be supported by the institution.

Biostatistician (10% effort, 1.2 calendar months) The biostatistician will perform technical biostatistics programming to address statistical needs throughout the course of the award. This will include development of statistical models: to assess semi-quantitative qualitative outcomes from treatment consultation transcripts; to analyze conjoint analysis data; and to analyze comparative outcomes from the pilot trial. This individual will assist in maintaining and cleaning the research database. He/she will use statistical software such as SAS, STATA, and R to execute analyses and maintain documentation of statistical coding to support further research. This individual will report data to the research team and provide support in interpretation of the statistical results as well as write technical reports of statistical analyses for IRB submission and publication. A portion of the biostatistician's salary and fringe will be supported by the institution

Fringe benefits are calculated at [REDACTED] negotiated rate of 26.90%, effective as of July 1, 2016.

Other Direct Costs

Travel: Travel expenses for meeting presentations will be cost-shared by the Department of Surgery.

Cint - Cint crowdsourcing services is required to execute online crowdsourcing of conjoint analysis, which is detailed in Aim 3 of the proposal. Cost for this service depends on volume and specificity of the surveyed population and is projected to cost ~\$40,000. [REDACTED] has agreed to cover these associated costs to ensure that the PI has all of the necessary resources to execute the outlined goals/aims of this project.

Indirect costs are fixed at 75.00% of direct costs, effective as of July 1, 2016.

[REDACTED]

August 4, 2017

Dear Department of Defense Physician Research Award Review Committee:

It is with great enthusiasm that I write this letter of support for Dr. [REDACTED], Assistant Professor of Surgery in the Division of Urology. Dr. [REDACTED] is a model for a young surgeon-scientist and a rising star within our Department. I believe he is an excellent candidate for the DoD Physician Research Career Development Award. I have read his proposal "A Patient-Centered Approach to Integration of Life Expectancy into Prostate Cancer Treatment Decision Making" with great interest, and I feel that Dr. [REDACTED] is ideally suited to conduct this research project in the Department of Surgery at [REDACTED]. This project will capitalize on Dr. [REDACTED] expertise in health services research and his interest in life expectancy and prostate cancer treatment decision making, an area in which he has published widely. Furthermore, Dr. [REDACTED] mentorship team is superb and well-suited to the intended research and Dr. [REDACTED] career development. I helped to recruit Dr. [REDACTED] who is an incredibly productive outcomes researcher in prostate cancer and has been a mentor to many young faculty members throughout his career; he will certainly be an excellent guiding force for Dr. [REDACTED] within the field of urologic oncology. [REDACTED] also convened a fantastic translational health services research faculty across numerous departments, including Drs. [REDACTED] and [REDACTED], and I am delighted that they have committed to mentoring Dr. [REDACTED] in his early academic career as a health services researcher. Pairing Dr. [REDACTED] ambition and intellect with this remarkable team will undoubtedly result in a highly productive collaboration.

Dr. [REDACTED] has the Departmental support for the necessary infrastructure for this project, including guaranteed 50% protected research time for the duration of this award as well as research staff, including a biostatistician and research coordinator. The Department will gladly cover any excess funding for his career development project that is not covered by this award. His health services research lab is located within the [REDACTED] Outcomes Research and Education, a suite of offices that houses all of the health services researchers within the institution; this allows for informal sharing of ideas as well as use of shared resources such as a state-of-the-art conference room with video conferencing capability, high-speed computers in each office, and licensed biostatistics software packages. Dr. [REDACTED] also has access to the biostatistics core, where his biostatistician is housed, that also has PhD-level biostatistics support available should he need it. The entirety of this departmental infrastructure and other core support services will be available to Dr. [REDACTED] to conduct this research project.

In summary, Dr. [REDACTED] is ideally suited for this type of research, he has full and uninterrupted use of world-class facilities and core support services and has our continued full Departmental and Institutional support for this application.

Sincerely,

[REDACTED]

Surgeon-in Chief

[REDACTED] Chair in Surgery
Chair, Department of Surgery

[REDACTED]

[REDACTED]

[REDACTED]

Dear Committee Members:

It is my utmost pleasure to write this letter of support for [REDACTED], MD, who is applying for a Department of Defense Prostate Cancer Physician Research Career Development Award. I am writing this letter in the capacity of co-primary career and research mentor for [REDACTED]. In short, I believe [REDACTED] has a unique constellation of skills that will propel him to become a national leader in health services research and an innovator across surgical disciplines, and I believe he is a superb candidate for this award. I say this having written many letters for CDA applicants, and having chaired the American College of Gastroenterology (ACG) research committee for over 9 years where I oversaw a CDA funding mechanism. It is with this background that I can definitively say that [REDACTED] application is truly exceptional across all three areas: the science, the mentorship, and the candidate himself.

[REDACTED] has been a part of the [REDACTED] Outcomes Research [REDACTED]—a twenty-member, multi-disciplinary health services research lab that I direct—since he joined the [REDACTED] faculty in [REDACTED]. I first met him when he was a student in my class at the [REDACTED] School of Public Health, where I teach cost-effectiveness analytics. He caught my attention as having a bright, eager and creative mind and I was thrilled when we were able to recruit [REDACTED] to our faculty and to work with our [REDACTED] team. [REDACTED] is the sole surgeon in our group and directs a number of original, innovative projects in collaboration with our team. His office is located in our suite two doors from mine, and he is adjacent to two similarly talented health services research junior faculty who are supported by national career development awards. I already gladly serve as [REDACTED] health services research mentor; we have weekly mentoring meetings to discuss research and career development, and we also talk several times a week more informally. I have helped [REDACTED] to begin building a research team; we have co-hired a clinical research coordinator and programmer to work on projects of mutual interest. Although I am a gastroenterologist, I have found our mentor-mentee relationship to be seamless and refreshing; in fact, I find that our dissimilarity in specialty allows us to focus on broader concepts that affect the entire field of medicine and pushes us to take a fresh look at issues within our own specialties.

[REDACTED] is a broadly talented individual who has the intellect, ambition, personal skills, and curiosity to become an academic leader. As testament to these traits: I recall on his first day on faculty at [REDACTED]. I gave an introductory talk to all of the new hires across the institution, which he attended. After the talk, he asked to briefly meet, and he articulated his vision for extending the reach of our health service research group to the Department of Surgery, which included plans to establish a multi-specialty surgical quality collaborative, develop digital health apps for monitoring patient reported outcomes (PROs), and to initiate surgical trials of wearable biosensor technology across the Department. A year and a half later, he has seen this vision through to reality: he convened 10 members of the surgical faculty to help create a surgical quality collaborative across the Department, providing annual individualized reports for [REDACTED] surgeons on their cost and quality of care; he is working with a programmer on our team to develop a patient-facing app to monitor of PROs in urology; and he is directing a pilot trial of wearable biosensors to monitor postoperative ambulation across 8 surgical divisions. [REDACTED] is someone who can efficiently accomplish what he sets his mind to, and he does it with genuine collegiality and humility.

In short, if CDAs are designed for anyone, then they are designed for [REDACTED]. He's done everything we would ever expect or desire out of an early career trainee. He's identified every opportunity he can to succeed and made a major impact in his field, all while still only being 1.5 years into his faculty tenure. Just amazing.

During his residency and fellowship, [REDACTED] developed a research niche looking at how life expectancy affects treatment decision making in men with prostate cancer. He has analyzed this issue using multiple health services research approaches, including studies assessing variation in treatment, comparative effectiveness, and basic epidemiology using secondary data. His disciplined focus on this single topic has allowed him to develop a strong thesis advocating for

improved inclusion of life expectancy in treatment decision making for patients with genitourinary malignancy. He has published over twenty high-quality articles on this topic over the last 7 years, including a first-author paper in *Annals of Internal Medicine*, and 90% of these articles have been first-author, original research. Even in his first year and a half here at [REDACTED] during his transition to a new faculty position, [REDACTED] has maintained his focus on this topic; he will have three podium talks at the [REDACTED] American Urological Association Annual meeting on new research in this area.

I am delighted that [REDACTED] has decided to pair his longstanding interest in life expectancy and cancer treatment decision making with some of our methodologic strengths within [REDACTED], namely qualitative analysis and online crowdsourcing of conjoint analysis. We have extensive experience with both of these approaches. Our lab has conducted extensive qualitative research, including with the NIH Patient Reported Outcome Measurement Information System (PROMIS), performing patient and provider focus groups and qualitative text analysis. We have also conducted a variety of conjoint analyses across disciplines, involving both patients and physicians. For example, we are currently working on a funded project to use online crowdsourcing of conjoint analysis for elicitation of community preferences for medical therapy of inflammatory bowel disease, a clinical scenario involving tradeoffs between major treatment-related side effects and disease/symptom management, similar to the clinical situation encountered with genitourinary malignancy. For that project, we developed conjoint models using Sawtooth software, used a third-party survey contractor to conduct the online crowdsourcing, and analyzed data using our in-house biostatisticians. This project provides the proof of concept behind the proposed analysis in this study; [REDACTED] application of these established methods to the question of life expectancy in urologic malignancy is appropriate, quite achievable, and undoubtedly will be of interest.

The techniques that [REDACTED] will learn in the course of his career development award will lay the foundation for future work. (1) Use of crowdsourcing and other digital outreach tools will inevitably become more valuable as research evolves to be more patient-centric (as exemplified by PCORI's emphasis on patient engagement and multiple recent grants our lab has received for conducting this type of work). (2) [REDACTED] idea to study quality of communication by qualitatively assessing outpatient visits is forward-thinking and generalizable to other scenarios. While many surgeons are trained to do quantitative analysis, few are trained in qualitative analysis; this plan will allow [REDACTED] to occupy a unique niche.

Tim is strongly supported by [REDACTED], the Department of Health Services, and the Department of Surgery. He has 40% protected time for research and has generous startup funding to develop a research team. He will have full access to the resources of the [REDACTED], including a PhD-level psychometrician, a masters-level biostatistician, 4 clinical research coordinators, multiple MD collaborators, and administrative support. He will also have access to all software and computing resources needed for this work, including Sawtooth conjoint analysis software (~\$10,000 software that we have already purchased for him). He will have ample mentorship in both urologic oncology and health services research, with a team that is bespoke to his research needs. His educational plan has been carefully selected, with local courses on qualitative analysis, risk analysis, and digital health at [REDACTED] Public Health, [REDACTED] and the Masters Program in Health [REDACTED] (for which myself and [REDACTED] are co-directors). [REDACTED] is committed to doing everything it can to help him succeed in building his career as a surgeon-scientist.

I most enthusiastically support [redacted] application for the DoD career development award. Please do not hesitate to contact me if you have any questions at all about [redacted], his proposal, or my role in his research and career development.

Sincerely,



10-10-68

Center for Outcomes Research

 Director, Division of Health Care Policy and Practice, National Institutes of Health

Journal of Management Education 35(1) 10-11

Gastroenterology Professor of Medicine

[REDACTED]

[REDACTED]

Dear Department of Defense Physician Research Award Selection Committee:

I am delighted to offer this very strong letter of support for Dr. [REDACTED], who is an outstanding applicant for the Physician Research Career Development Award, and specifically his project entitled "A Patient-Centered Approach to Integration of Life Expectancy into Prostate Cancer Treatment Decision Making."

After graduating *magna cum laude* in Biochemical Sciences from [REDACTED], [REDACTED] earned [REDACTED] Medical School [REDACTED]. He completed his urology residency at [REDACTED] and then went on to do a three-year combined health services/urologic oncology fellowship through the Robert Wood [REDACTED] Scholars Program—a noted health services research fellowship in public health, public policy, and health leadership—and the Institute of Urologic [REDACTED]. As a part of this fellowship, he earned his Masters of Science degree in Health Policy and Management (MSHPM) from the [REDACTED] School of Public Health [REDACTED]. During his training, [REDACTED] was mentored closely by [REDACTED], the chair of the Department of Urology [REDACTED] and a world leader in urological oncology health services, and he developed a strong portfolio of work around the role of comorbidity and life expectancy in prostate cancer decision making. As a mark of his productivity, he published >25 papers during his residency and fellowship many as first author, and his work was supported by grants from the American Cancer Society and Urology Care Foundation. We were delighted when [REDACTED] joined the [REDACTED] Division of Urology as Assistant Professor in August [REDACTED].

Since joining the faculty, [REDACTED] has been incredibly productive and has proven himself to be a valued asset to our prostate cancer research team at [REDACTED]. He has leveraged his comprehensive training in health services research, secondary data analysis, statistics methodology, and clinical urologic oncology to conceive and execute a variety of retrospective and prospective studies in a short period of time. In just under two years on faculty, [REDACTED] has already published 6 first-authored original research papers with an additional two under review, 2 editorial pieces, and is a coauthor on 6 additional manuscripts. He was also recently awarded a DoD Idea Development Award to investigate variation in timing of ADT across age and comorbidity in men with recurrence after radical prostatectomy, with me as a collaborator. He also conceived and is executing a prospective trial involving wearable biosensors, and is site PI for two industry-sponsored clinical trials in urologic oncology. With the support of [REDACTED], [REDACTED] has hired a biostatistician and clinical research coordinator to manage his increasing academic portfolio. He has done all of this while maintaining an active urologic oncology practice. [REDACTED]'s productivity over the last year speaks great lengths to his profound dedication, hard work, intellectual curiosity, and ability to lead a team. These are the important attributes of a successful surgeon scientist.

I have been a research mentor to [REDACTED] since he joined our faculty, and I have thoroughly enjoyed building research ideas around our common interests, including this project. [REDACTED] and I have weekly research meetings and have our outpatient clinics scheduled together, which has provided ample opportunity for idea generation. I have no doubt that the proposed project will also allow for a very fruitful collaboration, leveraging [REDACTED] strength in health services research, my expertise in outcomes research in urologic oncology, and the further experience of his mentorship team in relevant areas of digital health and health services research methodology. As outlined in the proposal, we will create and test a patient-centered approach to communication of life expectancy to patients with genitourinary malignancy by: defining current practice for such communication by analysis of treatment counseling discussions; identifying barriers to implementation from the patient and physician perspectives; predicting situations where life expectancy is relevant through online crowdsourcing of conjoint models; and conducting a pilot test of our targeted, patient-centered communication strategy. This work is timely and relevant, and is consistent with the broader goal of our field to minimize overtreatment and improve shared decision making for our growing older population.

This project is a natural extension of [REDACTED] previous work showing mismanagement of patients with limited life expectancy in the setting of prostate cancer, and it is well suited to expand his expertise beyond secondary data analysis, which has comprised most of his previous research. Over the last seven years, he has definitively shown that patients with limited life expectancy are over- and undertreated across a number of different urologic malignancies, and work by others has shown that this problem persists in an era where active surveillance and observational approaches are more widely accepted. The failure of physicians to appropriately manage these patients despite its role in guidelines calls for a more thorough investigation into why this is occurring and an evidence-based interventional strategy. While [REDACTED] has a robust experience with secondary data analysis, he has not yet had experience with qualitative research and trial design, which I feel are central to his development as a urologic oncologist and translational health services researcher.

In summary, I feel Dr. [REDACTED] is an ideal candidate for a DoD career development award. He has the ambition, knowledge, clinical and research acumen, and personal skills to become a national leader in the fields of health services research and urologic oncology. I do hope that you will strongly consider his application for this critical next step in his career development.

[illegible]



CEDARS SINAI
MEDICAL CENTER

Dea [REDACTED]

I am writing in support of your Physician Research Career Development Award proposal: "A Patient-Centered Approach to Integration of Life Expectancy into Prostate Cancer Treatment Decision Making." I am delighted to collaborate with you on your continuing effort to improve incorporation of life expectancy into decision making for patients with urologic malignancy. I have enjoyed watching you develop this idea over the last seven years as your attending at [REDACTED] and now as your colleague at [REDACTED], and I am looking forward to bringing my expertise in qualitative methodology to your work and to your overall training as a surgeon-scientist and health services researcher. Based on your past success, your superb mentorship team, and the importance of the project, I have no doubt that this will be an incredibly fruitful collaboration.

The intended research is very exciting. Although we as urologists understand that patients with limited life expectancy should not be treated for indolent cancers, data suggests that these patients are frequently overtreated. And despite directed interventions in which life expectancy information is provided, there seems to be little impact on critical outcomes like decisional conflict and overtreatment. This suggests the need for an empiric approach to implementation of life expectancy that establishes how it is currently communicated, engages key stakeholders to identify barriers to integration, uses robust methods to identify situations when it is relevant to discuss, then tests a targeted, patient-centered strategy for communication of patient-specific life expectancy estimates. This is a natural extension of your previous work, and it is exciting to see health services research that focuses on developing successful interventions rather than purely identifying the problems in our healthcare system.

For this project, I will provide my expertise in qualitative research methods, which I gained from my K23 [REDACTED] [REDACTED] now [REDACTED]. My experience in qualitative methods has spanned across many urologic conditions, addressing sensitive topics such as disease understanding, miscommunication with providers, shame and silence surrounding pelvic organ prolapse, and disparities among underserved Latinas with pelvic floor disorders. In addition, I will provide mentorship along with your other mentors on how to develop a robust health services research program while balancing a clinical workload. Having modeled my own career as a balance between a thriving clinical practice, health services research, and raising a family, I understand both the excitement and challenges that this path can present. Since my K award, I have competed successfully for multiple NIH grants, and I understand how an award like this can be formative to your academic development and provide the trajectory for your future as an independently funded surgeon-scientist. In being a member of your mentorship team, I hope to help to guide you to maximize the benefits of such an award and leverage it to building a national presence in urologic health services research.

Again, I offer my strongest recommendation for your candidacy for this award, and I look forward to our collaboration in the future.

Sincerely,

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]
[REDACTED]
[REDACTED]
[REDACTED]
[REDACTED]

I am pleased to serve as a collaborator and co-mentor for your DoD Physician Research Award, "A Patient-Centered Approach to Integration of Life Expectancy into Prostate Cancer Treatment Decision Making."

As a health services researcher and general internist, I can appreciate the scope and quality of your work on this topic during your residency, fellowship, and now as early faculty, showing that patients with limited life expectancy are often mismanaged in treatment of prostate cancer and other urologic malignancies. Having thoroughly examined variation in management, comparative effectiveness, and quality of life around life expectancy using secondary data, you are now advancing this work using qualitative analysis and testing an intervention as part of a pilot trial. Your accomplishments to date are impressive, and I believe that the training and experience you will receive with this award will help to expand your capabilities as a health services researcher and provide the protected time you need to realize your goal of becoming an independently funded health services surgeon-scientist.

I am qualified to provide mentorship and content guidance in the areas of stakeholder engagement, study design, health policy, and other topics. I am Associate Professor of Medicine and Director of the Division of General Internal Medicine at [REDACTED] and a faculty member of [REDACTED]

[REDACTED] I am an active health services researcher whose primary interest is in topics surrounding the quality and value of care. I have extensive experience with mixed methods studies that employ stakeholder engagement as a component of development of clinical quality measures. I led a national, multidisciplinary team who developed quality of care measures for carpal tunnel syndrome in 2011, and surgical appropriateness criteria for degenerative lumbar scoliosis in 2016. I have a particular interest in issues related to surgical care, because I completed a categorical internship in general surgery before choosing internal medicine.

I have been the Principal Investigator of three R01s to date, funded by the Agency for Healthcare Research and Quality; one is active and two were completed in 2016. I am also the former recipient of an AHRQ K08, in which I evaluated the effects of hospital-related patient safety interventions on key stakeholders, including financial effects on payers, employers, hospitals, and physicians. Currently, I am the primary mentor to the recipient of a K23 career development award with the National Institute of Aging. My experience with methodological approaches similar to those in the proposed study will certainly help to inform both the study design and conduct of the research.

Having known you as both a R. [REDACTED] Johnson Ch. [REDACTED] well as a colleague on the [REDACTED], I have no doubt that you will be highly productive during the course of this award. I look forward to offering my expertise to your research and career development.

Sincerely,

[REDACTED]

[REDACTED]
[REDACTED]
Assistant Professor of Surgery
[REDACTED]
[REDACTED]
[REDACTED]

Dear [REDACTED],

Please let this letter serve as an enthusiastic recommendation of your Physician Research award application, "A Patient-Centered Approach to Integration of Life Expectancy into Prostate Cancer Treatment Decision Making." As one of your mentors since you began your work on comorbidity assessment and life expectancy in prostate cancer treatment decision making over 5 years ago, I am excited to see how this work has progressed over time and fully agree with the idea of developing an evidence-based, patient-centered interventional approach as a next step. This work that seeks to comprehensively investigate the patient perspective on life expectancy's role genitourinary malignancy treatment decision making and integrate it as part of a targeted intervention will certainly help to advance our precision in management of older and sicker patients, who continue to suffer significant morbidity due to overtreatment. I am also happy to formalize our ongoing collaboration under this K award and to serve as a collaborator and mentor on this project.

I am well qualified to mentor you in the content areas of life expectancy and its interface with clinical management of patients with urologic malignancy. As you know, I am the [REDACTED]

[REDACTED] and I am a widely published health services researcher who has had a longstanding interest in comorbidity assessment and its impact on health and healthcare over the last 35 years. I am also a member of the Institute of Medicine and have been the [REDACTED] to Cancer Survivorship from [REDACTED]. I also have a track record of collaboration with urologists across the country on efforts to improve integration of age and comorbidity into cancer care, including [REDACTED], [REDACTED], [REDACTED], [REDACTED], and [REDACTED]. Given that urologists primarily deal with older patients who often have coexisting disease, I have found that my research goals have resonated with urologists seeking to improve decision quality and risk stratification of their patients. As a testament to our continued efforts in this area, I led a collaborative, multi-center effort involving [REDACTED], [REDACTED], [REDACTED], and [REDACTED] to investigate the relative impact of biological and patient complexity variables in predicting quality of life outcomes in patients with early-stage prostate cancer. This was one of six proposals funded in a statewide competition sponsored by the [REDACTED], and I was delighted that you agreed to serve as [REDACTED].

I also look forward to providing career mentorship to you in your transition to independent investigator. In addition to further strengthening our research collaboration, I will help to mold your research to provide infrastructure for future work, as I have with the [REDACTED] project. I will also help you to establish national connections within the broader health services research community both in and outside of the field of Urology. And I will also of course offer my career advice as an experienced health services researcher to help you develop a sustainable and fulfilling research career, as I have.

I look forward to our continued collaboration and give my highest recommendation for your application for a career development award.

Sincerely,

[Redacted signature block]

TECHNICAL ABSTRACT

Background. Life expectancy (LE) is a critical factor in treatment decision making for men with prostate cancer (PC), since limited LE predicts lower likelihood of sufficient longevity to benefit from treatment, higher morbidity after treatment, and decreased effectiveness of treatment. Despite a prominent role of LE in guidelines, patients with limited LE are often overtreated for indolent cancers. Data from non-cancer treatment settings suggest that this may be due to physician-level barriers precluding effective communication of LE. Yet, surprisingly little is known about how LE is actually communicated as well as physician and patient perspectives on how it should be communicated. Furthermore, patient/community opinions on what LE cutoffs are best suited to non-aggressive treatment are lacking, which precludes targeted interventions that seek to reduce overtreatment of men with limited LE.

Objective/Hypothesis. We will conduct a series of incremental studies to define how LE is currently communicated to men with PC, identify best practices for communication of LE data, determine community perspectives on when LE data is most relevant, and test a patient-centered approach to communication of LE in these situations. We hypothesize that: (1) communication of LE will be highly variable in terms of incidence of discussion, mode of communication, and emotive context; (2) both patient-level and physician-level barriers will exist to preclude optimal communication of LE; (3) community preferences on what LE cutoffs are best suited to “non-aggressive treatment” will differ from guidelines recommendations; and (4) a targeted, patient-centered approach to delivery of LE information will result in lower decisional conflict and improved decision making for men with newly diagnosed PC.

Specific Aims.

Aim 1: To characterize how LE is currently communicated to patients with newly diagnosed PC through qualitative analysis of treatment consultation discussions

Aim 2: To identify opportunities on how to optimize communication of LE to patients with PC through structured interviews of patients and specialty physician stakeholders

Aim 3: To identify community preferences regarding which LE cutoffs are best suited to nonaggressive treatment in early stage PC using online crowdsourcing of conjoint analysis tools

Aim 4: To determine if a patient-centered, targeted approach to communication of LE improves decisional conflict, quality of LE discussion, and reduces rates of overtreatment of patients with PC and limited LE

Study Design. First, we will delineate how LE is currently communicated to PC through qualitative analysis of treatment consultation transcripts of patients with early-stage prostate cancer. Second, we will engage patient and specialist physician stakeholders through structured interviews to identify barriers and opportunities to improve communication information about LE in PC treatment decision making. Third, we will use online crowdsourcing of conjoint analysis as a platform to study how patients, physicians, and the lay community value LE relative to other tradeoffs typically encountered in PC treatment decision making. We will analyze the data to determine how individual characteristics affect valuation of LE in the context of other tradeoffs and identify areas of consensus where LE appears to drive “non-aggressive” treatment choices. Fourth, we will conduct a randomized pilot trial to determine whether patient-centered communication of LE targeted to relevant situations improves decisional conflict and reduces overtreatment of PC.

Personnel. I am a surgeon-scientist specializing in urologic oncology with a goal of becoming a national leader in PC health services research. With this award, I am eager to expand my expertise beyond secondary data analysis to qualitative analysis, stakeholder engagement, and trial design. My proposal also incorporates cutting edge digital health techniques like conjoint analysis and online crowdsourcing, which will dovetail my primary research focus with a developing interest in digital health. I am privileged to have a highly qualified, dedicated, and diverse mentorship team co-led by Dr. [REDACTED], a urologic oncologist and nationally renowned prostate cancer outcomes researcher, and Dr. [REDACTED], a health services researcher and gastroenterologist who is a pioneer in digital health. With this award, I look forward to advancing knowledge in the field, increasing my breadth of expertise in health services research, and moving closer to my goal of becoming an independent federally funded surgeon-scientist.

Impact. This project has the potential to improve both the mental and physical well being of men with PC and limited LE. First, it will improve their ability to make informed decisions regarding whether to pursue aggressive or non-aggressive treatment by optimizing the communication between doctor and patient. Second, it will give patients a more prominent voice in determining which LE cutoffs are best suited for “non-aggressive” treatment, instead of relying on expert opinion. Last, our pragmatic intervention will reduce potentially morbid and unnecessary overtreatment for men with newly diagnosed PC and limited LE.

LAY ABSTRACT

Rationale. Men with limited life expectancy (LE) are at risk for overtreatment of low- and intermediate-risk prostate cancer (PC), since (1) they are much more likely to die of other causes than their PC and (2) aggressive treatment with surgery or radiation can cause substantial side effects such as impotence and urinary incontinence. Though guidelines recommend against surgery and radiation for men with limited LE, they are often overtreated due to poor incorporation of LE in treatment decision making. Very little is known about how LE is currently discussed with patients during treatment counseling and what are the best strategies for effective communication of LE information. Furthermore, patient perspectives on what LE cutoffs are best suited to non-aggressive treatment are lacking. Last, there have been sparingly few studies testing LE communication strategies to see if they reduce anxiety related to treatment choice and overtreatment in men with limited LE.

Objective. We will conduct a series of studies to define how LE is currently communicated to men with PC, identify best practices for communication of LE data, determine community perspectives on when LE data is most relevant to treatment decisions, and test a patient-centered approach to communication of LE in these situations. We hope that this targeted, patient-centered approach to communication of LE information will improve the ability of patients to make more informed decisions about treatment and ultimately reduce unnecessary and potentially harmful overtreatment.

Aims.

Aim 1: To characterize how LE is currently communicated to patients with newly diagnosed PC through qualitative analysis of treatment consultation discussions

Aim 2: To identify opportunities on how to optimize communication of LE to patients with PC through structured interviews of patients and specialty physician stakeholders

Aim 3: To identify community preferences regarding which LE cutoffs are best suited to nonaggressive treatment in early stage PC using online crowdsourcing of conjoint analysis tools

Aim 4: To determine if a patient-centered, targeted approach to communication of LE improves decisional conflict, quality of LE discussion, and reduces rates of overtreatment of patients with PC and limited LE

Applicability of Research. This research is directly applicable to patients with limited LE who are considering aggressive (surgery or radiation) versus non-aggressive (active surveillance/watchful waiting, observation) therapies for clinically localized prostate cancer. These patients—especially those with indolent low- and intermediate-risk tumors—are at highest risk of overtreatment with surgery and radiation. Improved communication of patient-specific LE information in the context of treatment counseling will help these patients make better decisions in line with their own preferences and values. Risks of our intervention include worsened anxiety due to the sensitive nature of LE data. This translational work is intended to be applied directly to the clinical setting and would be easy to apply in other similar clinical scenarios, such as early-stage kidney and breast cancers.

PI Career Goals in PC Research and Patient Care. I am a surgeon-scientist specializing in Urologic Oncology who seeks to become a national leader in PC health services research. This work will help me to gain expertise in health services research methods that I currently have no direct experience with, such as qualitative research (i.e. analysis of text-based data) and stakeholder engagement (i.e. structured interviews). In addition, I will gain experience with cutting-edge techniques in digital health such as online crowdsourcing and conjoint analysis, which will have become increasingly important tools for health services researchers specializing in decision making. I am privileged to have a highly qualified, dedicated, and diverse mentorship team co-led by Dr. [REDACTED], a urologic oncologist and nationally renowned prostate cancer outcomes researcher, and Dr. [REDACTED], a health services researcher and gastroenterologist who is a pioneer in digital health. With this award, I look forward to advancing knowledge in the field, increasing my breadth of expertise in health services research, and moving closer to my goal of becoming an independent federally funded surgeon-scientist.

Impact. We believe that this study will provide the foundation for a paradigm shift in how LE is incorporated into PC treatment decision making. We will provide novel insight into the current standard of care for how LE is communicated to patients with PC and identify best practices for communication of LE data to men with newly diagnosed PC. We will also generate insight into how patients and the community value LE in the context of other tradeoffs in PC treatment decision making and determine whether these values match with current treatment guidelines. Lastly, we will test a pragmatic approach to communicating LE to patients with newly diagnosed PC, which we hope will ultimately reduce anxiety related to treatment decision making and also reduce unnecessary overtreatment.

This generic Statement of Work document is intended to assist applicants with the format preferred by CDMRP. This particular SOW does not contain any specific scientific information and is intended to be easily modifiable for any project. Not all components will be applicable for every project; please consult your Program Announcement for specific award requirements.

STATEMENT OF WORK – [REDACTED] PROPOSED START DATE [REDACTED]

Site 1: [REDACTED]
[REDACTED]
[REDACTED]
[REDACTED]

| | | |
|--|-----------------|-------------------------------|
| Aim 1: To characterize how LE is currently communicated to patients with newly diagnosed prostate cancer through qualitative analysis of treatment consultation discussions | Timeline | CSMC |
| Major Task 1: Preparation & Data Collection | Months | |
| Subtask 1: Obtain IRB Approval | 1-3 | Dr. [REDACTED] |
| Subtask 2: Audio record and transcribe treatment counseling discussions for 30 men with newly diagnosed early-stage prostate cancers | 4-18 | Dr. [REDACTED] |
| Milestone Achieved: IRB Approval, data collected for analysis | 18 | Dr. [REDACTED] |
| Major Task 2: Analysis & Manuscript Preparation | | |
| Subtask 1: Use ATLAS.ti to extract segments of discussion related to life expectancy and code content using constant comparative method | 18-20 | Drs. [REDACTED] [REDACTED] |
| Subtask 2: Analyze data | 21-22 | Drs. [REDACTED] [REDACTED] |
| Subtask 3: Manuscript preparation | 23-24 | Dr. [REDACTED] |
| Milestone(s) Achieved: Manuscript addressing qualitative content of LE treatment discussions written and submitted for publication. | 24 | |
| Aim 2: To identify opportunities on how to optimize communication of LE to patients with prostate cancer through structured interviews of patients and specialty physician stakeholders | | |
| Major Task 1: Preparation & Data Collection | | |
| Subtask 1: Conduct structured interviews with 15 patients with PC and 15 physicians who treat PC, audiotape and transcribe interviews | 4-12 | Dr. [REDACTED] |
| Milestone(s): Data collected for analysis | 12 | Dr. [REDACTED] |
| Major Task 2: Analysis & Manuscript Preparation | | |
| Subtask 1: Code transcribed interviews using constant comparative method | 13-14 | Drs. [REDACTED] [REDACTED] |

This generic Statement of Work document is intended to assist applicants with the format preferred by CDMRP. This particular SOW does not contain any specific scientific information and is intended to be easily modifiable for any project. Not all components will be applicable for every project; please consult your Program Announcement for specific award requirements.

| | | |
|---|-------|--------------------------------|
| Subtask 2: Analyze data | 15-16 | Drs. [REDACTED] and [REDACTED] |
| Subtask 3: Manuscript preparation | 17-18 | Dr. [REDACTED] |
| Milestone(s) Achieved: Manuscript addressing patient and physician perspectives on communication of LE submitted for publication. | 18 | Dr. [REDACTED] |
| Aim 3: To identify community preferences regarding which LE cutoffs are best suited to nonaggressive treatment in early stage prostate cancer using online crowdsourcing of conjoint analysis tools | | |
| Major Task 1: Preparation and Data Collection | | |
| Subtask 1: Create conjoint analysis tool | 24-25 | Drs. [REDACTED] and [REDACTED] |
| Subtask 2: Deploy conjoint analysis tool to patient, physician, and crowdsourced groups | 26-31 | Dr. [REDACTED] |
| Major Task 2: Analysis & Manuscript Preparation | | |
| Subtask 1: Analyze data | 32-33 | Drs. [REDACTED] and [REDACTED] |
| Subtask 2: Manuscript Preparation | 34-35 | Dr. [REDACTED] |
| Milestone(s) Achieved: Manuscript addressing crowdsourced community preferences written and submitted for publication | 36 | Dr. [REDACTED] |
| Aim 4: To determine if a patient-centered, targeted approach to communication of LE improves decisional conflict, quality of LE discussion, and reduces rates of overtreatment of patients with prostate cancer and limited LE | | |
| Major Task 1: Data Collection | | |
| Subtask 1: Conduct pilot trial of targeted, patient-centered approach to communication of LE to patients with newly diagnosed PC | 34-46 | Drs. [REDACTED] |
| Major Task 2: Analysis & Manuscript Preparation | | |
| Subtask 1: Analyze data | 46-47 | Drs. [REDACTED] and [REDACTED] |
| Subtask 2: Manuscript Preparation | 47-48 | Dr. [REDACTED] |
| Milestone(s) Achieved: Manuscript describing pilot trial submitted for publication | 48 | Dr. [REDACTED] |

This generic Statement of Work document is intended to assist applicants with the format preferred by CDMRP. This particular SOW does not contain specific information and is intended to be easily modifiable for any project. Not all components will be applicable for every project; please consult your project-specific award requirements.

If human subjects are involved in the proposed study, please provide the projected quarterly enrollment in the table below.

No new human subject accrual is needed. The table below shows the number of subjects already accrued to the study as of the date of the SOW.

| | Year 1 | | | | Year 2 | | | | Year 3 | | | | |
|---------------------------------|--------|----|----|----|--------|----|----|----|--------|--------|--------|--------|--------|
| Target Enrollment (per quarter) | Q1 | Q2 | Q3 | Q4 | Q1 | Q2 | Q3 | Q4 | Q1 | Q2 | Q3 | Q4 | Q1 |
| Aim 1 | 0 | 6 | 6 | 6 | 6 | 6 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Aim 2 | 0 | 10 | 10 | 10 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Aim 3 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 50 | 30,050 | 0 | 0 | 0 |
| Aim 4 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 4 | 4 |
| Target Enrollment (cumulative) | 0 | 16 | 32 | 48 | 54 | 60 | 60 | 60 | 110 | 30,160 | 30,160 | 30,164 | 30,168 |

Note: The Government reserves the right to request a revised SOW format and/or additional information.

RESEARCHER DEVELOPMENT PLAN

Strategy. My proposed research will offer experience in areas in which I need substantial training and mentorship, including: qualitative analysis; stakeholder engagement/structured interviews; use of crowdsourcing and conjoint analysis; and trial design. I plan to gain expertise in these areas through a combination of coursework, mentorship, and conferences, as detailed below. In addition to this, I will plan to broaden my knowledge of prostate cancer through mentorship, conferences, and collaboration.

Coursework. I will take the following courses to gain knowledge in content areas relevant to my research. I expect that ~10% of my research time will be spent in coursework.

| Course | Location | Time | Description |
|--|------------------------------------|---------------|--|
| Qualitative Research | [REDACTED] | Year 1 Fall | Focus on semi-structured interviews, including: formulating a question, developing and conducting interviews, analyzing data, and presenting results |
| [REDACTED] Qualitative Research Methodology | [REDACTED] School of Public Health | Year 1 Spring | Learn qualitative research methods and techniques in research and evaluation related to healthcare |
| [REDACTED] Health Analytics: Identifying, Collecting, and Analyzing Big Data in Healthcare | [REDACTED] | Year 2 Fall | Gain familiarity with modern health analytic techniques, including ATLAS.ti and conjoint analysis. |
| Behavioral Economics | [REDACTED] Graduate School | Year 3 Fall | Explore anomalies in behavior of consumers, including risk aversion, over-weighting of small probabilities, inconsistent attribute weighting |
| [REDACTED]: Health Policy and the Aged | [REDACTED] School of Public Health | Year 4 Spring | Examine political, economic, and social forces that shape elderly health policy. |

Conferences. Society of Urologic Oncology (December); American Urological Association Annual Meeting (May); Academy Health (June); University of Michigan Health Services Research Symposium (September)

Mentorship. Most important to my development as a researcher will be my superb, diverse, and highly experienced mentorship team. My primary mentor, Dr. [REDACTED], will provide education and collaboration in projects related to prostate cancer. We currently hold a DoD PCRP idea development award analyzing variation in use of androgen deprivation therapy by age and comorbidity in men with advanced prostate cancer. As the editor-in-chief of a major prostate cancer journal, Dr. [REDACTED] also often requests my review of manuscripts across a variety of topics, which keeps my knowledge base broad and current. We also often collaborate on other projects using secondary data such as the SEARCH database and SEER-Medicare. Dr. [REDACTED], my secondary mentor, will provide education in the use of online crowdsourcing and conjoint analysis. He has substantial experience with a broad variety of health services methods and has conducted grant-funded research using online crowdsourcing and conjoint analysis in the context of inflammatory bowel disease using a similar approach to that proposed in this application. Drs. [REDACTED] will provide education in qualitative analysis and stakeholder engagement. Dr. [REDACTED] has used methods similar to those proposed in this application to study benign urologic conditions. The collaborative experience of my mentorship team will undoubtedly be crucial to the success of this project and my educational development.

Environment. [REDACTED] is an emerging center of excellence for prostate cancer research and employs a world-class faculty across numerous disciplines, including leaders in prostate cancer basic science (Drs. [REDACTED] and [REDACTED]), urologic oncology (Drs. [REDACTED] and [REDACTED]), medical oncology (Dr. [REDACTED]), and radiation oncology (Dr. [REDACTED]). The genitourinary (GU) oncology faculty comprises the Urologic division of the [REDACTED] Cancer Institute [REDACTED], which currently offers over 20 active GU clinical trials, of which I serve as PI on two. The [REDACTED] supports multiple weekly educational seminars and a weekly tumor board, and provides resources for investigator initiated trial development. I recently was awarded [REDACTED] developmental funding for a clinical trial investigating the role of PET-MRI imaging for HIFU mapping. There is undoubtedly ample opportunity for collaboration and educational development surrounding prostate cancer at [REDACTED].

IMPACT STATEMENT

Impact. We believe that this study will provide the foundation for a paradigm shift in the way life expectancy (LE) is incorporated into prostate cancer (PC) treatment decision making. Our previous work has clearly shown that LE is critical to treatment decision making, since it is a strong predictor of treatment effectiveness and treatment-related morbidity. Despite this, LE is poorly incorporated into decision making, as evidenced by high rates of overtreatment of men with limited LE for low- and intermediate-risk disease. There is currently a lack of information regarding how LE is addressed in PC treatment consultation discussions, and by conducting textual analysis of transcribed treatment counseling discussions (**Aim 1**), we will begin to fill this void by defining the current standard of care for communication of LE. By engaging the relevant stakeholders in shared decision making (**Aim 2**)—men with PC and their physicians—we will also identify best practices for how physicians should deliver this information to patients in a meaningful way.

This study will also help to address the disconnect between patients, the community, and physicians regarding what are relevant LE cutoffs for pursuing “non-aggressive” treatment in the context of other tradeoffs. Currently we rely on expert opinion (in the form of guidelines) to define what is a reasonable LE cutoff to pursue observation rather than aggressive treatment for a given tumor risk profile. By analyzing crowdsourced conjoint analysis data of patients and individuals in the community, we will be able to determine if patients and the community agree with guidelines on what is a relevant LE cutoff for pursuing non-aggressive treatment across different tumor risk subtypes (**Aim 3**).

The final aim of the project will test a pragmatic approach to integration of LE into PC treatment decision making (**Aim 4**), capitalizing on lessons learned in the first two aims and targeted to clinical scenarios deemed most high-yield by patients (**Aim 3**). This will provide an opportunity for direct translation of the information gleaned from the first three aims to the point-of-care. This study will provide the pilot data for a larger clinical trial that will provide a definitive answer regarding whether LE information reduces decisional conflict and helps patients make better choices regarding treatment of early-stage PC. Ultimately, we believe that better incorporation of LE into treatment counseling will reduce unnecessary and potentially harmful overtreatment of indolent tumors in men with limited LE.

PCRP Overarching Challenges. This proposal directly aligns with the PCRP overarching challenge to **develop strategies to optimize the physical health of men with prostate cancer**. In developing a pragmatic approach to improve the integration of LE into decision making, we hope to reduce the overtreatment of men with limited LE for low- and intermediate-risk PC. We have previously shown that treatment of men with limited LE with surgery and radiation therapy is not only unnecessary but also results in worse treatment-related morbidity compared with healthier men. Therefore efforts to reduce overtreatment are critical to reducing harm and improving overall health of these men.

PCRP Focus Areas. Our proposal addresses two of the PCRP focus areas. We will address **population science** by using crowdsourced, population-based conjoint analysis data to determine how the community values LE in the context of other tradeoffs typically encountered in PC decision making. Crowdsourced data will also be used to target our LE intervention to clinical scenarios where patients believe this information is relevant. We will also address **survivorship, including psychosocial impact on the patient and family** by identifying ways to optimize communication of LE to patients and their families during treatment counseling discussions, with the ultimate goal of reducing decisional conflict and stress around treatment choice.

BIOGRAPHICAL SKETCH

NAME: [REDACTED]

eRA COMMONS USER NAME (credential, e.g., agency login): [REDACTED]

POSITION TITLE: Assistant Professor of Urology

EDUCATION/TRAINING

| INSTITUTION AND LOCATION | DEGREE (if applicable) | Completion Date MM/YYYY | FIELD OF STUDY |
|---|---------------------------|----------------------------|----------------------|
| [REDACTED] Medical School, [REDACTED] | A.B. | [REDACTED] | Biochemical Sciences |
| [REDACTED] Internship, [REDACTED] | M.D. | [REDACTED] | Medicine |
| [REDACTED] Residency, University [REDACTED] | - | [REDACTED] | General Surgery |
| [REDACTED] Fellowship, [REDACTED] | - | [REDACTED] | Urology |
| [REDACTED] Fellowship, [REDACTED] | M.S.H.P.M. | [REDACTED] | Health Svcs Research |
| [REDACTED] Fellowship, [REDACTED] | - | [REDACTED] | Urologic Oncology |

Personal Statement

I am a surgeon-scientist specializing in urologic oncology and health services research who has devoted my academic career to understanding how age, comorbidity, and life expectancy affect genitourinary cancer risk stratification and treatment choice. The overarching goal of my research is to promote integration of these factors into treatment decision making, since they predict not only the likelihood of living long enough to benefit from treatment but also the morbidity associated with treatment and the effectiveness of treatment itself. Over the past seven years, I have applied my health services training in epidemiology, survival analysis, and comparative effectiveness methodology to show that patients with limited life expectancy are often overtreated across multiple GU malignancies, to prove that long-term survival benefits of treatment diminish with increasing comorbidity burden at diagnosis, and to create a life expectancy prediction tool for use at the point of care for men with prostate cancer. Though my research to date has been rooted in secondary data analysis, I am eager to expand my expertise to qualitative analysis, stakeholder engagement, and trial design using this career development award as a springboard; ultimately these will be the methods that I will need to bring my work from bench to bedside. My proposal also incorporates cutting edge digital health techniques like conjoint analysis and online crowdsourcing, which will dovetail my primary research focus with a developing interest in digital health. I am privileged to have a highly qualified, dedicated, and diverse mentorship team co-led by Dr. [REDACTED], a urologic oncologist and nationally renowned prostate cancer outcomes researcher, and Dr. [REDACTED], a health services researcher and gastroenterologist who is a pioneer in digital health. I feel qualified to conduct this research given my previous experience with the subject matter and my foundational training in health services research through the [REDACTED] Clinical Scholars Program. With this award, I look forward to advancing knowledge in the field, increasing my breadth of expertise in health services research, and moving closer to my goal of becoming an independent federally funded surgeon-scientist.

Positions and Employment

8/2015–Present Assistant Professor, [REDACTED] Center, Los Angeles, CA
8/2015–Present Director of Health Services Research, Dept of Surgery, [REDACTED] MD, VA, CA
7/2012–6/2015 Volunteer Clinical Faculty, Urology, [REDACTED] VA Medical Center
12/2012–6/2015 Volunteer Clinical Faculty, Urology, [REDACTED] Medical Center, [REDACTED]
7/2013–6/2014 Staff Urologist, [REDACTED] Medical Center, [REDACTED]
7/2012–8/2013 Staff Urologist, [REDACTED] Student Clinic, [REDACTED]
2/2005–2/2006 [REDACTED] GU Oncology Research Fellow, [REDACTED]
6/2002–8/2002 Gene Therapy Research Fellow, [REDACTED]

2/2000–9/2001 Transplant Immunology Research Fellow, [REDACTED]
 6/1999–9/1999 Transplant Immunology Research Fellow, [REDACTED]
 6/1998–9/1998 Transplant Biochemistry Research Fellow, [REDACTED]

Other Experiences and Professional Membership

2017 Peer Reviewer, AUA Localized Prostate Cancer Guidelines
 2014-2016 Resident Wellness Task Force, ACGME
 2013-2015 Chair, ACGME Council for Review Committee Residents
 2013-2015 Resident Director, ACGME Board of Directors
 2013-2015 Awards Subcommittee, ACGME Board of Directors
 2013-2015 Committee on Requirements, ACGME Board of Directors
 2011-2013 American Urological Association Residents Committee
 2010-2013 Resident Member, Residency Review Committee for Urology
 2010-2013 Member, ACGME Council for Review Committee Residents

Honors

2014 Best Abstract, Society of Urologic Oncology Annual Meeting, YUO Selection
 2013-2015 Collaborator, RAND Urologic Diseases in America Project
 2012 Best Poster, American Urological Association 2012 Annual Meeting
 2011 [REDACTED] Award, [REDACTED] Department of Urology
 2008 Resident of the Year in General Surgery
 2005 [REDACTED] Oncology Research Fellowship
 2002 [REDACTED] Research Fellowship
 1997 [REDACTED] Transplantation Biology Research Scholarship
 2000, 2001 [REDACTED] Scholarship for Academic Excellence
 1998, 1999 [REDACTED] Scholarship for Academic Excellence
 1997 National Merit Scholarship

Contributions to Science

COMORBIDITY, LIFE EXPECTANCY, AND TREATMENT DECISION MAKING IN PROSTATE CANCER

My main contribution to the scientific community has been to improve understanding of the importance of comorbidity and life expectancy in treatment decision making for men with prostate cancer. My work has shown that older men with even modest comorbidity burdens have such a high likelihood of death from other causes that aggressive treatment with surgery or radiation for lower-risk prostate cancer is seldom beneficial and often harmful, while treatment for high-risk disease may be reasonable given high rates of short-term mortality. Since beginning this work five years ago: (1) I quantified the risks of other-cause mortality by age and comorbidity for men with prostate cancer in both the VA ([REDACTED] et al, *Cancer* 2011) and nationally representative samples ([REDACTED] et al, *Ann Int Med* 2013); (2) I described patterns of overtreatment of men with limited life expectancy in both the VA ([REDACTED] et al, *Cancer* 2011, 2013) and nationally representative samples ([REDACTED] et al, *Cancer* [REDACTED]); (3) I showed that men with worse comorbidity burdens have poorer quality of life after aggressive treatment than their healthier peers ([REDACTED] et al, *Pros Can Pros Dis*, 2010); and (4) I showed that the effectiveness of aggressive treatment decreases as comorbidity increases (eventually becoming ineffective) and quantified this relationship ([REDACTED] et al, *Cancer* 2014). Ongoing research efforts focus on patient engagement around life expectancy in treatment decision making for indolent malignancies and finding ways to optimize comorbidity assessment for translation to the point of care.

1. [REDACTED], [REDACTED], [REDACTED], [REDACTED], [REDACTED], [REDACTED], [REDACTED], [REDACTED], [REDACTED], [REDACTED]. Overtreatment of men with low-risk prostate cancer and significant comorbidity. *Cancer*, 117:2058-66 (2011).
2. [REDACTED], [REDACTED]. Impact of Age, Tumor Risk, and Comorbidity on Competing Risks for Survival in a U.S. Population-Based Cohort of Men with Prostate Cancer. *Ann Intern Med*, 158: 709-17 (2013).

Completed Research Support:

PI: [REDACTED] (Dr. Mark S. Litwin, Mentor)

01/01/2013 – 06/30/2015

American Cancer Society Postdoctoral Fellow Award: The Role of Age & Comorbidity in Prostate Cancer Treatment Decision Making

Funding: \$98,000

PI: [REDACTED] (Dr. Mark S. Litwin, Mentor)

07/01/2012 – 06/30/2014

American Urological Association Foundation Postdoctoral Research Grant: The Impact of Age and Comorbidity on Survival and Treatment Decision Making in Men with Early-Stage Prostate Cancer

Funding: \$88,000

BIOGRAPHICAL SKETCH

Provide the following information for the Senior/key personnel and other significant contributors.
Follow this format for each person. **DO NOT EXCEED FIVE PAGES.**

NAME: [REDACTED] MD

eRA COMMONS USER NAME (credential, e.g., agency login): [REDACTED]

POSITION TITLE: Professor of Urology

EDUCATION/TRAINING (Begin with baccalaureate or other initial professional education, such as nursing, include postdoctoral training and residency training if applicable. Add/delete rows as necessary.)

| INSTITUTION AND LOCATION | DEGREE (if applicabl e) | Completion Date MM/YYYY | FIELD OF STUDY |
|-------------------------------|----------------------------------|-------------------------------|----------------------|
| [REDACTED] | BS | 06/93 | Biology |
| [REDACTED] | MD | 06/97 | Medicine |
| [REDACTED] School of Medicine | | 06/03 | Resident, Urology |
| | | 10/05 | Urological, Oncology |

A. Personal Statement

I am a urological surgeon scientist devoted to improving outcomes for patients with urological cancers. My primary focus to date has been on prostate cancer research. I am especially interested in prostate cancer risk stratification, health disparities, and the role of obesity/lifestyle and its relationship with prostate cancer. To address these questions, I run a basic science lab, conduct epidemiological research, as well as lead clinical trials. I have funding from several sources including NCI, DOD, and CDC. I am also the co-Director of the Cancer Prevention and Genetics Program, Director of the Center for Integrated Research on Cancer and Lifestyle ([REDACTED]) and Associate Director for Faculty Development of the [REDACTED] Cancer Institute. I am delighted to have been [REDACTED]'s research mentor since he joined the [REDACTED] faculty in August 2015 and now the co-primary mentor for his career development award. [REDACTED] and I have similar interests in prostate cancer risk stratification and outcomes research, and we have already collaborated on several papers on topics related to comorbidity and risk assessment in early-stage prostate cancer. I have helped [REDACTED] to develop this career development award application through our weekly meetings, and I strongly believe that the work will substantively contribute to the field. I feel that I am well qualified to provide career guidance and scientific mentorship to [REDACTED] as a urologic oncologist and surgeon-scientist.

B. Positions and Honors**Positions and Employment**

2000 – Present National Principal Investigator, SEARCH Database Study Group
 2003 – 2005 Instructor in Urology, [REDACTED] School of Medicine
 2005 – 2008 Assistant Professor, Division of Urologic Surgery, Departments of Surgery and Pathology, [REDACTED] University School of Medicine
 2005 – Present Staff Physician, [REDACTED] Medical Center
 2006 – 2009 Director of Outcomes and Translational Research, Division of Urology, Departments of Surgery, [REDACTED] University School of Medicine
 2008 – 2014 Associate Professor, Division of Urologic Surgery, Departments of Surgery and Pathology, [REDACTED] University School of Medicine
 2009 – 2012 Vice Chief of Research, Division of Urology, [REDACTED] University
 2010 – 2012 Associate Director for Clinical Research, Genitourinary Program, [REDACTED] Cancer Institute

2015 – Professor of Surgery (Urology), [REDACTED] Medical Center
 2015 – Director, Center for Integrated Research on Cancer and Lifestyle, [REDACTED]
 2015 – Co-Director, Cancer Prevention and Genetics Program, [REDACTED] Comprehensive Cancer Institute, [REDACTED] Medical Center
 2015 – Associate Director, [REDACTED] Cancer Institute, Cedars-Sinai

Other Experience and Professional Memberships

1998 – Present American Urological Association — Active Member
 2004 – Present American Association for Cancer Research – Active Member
 2005 – Present American Society for Clinical Oncology – Active Member
 2008 – 2015 Durham VA Institutional Review Board – Active Member
 2010 – Present Editor-in-Chief, Prostate Cancer and Prostatic Diseases
 2011 – 2014 North American Editor, European Urology
 2011 – Present Member, AUA Guideline panel on prostate cancer screening
 2011 – Present Member, AUA Guideline panel on castrate resistant prostate cancer
 2014 – Present Member, AUA Guideline panel on clinically localized prostate cancer
 2014 – 2015 Co-Chair, ASCO Guideline panel on adjuvant and salvage radiotherapy after prostatectomy

Honors

1989 Eagle Scout, Boy Scouts of America
 1993 Phi Beta Kappa, [REDACTED]
 2001 1st and 2nd Prize, Miley B. Wesson Resident Essay Competition, Western Section of the AUA
 2002 2nd Prize, AUA/ACMI Prize Essay Contest, Laboratory Research Category, National AUA
 2002 3rd Prize, Miley B. Wesson Resident Essay Competition, Western Section of the AUA
 2003 1st Prize Miley B. Wesson Resident Essay Competition, Western Section of the AUA
 2005 2nd and 3rd Prize, AUA/ACMI Prize Essay Contest, Clinical Research Category, National AUA
 2005 Alfred Blalock Research Award, Johns Hopkins Young Investigator's Day
 2006 Rising Star in Urology Award, American Urological Association Foundation
 2006 3rd Prize, AUA/ACMI Prize Essay Contest, Laboratory Research Category, National AUA
 2006 3rd Prize, AUA/ACMI Prize Essay Contest, Clinical Research Category, National AUA
 2007 1st Prize, AUA/ACMI Prize Essay Contest, Clinical Research Category, National AUA
 2007 2nd Prize, AUA/ACMI Prize Essay Contest, Laboratory Research Category, National AUA
 2007 Young Investigator Award, Society of Basic Urological Research
 2009 1st Prize, AUA/ACMI Prize Essay Contest, Laboratory Research Category, National AUA

C. Contributions to Science

1. Risk stratification for prostate cancer

As a practicing urologist, I am constantly faced with the dilemma of determining how aggressive a patient's cancer is. To address this problem, I focused much of my energies on risk stratification for prostate cancer at every point in the prostate cancer continuum from prior to diagnosis all the way to the time of development of castrate resistant prostate cancer and every step in between. Most of my work used standard variables available in all men – PSA, stage, grade, and PSA kinetics. However, we have also studied multiple molecular markers for their ability to better identify men with aggressive prostate cancer. Based upon my work in this area, I was the only person selected to serve on all 3 recent guideline panels for the American Urological Association – castrate resistant prostate cancer, prostate cancer detection, and clinically localized prostate cancer. In addition, I was asked to write a section for UpToDate on molecular tests for early stage prostate cancer.

- a. [REDACTED], [REDACTED], [REDACTED], [REDACTED], [REDACTED], and [REDACTED]. Risk of prostate cancer specific mortality following biochemical recurrence after radical prostatectomy. *JAMA* 2005;293:133-40.
- b. [REDACTED], [REDACTED], [REDACTED], [REDACTED], [REDACTED], and [REDACTED]. Death in patients with recurrent prostate cancer after radical prostatectomy: PSADT subgroups and their associated contributions to all-cause mortality. *J Clin Oncol* 2006;24:1000-10.

- c. Freedland SJ, Gerber L, Reid J, Welbourn W, Tikishvili E, Park J, Younus A, Guin A, Sangale Z, Lanchbury JS, Salama JK, and Stone S. Prognostic utility of CCP score in prostate cancer after primary external beam radiation therapy. *Int J Rad Bio Ther*. 2013;86: 848-53.
- d. Freedland SJ, Choeng V, Howard L, De Hoedt A, du Plessis M, Yousefi K, Lami LL, Buerki C, Robbins B, Trabulsi EJ, Shah NL, Abdollah F, Feng FY, Davicioni E, Dicker AP, Kames RJ and Den RB. Utilization of a genomic classifier for prediction of metastasis following salvage radiation therapy after radical prostatectomy. *Eur Urol*. In press.

2. Impact of lifestyle and obesity on prostate cancer

Obesity is rampant in Western society and we were one of the leading groups in the world to highlight both the adverse effects of obesity on prostate cancer but also the challenges in appropriately diagnosing prostate cancer in obese men. We have further examined other lifestyle features (smoking, alcohol, exercise) and how they link with prostate cancer as well as explored mechanisms linking lifestyle and prostate cancer both through observational studies, but also basic science work.

- a. Freedland SJ, Aronson WJ, Kane CJ, Presti JC Jr, Aming CL, Elashoff D, and Terris MK. Impact of obesity on biochemical control after radical prostatectomy for clinically localized prostate cancer. *J Clin Onc* 2004;22: 446-453.
- b. Banez LL, Hamilton RJ, Partin AW, Vollmer RT, Sun L, Rodriguez C, Wang Y, Terris MK, Aronson WJ, Presti JC Jr, Kane CJ, Aming CL, Moul JW, and Freedland SJ. Obesity-related plasma hemodilution and PSA concentration among men with prostate cancer. *JAMA* 2007;298: 2275-2280.
- c. Masko EM, Thomas JA, Antonelli JA, Lloyd JC, Phillips TE, Poulton SH, Dewhirst MW, Pizzo SV, and Freedland SJ. Low-carbohydrate diets and prostate cancer: how low is "low-enough"? *Cancer Prev Res*. 2010;3: 1124-1131.
- d. Islami F, Moreira DM, Boffetta P, and Freedland SJ. A systematic review and meta-analysis of tobacco use and prostate cancer mortality and incidence in prospective cohort studies. *Eur Urol*. 2014;66: 1054-64.

3. Racial disparity in prostate cancer

Black men in the US have the highest one of the highest incidence and mortality rates from prostate cancer in the world. The exact mechanisms underlying this association are unclear. We have focused on men diagnosed and treated with the VA health system, an equal access medical system, wherein barriers to care are minimized. Within this population, we still find differences in cancer recurrence rates after surgery and biological differences in tumor biology. On-going work in our groups is focused on understanding the molecular basis for the more aggressive prostate cancers in black men.

- a. Kim H, Moreira D, Jayachandran J, Gerber L, Banez LL, Vollmer R, Lark A, Donovan M, Powell D, Khan F and Freedland SJ. Prostate biopsies from African-American men express higher levels of aggressive disease biomarkers than prostate biopsies from Caucasian men. *Pros Can Pros Dis*, 2011;14: 262-265.
- b. Gaines AR, Turner EL, Moorman PG, Freedland SJ, Keto CJ, McPhail ME, Grant DJ, Vidal AC, and Hoyo C. The association between race and prostate cancer risk on initial biopsy in an equal access, multiethnic cohort. *Cancer Causes Control*. 2014;25: 1029-35.
- c. Allott EH, Howard LE, Song HJ, Sourbeer KN, Koontz BF, Salama JK, Freedland SJ. Racial differences in adipose tissue distribution and risk of aggressive prostate cancer among men undergoing radiation therapy. *Cancer Epi Biom Prev*. 2014;23: 2404-12.
- d. Allott EH, Howard LE, Aronson WJ, Terris MK, Kane CJ, Aming CL, Cooperberg MR, Freedland SJ. Racial differences in the association between preoperative serum cholesterol and prostate cancer recurrence: results from the SEARCH database. *Cancer Epi Biom Prev*. In press.

My Bibliography (426 publications):

<http://www.ncbi.nlm.nih.gov/sites/myncbi/stephen.freedland.1/bibliography/44054643/public/?sort=date&direction=ascending>

D. Research Support

Ongoing Research Support

Centers for Diseases Control U01DP006079 (Freedland/Anger/Kim) 9/30/15-9/29/20
The Epidemiology of Interstitial Cystitis in a Nationwide Multiethnic VA Cohort
The purpose of this study is to estimate the national prevalence of interstitial cystitis using a VA cohort

NIH R01 CA179115 (Vickers) 8/1/14-7/31/19
Dynamic, multi-cohort prediction modeling of prostate biopsy outcome
This is a multi-center study to analyze data from men undergoing prostate biopsy to develop a dynamic prediction model for assessing prostate cancer risk

DOD PC150978 (Freedland) 9/30/16-9/29/18
Racial differences in systemic and prostatic inflammation
The goal of this study is to examine racial differences in serum immune markers and inflammatory cells within the prostate to help understand racial differences in prostate cancer risk and aggressiveness

DOD W81XWH-16-1-0397 (Di Vizio) 9/1/16-8/31/18
Large Oncosomes: A Novel Liquid Biopsy for Genetic Profiling in Patients with Castration Resistant Prostate
The goal of this grant is to examine the ability of large oncosomes to be novel prostate cancer biomarkers

DOD PC141010 (Fowke) 9/1/15-8/31/17
Magnesium predicts high-grade prostate cancer
This is a DOD health disparity grant to analyze the role of magnesium in predicting high-grade prostate cancer and in helping to explain racial disparities in prostate cancer using samples from the Durham VA hospital

Completed Research Support

NIH K24 CA160653-01 (Freedland) 8/1/12-7/31/17
Midcareer Investigator Award
This is a midcareer investigator award to Dr. Freedland to support his efforts for his own research program in patient oriented research and to mentor junior investigators

American Institute for Cancer Research (Smith-Warner) 7/1/16-6/30/17
Carbohydrate quantity and quality
The goal of this study is to understand the relationship between dietary carbohydrate quantity and quality and prostate cancer aggressiveness using large epidemiological data as well as animal data

DOD PC110097 (Freedland) 5/1/16-4/30/17
Disparities in Intratumoral Steroidogenesis
The goal of this study is to examine the relationship between serum cholesterol levels and intratumoral steroidogenesis and to test whether these associations differ by race

NIH U01 CA158431-01 (Ostrer) 9/1/11-8/31/16
Genomics and Predictive Modeling of Prostate Cancer Health Disparity
The goal of this study is to determine genetic predictors of aggressive prostate cancer biology and see how these signatures differ by race.

Atkins Foundation (Freedland) 4/1/08-3/31/16
Randomized Controlled Clinical Trial of Carbohydrate Restriction among Men Initiating Androgen Deprivation Therapy for Prostate Cancer
This is a three-site randomized trial of carbohydrate restriction with exercise vs. no intervention control among 100 men initiating androgen deprivation therapy for advanced prostate cancer with the goal of preventing impaired insulin sensitivity that often occurs after starting androgen deprivation therapy. I am overall PI.

Department of Defense PC110974 (Ostrer) 7/1/12-6/30/15
Genomic Basis of Prostate Cancer Health Disparity Among African-American Men

The goal of this study is to determine genetic predictors of aggressive prostate cancer biology and see how these signatures differ by race.

Department of Defense (Pizzo/Bachelder)

10/1/12-12/31/14

The goal of this study to examine the role of GRP78 as a marker of prostate cancer stem-like cells and explore GRP78 as a potential therapeutic target for prostate cancer

Atkins Foundation (Freedland)

7/1/13-12/31/14

Randomized Controlled Clinical Trial of Carbohydrate Restriction among Men with a Rising PSA after Failed Primary Therapy for Prostate Cancer

This is a one-site randomized trial of carbohydrate restriction vs. no intervention control among 60 men with a rising PSA after failed primary therapy with the goal of slowing the rate of PSA rise.

NIH K12 (K100024-01 (Freedland)

8/1/13-12/31/14

KURE Program

This is a K12 to help train the next generation of urological researchers

NIH R01 (CA131235 (Freedland/Pizzo)

9/15/08-9/14/14

Resveratrol, Carbohydrate Restriction and Prostate Cancer Progression

The goals of this grant are: 1) Determine the maximum carbohydrate amount that is still a "low-carbohydrate diet" and delays prostate cancer growth 2) optimize resveratrol to inhibit prostate cancer growth, and 3) test if there is synergy between resveratrol and low-carbohydrate diets for slowing prostate cancer xenograft growth

Department of Defense (PC100266 (Shinohara)

8/1/11-7/31/14

Targeting a Novel Intracellular Isoform of Osteopontin in Innate Immunology to Suppress Prostate Cancer Progressions

The goal is to test the role of inhibiting a novel intracellular isoform of osteopontin and assessing its impact on immune function and prostate cancer development in various animal models of prostate cancer.

NIH 5 (R01CA134425-03 (Walter)

7/1/2009-4/30/13

Prostate Specific Antigen Practices and Outcomes in the Elderly

The goal of this study is to examine the downstream consequences of excessive PSA testing in older men

Department of Defense (PC094163 (Muller, Freedland Mentor)

4/15/09-4/14/13

Recurrence after Radical Prostatectomy: Is it Different in Black Men?

This is a Health Disparity Research – Prostate Scholar Award to Dr. Muller to study the natural history of recurrence after surgery and examine whether it differs by race. Dr. Freedland serves as Dr. Muller's mentor.

NIH U54 (CA156735-01 (Richardson)

9/1/10-8/31/13

CB2 Cannabinoid Receptor-mediated Regulation of Prostate Cancer Growth

The goal is test the anti-prostate cancer properties of targeting the CB2 Cannabinoid Receptor using both in vitro and in vivo prostate cancer models.

Pom Wonderful, LLC (Freedland)

5/15/09-10/14/13

Effects of Pomegranate Pills in Men with Prostate Cancer

Test the effect of pomegranate pills (POMx) on prostate cancer biomarkers in men undergoing surgery

BIOGRAPHICAL SKETCHNAME: **Shrennan M.R. Spiegel, MD, MSHS, FACG, AGAF**eRA COMMONS USER NAME: **uspiegel**POSITION TITLE: **UCLA Professor of Medicine and Public Health in Residence; Director of Health Services Research Cedars-Sinai Health System****EDUCATION/TRAINING**

| INSTITUTION AND LOCATION | DEGREE (if applicable) | Completion Date MM/YYYY | FIELD OF STUDY |
|--|---------------------------|----------------------------|------------------|
| Tufts University, Medford, Massachusetts | B.A. | 1994 | Philosophy |
| Tufts University, Medford, Massachusetts | Certificate | 1994 | Community Health |
| New York Medical College, New York | M.D. | 1998 | Medicine |
| UCLA School of Public Health, Los Angeles, Calif | M.S.H.S. | 2004 | Health Services |

A. Personal Statement

I am a Professor of Medicine and Public Health at **UCLA** and Director of Health Services Research for **Cedars-Sinai Health System**. I am trained in health services research methods, including biostatistics, psychometrics, decision analysis, meta-analysis, "big data" analytics, epidemiology and clinical trial design. I direct the **Cedars-Sinai Center for Outcomes Research and Education (CS-CORE)**, a health services research unit that develops and tests value-based healthcare delivery innovations. **CORE** focuses on how to use digital health and information technologies in a cost-effective manner while optimizing patient outcomes. My research combines traditional health services techniques with digital health platforms, including: (1) mobile health (mHealth) smartphone applications; (2) electronic health records (EHRs); (3) patient-provider portals with clinical decision support (CDS); (4) social media analytics to conduct clinical research while gaining insight into patient knowledge, beliefs, and behaviors; and (4) wearable biosensors to monitor health outside of the traditional provider visit. I have been a Principal Investigator for VA and NIH projects that evaluate novel informatics for healthcare delivery, including using big data approaches to predict patient outcomes, use of e-portals to connect patients and providers, and techniques to electronically monitor patient reported outcomes (PROs). For example, I was the PI of a VA Merit Award to conduct a pragmatic clinical trial in colon cancer screening using novel educational and informatics approaches. Similarly, I was PI on a VA Merit evaluating CDS for safe prescribing of nonsteroidal anti-inflammatory drugs. I have served as PI of an NIH grant to develop EHR-based PROs via the Patient Reported Outcome Measurement Information System (PROMIS®) U01 consortium. Most recently, I was PI on a large analysis of social media data to understand patient knowledge, attitudes, and beliefs around opioid use in America – a study that received broad media attention. I am currently a site leader for the **UCLA Clinical and Translational Science Institute (CTSI)**, a member of the Food and Drug Administration Gastroenterology Field Advisory Board, and Editor-in-Chief for the American Journal of Gastroenterology. I also have expertise with conjoint analysis; we are concurrently working on a project that uses online crowdsourcing of conjoint analysis to analyze community preferences regarding treatment options for inflammatory bowel disease, using similar methods to those described in Dr. **Daskivich** proposal. Dr. **Daskivich** has been a member of the **CS-CORE** and my mentee since he joined the faculty in August 2015. Over the past year and a half, we have worked together on writing several manuscripts and have developed a pilot trial of wearable biosensors for monitoring postoperative ambulation across 8 surgical specialties. I am delighted to be Dr. **Daskivich** co-mentor on his career development award, and I feel well qualified to offer guidance for his career development as a health services researcher as well as content expertise in the areas of qualitative analysis, digital health, and conjoint analysis.

B. Positions and Honors

Positions

| | |
|----------------|--|
| 2004 – Present | Professor, UCLA School of Medicine and Public Health |
| 2014 – Present | Director, Health Services Research Cedars-Sinai Health System |
| 2016 | Site Leader UCLA Clinical and Translational Science Institute (CTSI) |
| 2010 – 2015 | Principal Investigator, NIH/NIAMS PROMIS® Steering Committee |
| 2015 – Present | Editor-in-Chief, American Journal of Gastroenterology |
| 2012 – Present | Board Member, American College of Gastroenterology (ACG) |
| 2012 – 2015 | Chair, ACG Research Committee |
| 2008 – Present | Member, FDA GI Field Advisory Committee |

Selected Honors

| | |
|------|--|
| 1997 | Alpha Omega Alpha National Honors Society, Iota Chapter, New York Medical College |
| 1998 | Leo G Rigler Award for “Resident of the Year” at Cedars-Sinai Medical Center |
| 2003 | Janssen Research Excellence in Gastroenterology and Liver (REGAL) Award |
| 2004 | CURE Digestive Diseases Research Center Named New Investigator Award |
| 2004 | AstraZeneca Emerging Leaders in Gastroenterology Award |
| 2004 | VA HSR&D Entry Level Career Development Award |
| 2005 | AGA/FDHN Outcomes Research Award |
| 2007 | VA HSR&D Career Development Transition Award (CDTA) |
| 2007 | International Foundation for Functional Gastrointestinal Disorders (IFFGD) Distinguished Clinical Investigator Award |
| 2007 | VA HSR&D Career Development Transition Award (CDTA) |
| 2008 | Appointed as Fellow of the American College of Gastroenterology (FACG) |
| 2008 | Appointed to FDA Gastroenterology Field Advisory Board |
| 2009 | American College of Gastroenterology Leadership Award |
| 2011 | Appointed as Fellow of the American Gastroenterological Association (FAGA) |
| 2014 | Outstanding Manuscript of 2013, American Society of Gastrointestinal Endoscopy (ASGE) |
| 2015 | Elected Co-Editor-in-Chief, American Journal of Gastroenterology |

C. Contribution to Science

1. Advancing the Science of Digital Health

Fueled by population health mandates from the Affordable Care Act, and buoyed by advances in computing technologies, healthcare delivery now relies heavily on digital platforms, including electronic health records (EHRs), smartphone applications, mobile health (mHealth) devices, and wearable biosensors. However, it is hard for patients and their providers to know how to interpret and act upon the overwhelming amount of data streaming from these platforms. My research lab studies the impact of digital health on patient and provider outcomes. I combine expertise in medicine, psychology, epidemiology, decision science, information technology, and statistics to develop, test, and implement digital technologies in the “clinical trenches.” For example, I developed and validated an abdominal biosensor that non-invasively monitors digestion and helps make decisions about hospital discharge. I also developed an mHealth application that can outperform a doctor in obtaining a patient history. My team also uses social media to conduct epidemiologic research across wide populations. Forbes Magazine [recently profiled](#) our work in digital health, emphasizing our mission to advance the science and provide an honest accounting of where technologies can – and cannot – improve the value of care. I also teach this material in a class called [Health Analytics](#) at the [UCLA School of Public Health](#). The articles, below, offer examples of our work in developing and testing digital health technologies.

- a) [Whitman CB, Reid MW, Arnold C, Patel B, Ussos L, Salasanta, Pourmorady J, Spiegel BM](#). Balancing opioid-induced gastrointestinal side effects with pain management: insights from the online community. *J Pain Management* [2015 Sep-Oct;11\(5\):383-91](#)

- b) Almaro CV, Chey WD, Iriana S, Dalley F, Robbins K, Patel AV, Reid M, Whitman C, Fuller G, Bolus R, Dennis B, Encarnacion R, Martinez B, Soares J, Modi R, Agarwal N, Lee A, Kubomoto S, Sharma G, Bolus S, Chang L, Spiegel BM. Computer versus physician identification of gastrointestinal alarm features. *Int J Med Inform.* 2015 Jul 26; pii: S1386-5056(15)30023-X. doi: 10.1016/j.ijmedinf.2015.07.006. [Epub ahead of print] PubMed PMID: 26254875. [Spiegel=corresponding author]
- c) Almaro CV, Chey W, Kaung A, Whitman C, Fuller G, Reid M, Nguyen K, Bolus R, Dennis B, Encarnacion R, Martinez B, Talley J, Modi R, Agarwal N, Lee A, Kubomoto S, Sharma G, Bolus S, Chang L, Spiegel BM. Computer-generated vs. physician-documented history of present illness (HPI): results of a blinded comparison. *Am J Gastroenterol.* 2015 Jan;110(1):170-9. doi: 10.1038/ajg.2014.356. Epub 2014 Dec 2. PubMed PMID: 25461620; PubMed Central PMCID: PMC4289091. [Spiegel=corresponding author]
- d) Spiegel BM, Kaneshiro M, Russell MM, Lin A, Patel A, Tashjian VC, Zegarski V, Singh D, Cohen S, Reid MW, Whitman CB, Talley J, Martinez BM, Kaiser W. Validation of an acoustic gastrointestinal surveillance biosensor for postoperative ileus. *J Gastrointest Surg.* 2014 Oct;18(10):1795-803. doi:10.1007/s11605-014-2597-y. Epub 2014 Aug 5. PubMed PMID: 25091837

2. Integrating Patient Reported Outcomes (PRO) Into Clinical Practice

Because we ultimately judge the value of healthcare by its impact on the patients we serve, I have long been interested in measuring patient reported outcomes (PROs) in clinical practice. I have published extensively on the development and validation of health related quality of life (HRQOL) instruments and have expertise in psychometric techniques. However, my focus has been to *integrate* PROs into everyday clinical practice to improve shared decision making, enhance patient satisfaction, and maximize outcomes. As a PI for the NIH PROMIS® consortium, I developed computer-administered item banks to monitor symptoms in patients with chronic illness. I also created short-form questionnaires for use in clinical practice, created an online library of PROs for research and clinical care, and am conducting an NIH-funded study comparing PROMIS® vs. usual care in everyday clinical practice. The articles, below, offer examples of this work.

- a) Spiegel BM, Hays RD, Bolus R, Melmed GY, Chang L, Whitman C, Khanna PP, Paz SH, Hays T, Rouse S, Khanna P. Development of the NIH Patient-Reported Outcomes Measurement Information System (PROMIS) Gastrointestinal Symptom Scales. *Am J Gastroenterol.* 2014 Nov;109(11):1304-14.
- b) Spiegel BM, Khanna D, Bolus R, Agarwal N, Khanna P, Chang L. Understanding gastrointestinal distress: a framework for clinical practice. *Am J Gastroenterol.* 2011 Mar;106(3):380-5.
- c) Khanna P, Agarwal N, Khanna D, Hays RD, Chang L, Bolus R, Melmed G, Whitman CB, Kaplan RM, Ogawa R, Snyder B, Spiegel BM. Development of an online library of patient-reported outcome measures in gastroenterology: the GI-PRO database. *Am J Gastroenterol.* 2014 Feb;109(2):234-48.
- d) Spiegel B, Camilleri M, Bolus R, Andresen V, Chey WD, Fehnel S, Mangel A, Talley NJ, Whitehead WE. Psychometric evaluation of patient-reported outcomes in irritable bowel syndrome randomized controlled trials: a Rome Foundation report. *Gastroenterology.* 2009 Dec;137(6):1944-53.e1-3.

3. Making More Cost-Effective Decisions

I have a longstanding interest in measuring how best to use healthcare resources in cost-constrained environments. I have expertise in decision science and teach a class in cost-effectiveness analysis at the UCLA School of Public Health. I have used this training to study how to make more cost-effective decisions in healthcare. For example, I published a widely cited paper in *Ann Intern Med* demonstrating that cox-2 inhibitors like celecoxib are not cost-effective under most any scenario. I also published a decision model that revealed the national dyspepsia management guidelines to be highly cost-ineffective; the subsequent guidelines cited this work and adopted the strategy identified in our paper. I published another high impact study showing that patients with irritable bowel syndrome (IBS) should first be tested for celiac sprue before confirming the IBS diagnosis; this has also been featured in national guidelines. My highest cited analyses are listed, below.

- a) Spiegel BMR, Vakil NB, Ofman JJ. Dyspepsia management in primary care: a decision analysis of competing strategies. *Gastroenterology.* 2002;122:1270-1285.
- b) Spiegel BMR, DeRosa VP, Grajek JM, Wang V, Dulai GS. Testing for celiac sprue in diarrhea-predominant irritable bowel syndrome: a cost-effectiveness analysis. *Gastroenterology.* 2004;126:1721-32.

- c) Spiegel BMR, Targownik LE, Dulai GS, Gralnek IM. The cost-effectiveness of cyclooxygenase-2 selective inhibitors in the management of chronic arthritis. *Annals of Internal Medicine* 2003;138:795-806
- d) Kanwal F, Gralnek IM, Martin P, Dulai GS, Martin P, Spiegel BMR. The cost-effectiveness of competing therapies in the management of chronic infection with hepatitis B virus. *Annals of Internal Medicine* 2005;142:821-31

4. Improving Quality and Education around Cancer Screening

As a physician and professor of public health, I am deeply interested in providing high-quality cancer screening. For example, I published a series of studies that identified opportunities for improving quality in colorectal cancer (CRC) screening, including a study that found a relationship between time of day and polyp detection with colonoscopy: hour-by-hour as the day goes on, colonoscopists find fewer and fewer polyps. Subsequent investigators confirmed this work. In a series of two VA Merit Awards, we first developed an educational booklet that improved patient preparation for colonoscopy, and then created "big data" analytics approach to predicting "no shows" for colonoscopy; the latter study tested a novel scheduling technique to conditionally overbook slots to improve patient throughput. Most recently our group published a series of studies demonstrating that African American patients are less likely than others to obtain CRC screening, and developed a blueprint for addressing this inequity. Selected studies are provided, below.

- a) Spiegel BM, Talley J, Shekelle P, Agarwal N, Snyder B, Bolus R, Kurzbard N, Chan M, Ho A, Kangshing M, Cordasco K, Cohen H. Development and validation of a novel patient educational booklet to enhance colonoscopy preparation. *Am J Gastroenterol*. 2011 May;106(5):875-83. Epub 2011 Apr 12.
- b) May FP, Bromley EG, Reid MW, Baek M, Yoon J, Cohen E, Lee A, van Oijen MG, Spiegel BM. Low uptake of colorectal cancer screening among African Americans in an integrated Veterans Affairs health care network. *Gastrointest Endosc*. 2014 Aug;80(2):291-8. Doi PubMed PMID: 24674351
- c) Reid MW, Cohen S, Wang H, Kaung A, Patel A, Tashjian V, Williams D, Martinez B, Spiegel BM. Preventing patient absenteeism: Validation of a predictive overbooking model. *Am J Manag Care*. 2015 Dec;21(12):902-10
- d) Chan M, Cohen H, Spiegel BM. Colonoscopy yields fewer polyps as the day progresses: Experience in a Veteran Administration teaching hospital. *Clinical Gastroenterology and Hepatology* 2009 Nov;7(11):1217-23

Please [click here](#) for my complete list of published work in Pubmed (H-Index=40+)

D. Research Support (Representative sample selected from 15 research grants from past 3 years)

| INSTITUTION AND LOCATION | RESPONSIBILITIES | DATE |
|--|--|------------------|
| "Maximizing VA Colonoscopy Capacity through No-Show Predictive Overbooking", VA, HSR&D Merit Award, Veteran's Administration, West Los Angeles VA, Los Angeles, California | Principal Investigator. Pragmatic clinical trial comparing a novel scheduling technique called "no-show predictive overbooking" vs. status quo "one patient, one slot" scheduling for colonoscopy appointments. | 4/1/12-3/31/16 |
| "Development and Initial Validation of PROMIS GI Distress Scale", NIH/NIAMS, UCLA-West LA VA, Los Angeles, California | Principal Investigator. Developed a GI Symptom Scale for the NIH PROMIS® consortium. | 9/30/09-7/31/14 |
| "Validation of GI PROMIS® Measures in Clinical Practice: A Pragmatic Trial Using a Novel e-Platform", U01 NIH/NIAMS Supplemental, Cedars-Sinai Medical Center, Los Angeles, California | Principal Investigator. Testing implementation of NIH PROMIS® scale in clinical practice setting | 7/1/14 - 6/30/16 |
| UCLA Clinical and Translational Science Institute (CTSI) | Site Leader, Cedars-Sinai Multi-site collaborative research partnership that brings biomedical innovations to bear on the greatest health needs of | 7/1/16 |

| | | |
|--|---|--------------------|
| | Los Angeles. | |
| "Harnessing Social Media to Estimate Health-Related Quality of Life (HRQOL)," Robert Wood Johnson Foundation, Cedars-Sinai, Los Angeles, California | Principal Investigator. Developing an algorithm to estimate HRQOL in patients using Twitter | 12/15/14 – 6/14/16 |
| "Developing the Automated Evaluation of Gastrointestinal Symptoms (AEGIS) Platform", Ironwood Pharm., Cedars-Sinai Medical Center, Los Angeles, California | Principal Investigator. Developing a mobile health application to improve patient provider communication for digestive diseases | 10/2011-9/2016 |
| "Transforming the Care of Patients with GI Conditions through Education on Diet and Nutrition", Nestle Global, UCLA, Los Angeles, California | Principal Investigator. Developing a platform module pertaining to diet and nutrition using biosensor technology and patient reported outcomes (PROs). | 12/2013-11/2016 |
| "Concept Development of a Smartphone-Biosensor Intervention to Improve Adherence among Prescription Medication Users with Rheumatoid Arthritis", Amgen Pharm., Cedars-Sinai, Los Angeles, California | Principal Investigator. Developing and conducting an intervention pertaining to adherence to prescription drugs using biosensor technology and patient reported outcomes (PROs). | 10/2013- 9/2015 |
| "Navigating Use of Biologics in IBD: Development of A Novel Patient Decision Aid Using Conjoint Analysis and Social Media Insights", Takeda Pharmaceuticals, Cedars-Sinai, Los Angeles, California | Principal Investigator. Using conjoint analysis to develop an online decision aid to help patients with IBD make better decisions when starting biologic therapies | 3/15/15 – 3/31/16 |
| Integrating the Patient Voice into a Comparative Effectiveness Trial of Communication Strategies in the Management of Chronic Pain, Patient-Centered Outcomes Research Institute (PCORI), Washington, DC | Principal Investigator. Analyzing the effectiveness of Clinical Decision Support System and a patient education tool to reduce high-risk and inappropriate opioid prescriptions in the primary care setting. | 3/13/17 - 3/12/20 |
| Early Prediction of Major Adverse Cardiovascular Event Surrogates Using Remote Monitoring with Biosensors, Biomarkers, and Patient-Reported Outcomes, California Initiative for the Advancement of Precision Medicine (CIAPM), San Francisco, CA | Principal Investigator. leveraging a unique informatics and biosystems infrastructure to develop a clinical algorithm for remote early detection of MACE, facilitating early and cost-efficient treatment. | 2/1/17 – 7/31/18 |

BIOGRAPHICAL SKETCH

Provide the following information for the Senior/key personnel and other significant contributors.
Follow this format for each person. **DO NOT EXCEED FIVE PAGES.**

NAME: Jennifer Tash Anger, M.D., M.P.H.
Maiden name: Jennifer Allison Tash, M.D.

eRA COMMONS USER NAME (credential, e.g., agency login): JANGER2

POSITION TITLE: Associate Professor of Urology and Gynecology
Associate Director of Urological Research
Cedars-Sinai Department of Surgery and Obstetrics & Gynecology
Adjunct Assistant Professor of Urology UCLA

EDUCATION/TRAINING (Begin with baccalaureate or other initial professional education, such as nursing, include postdoctoral training and residency training if applicable. Add/delete rows as necessary.)

| INSTITUTION AND LOCATION | DEGREE (if applicable) | Completion Date MM/YYYY | FIELD OF STUDY |
|---|------------------------------|-------------------------------|--|
| University of California, Berkeley | AB | 1988-1992 | Integrative Biology, Spanish minor |
| University of Southern California | MD | 1993-1997 | Medicine |
| New York Weill Cornell Medical Center | Surgery | 1997-1999 | Surgery Junior Residency |
| Duke University Medical Center | Urology Fellowship | 1999-2003 2003-2004 | Urology Residency Urologic Reconstruction, Female Urology, and Urodynamics |
| UCLA Department of Urology and School of Public Health | MPH | 2004-2006 | Urological Health Services Research |

Please refer to the Biographical Sketch sample in order to complete sections A, B, C, and D of the Biographical Sketch.

A. Personal Statement

I am a dual fellowship-trained reconstructive urologist with extensive experience treating pelvic floor disorders and health services researcher. I completed my Master's degree in Public Health at UCLA under the mentorship of Dr. Mark Litwin, a pioneer in urological health services research. I have over ten years of research experience using administrative claims, including data from the VA. In October of 2007, I received a mentored career development award (K23) from the NIDDK to develop and test a set of quality-of-care indicators for women with pelvic floor disorders, "Evaluating the Quality of Urinary Incontinence and Prolapse Treatment" (EQUIPT). In 2009 I was awarded a challenge grant as lead PI for a randomized trial comparing costs of robotic vs. laparoscopic prolapse surgery. This specific trial attests to my ability to conduct complex multi-site trials in which patients are randomized on the morning of surgery, blinded to surgical technique, and followed diligently for one year after surgery. I am currently administrative PI for the Cedars-Sinai Multi-disciplinary Approach to the Study of Chronic Pelvic Pain (MAPP) site (U01 DK103260), where I oversee and coordinate the work of two basic science laboratories to study proteomics and the microbiome (including the mycobiome) of patients with chronic pelvic pain.

I am honored to serve as a collaborator and part of the mentorship team of Dr. Tim Daskivich in his application for a career development award. Although we have different clinical areas of expertise, Dr. Daskivich and I share a common fellowship mentor in Dr. Mark Litwin, who trained us similarly on our early road to academics. As a former career development award recipient, I am uniquely qualified to mentor Dr.

Deskwork by pacing his training and timing his project such that he has the skills needed to conduct each aim. I will also assist in carrying out his proposal where I can provide my expertise, specifically with conducting structured interviews and qualitative data analysis. I will also mentor him in transitioning to achieving academic independence through independent publications and his future application for independent funding.

B. Positions and Honors

POSITIONS

THE NEW YORK PRESBYTERIAN HOSPITAL-
WEILL MEDICAL COLLEGE OF CORNELL UNIVERSITY

New York, NY
1997-2003

Resident, Department of General Surgery 1997-1999

Resident, James Buchanan Brady Department of Urology
1999-2003

DURHAM UNIVERSITY MEDICAL CENTER

Durham, NC
2003-2004

Clinical Fellow, Urologic Reconstruction, Female Urology
and Urodynamics with Dr. George D. Webster

UCLA MEDICAL CENTER

Los Angeles, CA
2004-2006

Research Fellow and MPH candidate, Urological Health
Services Research with Dr. Mark Litwin

Assistant Professor of Urology

2006 to November of 2010

HONORS

American Urological Association Foundation Research Scholar Award, 2005-2006

American Urological Association Foundation Medical Student Summer Fellowship Award, 2011

Role: Mentor (Mentee: Claudia Sevilla)

Young Investigators Showcase, presentation on behalf of the Society for Urodynamics and Female Urology,
American Urological Association Annual Meeting, 2011

Best Quality of Life Paper, American Urogynecologic Society Annual Meeting, October, 2012

Zimskind Award, Society of Urodynamics, Female Pelvic Medicine and Urogenital Reconstruction, 2013

UCLA Medical Student Training in Aging Research (MSTAR) Mentorship Award, 2008 and 2013

PROFESSIONAL SOCIETIES

American Urological Association

Society of Urodynamics, Female Pelvic Medicine and Urogenital Reconstruction

American Urogynecologic Society

Society of Genitourinary Reconstructive Surgeons

COMMITTEES & EDITORIAL WORK

1. Planning Committee for NIDDK New Research Directions in Urinary Incontinence Symposium, Bethesda, MD, January 7-9, 2009 2008 – 2009
2. National Institutes of Health Review Panel ZRG1 DKUS-G 12 2010
3. American Medical Association's Physician Consortium for Performance Improvement: American Urological Association's and the American College of Obstetricians and Gynecologists' Stress Urinary Incontinence Measure Development Panel 2010 - present
4. Editorial Board, Female Pelvic Medicine and Reconstructive Surgery 2011- present
5. National Institutes of Health Review Panel ZDK1 GRB-G O3 2011
6. American Urological Association Data Panel 2012-present
7. Cedars-Sinai Medical Center Ethics Committee 2016-present
8. National Institutes of Health Review Panel 2016/10 ZRG1 DKUS-G (55) S 2016

C. Contribution to Science

My diverse research background stems from my mixed fellowship training in FPMRS and urological health services research. In addition, close collaborators across the country have led me to broaden my research interests into the realm of health disparities and basic science research.

Multi-Site Randomized Clinical Trials

My experience most relevant to our PFDN application is my role as administrative PI on a multi-center randomized trial analyzing comparative effectiveness of robotic vs. laparoscopic sacrocolpopexy for vaginal

prolapse. This study randomized women on the morning of surgery to robotic vs. laparoscopic surgery, and women were blinded to surgical technique. We found that costs of robotic sacrocolpopexy were higher than laparoscopic, whereas short-term outcomes and complications were similar. However, the primary cost differences resulted from robot maintenance and purchase costs rather than the costs associated with each procedure.

1. Anger JT, Mueller ER, Tarnay C, Smith B, Stroupe K, Rosenman A, Brubaker L, Bresce C, Kenton K. Robotic compared with laparoscopic sacrocolpopexy: a randomized controlled trial. *Obstetrics and gynecology*. 2014;123(1):5-12. Epub 2014/01/28. doi: 10.1097/aog.0000000000000006. PubMed PMID: 24463667; PubMed Central PMCID: PMC4266590.
2. Mueller ER, Kenton K, Tarnay C, Brubaker L, Rosenman A, Smith B, Stroupe K, Bresce C, Pantuck A, Schulam P, Anger JT. Abdominal Colpopexy: Comparison of Endoscopic Surgical Strategies (ACCESS). *Contemporary clinical trials*. 2012;33(5):1011-8. Epub 2012/05/31. doi: 10.1016/j.cct.2012.05.007. PubMed PMID: 22643040; PubMed Central PMCID: PMC4203307.

Health Services Research and Epidemiology

My NRSA-funded fellowship provided me experience with interpreting claims-based analyses. Using a 5% national random sample of data on Medicare beneficiaries, my work revealed that outcomes in the general population are inferior to that reported in the clinical literature. We also identified volume- and specialty- effects in that women who underwent sling surgery by a urologist or by a low volume surgeon were less likely to undergo concomitant prolapse surgery, and therefore were more likely to undergo an early repeat operation for prolapse. Our findings of variation in care led to my career development award, in which we developed and tested a set of quality of care indicators for women with urinary incontinence and pelvic organ prolapse.

1. Anger JT, Litwin MS, Wang Q, Pashos CL, Rodriguez LV. Complications of sling surgery among female Medicare beneficiaries. *Obstetrics and gynecology*. 2007;109(3):707-14. Epub 2007/03/03. doi: 10.1097/01.AOG.0000255975.24668.f2. PubMed PMID: 17329524.
2. Anger JT, Rodriguez LV, Wang Q, Chen E, Pashos CL, Litwin MS. Racial disparities in the surgical management of stress incontinence among female Medicare beneficiaries. *The Journal of urology*. 2007;177(5):1846-50. Epub 2007/04/18. doi: 10.1016/j.juro.2007.01.035. PubMed PMID: 17437833.
3. Anger JT, Scott VC, Kiyosaki K, Khan AA, Weinberg A, Connor SE, Roth CP, Wenger N, Shekelle P, Litwin MS. Development of quality indicators for women with urinary incontinence. *Neurourology and urodynamics*. 2013;32(8):1058-63. Epub 2013/10/10. doi: 10.1002/nau.22353. PubMed PMID: 24105879; PubMed Central PMCID: PMC3857939.
4. Anger JT, Scott VC, Kiyosaki K, Khan AA, Sevilla C, Connor SE, Roth CP, Litwin MS, Wenger NS, Shekelle PG. Quality-of-care indicators for pelvic organ prolapse: development of an infrastructure for quality assessment. *International urogynecology journal*. 2013;24(12):2039-47. Epub 2013/05/07. doi: 10.1007/s00192-013-2105-z. PubMed PMID: 23644812; PubMed Central PMCID: PMC3890317.

I am administrative PI of the Cedars-Sinai site for the Multidisciplinary Approach to the Study of Chronic Pelvic Pain (MAPP). At Cedars-Sinai, we will soon conduct studies of MAPP specimens in two laboratories. First, we will study the microbiome of the urine of patients with pelvic pain (with and without symptom flares). We will also perform sophisticated proteomics on urine and blood of MAPP specimens and controls. To date, we have identified a broad spectrum of fungal species in the urine of catheterized, asymptomatic women (unpublished data), revealing that we still know very little about the potential infectious agents contributing to disease symptoms. To date, we have found elevations of the pain modulator tyramine in the urine of patients with painful bladder syndrome.

1. You S, Yang W, Anger JT, Freeman MR, Kim J. 'Omics' approaches to understanding interstitial cystitis/painful bladder syndrome/bladder pain syndrome. *International neurourology journal*. 2012;16(4):159-68. Epub 2013/01/25. doi: 10.5213/inj.2012.16.4.159. PubMed PMID: 23346481; PubMed Central PMCID: PMC3547176.
2. Wen H, Lee T, You S, Park SH, Song H, Eilber KS, Anger JT, Freeman MR, Park S, Kim J. Urinary metabolite profiling combined with computational analysis predicts interstitial cystitis-associated candidate biomarkers. *Journal of proteome research*. 2014;14(1):541-8. Epub 2014/10/30. doi: 10.1021/pr5007729. PubMed PMID: 25353990; PubMed Central PMCID: PMC4286193.

Health Disparities Research

During my career development period, my qualitative work addressing overactive bladder among aging women led to a collaboration with Dr. Rebecca Rogers at the University of New Mexico. Together with her group, we conducted focus groups among women with pelvic organ prolapse, both in English and in Spanish in Los Angeles and Albuquerque. Our work resulted in numerous publications that explored women's experiences with pelvic organ prolapse. We identified prolapse as a disease of silence and shame that is not discussed publicly. We found that Spanish-speaking women faced additional barriers to care as a result of their need to use an interpreter in their care. We also identified disparities in care for women seeking care for pelvic floor disorders in the public hospital setting.

1. Wieslander CK, Alas A, Dunivan GC, Sevilla C, Cichowski S, Maliski S, Ellber K, Rogers RG, Anger JT. Misconceptions and miscommunication among Spanish-speaking and English-speaking women with pelvic organ prolapse. *International urogynecology journal*. 2015;26(4):597-604. Epub 2014/12/18. doi: 10.1007/s00192-014-2562-5. PubMed PMID: 25516231. PubMed Central PMCID: PMC4550586.
2. Khan AA, Sevilla C, Wieslander CK, Moran MB, Rashid R, Mittal B, Maliski SL, Rogers RG, Anger JT. Communication barriers among Spanish-speaking women with pelvic floor disorders: lost in translation? *Female pelvic medicine & reconstructive surgery*. 2013;19(3):157-64. Epub 2013/04/25. doi: 10.1097/SPV.0b013e318288ac1c. PubMed PMID: 23611934. PubMed Central PMCID: PMC3826442.
3. Sevilla C, Wieslander CK, Alas AN, Dunivan GC, Khan AA, Maliski SL, Rogers RG, Anger JT. Communication between physicians and Spanish-speaking Latin American women with pelvic floor disorders: a cycle of misunderstanding? *Female pelvic medicine & reconstructive surgery*. 2013;19(2):90-7. Epub 2013/02/28. doi: 10.1097/SPV.0b013e318278cc15. PubMed PMID: 23442506. PubMed Central PMCID: PMC3584345.
4. Anger JT, Lee UJ, Mittal BM, Pollard ME, Tarnay CM, Maliski S, Rogers RG. Health literacy and disease understanding among aging women with pelvic floor disorders. *Female pelvic medicine & reconstructive surgery*. 2012;18(6):340-3. Epub 2012/11/13. doi: 10.1097/SPV.0b013e31826fb8d3. PubMed PMID: 23143427. PubMed Central PMCID: PMC3723393.

Complete List of Published Work in MyBibliography:

<http://www.ncbi.nlm.nih.gov/sites/myncbi/jennifer.anger.1/bibliography/40959412/public/?sort=date&direction=ascending>

D. Research Support

Ongoing Research Support

National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK)

9/10/2014-6/30/2019

1U01DK103260-01

Title: Microbiome and Proteome as Predictive Biomarkers of UCPPS

Role: Principal Investigator (Anger/Freeman/Kim)

I am the administrative PI for the Cedars-Sinai Medical Center site for the Multi-Disciplinary Approach to the Study of Chronic Pelvic Pain (MAPP) Network. We seek to phenotype patients with chronic prostatitis/chronic pelvic pain syndrome (UCPPS) using advanced proteomics technologies. We will also identify disease-specific changes in commensal bacterial and fungal communities in urine in UCPPS patients using Next Generation Sequencing.

Center of Disease Control and Prevention

9/30/2015-9/29/2020

Title: The Epidemiology of Interstitial Cystitis in a Nationwide Multiethnic VA Cohort

Role: Principal Investigator (Freedland/Anger/Kim)

Our goals in this proposed study are to estimate the US nationwide prevalence and incidence of interstitial cystitis (IC) using a large, heterogeneous cohort from the Veterans Affairs healthcare system, the largest integrated health care system in the US. We will also prospectively enroll subjects to identify the impact of IC on individual health and test the value of a specific set of urine biomarkers already identified by our research team to diagnose IC.

Department of Defense, PRMRP PI (Kim)

9/1/15- 8/31/18

W81XWH-15-1-0415

Title: A Noninvasive Urine Marker of Interstitial Cystitis

Role: Co-Investigator (Kim)

This study seeks to quantify potential biomarkers for IC diagnosis. In particular, we aim to determine the epigenetic regulation on expression of cannabinoid 2 receptor (CNR2) and to qualify the promoter CpG methylation levels of CNR2 that predict the diagnostic value in IC.

National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK)

9/16/2013- 9/26/2016
(NCE)

1R01 DK100974-01

Title: Urinary metabolites in IC/PBS Diagnosis

Role: Principal Investigator (Kim/Anger)

This is a multiple PI prospective study evaluating the role of urinary metabolites in patients with interstitial cystitis/painful bladder syndrome (IC/PBS). The goal of this study is to identify/validate sensitive and non-invasive diagnostic biomarkers using urine specimens that stratify IC/PBS patients from healthy subjects.

Boston Scientific Corp.

3/14/2014- 12/30/2017

Title: A Prospective, Non-Randomized, Parallel Cohort, Multi-center Study of the Solyx™ Single Incision Sling System vs. the Obtryx™ II Sling System for the Treatment of Women with Stress Urinary Incontinence

Role: Co-Investigator

This multi-center study is to compare the treatment device (Solyx) to a different mesh sling or control device (Obtryx II) for the treatment of stress urinary incontinence. Safety information and patient outcomes are collected for three years and evaluated. Recruitment has been completed.

Completed Research Support

National Institute of Biomedical Imaging and Bioengineering (NIBIB)

4/01/2014-3/31/2016

1R03 EB017447-01

Title: Improving the Delivery of Robotic Surgery

Role: Principal Investigator (Catchpole/Anger)

The major goal of this project is to apply human factors systems analysis techniques to robotic surgery in order to better understand where care is inefficient or suboptimal.

National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK)

9/30/2007-8/31/2012

Patient-Oriented Research Career Development Award 1 K23 DK080227-01

NIDDK ARRA Supplement 5K23DK080227-03

2009-2011

NIH Loan Repayment Award

2007-2011

Title: *Quality of Care Indicators for Overactive Bladder Symptoms*

Role: Principal Investigator/Mentee

This project involves the development and pilot testing of candidate quality indicators for women with overactive bladder symptoms. The research includes systematic literature reviews, patient focus groups, physician expert interviews, an expert panel to rate the candidate indicators, and pilot testing the candidate indicators to rate their feasibility.

National Institute of Biomedical Imaging and Bioengineering (NIBIB) Recovery Act

10/1/2009-8/31/2012

Limited Competition: NIH Challenge Grant 1 RC1 EB010649-01

Title: *Abdominal Colpopexy: Comparison of Endoscopic Surgical Strategies (ACCESS)*

Role: Principal Investigator (Anger/Kenton/Mueller)

This is a randomized comparative effectiveness trial at UCLA, Cedars-Sinai, and Loyola University Medical Center in Chicago. Patients were randomized to undergo laparoscopic vs. robotic approaches to sacrocolpopexy for vaginal prolapse.

Kidney & Urology Foundation of America, Inc., Research Fellowship Award

2004-2005

NIH/NIDDK Individual National Research Service Award F32 DK072874-01

2005-2006

American Urological Association Foundation (AUAF) Health Policy Award

2005-2006

NIH Loan Repayment Award

2005-2007

BIOGRAPHICAL SKETCH

Provide the following information for the Senior/key personnel and other significant contributors.
Follow this format for each person. **DO NOT EXCEED FIVE PAGES.**

NAME: Nuckols, Teryl Kirsten

eRA COMMONS USER NAME: NUCKOLS1

POSITION TITLE: Director, Division of General Internal Medicine

EDUCATION/TRAINING

| INSTITUTION AND LOCATION | DEGREE (if applicable) | Completion Date MM/YYYY | FIELD OF STUDY |
|---|---------------------------|----------------------------|-----------------|
| Cornell University, Ithaca, NY | B.A. | 1989 | Biology |
| University of California, San Diego School of Medicine; La Jolla, CA | M.D. | 1997 | Medicine |
| UCLA Fielding School of Public Health; University of California, Los Angeles; Los Angeles, CA | M.S.H.S. | 2003 | Health Services |

A. Personal Statement

I am an Associate Professor of Medicine and Director of General Internal Medicine at Cedars-Sinai Medical Center who has a research interest in the clinical and financial implications of improving the quality and safety of healthcare. I am currently PI of an AHRQ R01 that is examining the effects of the Medicare Hospital Readmissions Reduction Program on outcomes among seniors hospitalized with heart failure, myocardial infarction, and pneumonia, including survival after hospitalization, hospital readmissions, use of post-acute care, and Medicare payments after hospitalization. I recently completed two additional AHRQ-funded R01s in 2016 and also completed an AHRQ-funded K08 Career Development Award in 2014, in which I evaluated the effects of hospital-related patient safety interventions on key stakeholders, including financial effects on payers, employers, hospitals, and physicians. I feel well qualified to serve on the mentorship team for Dr. Daskivich's career development award, given my expertise in stakeholder engagement, health services research, and health policy. I am also qualified to counsel Dr. Daskivich in his transition to becoming an independent federally funded health services researcher.

B. Positions and HonorsPositions and Employment

1997-1998 Categorical Internship, Department of General Surgery, University of Michigan, Ann Arbor, MI
 1998-2001 Internship and Residency, Center for Health Sciences, Internal Medicine, Department of Medicine, University of California, Los Angeles, CA (UCLA)
 2001-2003 Primary Care Research Fellowship, National Research Service Award (NRSA), and Clinical Instructor; Division of General Internal Medicine and Health Services Research, UCLA
 2003-2011 Assistant Professor, Division of General Internal Medicine and Health Services Research, Department of Medicine, UCLA
 2011-2014 Associate Professor, Division of General Internal Medicine and Health Services Research, Department of Medicine, UCLA
 2003-present Health Services Researcher, RAND Corporation, Santa Monica, CA; Secondary appointment.
 2014-present Director, Division of General Internal Medicine, Cedars-Sinai Medical Center, Los Angeles, CA
 2017-present Associate Professor, Department of Medicine, Cedars-Sinai Medical Center, Los Angeles, CA

Academic Leadership:

- 2003 – 2009 Site Director, Center for Health Sciences, Discussion of Major Diseases, Inpatient Internal Medicine Clerkship, [REDACTED] School of Medicine.
- 2011-present Core Faculty of the NRSA Primary Care Research Fellowship in the Division of General Internal Medicine and Health Services Research.
- 2015-present Core Faculty [REDACTED] RAND Pardee Graduate School
- 2015-present Faculty Mentor and Selection Committee Member, National Clinician Scholars Program [REDACTED] Los Angeles, CA
- 2015-present Committee Member and Faculty Mentor, Clinical Scholars Program, [REDACTED] Cedars-Sinai Medical Center, Los Angeles, CA
- 2015-present Associate Director [REDACTED] Cedars-Sinai Graduate Program, Masters' Program in Health Delivery Science (accreditation pending, anticipated start Fall 2017).

Honors and Certifications

- 1989 [REDACTED] Cornell University College of Arts and Sciences, "Distinction in All Subjects."
- 1993-1997 [REDACTED] UCSD School of Medicine, Honors in 14 pre-clinical and clinical courses (no AOA Chapter).
- 2001-present Board Certification and Recertification, American Board of Internal Medicine.

C. Contributions to Science and Public Policy

Complete List of Published Work in [MyBibliography](#)

Understanding How Improving Quality of Care Affects Costs

The quality of health care and the financial costs affected by receiving care represent two fundamental dimensions for judging health care performance, and the U.S. health care system does not function well on either dimension. Policymakers and observers have become hopeful about the possibility of improving quality while simultaneously saving money. Accordingly, many studies have examined the costs attributable to poor quality, the relationship between performance on quality measures and health care expenditures, and the costs and health effects associated with improvement programs. Several policies have been designed to spur improvements in quality and reduce costs. Yet numerous analyses purport to examine quality and costs but each analysis measures something different from the next. Consequently, I led the development of the Quality-Cost Framework, which describes how quality of care affects health care and other costs.

1. **Nuckols TK, Escarce JJ, Asch SM.** The Effects of Quality of Care on Costs: A Conceptual Framework. *Milbank Q.* 2013 Jun; 91(2):316-53.

I have also conducted several economic evaluations of interventions including computerized physician order entry systems, a hospital-based fall-prevention intervention, intravenous infusion pumps with embedded clinical decision support software, limits on the work-hours of resident physicians, and improvements in care for patients with diabetes and hypertension. In addition, I led systematic reviews of economic evaluations of interventions designed to reduce central-line-associated blood stream infections, hospital readmissions, catheter-associated urinary tract infections, and glycemic control for diabetes mellitus (manuscripts in preparation or under review).

2. **Teryl K. Nuckols, Emmett Keeler, Sally C. Morton, Laura Anderson, Brian Doyle, Marika Booth, Roberta Shanman, Paul Shekelle.** Economic Evaluation of Quality Improvement Interventions for Bloodstream Infections Related to Central Catheters: A Systematic Review. *JAMA Intern Med.* Published online October 24, 2016. doi:10.1001/jamainternmed.2016.6610
3. **Nuckols TK, Asch SM, Patel V, Keeler E, Anderson L, Buntin MB, Escarce JJ.** Implementing Computerized Provider Order Entry in Acute Care Hospitals in the United States Could Generate Substantial Savings to Society. *Jl Comm J Qual Patient Saf.* 2015;41(8):341-1.

Assessing Quality of Care and Evaluating Approaches to Improving Quality in the Hospital Setting

As a practicing hospitalist, I am particularly interested in strategies for improving the quality and safety of care in the inpatient setting. In addition to the two recent citations listed below, I have led studies that identified preventable adverse drug events in the inpatient setting, assessed the effectiveness of IV pumps with embedded clinical decision support software, examined the potential usefulness of hospital incident reports, and evaluated the effectiveness of fall prevention interventions.

1. McNair N, Balci J, Grogan TR, Walsh CM, Liang LJ, Worobal-Luk P, Needleman J, Nuckols TK. Is Spending More Time Associated With Less Missed Care?: A Comparison of Time Use and Missed Care Across 15 Nursing Units at 2 Hospitals. *J Nurs Adm.* 2016 Sep 46(9):426-37.
2. Nuckols TK, Smith-Spangler C, Morton SC, Asch SM, Patel VM, Anderson LJ, Delchase EL, Shekelle PG. The Effectiveness of Computerized Order Entry at Reducing Medication Errors and Adverse Drug Events in Hospital Settings: A Systematic Review and Meta-Analysis. *Syst Rev.* 2014 Jun 4; 3(1):56.

Examining the Effect of Quality on Clinical Outcomes and Costs in Workers' Compensation Settings

Workers' compensation is unique in the U.S. healthcare system in that payers are responsible for both medical and disability costs. This creates a natural alignment of financial incentives such that improving quality has a higher likelihood of being cost saving. For more than a decade, I have worked with California policymakers and national specialty societies on issues related to quality of care for musculoskeletal conditions.

In 2004, colleagues at RAND and I evaluated clinical practice guidelines for the California workers' compensation system, in response to specific legislation. With input from stakeholders and experts, we also formulated recommendations on guideline implementation. In the ensuing years, California policymakers implemented the guideline that performed best in our analysis and also multiple policy changes that were consistent with our recommendations. Policymakers in many other states considered our analysis when deciding which guideline to implement. Australian policymakers asked us to update this work in 2016.

1. Nuckols TK, Wynn BO, Lim YW, Shaw R, Matke S, Wickizer T, Harber P, Wallace P, Asch SM, MacLean C, Hansenfeld Garland R. Evaluating Medical Treatment Guideline Sets for Injured Workers in California. RAND, MG-400-ICJ, 2005. Available at: <http://www.rand.org/publications/MG/MG400/>
2. Nuckols TK, Lim YW, Wynn BO, Matke S, MacLean CH, Harber P, Wallace P, Garland RH, Asch S. Rigorous Development Methods Do Not Ensure That Guidelines Are Acceptable to A Panel of Knowledgeable Providers. *Journal of General Internal Medicine.* 2008 Jan; 23(1):37-44.

Subsequently, colleagues and I worked with the California policymakers, insurers, and provider groups to develop and test quality measures for work-associated carpal tunnel syndrome, leading to the AHRQ R01 discussed above.

1. Nuckols T, Conlon C, Robbins M, Dworsky M, Lai J, Roth CP, Levitan B, Seabury S, Seelam R, Asch SM. Quality of Care for Work-Associated Carpal Tunnel Syndrome. *J Occup Environ Med.* 2017 Jan; 59(1):47-53. doi: 10.1097/JOM.0000000000000916. PMID: 28045797.
2. Conlon C, Asch S, Hanson M, Avins A, Levitan B, Roth C, Robbins M, Dworsky M, Seabury S, Nuckols T. Assessing the Value of High-Quality Care for Work-Associated Carpal Tunnel Syndrome in a Large Integrated Health Care System: Study Design. *Perm J.* 2016 Fall; 20(4):87-95. doi: 10.7812/TPP/15-220. PubMed PMID: 27723446. PubMed Central PMCID: PMC5101093.

In other projects, I have led the development of appropriateness criteria for surgical procedures related to musculoskeletal conditions, and evaluated guidelines for opioid prescribing.

3. Chen PG, Daubs MD, Berven S, Raeh LB, Anderson AT, Asch SM, Nuckols TK, and the Degenerative Lumbar Scoliosis Appropriateness Group.* Surgery for Degenerative Lumbar Scoliosis: The Development of Appropriateness Criteria. *Spine (Phila Pa 1976).* 2016 May; 41(10):910-8. *Senior author.
4. Nuckols TK, Anderson L, Popescu I, Diamant AL, Doyle B, Di Capua P, Chou R. Opioid Prescribing: A Systematic Review and Critical Appraisal of Guidelines for Chronic Pain. *Ann Intern Med.* 2013 Nov 12.

D. Research Support

Ongoing Research Support

R01HS024284

Nuckols (PI)

9/30/2015 – 9/29/2017

AHRQ to Cedars-Sinai

Medicare Readmissions Reduction Program: Outcomes, Costs, and Inadvertent Effects

This project seeks to evaluate the Centers for Medicare and Medicaid Services Hospital Readmissions Reduction Program's effects on survival, healthcare utilization, and payments by Medicare, and to consider potential inadvertent effects.

1R01 HL130500 - 01A1 [redacted] Moran [redacted] 7/1/2016-6/30/2020
NHLBI to [redacted] Columbia [redacted]

Model Based Approach to Improving Hypertension Control in Populations

This project will estimate the long-term clinical and economic effects of efforts to improve blood pressure control, including among high-risk populations with heart disease and cerebrovascular disease.

Role: Co-Investigator

1K23 AG049181-01 [redacted] Pevnick [redacted] 8/01/2015-3/31/2019
NIA to [redacted] Cedars-Sinai [redacted]

Minimizing Errors in Medication Histories Obtained at Hospital Admission

The major goal of this project is to use mentoring, training, and my own research project to develop myself into an independent investigator. The training plan focuses on geriatrics. The research project examines the potential benefit of pharmacists, pharmacy technicians, and electronic pharmaceutical claims data to increase the accuracy of medication histories obtained at hospital admission.

Role: Co-Mentor

Completed Research Support

1R01 HS22644-01 [redacted] Nuckols (PI) [redacted] 10/1/2013 – 9/30/2016
AHRQ to [redacted] Cedars-Sinai [redacted]

When is Quality Improvement Cost Saving, Cost Effective, or Not a Good Value?

The goal of this project is to evaluate existing economic analyses to determine how often efforts to improve quality do save money, and the types of quality improvement interventions and specific quality problems that make savings vs. increased costs more likely.

R01HS0189802-01 [redacted] Nuckols (PI) [redacted] 9/17/2010 – 7/30/2016
AHRQ to [redacted] RAND [redacted]

"The Value of High Quality Medical Care for Work-Associated Carpal Tunnel Syndrome"

The major objective of this project is to assess quality of care for individuals with new workers' compensation claims for carpal tunnel syndrome, and to assess the relationships between quality of care and clinical and economic outcomes.

5K08 HS017954 [redacted] Nuckols (PI) [redacted] 4/1/2009 – 3/31/2014
AHRQ [redacted]

"The Value of Hospital-Related Patient-Safety Interventions to Key Stakeholders"

The major objective of this Career Development Award is to develop knowledge and skills in the fields of quality of care, health economics, and cost-effectiveness analysis.

No award # available [redacted] Nuckols, Walsh (Co-PIs) [redacted] 9/1/2011 – 06/30/2015
[redacted] University of California [redacted]

Center for Health Quality & Innovation

"Individualizing the Assessments of Risk to Reduce Falls in U.C. Hospitals"

The primary objective of this project is to have nurses at three University of California [redacted] medical centers critically and uniformly incorporate preventing falls into their current hourly rounding practices, thereby assessing individual patients' risks of falling and mitigating those risks on an ongoing basis during hospitalization. A secondary objective is to improve collaboration among providers with the ability to influence fall risk during hospitalization.

BIOGRAPHICAL SKETCH

Provide the following information for the Senior/key personnel and other significant contributors.
Follow this format for each person. **DO NOT EXCEED FIVE PAGES.**

NAME: Greenfield, Sheldon

eRA COMMONS USER NAME (credential, e.g., agency login): shgreenfield

POSITION TITLE: Bran Professor

EDUCATION/TRAINING (Begin with baccalaureate or other initial professional education, such as nursing, include postdoctoral training and residency training if applicable. Add/delete rows as necessary.)

| INSTITUTION AND LOCATION | DEGREE (if applicable) | Completion Date MM/YYYY | FIELD OF STUDY |
|--------------------------------|---------------------------|----------------------------|----------------|
| Harvard College, Massachusetts | BA | 1960 | Biochemistry |
| University of Cincinnati, Ohio | MD | 1964 | Medicine |

A. Personal Statement

I am a general internist with more than 30 years experience in clinical and health services research mainly in the areas of diabetes, prostate cancer, heterogeneity of treatment effects, quality of care, measurement of comorbidity, and patient participation in care. Our research group developed the patient reported measure of comorbidity, the Total Illness Burden Index, now being used to identify patients who can or cannot respond to treatment. I chaired the IOM report on Cancer Survivors: Lost in Transition; I chaired the IOM report on Clinical Practice Guidelines We Can Trust; and I co-chaired, with Hal Sox, the IOM report Initial Priorities for Comparative Effectiveness Research. I am Executive Co-Director of the Health Policy Research Institute at University of California, Irvine. I maintain a small practice in general internal medicine one day a week. I am a member of the Medi-Cal Performance Advisory Committee, which oversees the quality of care assessment for the California Medicaid program. As can be seen, I have had a career long interest in the role of the patient as a participant and as a reporter of health and disease status. I am proud to have served as a mentor to Dr. Daskivich during his residency and fellowship, and I am more than happy to continue to be part of his career development through this DoD Physician Research award. I will provide career guidance, content expertise in mortality prediction and translational health services research, and will help to develop Dr. Daskivich's national presence in this area.

B. Positions and Honors

1964 – 1966 Intern, Junior Assistant Resident in Medicine: V and VI Medical Sciences, Boston University, Boston City Hospital

1966 – 1968 Epidemic Intelligence Service Officer, Epidemiology Program, National Communicable Disease Center (now Centers for Disease Control), USPHS, and Fellow, Department of Preventive Medicine, State University of New York, Upstate Medical Center, Syracuse, New York

1968 – 1969 Senior Assistant Resident, Medicine, Beth Israel Hospital, Harvard Medical School, Boston, MA

1971 – 1972 Chief Resident in Medicine, Beth Israel Hospital, Instructor in Medicine, Harvard Medical School, Boston, MA

1978 – 1984 Associate Professor of Medicine and Public Health, UCLA School of Medicine, Los Angeles, California

1982 – 1988 Co-Director, RAND/UCLA Center for Health Policy Study

1984 – 1988 Professor of Medicine and Public Health, [REDACTED] School of Medicine & [REDACTED] School of Public Health
 1984 – 1996 Medical Director, National Study of Medical Outcomes
 1986 – 1988 Director, Joint [REDACTED] RAND/UCLA Health Services Research Training Program, Natl. Ctr. For Health Services Research
 1986 – 1990 Chairman, Health Care Technology Study Section, Natl. Ctr. for Health Svcs. Research, and Health Care Technology
 1988 – 2003 Director, The Primary Care Outcomes Research Institute, [REDACTED] Medical Center Hospitals and [REDACTED] Tufts University School of Medicine
 1988 – 2003 Professor of Medicine and Community Health, Departments of Medicine and Community Health, [REDACTED] Tufts University School of Medicine
 2003 – Present Professor, Department of Medicine; Director, Health Policy Research Institute, University of [REDACTED] California, [REDACTED] Irvine
 2011 – Present Senior Co-Editor, Journal of Comparative Effectiveness Research

C. Contributions to Science

An area in which my research has made a major contribution is that of heterogeneity of treatment effects: recognizing that patients with the same condition may respond differently to the same treatment. Our studies focused on the patient's comorbidity as a modifier of patient outcomes. We showed that lowering the blood sugar had little impact on patients with high levels of comorbidity. Those findings have been extended to other diseases, such as early prostate cancer. We also showed that adding functional status, depression, and an ability to work closely with the physicians, allowed the creation of a composite measure, the Potential for Benefit Scale, which predicted both self-reported adherence and blood sugar control in diabetes patients.

- a. [REDACTED] Greenfield S, Aronow H, Elashoff R, Watanabe D. Flaws in Mortality Data: The Hazards of Ignoring Comorbid Disease. *JAMA* 1988; 260:2253-55.
- b. [REDACTED] Litwin M, Greenfield S, Elkin E, Luback D, Broering J, Kaplan S. Assessment of prognosis with the Total Illness Burden Index for Prostate Cancer (TIBI-CaP): validation of an instrument to aid clinicians in treatment choice. *Cancer* 2007; 109(9):1777-1783.
- c. [REDACTED] Greenfield S, Billimek J, Pellegrini E, Franciosi M, De Berardis G, Nicolucci A, Kaplan SH. Comorbidity affects the relationship between glycemic control and cardiovascular outcomes in diabetes: a cohort study. *Ann Intern Med*. 2009 Dec 15; 151(12):854-60.
- d. [REDACTED] Daskivich T, Sadelsky N, Kaplan SH, Greenfield S, Litwin M. Less Is More: Severity of Comorbidity and Non-Prostate Cancer Mortality in Men With Early-Stage Prostate Cancer. *Archives of Internal Medicine* 2010; 170(15): 1396-1397.

I have been performing clinical research since the 1970s. In the 1980s, my colleague, [REDACTED] Sherrie Kaplan, and I, she as a PhD social scientist and I as a clinician, began a series of studies to evaluate an intervention designed to increase patient participation in treatment decisions. These widely cited studies showed that it was possible, in a brief and feasible way, to improve patient outcomes compared to an active control group. In an analysis led by [REDACTED] Dr. Kaplan, we showed that the doctor-patient interaction was associated with patient outcomes. These were the pioneering studies in this field and are now widely cited. From an NIDDK grant, we then studied whether coaching by community patients with diabetes would have similar effects in patients of low economic status. Again, there was an effect on lowering hemoglobin a1c with an active control group and with high quality of diabetes care by the physicians. This work was in an abstract presented at a national ADA meeting and is being prepared for publication. This research has become the major basis for the current proposal, with the realization that building on a platform of activation, the patients need training in various spheres in modern day healthcare.

- a. [REDACTED] Greenfield S, Kaplan SH, Ware JE Jr. Expanding Patient Involvement in Care: Effects on Patients Outcome. *Annals of Internal Medicine* 1985; 102(4):520-28.
- b. [REDACTED] Greenfield S, Kaplan SH, Ware JE, Jr., Martin E, Frank H. Patient Participation in Medical Care: Effects on Blood Sugar Control and Quality of Life in Diabetes. *Journal General Internal Medicine* 1988; 3:443-47.
- c. [REDACTED] Kaplan SH, Greenfield S, Ware JE Jr. Assessing the Effects of Physician-Patient Interactions on the Outcomes of Chronic Disease. *Medical Care* 1989; S110-S127.
- d. [REDACTED] Kaplan SH, Ngo-Metzger Q, Sorkin DH, Billimek J, Greenfield S. Reducing Racial Disparities in Diabetes: The Coached Care (R2D2C2) Project. *J Gen Intern Med*. 2013 Oct; 28(10): 1340-9.

A third area of high impact research started with the Medical Outcomes study of which I was Medical Director. That study led, in a major way, to the use of patient reported outcomes, partially functional status, using those outcomes to compare systems and specialties. In data from that study, again led by Dr. Kaplan, we showed that a physician's style for facilitating patient participation was associated with both increased activation and better outcomes.

- a. Stewart AL, Greenfield S, Hays RD, et al: Functional Status and Well-being of Patients with Chronic Conditions: Results from The Medical Outcome Study. JAMA 1989; 262(7):907-13.
- b. Greenfield S, Rogers WH, Mangotich M, Carney MF, Tarlov AR: Outcomes of Patients with Hypertension and Non Insulin Dependent Diabetes Mellitus Treated by Different Systems and Specialties: Results from the Medical Outcomes Study. JAMA 1995; 274(18):1436-44.
- c. Kaplan SH, Greenfield S, Gandek B, Rogers WH, Ware JE: Patient and Visit Characteristics Related to Physician's Participatory Decision-Making Style: Results from the Medical Outcomes Study. Medical Care 1995; 33(12):1176-87.
- d. Kaplan SH, Gandek B, Greenfield S, Rogers WH, Ware JE: Characteristics of Physicians With Participatory Decision-Making Styles. Annals of Internal Medicine 1996; 124:497-504.

My research in translation led to me being co-chair of the IOM report on Comparative Effectiveness Research, chair of the IOM Committee to design the National Healthcare Disparities Report to Congress, and chair of the IOM report on Clinical Practice Guidelines.

Complete List of Published Work in MyBibliography:

<http://www.ncbi.nlm.nih.gov/sites/myncbi/14wmPrxI985o/bibliography/44926242/public/?sort=date&direction=descending>

D. Additional Information: Research Support and/or Scholastic Performance

Ongoing Research Support

ACTIVE

Award # ME-1306-01715 6/1/14 – 08/31/17
PCORI

Computer-administered animation as a new method for measuring young children's health outcomes. This study seeks to develop a new online animated application for use with iPads to measure children's health status and well-being.

Role: Co-Investigator

VUMC42001 (Kaplan) 09/01/13 - 09/29/18

Subcontract with Vanderbilt University
1R01HS022640-01 (Prime PI: Penson)

Agency for Healthcare Research and Quality

Comparative Effectiveness of Modern Therapies for Localized Prostate Cancer

This study compares the effectiveness of commonly used modern therapies (including surgery, radiation and active surveillance) for the treatment of localized prostate cancer, the most common solid tumor among American men. As all therapies have differing effects of cancer control and quality of life, men newly diagnosed with this condition will be able to use the information gathered in this study to make more informed and personal decisions about the best way to treat their cancer.

Role: Co-Principal Investigator

POR14109 (Greenfield and Kaplan) 02/01/17 – 8/31/18

California initiative to Advance Precision Medicine

Precision Medicine for Early Prostate Cancer: Integrating Biological and Patient Complexity Variables to Predict Treatment Response

This proposal focuses on improving risk predictions for differential responses to treatment for early stage prostate cancer to aid physicians and patients in personalizing prostate cancer treatment decisions thereby maximizing effectiveness for individual patients.

Role: Co-Principal Investigator

COMPLETED

20132152 (Greenfield)

09/01/12 – 06/30/17

Subcontract with University of California, Los Angeles (Prime PI: Ong)
Innovation Evaluation Center

The goal of this project is to evaluate CHQI and CHQI QERM funded projects to help the leadership determine the advisability of scaling up the projects and/or sustaining the existing projects.

Role: Principal Investigator

ONC-MA-1004 (Greenfield and Kaplan)

04/08/15 – 06/30/16 (pending extension)

Astellas Pharma Europe Ltd.

This project incorporates a precise measure of non-cancer illness severity, the Total Illness Burden Index, into the baseline evaluation of patients in a data registry.

Role: Co-Principal Investigator

VUMC41762 (Kaplan)

09/01/13 – 08/31/16

Subcontract with Vanderbilt University Medical Center

CE-12-11-4667 (CORI (Prime PI: Penson)

Generating Critical Patient-Centered Information for Decision Making in Localized Prostate Cancer

This study seeks to develop an ongoing Comparative Effectiveness Assessment of Surgery and Radiation (CEASAR) study, a prospective, population-based cohort study designed to determine treatments which yield most favorable outcomes

Role: Co-Principal Investigator

PSSA-102827 (Kaplan)

02/25/14 – 08/30/15

Eli Lilly & Co.

TIBI: Total Illness Burden Score

This study seeks to use the Total Illness Burden Index to assess co-morbid conditions and verify eligibility in a clinical trial at Eli Lilly study site.

Role: Co-Principal Investigator

Subcontract with University of California, Los Angeles (Ong)

09/01/2010 – 08/30/2013

Agency for Healthcare Research and Quality (AHRQ)

Variations in Care: Comparing Heart Failure Care Transition Intervention Effects

The goal of this project is to identify and collect data from local Health Management departments and provide reports to the data analysis team at UCLA

MA-10998-2009 (Kaplan/Greenfield)

03/20/2009 – 06/30/2013

Eli Lilly and Company

Heterogeneity in Treatment Response

The goal of this project is to implement a tailored therapeutic strategy--providing the right drug to the right patient and the right time.

UCLA-13633 (Greenfield)

09/30/2010 – 09/29/2013

Subcontract with University of California, Los Angeles (Ong) 09/01/2010 – 08/30/2013

Agency for Healthcare Research and Quality (AHRQ)

Variations in Care: Comparing Heart Failure Care Transition Intervention Effects

The goal of this project is to identify and collect data from local Health Management departments and provide reports to the data analysis team at UCLA

DK1059846 (Greenfield)

04/01/2007 – 03/31/2013

National Institute of Diabetes and Digestive and Kidney Disease

Reducing diabetes disparities using community coaches

The goal of this project is to increase patient participation in treatment decisions on diabetes quality of care through intervention by using volunteer coaches from the patient's community who themselves have diabetes.

**Department of Defense
U.S. Army Medical Research and Materiel Command
Congressionally Directed Medical Research Programs
Fiscal Year 2017 Prostate Cancer Research Program
Physician Research Award
Peer Review Summary Statement**

CDMRP Log Number: PC171088
Grants.gov ID Number: GRANT12465228
Meeting Dates: 10/02/2017-10/03/2017
Review Panel: Development - Clinical and
Experimental Therapeutics

Project Duration: 48 months
Total Budget Requested: \$908,272
Direct Costs: \$519,012
Indirect Costs: \$389,260

Title: A Patient-Centered Approach to Integration of Life Expectancy into Treatment Decision-Making for Patients with Genitourinary Malignancy

Principal Investigator: Timothy Daskivich

Performing Organization: Cedars-Sinai Medical Center

Contracting Organization: Cedars-Sinai Medical Center

Overview

The Principal Investigator (PI), Dr. Timothy Daskivich, of this application is currently an assistant professor urologist at Cedars-Sinai in Los Angeles. His mentor is Dr. Stephen Freedland, a professor and urological oncologist at Cedars-Sinai with research expertise in prostate cancer outcomes. His comentor is Dr. Brennan Spiegel, a professor at Cedars-Sinai with expertise in health services research and gastroenterology. The PI's career goals as a researcher and clinician are to become a national leader in prostate cancer (PC) health services research. The research project's objectives are to define how life expectancy (LE) is currently communicated to men with PC, identify best practices for communication of LE data, determine community perspectives on when LE data are most relevant, and test a patient-centered approach to communication of LE in these situations, based on the hypotheses that (1) communication of LE will be highly variable in terms of incidence of discussion, mode of communication, and emotive context; (2) both patient-level and physician-level barriers will exist to preclude optimal communication of LE; (3) community preferences on what LE cutoffs are best suited to "non-aggressive treatment" will differ from guideline recommendations; and (4) a targeted, patient-centered approach to the delivery of LE information will result in lower decisional conflict and improved decision-making for men with newly diagnosed PC. The project's specific aims are (1) to characterize how LE is currently communicated to patients with newly diagnosed PC through qualitative analysis of treatment consultation discussions; (2) to identify opportunities on how to optimize communication of LE to patients with PC through structured interviews of patients and specialty physician stakeholders; (3) to identify community preferences regarding which LE cutoffs are best suited to nonaggressive treatment in early-stage PC using online crowdsourcing of conjoint analysis tools; and (4) to determine if a patient-centered, targeted approach to communication of LE improves decisional conflict and quality of LE discussion and reduces rates of overtreatment of patients with PC and limited LE.

| | Average Score | Standard Deviation |
|--|----------------------|--------------------|
| Overall Evaluation | 1.2 | 0.1 |
| Rating Scale: 1.0 (highest merit) to 5.0 (lowest merit) | (Outstanding) | |
| | | |
| Evaluation Criteria | Average Score | |
| Rating Scale: 10.0 (highest merit) to 1.0 (lowest merit) | | |
| Principal Investigator | 9.8 | |
| Mentor(s) | 9.6 | |
| Research Project | 8.3 | |
| Researcher Development Plan and Environment | 8.9 | |
| Impact | 9.6 | |

SCORED EVALUATION CRITERIA

Principal Investigator

Average Score: 9.8

Scientist Reviewer A

The PI, Timothy Daskalych, received an MD in 2006 from Harvard Medical School. He completed a general surgery internship (2007) and a urology residency (2012) at the University of California, Los Angeles, and fellowships in health services research (2014) as a Robert Wood Johnson Clinical Scholar and in urologic oncology (2015) at the University of California, Los Angeles. He is currently an assistant professor of urology at the Cedars-Sinai Medical Center.

Strengths: This is an already accomplished assistant professor in surgery (urology) at Cedars-Sinai. He has more than 25 publications, with many as first author and in high-impact journals (*Cancer*, *Annals of Internal Medicine*). He also has some current grant funding as PI and as Coinvestigator with members of the mentorship team. It is clear from the current biosketch that this applicant is committed to a career in urology and social science research specifically applied to urology cancer care. The letters of recommendation are very strong; one describes the applicant as a rising star in the department. Although it is not discussed, surgery clinical commitments may cause time conflicts; however, this does not appear to be a problem for this already productive researcher.

Weaknesses: No weaknesses were noted.

Scientist Reviewer B

Strengths: The PI is well trained. He completed a Robert Wood Johnson Clinical Scholars Program (received a master's degree) and fellowship at the University of California, Los Angeles (UCLA). He has an excellent publication record, with first-author, high-impact publications in *Annals of Internal Medicine* and *European Urology*. The stated career goals are to become a "national leader in PC health services research." The stated 40% effort in all years is appropriate for completion of the proposed work.

Weaknesses: No weaknesses were noted.

Mentor(s)

Average Score: 9.6

Scientist Reviewer A

The mentor, Dr. Stephen Freedland, is a professor and urological oncologist at Cedars-Sinai Medical Center. The comentor is Dr. Brennan Spiegel, a professor at Cedars-Sinai Medical Center.

Strengths: An outstanding mentorship team has been assembled, and each has provided a strong letter of support.

Weaknesses: No weaknesses were noted.

Scientist Reviewer B

Strengths: Dr. Freedland (primary mentor) is a renowned researcher in PC, with a superb track record for funding. Additional mentors provide complementary expertise in qualitative analysis, stakeholder engagement, and health services research. Exceptional letters of support are provided.

Weaknesses: No weaknesses were noted.

Research Project

Average Score: 8.3

Scientist Reviewer A

Strengths: This application makes it relatively easy to understand how the project will be conducted and how data will be interpreted and reported. Much of the design and concept overlaps with a clinical quality improvement type of effort, but as this is research that will be reported, there are some interesting questions raised by the approach.

Weaknesses: It is not clear how consent will be achieved for both patients and doctors whose conversations will be recorded.

Scientist Reviewer B

Strengths: A major strength is that the 4 aims are logical and build toward achieving the overall goal, which is to provide necessary preliminary data for an R01 application to conduct a larger study. The described approaches are appropriate and feasible.

Weaknesses: Aim 4 sample size (15) seems low. A larger sample size will provide better estimates to inform the future R01/multicenter trial.

Researcher Development Plan and Environment

Average Score: 8.9

Scientist Reviewer A

Strengths: A very good training plan is described, with supplemental course work that may be more than what is needed for such a proposal but is still very commendable. This reflects an unusual respect for the disciplines of social sciences coming from a physician-surgeon and is refreshing.

Weaknesses: No weaknesses were noted.

Scientist Reviewer B

Strengths: This is a detailed development plan including appropriate course work, specific conferences to attend, and justification for the need for additional training/development (to develop skills in qualitative analysis). The PI makes a strong argument of a need to have training in qualitative science. Cedars-Sinai seems an exceptional environment to conduct the proposed work, and there appears to be appropriate use of local resources (course work at RAND and UCLA).

Weaknesses: No weaknesses were noted.

Impact

Average Score: 9.6

Scientist Reviewer A

Strengths: This research is necessary for an evidence-based approach to clinical care today. The impact is essentially indispensable. Impact might be limited by the pilot nature of the study, where definitive answers may not yet be reached; however, it seems likely to provide impact anyway, especially given the applicant's track record of contribution to the literature.

Weaknesses: No weaknesses were noted.

Scientist Reviewer B

Strengths: Incorporation of LE into discussions about PC treatment is critically important. This study has the potential to lead to a future R01 that will ultimately make a major impact in the care of patients with PC. This proposed project/experience will bring the PI to the forefront of PC research.

Weaknesses: No weaknesses were noted.

Consumer Reviewer

Strengths: This proposal directly aligns with the PCRP overarching challenge to develop strategies to optimize the physical health of men with PC. In developing a pragmatic approach to improve the integration of LE into decision-making, this project has the potential to have a highly significant impact to improve both the mental and physical well-being of men with PC and limited LE. The proposal addresses 2 of the PCRP focus areas by addressing population science, survivorship, and decisional conflict questions. First, it will improve the ability to make informed decisions regarding whether to pursue aggressive or nonaggressive treatment by optimizing the communication between doctor and patient. Second, it will give patients a more prominent voice in determining which LE cutoffs are best suited for "non-aggressive" treatment, instead of relying on expert opinion. Lastly, intervention will reduce potentially morbid and unnecessary overtreatment for men with newly diagnosed PC and limited LE.

Weaknesses: No weaknesses were noted.

UNSCORED EVALUATION CRITERIA

Responsiveness to Overarching Challenges and Focus Areas

Scientist Reviewer A

The application addresses “develop strategies to optimize the physical health of men with prostate cancer” very well.

Scientist Reviewer B

The application is responsive to developing strategies to optimize the physical and mental health of men with prostate cancer.

Budget

Scientist Reviewer A

No concern was noted regarding the budget.

Scientist Reviewer B

No concerns were noted regarding the budget.

Application Presentation

Scientist Reviewer A

The application presentation is outstanding.

Scientist Reviewer B

The PI provides an outstanding presentation of the application.

ABSTRACT

Background. Life expectancy (LE) is a critical factor in treatment decision making for men with prostate cancer (PC), since limited LE predicts lower likelihood of sufficient longevity to benefit from treatment, higher morbidity after treatment, and decreased effectiveness of treatment. Despite a prominent role of LE in guidelines, patients with limited LE are often overtreated for indolent cancers. Data from non-cancer treatment settings suggest that this may be due to physician-level barriers precluding effective communication of LE. Yet, surprisingly little is known about how LE is actually communicated as well as physician and patient perspectives on how it should be communicated. Furthermore, patient/community opinions on what LE cutoffs are best suited to non-aggressive treatment are lacking, which precludes targeted interventions that seek to reduce overtreatment of men with limited LE.

Objective/Hypothesis. We will conduct a series of incremental studies to define how LE is currently communicated to men with PC, identify best practices for communication of LE data, determine community perspectives on when LE data is most relevant, and test a patient-centered approach to communication of LE in these situations. We hypothesize that: (1) communication of LE will be highly variable in terms of incidence of discussion and mode of communication; (2) both patient-level and physician-level barriers will exist to preclude optimal communication of LE; (3) community preferences on what LE cutoffs are best suited to "non-aggressive treatment" will differ from guidelines recommendations; and (4) a targeted, patient-centered approach to delivery of LE information will result in lower decisional conflict in men with newly diagnosed PC.

Specific Aims.

Aim 1: To characterize how LE is currently communicated to patients with newly diagnosed PC through qualitative analysis of treatment consultation discussions

Aim 2: To identify opportunities on how to optimize communication of LE to patients with PC through structured interviews of patients and specialty physician stakeholders

Aim 3: To identify community preferences regarding which LE cutoffs are best suited to nonaggressive treatment in early stage PC using online crowdsourcing of conjoint analysis tools

Aim 4: To determine if a patient-centered, targeted approach to communication of LE improves decisional conflict, quality of LE discussion, and reduces rates of overtreatment of patients with PC and limited LE

Study Design. First, we will delineate how LE is currently communicated to PC through qualitative analysis of treatment consultation transcripts of patients with early-stage prostate cancer. Second, we will engage patient and specialist physician stakeholders through structured interviews to identify barriers and opportunities to improve communication information about LE in PC treatment decision making. Third, we will use online crowdsourcing of conjoint analysis as a platform to study how patients, physicians, and the lay community value LE relative to other tradeoffs typically encountered in PC treatment decision making. We will analyze the data to determine how individual characteristics affect valuation of LE in the context of other tradeoffs and identify areas of consensus where LE appears to drive "non-aggressive" treatment choices. Fourth, we will conduct a randomized pilot trial to determine whether patient-centered communication of LE targeted to relevant situations improves decisional conflict and reduces overtreatment of PC.

Mentoring Plan. With this award, I am eager to expand my expertise beyond secondary data analysis to qualitative analysis, stakeholder engagement, decision analysis, and trial design. I am privileged to have a highly qualified, dedicated, and diverse mentorship team co-led by Dr. **Stephen Freedland**, a urologic oncologist and nationally renowned prostate cancer outcomes researcher, and Dr. **Brennan Spiegel**, a health services researcher and gastroenterologist who is a pioneer in digital health. I will meet with both Dr. **Freedland** and Spiegel weekly and share clinic/research space with them, which will facilitate formal and informal mentoring. These mentors and my team of collaborators will provide content expertise specific to my project aims, including qualitative analysis (**Spiegel, Anger**); stakeholder engagement (**Nuckols**); decision analysis (**Spiegel**); and trial design (**Freedland, Greenfield**).

Impact on Patients and Career Development. This project has the potential to substantially improve quality of life of men with limited LE by reducing overtreatment of indolent PC. We also hope that this work will serve as a paradigm for how to integrate LE into decision making for other indolent cancers, such as early-stage breast, kidney, and thyroid cancers. For my career development, this project will enable me to complete the research arc from "bench to bedside" and achieve my goal of becoming a translational HSR surgeon-scientist. Through this project, I will also gain valuable and unique skills in qualitative analysis and decision analysis that I can bring to the field and will help me to compete successfully for PCORI or R-level funding in the future.

RELEVANCE STATEMENT

Relevance to NURA Research Priorities. This project uniquely addresses the NURA research priority for prostate cancer (PC) seeking “to develop biomarkers that allow for identification of truly indolent disease.” We strongly feel that life expectancy (LE) is an unrealized biomarker that can reliably identify men in whom no treatment is necessary. Our previous work shows that LE not only predicts likelihood of sufficient longevity to reap survival benefits from aggressive treatment, but also the effectiveness of treatment itself. Unfortunately, through our work and others, it has also been shown that this very basic component of an individualized/precision medicine approach to PC care is often ignored, given high rates of overtreatment based on LE. With this career development award, we hope to improve uptake of this very basic and critical biomarker that already has guidelines approval.

This project will provide the foundation for a paradigm shift in the way LE is incorporated into PC treatment decision making. There is currently a lack of information regarding how LE is addressed in PC treatment consultation discussions, and by conducting textual analysis of transcribed treatment counseling discussions (**Aim 1**), we will begin to fill this void by defining the current standard of care for communication of LE. By engaging the relevant stakeholders in shared decision making (**Aim 2**)—men with PC and their physicians—we will also identify best practices for how physicians should deliver this information to patients in a meaningful way.

This study will also help to address the disconnect between patients, the community, and physicians regarding what are relevant LE cutoffs for pursuing “non-aggressive” treatment in the context of other tradeoffs. Currently we rely on expert opinion (in the form of guidelines) to define what is a reasonable LE cutoff to pursue observation rather than aggressive treatment for a given tumor risk profile. By analyzing crowdsourced conjoint analysis data of patients and individuals in the community, we will be able to determine if patients and the community agree with guidelines on what is a relevant LE cutoff for pursuing non-aggressive treatment across different tumor risk subtypes (**Aim 3**).

The final aim of the project will test a pragmatic approach to integration of LE into PC treatment decision making (**Aim 4**), capitalizing on lessons learned in the first two aims and targeted to clinical scenarios deemed most high-yield by patients (**Aim 3**). This will provide an opportunity for direct translation of the information gleaned from the first three aims to the point-of-care. This study will provide the pilot data for a larger clinical trial that will provide a definitive answer regarding whether LE information reduces decisional conflict and helps patients make better choices regarding treatment of early-stage PC. Ultimately, we believe that better incorporation of LE into treatment counseling will reduce unnecessary and potentially harmful overtreatment of indolent tumors in men with limited LE.

APPLICANT CAREER PLAN

Overview and Career Goals. I am an Assistant Professor of Urology at Cedars-Sinai Medical Center (CSMC), specializing in surgical urologic oncology. I am fellowship-trained in both urologic oncology and health services research (HSR) with an emphasis on epidemiology, variation in care, and comparative effectiveness. My research to date has shown that life expectancy (LE)—a critical, guideline-endorsed determinant for triage of aggressive vs. conservative management of genitourinary (GU) malignancies—is poorly incorporated into decision making, leading to both overtreatment and undertreatment. I plan to devote my next five years as junior faculty building expertise in qualitative analysis, conjoint analysis, and decision analysis techniques to inform a pilot trial of a patient-centered intervention to address this issue. **My overarching career goal is to become a surgeon-scientist who integrates my surgical practice with translational HSR focusing on development and testing of patient-centered interventions to improve treatment decision making for patients with GU malignancies.**

Coursework. I will take the following courses to gain knowledge in content areas relevant to my career development award, concurrent with the stages of the project: qualitative analysis* (years 1-2); decision analysis** (year 3); and trial design*** (year 4). I expect that ~10% of my time will be spent in coursework.

| Course | Location | Schedule | Duration | Time Commitment | Skills learned |
|---|---|---------------|----------|------------------------------|---|
| Qualitative Research* | RAND Pardee Graduate School | Year 1 Fall | 3 months | 2 hours twice weekly (Tu Th) | Learn to conduct semi-structured interviews, analyze textual data, and report results |
| M281/CHS216: Qualitative Research Methodology* | UCLA Fielding School of Public Health | Year 1 Spring | 3 months | 2 hours twice weekly (Tu Th) | Provides foundational knowledge in qualitative theory |
| HDS 200A: Health Analytics: Analyzing Big Data in Healthcare** | Cedars-Sinai Masters Program in Health Delivery Science | Year 2 Fall | 3 months | 2 hours twice weekly (W F) | Learn modern health analytic techniques, including ATLAS.ti and conjoint analysis |
| Behavioral Economics** | RAND Pardee Graduate School | Year 3 Fall | 3 months | 2 hours twice weekly (Tu Th) | Provide foundation in consumer behavior, including risk aversion, attribute weighting |
| Biomath M260A: Methodology in Clinical Research I: Clinical Trials*** | UCLA CTSI | Year 4 Fall | 3 months | 2 hours twice weekly (M W) | Learn clinical trial design with emphasis on biostatistics and epi |
| Biomath M259: Controversies in Clinical Trials*** | UCLA CTSI | Year 4 Winter | 3 months | 2 hours once weekly (M) | Learn critical evaluation of clinical trial design |

Conferences. Society of Urologic Oncology (December); American Urological Association Annual Meeting (May); Academy Health (June); University of Michigan Health Services Research Symposium (September)

Mentorship/Collaboration. Most important to my development as a researcher will be my superb, diverse, and highly experienced mentorship and collaboration team. My primary mentor, Dr. Freedland, will provide career mentorship and education related to prostate cancer. We currently hold a DoD PCRP idea development award analyzing variation in use of androgen deprivation therapy by age and comorbidity in men with advanced prostate cancer. As the editor-in-chief of a major prostate cancer journal, Dr. Freedland also often requests my review of manuscripts across a variety of topics, which keeps my knowledge base broad and current. We also often collaborate on secondary data projects such as the SEARCH database, NCDB, and SEER-Medicare. Dr. Spiegel, my co-primary mentor, will provide education in health services methodologies such as decision analysis, including the use of online crowdsourcing and conjoint analysis. He has previously conducted grant-funded research using online crowdsourcing and conjoint analysis using a similar approach to that proposed in this application. Drs. Anger and Nuckols will provide experience and content expertise in qualitative analysis and stakeholder engagement, respectively. Both of these collaborators have used methods similar to those proposed in this application to study other conditions. Dr. Greenfield, a member of the IOM and pioneer in life expectancy assessment, will provide content expertise in these areas as well as mentorship in creating a national network. He and I are currently hold a grant for establishment of a prostate cancer registry across 5 Southern California hospitals. The experience of my mentorship team will undoubtedly be crucial to the success of this project and my educational development.

AUA Office of Research Early Career Investigators Workshop

Mock Study Section Grant Application

***“Mechanisms of Fatty Acid Metabolism
in Prostate Differentiation and Disease”***

A NOT-funded K01 application and its review (PI redacted)

PROJECT SUMMARY/ABSTRACT

The overall goals of this proposal are to determine whether alterations in fatty acid metabolism affect prostatic differentiation and immunomodulation and to develop my technical and professional abilities to become an independent investigator. My professional goal is to acquire a position as a faculty member at a top-tier academic institution capable of providing a fully supportive research environment for my pursuit of the molecular links between systemic metabolic stress and chronic urogenital dysfunction. I will continue to foster strong interdisciplinary relationships with epidemiologists, diabetologists and molecular biologists to capitalize on the novel models of stromal-epithelial interactions developed in our laboratory. To achieve these goals, I will enroll in research training activities provided by the NIH MMPC/NIDDK, the ~~Vanderbilt University Medical Center Biomedical Research and Training~~ office and the ~~Vanderbilt~~ Institute for ~~Clinical Training and Research~~. These include meetings on mouse and experimental models for metabolic research, grantsmanship, biostatistics and proteomics analysis. The ~~Vanderbilt Diabetes and Research Training Center~~ also provides funding opportunities, meetings and seminars in conjunction with its ~~Enrichment, Training and Outreach~~ Program, with which I will continue to be actively involved. Additional career development mechanisms will include instructive seminars at national meetings, mentored guidance in grant and manuscript preparation, and academic job interviewing. An advisory committee will evaluate the completion of both my scientific and career development milestones and facilitate my transition to an independent investigator.

Benign prostatic hyperplasia and associated lower urinary tract symptoms (BPH/LUTS) are a severe physical and financial burden, which, given their association with metabolic dysfunction, will continue to grow in the number affected. Moreover, clinical management of BPH/LUTS has reached limitations in efficacy, predominantly due to a lack of understanding of basic prostatic metabolism. Therefore, increased focus on the fundamental molecular mechanisms governing prostatic differentiation and immunomodulation is needed in order to identify new targets for preventing benign growth and inflammation in obese and diabetic patients. Based on our preliminary studies, I hypothesize that fatty acid metabolism is a key mediator of the differentiation program driven by stromal-epithelial interactions and that chronic obesity and type II diabetes disrupt the normal metabolic hierarchy governing differentiation and immunomodulation. The specific aims of this study are as follows: Aim 1: Determine how stromal fatty acid metabolism mediates prostate epithelial differentiation. Aim Two: Determine how monounsaturated fatty acid metabolism mediates luminal differentiation. Aim Three: Determine how CD36-mediated fatty acid uptake *in vivo* mediates differentiation and obesity-induced prostatic inflammation.

PROJECT NARRATIVE

The mechanisms responsible for development of benign prostatic hyperplasia (BPH) remain unclear despite recent strong epidemiological correlations with metabolic syndrome and type II diabetes. It is anticipated that the results of this proposal will provide a molecular rationale for targeting systemic or local fatty acid metabolism to reduce prostatic hyperplasia and inflammation. Successful completion of this proposal will address deficiencies in current therapeutic modalities for BPH by deepening our understanding of the basic metabolic machinery mediating prostatic differentiation and immunomodulation.



EQUIPMENT

Cell culture room: two hoods, eight incubators, refrigerated centrifuge, Axiovert 200 fluorescent microscope with attached Axiocam digital camera and dedicated computer.

Laboratory: liquid nitrogen, -80, -20 and 4C storage, Gene quant spectrophotometer, transilluminator with dedicated digital camera, MJ Research thermocycler with twin and gradient heads, Perkin Elmer VICTOR 2 luminometer/fluorometer/visible microplate reader with dedicated PC and automated injection, Leica RM 2135 microtome with dedicated waterbath and slide dryers, microwave and pressure cooker for epitope retrieval. Three Zeiss dissecting microscopes, one with a c-mount including a dedicated digital camera and a Leica dual headed bright field microscope.

An adjacent 120 sq. ft. internal room houses a Zeiss Axio M1 upright Imaging microscope equipped for bright field, DIC and fluorescence. This microscope has an attached Axiocam Hrc camera and dedicated PC.

BUDGET JUSTIFICATION

Key Personnel

Douglas W. Strand, Ph.D. – Principal Investigator (9.0 calendar months salary and effort)

Postdoctoral Fellow, Department of Urologic Surgery

Dr. Strand will be responsible for the day to day research and career development activities described in this application. He will be responsible for establishing cell lines, in vitro characterization of these cell lines and in vivo characterization of their use in tissue regeneration experiments. Additionally, Dr. Strand will be responsible for the establishment, maintenance and characterization of the proposed mouse models of obesity and diabetes described in this application. Dr. Strand will complete didactic coursework in *Experimental Targeting of the Metabolic Syndrome* (http://www.ummc.edu/shared/metabolic_syndrome), which will provide expertise on *in vitro* and animal modeling of metabolism. He will attend and present at the Society for Basic Urologic Research (SBUR) annual meeting. He will be responsible for the timely publication and sharing of research related data. Salary in years one and two are based on NIH stipend rates with level increases applied at reappointment. Salary in years three through five are based on anticipated faculty appointment.

Simon W. Hayward, Ph.D. – Mentor (salary and effort not requested)

Professor of Urologic Surgery and Cancer Biology

Dr. Hayward has over 20 years of experience in the study of stromal-epithelial interactions with many studies performed using the tissue recombination xenografting system. Dr. Hayward and his former mentor, Dr. Gail Cunha, are internationally renowned for their expertise in this area. Dr. Hayward is also an expert on establishment of human cell lines and has established the *Vanderbilt Center for Benign Urologic Disease*, which is integrated into multiple *Vanderbilt* Centers, notably including the *Obesity and the Diabetes and Research Training* Centers. Dr. Hayward will mentor Dr. Strand's research and career development plan. He will be available on a daily to weekly basis.

Non-Key Personnel

Robert J. Matusik, Ph.D. – Consultant (salary and effort not requested)

Professor of Cancer Biology and Cell and Developmental Biology

Director of the Vanderbilt Prostate Cancer Center

William L. Bray Chair in Urologic Surgery

Dr. Matusik's laboratory has been engaged in studies on androgen action, molecular genetics of prostate cancer and genetically engineered mouse models of prostate cancer for the past 30 years. He also has extensive expertise in targeting transgenes to the prostate due to his discovery of the probasin (PB) promoter. The PB promoter has been used to create and characterize transgenic mouse models for prostate disease by his laboratory. He also has expertise in xenograft models for human prostate cancer grafted in immune compromised mice. Dr. Matusik will mentor Dr. Strand's career development and is available on a daily to weekly basis.

David L. Hachey, Ph.D. – Consultant (salary and effort not requested)

Professor of Pharmacology, Biochemistry and Chemistry

Director, Proteomics and Mass Spectrometry within the Mass Spectrometry Research Center

His primary research interests are in lipid, sterol and lipoprotein metabolism, the pharmacology of drugs affecting lipid metabolism, and mathematical modeling of complex physiologic systems using stable isotopic tracers. Dr. Hachey will assist Dr. Strand in the metabolic tracing of lipid metabolites in stromal-epithelial interactions as well as lipidomics analysis of tissues from animal models. Dr. Hachey will also serve with Drs. Hayward and Matusik on Dr. Strand's career advisory committee and is available on a daily to weekly basis.

Nada Abumrad, Ph.D. - Consultant (salary and effort not requested)

Dr. Robert C. Atkins Professor of Medicine and Obesity Research

Director of the Adipocyte Biology and Molecular Nutrition Core for IIR-ORC, Washington University

The research of Dr. Abumrad's group deals with the molecular mechanisms regulating utilization of fatty acids and how abnormalities in fatty acid metabolism can result in metabolic disease such as obesity and diabetes. She identified the plasma membrane protein CD36 as a facilitator of cellular fatty acid uptake and is now examining the role of CD36 in modulating tissue adaptation to nutrient fluctuations and other stresses. Mice models of tissue-specific variations in CD36 gene level are being studied but the goal is to translate findings in rodents to humans where polymorphisms in the CD36 gene are common. Dr. Abumrad will act as a consultant and will provide resources for Dr. Strand's study of the effects of CD36 on prostate differentiation and inflammation by making use of knockout mice, constructs and technical expertise in lipidomics.

James Ntambi, Ph.D. - Consultant (salary and effort not requested)

Professor of Biochemistry, University of Wisconsin-Madison

Steenbock Professor and Chair, Dept. of Nutritional Sciences

Dr. Ntambi is the world expert on SCD1 and studies its role during obesity for increasing the risk for developing insulin resistance and several chronic conditions such as diabetes, cardiovascular diseases, inflammation and non-alcoholic fatty liver disease. He has developed several tissue-specific animal models of SCD1 knockout and has extensive experience in lipidomics techniques. Dr. Ntambi will act as a consultant and provide resources for Dr. Strand's study of SCD1 in prostate differentiation.

Bonnie F. Sloane, Ph.D. - Consultant (salary and effort not requested)

Distinguished Professor and Chair, Department of Pharmacology, Wayne State University School of Medicine

and Distinguished Professor, Karmanos Cancer Institute

Dr. Sloane's laboratory has a longstanding interest in the roles of proteases in development and progression of cancer. The Sloane group has been a leader in applying live-cell imaging to the protease field, in our case to the study of tissue interactions in prostate differentiation. For this purpose, she has established new assays to study tissue morphology in real time as they form 3-dimensional structures. Dr. Sloane has ongoing collaborations with Dr. Hayward and thus represents a seamless addition to my team of consultants. She will provide resources and expertise on 3D modeling of tissue interactions.

Supply Expense

General Laboratory Supplies

Funds are requested for the purchase of supplies necessary for the functioning of the laboratory. These include gloves, tips, dissecting instruments, and other general laboratory items.

Year 1 - \$2,612

Year 2 - \$2,952

Year 3 - \$2,274

Year 4 - \$1,996

Year 5 - \$2,685

Molecular Biology/Cell Culture Supplies

Funds are requested for the purchase of molecular biology supplies including retroviral packaging and production supplies for constructs, cell culture supplies and media including serum, medium, antibiotics and biochemical selection reagents. Costs are frontloaded for development of cell lines in years 1 and 2.

Year 1 - \$9,041

Year 2 - \$3,763

Year 3 - \$1,800

Year 4 - \$1,800

Year 5 - \$1,800

Antibodies, Immunohistochemistry and Biochemistry Supplies

Funds are requested for the purchase of supplies for preparation of samples and for histochemistry and immunohistochemical analysis of tissue sections from mice as well as antibodies for IHC, Western blot and FACS.

Year 1 - \$5,500

Year 2 - \$3,200

Year 3 - \$3,200

Year 4 - \$3,200

Year 5 - \$3,200

Mouse Chow

Funds are requested to purchase specially formulated high fat diet mouse chow. Estimated cost in years two through four are based on a price of \$45.25/kg.

Year 1 - 0

Year 2 - \$2,036 based on 9, 5kg purchases

Year 3 - \$2,715 based on 12, 5kg purchases

Year 4 - \$2,715 based on 12, 5kg purchases

Year 5 - \$0

Animal Purchases

Mice will be used for experiments in each aim. Funding is requested for the purchase of nude mice and pregnant rats.

Year 1 (Aim 1) - **Total Year 1 - \$1,155**

12 adult male nude mice @ \$62.35 - \$748.20

4 pregnant rats @ \$101.80 - \$407.20

Year 2 (Aims 1 and 2) - **Total Year 2 - \$2,980**

24 adult male nude mice @ \$63.60 - \$1,526.40

14 pregnant rats @ \$103.85 - \$1,453.90

Other Expenses

Animal Per Diem

Estimated cage costs in year 1 and 2 reflect animals for xenografting experiments in Aim 1 and breeding of GEM for Aims 2 and 3 while years 3 through 5 reflect the use of GEM for dietary experiments.

Year 1 - 9 cages for 365 days @ \$0.85/cage/day = \$2,792

Year 2 - 21 cages for 365 days @ \$0.87/cage/day = \$6,669

Year 3 - 21 cages for 365 days @ \$0.89/cage/day = \$6,822

Year 4 - 21 cages for 365 days @ \$0.91/cage/day = \$6,975

Year 5 - 12 cages for 365 days @ \$0.93/cage/day = \$4,073

Core Services

Cell Sorting/FACS

Costs are based on a rate of \$65/hour for unassisted acquisition. Year 3 includes an additional \$200 fee for attendance to the **Flow Cytometry** training course.

Year 1 - \$0

Year 2 - \$0

Year 3 - \$1,175 (15 x \$65) and \$200 training fee

Year 4 - \$1,300 (20 x \$65)

Year 5 - \$1,300 (20 x \$65)

Van Damm Hormone Assay and Analytical Services Core

Funds are requested to support the use of the Hormone Assay and Analytical Services Core for assays of fatty acid analysis of various lipid species in mouse samples.

Year 1 - \$0

Year 2 - \$0

Year 3 - \$3,564 (162 x \$22)

Year 4 - \$3,564 (162 x \$22)

Year 5 - \$8,492 (386 x \$22)

Molecular Cell Biology Resource Core

Funds are requested for the use of quantitative PCR machines in MCBR core.

Year 1 - \$100

Year 2 - \$100

Year 3 - \$150

Year 4 - \$150

Year 5 - \$150

Training

An Organ Systems Approach to Experimental Targeting of the Metabolic Syndrome

Funds are requested for the PI to enroll and attend training in animal models of metabolic syndrome hosted by the NIDDK/MMPC. The objective of this course is to give trainees the tools needed to assess whether an experimental intervention alters macronutrient metabolism, energy balance, cardiovascular homeostasis or animal behavior. This course is held at [REDACTED] and uses a combination of lectures, hands on laboratory demonstrations and data problem sessions to give you the tools necessary to probe physiological systems.

Year 1 - \$500

Publications

Funds are requested to support the publication of manuscripts emerging from the proposed work.

Year 1 - \$1,500

Year 2 - \$1,500

Year 3 - \$1,500

Year 4 - \$1,500

Year 5 - \$1,500

Travel

The PI requests funds to attend the annual SBUR meeting.

Year 1 - \$1,800

Year 2 - \$1,800

Year 3 - \$1,800

Year 4 - \$1,800

Year 5 - \$1,800

Fringe Rate

Current Institutional Fringe Benefit Rate

Medical Center Non-Hospital Staff (years 1 and 2) 24.8%

Medical Center Non-Clinical Faculty/Senior Staff (years 3-5) 21.5%

CAREER GOALS AND OBJECTIVES

My professional goal is to obtain a tenure-track position as an Assistant Professor at an elite academic institution that will enable me to conduct cutting-edge urogenital disease research. I intend to translate my laboratory studies into an improved standard of care for patients with BPH/LUTS. In addition to obtaining technical and scientific training in the implementation of lipidomics of the prostate and didactic training in experimental models of metabolic diseases, I will also increase my exposure to clinically trained investigators treating patients with BPH/LUTS, which I believe will be essential for my transition to a successful independent investigator.

Potential to develop into an independent investigator: I have been funded through a highly competitive Postdoctoral Fellowship from the DoD, a T32 Microenvironmental influences in Cancer Fellowship, a NIH Loan Repayment Program (LRP) grant and [REDACTED] federally funded Institute for [REDACTED] and [REDACTED] Research (VICTR) program. Since I started my postdoctoral fellowship, I have either first authored or co-authored 11 publications. I have two additional publications to be submitted. My research training, publications, ongoing collaborations, mentoring program, and the letters of reference from three prominent scientists demonstrate my potential to develop into a successful independent investigator.

To work with and lead a group of professionally diverse individuals in the pursuit of a common research goal: I have established collaborations with basic scientists and clinicians at [REDACTED] University [REDACTED] Medical Center [REDACTED] to pursue studies regarding the role of FAs in prostate differentiation and progression to BPH. This experience has been especially rewarding, as it required that I engage with individuals from diverse backgrounds, training, and skill. I believe that the interactions between groups of basic scientists and clinical professionals lie at the heart of translational research. The establishment of these collaborations has greatly increased my productivity and educated me in the regulatory processes and approvals required for the acquisition of fresh and archived human tissue. These collaborations have resulted in the banking and categorization of over a 150 clinically annotated human prostate BPH specimens that have been paraffin-embedded, OCT-embedded, and flash frozen for lipidomic analysis. Recently, we have also begun to collect serum samples for analysis of diabetic parameters that relate to our genetic and lipidomic findings, which will further support my on-going research efforts. These experiences have helped me develop the leadership, communication, and scientific skills required to be a successful independent investigator.

Ability to obtain funding: I obtained competitive funding for a DoD Postdoctoral Training Fellowship in [REDACTED] to study the role of the microenvironment in prostate disease. I received internal pilot funding from [REDACTED] (see Resources Page) for genetic and lipidomic analysis of human prostate. I received a competitive NIH LRP grant in [REDACTED], which requires a translational research commitment to human disease and am awaiting news of a two-year renewal. I have assisted in writing grants with my mentor that were funded through NIH and DoD as well as internally to [REDACTED] Diabetes and Research Training Center (unfunded). I helped write a successful [REDACTED] Medical Scholars Program grant with a medical student who I will mentor 2012-13. In consultation with my mentors and peers, I developed the ideas in this proposal, and Drs. [REDACTED] and [REDACTED] will continue to train me in the nuances of grant preparation.

Research skills and experience: I received my PhD in the field of the prostate microenvironment in [REDACTED] in the laboratory of Dr. [REDACTED] (Department of Medicine) and published 2 first author papers on the role of TGF beta and FGF2 in prostatic stroma, which included the development of a new transgenic mouse model. I am continuing my research in urology as a postdoctoral fellow in Dr. [REDACTED] lab, where I have developed or collaborated to develop *in vitro*, *in vivo* and *in silico* models for the study of tissue interactions in differentiation

and disease. I have also worked with Dr. [REDACTED] in the characterization of an inducible CK14-Cre^{ERT2} mouse model to study the impact of basal cells on prostate differentiation *in vivo*. The mentorship of Dr. [REDACTED] and interactions with urologists within our clinical department will add to my research skills and address deficiencies in training.

CAREER DEVELOPMENT/TRAINING ACTIVITIES DURING AWARD PERIOD

Mentorship structure and enhanced technical training: My experience as a postdoctoral fellow in Dr. [REDACTED] laboratory has been a true collaboration. Dr. [REDACTED] has given me the space, time, and resources to develop my own ideas about benign prostate disease, an area for which he and my graduate mentor, Dr. [REDACTED], have been funded for years. My work in Dr. [REDACTED] laboratory has resulted in numerous grant applications to our mutual benefit. I learned how to think and write about my hypotheses, and also how to organize projects and budgets. To overcome any further deficiencies I have the support of my collaborators who will provide technical training in lipodomics and animal modeling of obesity and diabetes, which will facilitate my goal of studying the role of obesity and diabetes in BPH/LUTS. The five years of mentorship provided by this K01 will allow me to hone the technical expertise and grant writing skills necessary to develop an independent career focused on understanding how systemic metabolic diseases contribute to prostatic inflammation and hyperplasia.

I designed the research plan in this proposal, which balances supervised research while fostering independence, in close consultation with Drs. [REDACTED], [REDACTED], and [REDACTED]. I have unlimited access to Dr. [REDACTED] and I will interact with career advisors [REDACTED] and [REDACTED] by attending lab meetings to evaluate research progress and interact with their lab personnel for technical training as needed. Consultants for technical training and resources include Dr. [REDACTED] (Professor of [REDACTED] Cell Biology & Physiology, [REDACTED] University) and Dr. [REDACTED] (Professor of [REDACTED] Biochemistry and Nutritional Sciences, University of Wisconsin-Madison) who will provide advice and resources on animal models of obesity and diabetes. Dr. [REDACTED] (Mass Spectrometry Research Center, Professor of Biochemistry and Pharmacology, VUMC) will provide expertise in tandem HPLC/Mass Spec and metabolic tracing studies. Dr. [REDACTED] (Distinguished Professor and Chair of Pharmacology, Wayne State University) will provide expertise in 3D/4D co-culture modeling. I believe this added technical expertise and mentorship will provide a strong foundation enabling my transition to an independent investigator. Additional technical training will be attained through coursework on proteomics analysis and the NIH and MMPC/NIDDK co-sponsored meeting on Experimental Targeting of the Metabolic Syndrome.

Grant writing skills and didactic coursework: The [REDACTED] Biomedical Research [REDACTED] and Training [REDACTED] office (<https://med.vanderbilt.edu/btr/>) supports and coordinates graduate education and postdoctoral training at [REDACTED]. I have attended a [REDACTED] office sponsored Grant Writing workshop consisting of presentations on navigating the NIH and peer review process, identifying and competing successfully for non-NIH funding, and writing a compelling research plan. I have also attended [REDACTED] Responsible conduct of research course, and will take a refresher course pending K award funding. The [REDACTED] Institute for Clinical and Translational Research (ICTR) (<http://www.mc.vanderbilt.edu/ict/>) was awarded a Clinical and Translational Science Award (CTSA), which provides multiple programmatic resources for training (<http://www.vanderbilt.edu/ict/pub/ucts/index.html?site=10>), notably including an Education, Training and Career Development Program for coursework in biostatistics and clinical trial design, implementation, and interpretation available to Post-Doctoral Ph.D. and M.D. enrolled in the Masters of Science in Clinical Investigation program at VUMC (<https://med.vanderbilt.edu/mcsc/2016/03/31/>), led by [REDACTED], [REDACTED], Catherine McLaughlin, [REDACTED] Vascular Biology. I will audit the Analytical Proteomics (BCHM 362) and Bioinformatics (BMIF 310 and 311) courses taught by Drs. [REDACTED] and [REDACTED].

The [REDACTED] Diabetes and Research Center (DRC) is an interdisciplinary program involving 98 participating faculty distributed among 18 departments in two schools and four colleges at [REDACTED] and at neighboring Meharry Medical College (http://www.mc.vanderbilt.edu/diabetes/drc/about_drc.php). In addition to access to numerous core facilities (<http://www.mc.vanderbilt.edu/diabetes/drc/core-facilities.php>), two of the [REDACTED] programs are particularly relevant for my own training. The [REDACTED] Pilot and Feasibility Program facilitates the

development of new investigators into fully independent scientists and encourages scientists in other fields to enter the field of diabetes research by providing pilot funding, which I have recently applied to receive. I have also been involved in the ~~Enrichment, Training, and Outreach~~ Program, which fosters an environment conducive to collaborative, interdisciplinary research ~~DRTC Seminar Series, Vanderbilt Diabetes Center, Diabetes Day,~~ and to training new diabetes scientists.

Scientific and career development advisory committee: In order to evaluate my progress during the proposal period, I have formed an advisory committee that will meet every six months to provide guidance on the completion of both scientific and career development milestones detailed above. This committee will consist of my primary mentor Dr. ~~Simon Hayman~~ and collaborative scientific contributors Drs. ~~David Hasley~~ and ~~Robert Johnson~~. These individuals have a long record of peer-reviewed grant support as well as training and mentoring of graduate students, postdoctoral fellows, residents, and clinical fellows. The committee will focus on my professional development providing an overall assessment of my ability to implement proposed training, timing of grant/and manuscript submissions, and applications for tenure track academic appointments. I anticipate submitting at least 3 first author manuscripts during this 5 year time period. I will compete for initial NIH R01 funding opportunities during the fourth and fifth years of the project phase.

Academic job search: I plan to search for an independent academic position approximately 2 years into funding. To prepare for this, I will complete a Seminar Series offered by the ~~Vanderbilt Clinical and Translational Scientist Development~~ program ~~(http://www.mc.vanderbilt.edu/clinicaltranslational/)~~. Seminars include (among others): *Preparing for an Academic Job Search*, *Negotiating your First Academic Position*, and *Obtaining your First R01*.

SPECIFIC AIMS

Current treatments for benign prostatic hyperplasia and associated lower urinary tract symptoms (BPH/LUTS) display limited efficacy. BPH/LUTS is linked to obesity and type II diabetes (T2D), but unlike other well-characterized tissues adversely affected by systemic metabolic disease, local effects on prostate differentiation and immunomodulation are poorly understood. Defects in glucose and fatty acid metabolism are major contributors in systemic insulin insensitivity and inflammation. Despite strong epidemiological links, little is known about such defects on prostate differentiation. In a manuscript currently under review, we demonstrate that peroxisome proliferator activated receptor gamma 2 (PPAR γ 2) increases basal and luminal epithelial differentiation and decreases lipogenesis and oxidative stress. PPAR γ agonists (TZDs) have been used in the treatment of T2D and act predominantly through adipose and muscle to relieve whole body insulin insensitivity by increasing fatty acid oxidation. Similar to these other tissues, PPAR γ 2 drives fatty acid transport and β -oxidation while decreasing glucose oxidation, which positively correlates with prostatic differentiation markers. Key regulators of fatty acid and glucose metabolism including CD36 and SCD1 were regulated by PPAR γ 2, and each is compartmentalized into a specific cell type. Whether these genes mediate differentiation through a metabolic stromal-epithelial interaction will be tested. Furthermore, we show that while TZD treatment increased prostatic epithelial differentiation, a high fat diet caused hyperplasia and inflammation. Obesity and T2D cause defective fatty acid metabolism resulting in insulin insensitivity and inflammation in numerous tissues, but it is unclear whether similar effects drive inflammation in prostate. Newly developed mouse and human models of cellular differentiation, obesity and inflammation will determine whether alterations to fatty acid metabolism disrupt prostatic differentiation and immunomodulation. To address the **hypothesis** that *fatty acid metabolism mediates prostate differentiation and immunomodulation*, the following aims are proposed:

Aim One: Determine how stromal fatty acid metabolism mediates prostate epithelial differentiation.

Rationale: Preliminary data demonstrate that ectopic expression of the nuclear receptor PPAR γ 2 in vitro or PPAR γ agonist treatment in animals regulated numerous downstream genes involved in fatty acid metabolism including CD36, which were highly expressed in prostate smooth muscle, and were associated with basal and luminal epithelial differentiation. These data suggest a potential metabolic mechanism mediating stromal-epithelial interactions in prostate differentiation. To test the **hypothesis** that *stromal fatty acid metabolism drives prostate epithelial AR expression and function*, tissue regeneration and 3D co-culture models of epithelial differentiation have been established. Prostate stromal cell lines genetically modified to overexpress PPAR γ 2 or CD36 will be co-cultured or recombined *in vivo* to determine their effect on epithelial differentiation.

Aim Two: Determine how monounsaturated fatty acid metabolism mediates luminal differentiation.

Rationale: Preliminary data indicate that PPAR γ 2 upregulates the fatty acid desaturase SCD1 in basal cells, which is induced by insulin and saturated fat in other tissues and maintains differentiation in adjacent cells through lipogenesis of monounsaturated fatty acids. To test the **hypothesis** that *basal cell SCD1 expression regulates luminal differentiation through paracrine MUFA production*, metabolic tracing studies will be performed using HPLC/MS on deuterium-labeled human basal epithelial cells over-expressing or knocking down SCD1. Effects of altered basal cell SCD1 on luminal cell differentiation will be tested using 3D and tissue recombination techniques. TZD-induced prostatic differentiation in control vs. SCD1-ablated animals will determine the contribution of SCD1 to prostatic differentiation *in vivo*.

Aim Three: Determine how CD36-mediated fatty acid uptake *in vivo* mediates differentiation and obesity-induced prostatic inflammation.

Rationale: Basal levels of fatty acid uptake and oxidation are important for proper function, but over-activation in obesity is pro-inflammatory in multiple tissues. While TZD treatment of animals increased prostatic differentiation, obese animals demonstrated prostatic hyperplasia and inflammation with increased CD36, which induces inflammation through eicosanoid production in other tissues. To test the **hypothesis** that *CD36 mediates prostatic differentiation and inflammation*, CD36-ablated animals will be fed a high fat diet and prostatic differentiation, lipid profiles and inflammation will be assessed.

RESEARCH STRATEGY

Significance: BPH is an enlargement of the periurethral area of the prostate gland that affects 40% of men over 60 years old, 20-30% of whom require surgical intervention by age 80, which is associated with numerous negative side-effects (1). The etiology of BPH is still unknown, although analyses of associated co-morbidities have revealed insights into potential molecular mechanisms involving sequelae of metabolic syndrome (2, 3). The global prevalence of glycemia and diabetes increased from 153 million affected individuals in 1980 to 347 million in 2008 (4), which will likely increase the incidence of BPH/LUTS. Current medical therapies for BPH include α_1 -adrenergic blockers to relax smooth muscle contraction and relieve urinary flow symptoms and 5 α -reductase inhibitors, which slowly decrease prostatic volume by inhibiting dihydrotestosterone production. Even under combination therapy, 33% of patients do not respond. Further, a significant proportion of patients eventually progress to require surgery despite receiving one or both drug therapies (5), warranting a new strategy targeting the cause vs. symptoms. This necessitates a wider understanding of prostate disease as a consequence of metabolic dysfunction, which requires an understanding of normal prostate metabolism.

This proposal will integrate benign urologic disease research, specifically in the area of BPH/LUTS, with work in the fields of obesity and diabetes. BPH is the most common symptomatic benign condition in men resulting in considerable morbidity and associated clinical expenses. Co-morbidities including obesity and type 2 diabetes are commonly seen in patients with BPH/LUTS and have been recognized for decades (6). BPH patients have a higher incidence of Type 2 diabetes (T2D), and the progression and severity of LUTS in diabetic patients is greater compared to non-diabetic BPH patients (7, 8). Obesity, as measured by waist to hip ratio, is also strongly correlated with the incidence and severity of BPH (9). Diabetes mellitus, hypertension, obesity, ischemic heart disease, excess intake of carbohydrates and fats, insulin resistance with hyperinsulinemia and dyslipidemia are associated with BPH (2).

The 2008 NIDDK Prostate Research Strategic Plan specifically describes the need for community based and prospective studies, methods to identify high-risk men, characterization of modifiable risk factors, development of new biomarkers of BPH, and basic research to understand mechanisms relating metabolic factors to prostate inflammation and BPH progression (<http://www2.niddk.nih.gov/NR/rdonlyres/318606D2-A9D1-4CAD-B9BF-8EB3009C83BE/0/NIDDKProstateStrategicPlan.pdf>). This proposal addresses basic research methods to determine the molecular relationships among FA metabolism, prostatic differentiation and immunomodulation as a means to understand the molecular mechanisms underlying the susceptibility of patients with obesity and diabetes to develop symptomatic BPH.

The goal of the proposed research is to create a training plan consisting of mentored research, didactic coursework, and technical instruction to enable my transition to an independent urogenital disease investigator by capitalizing on the supportive environment created by my mentors and the [REDACTED] community at large. Successful completion of this proposal will not only train a new investigator in a disease impacting *increasing* numbers of aging men with obesity and T2D, it will also lay the groundwork for a molecular understanding of nutritional metabolism governing stromal-epithelial interactions in prostate differentiation, thereby providing insights into etiological factors of hyperplasia and inflammation in BPH/LUTS.

Innovation: This proposal is innovative because it explores a potential metabolic mechanism driving stromal-epithelial interactions in prostate differentiation, an axis that is proposed to become dysfunctional in BPH leading to inflammation and hyperplasia. Successful completion of this proposal will significantly change the way we view prostate differentiation and disease, as shown in Figure 2a where storage of neutral lipids in prostate basal cells has been identified for the first time. These data will potentially produce a new paradigm of therapeutic intervention targeted to lipid metabolism in smooth muscle or basal cells. The refinement of models of metabolic crosstalk between tissues will advance our understanding of diseases in multiple organs.

Approach: This proposal integrates a variety of disciplines and mentors to both develop a personalized training program and to address a fundamental question in prostate biology. The premise is that stromal and basal cell FA metabolism are fundamental metabolic activities driving prostate epithelial differentiation, in obesity the oversupply of FAs leads to elevation of toxic FA metabolites and inflammation. The far-reaching implications lay a foundation for a career as an independent investigator in the metabolism of BPH/LUTS. In the first 2 years of funding and mentorship, I will become proficient in metabolic tracing and HPLC/MS [REDACTED] characterization of dietary and transgenic animal models of obesity and diabetes ([REDACTED] and [REDACTED]) and 3D co-culture modeling ([REDACTED]). I will also complete didactic coursework in mouse models of metabolic disease, proteomic analysis, grantsmanship and training in the responsible conduct of research. During year 4, I will submit an R01 to study effects of metabolic disease on BPH/LUTS.

Potential stromal-epithelial metabolic interactions regulating prostate differentiation: We recently showed that re-expression of PPAR γ 2 in a PPAR γ KO prostate epithelial cell line resulted in paracrine action to increase the percentage of basal cells as well as the expression and function of luminal epithelial androgen receptor (AR) (10). Identifying prostate-specific targets of obesity and T2D necessitates a more complete understanding of the impacts FA metabolism on prostate differentiation. Our recent data suggest that PPAR γ 2-mediated FA metabolism is sufficient to drive basal and luminal differentiation (10). What is yet unclear is whether paracrine regulation of luminal epithelial differentiation is (a) directly related to the increase in paracrine stromal FA metabolism and/or (b) indirectly related to a stromal-mediated increase in basal cell differentiation. It has been known for some time that the microenvironment is important for prostatic differentiation (11), but the mechanism(s) by which these cells communicate remain unidentified. This proposal seeks to determine the broader implications of dysfunctional FA metabolism on prostate differentiation and immunomodulation under systemic metabolic dysfunction by focusing on the hypothesis that stromal-epithelial interactions in prostate differentiation are mediated by a metabolic crosstalk between tissue compartments. Evidence from mass spectrometry analysis of human prostate tissue suggests that multiple lipid species are altered in BPH (Figure 1). We also show for the first time that neutral lipids are a normal part of prostate metabolism (Figure 2a), which may be lost in BPH (Figure 2b). Although preliminary data for this proposal were generated with the available PPAR γ tools (knockouts, cell lines, etc.) in our laboratory, the widely recognized risk of PPAR γ -agonist usage (12) mandates that we pursue a newer, deeper understanding of the mechanisms governing cellular differentiation and disease for the purpose of developing new drugs and/or re-purposing existing ones. Therefore, this proposal is focused on PPAR γ 2 and two downstream genes that are actively regulated in obesity, diabetes and inflammation and are strongly expressed in normal human prostate: SCD1 is a lipogenic gene expressed in prostate basal cells (Figure 2c) and CD36 is a FA transporter expressed in smooth muscle and epithelium (Figure 2d).

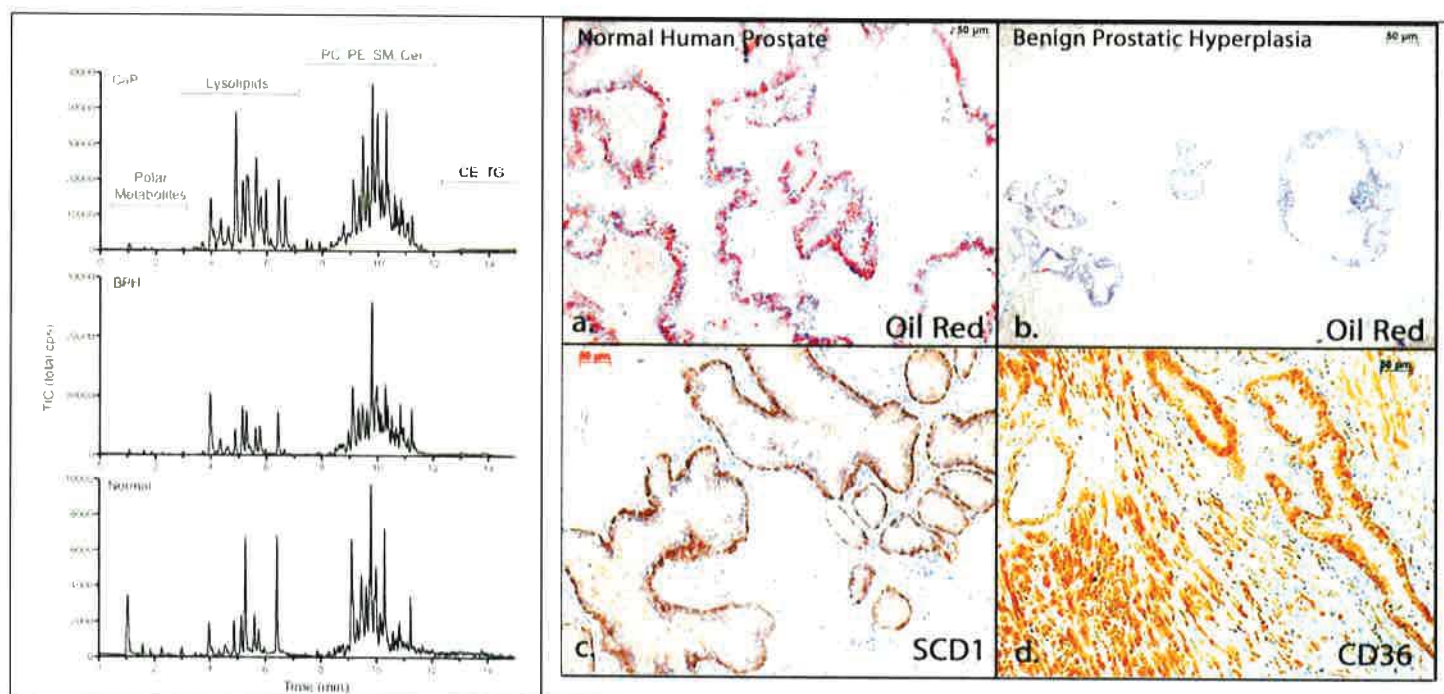


Figure 1. Representative total ion chromatograms of human prostate lipid extracts from normal (bottom), BPH (middle) and cancer (top) shows shifts in multiple unidentified lipids.

Figure 2. Oil red O staining of normal (a) vs. BPH (b) human tissue demonstrates loss of neutral lipids in BPH epithelium, perhaps due to insulin insensitivity and decreased basal cell lipogenesis. (c) SCD1 immunoreactivity is localized to basal epithelium. (d) CD36 immunoreactivity is localized to smooth muscle and epithelium.

BPH/LUTS as sequelae of metabolic disorders: When the limits of adipocyte expansion are reached at morbid obesity, ectopic lipids accumulate in secondary organ sites causing dyslipidemia, lipotoxicity and inflammation, usually concurrent with insulin resistance in multiple tissues (13). The consequence of insulin resistance is T2D, which results in hyperinsulinemia and hyperglycemia in a chronic setting. Even in non-

diabetic patients, hyperinsulinemia and dyslipidemia are independent risk factors for BPH (14). Recent studies suggest that PPAR γ agonists called thiazolidinediones (TZDs) restore insulin sensitivity in skeletal muscle by alleviating local hyperlipidemia with storage of excess FAs in intramuscular adipocytes (15). Pathophysiologic links between insulin sensitivity, lipotoxicity, and inflammation are well-recognized and are particularly relevant to the therapeutic efficacy of TZDs (16). We have been exploring the role of PPAR γ in prostate lipid metabolism and glucose homeostasis and have shown that PPAR γ knockout in mouse prostate epithelium causes hyperplasia, inflammation and autophagy (17). Our recent hypothesis that PPAR γ provides a molecular link between prostatic health and systemic metabolic alterations (3) is based on data that PPAR γ isoforms regulate distinct aspects of mouse prostate epithelial glucose and lipid metabolism to drive differentiation (10), which may indicate the need for the development of isoform-specific agonists. Specifically, PPAR γ 1 increased triglyceride levels and saturation through downregulation of basal cells and SCD1, while PPAR γ 2 increased FA oxidation, desaturation and differentiation. Furthermore, knockdown of PPAR γ 2 in human prostate epithelia results in a loss of prostate differentiation with a corresponding saturation of diglycerides (manuscript in preparation). Changes to FA saturation ratios are an important metric for inflammation, insulin sensitivity (18, 19) and prostate disease progression (20, 21), suggesting that a deeper understanding of the role of FA metabolism in prostate differentiation could provide insights into the etiology of inflammation in BPH.

Preliminary Studies: PPAR γ is a nuclear receptor that acts as a rheostat to modulate the supply of glucose and FAs through two isoforms (22). PPAR γ 1 is widely expressed while PPAR γ 2 expression is restricted (23). We show that PPAR γ 2 is highly enriched in prostate smooth muscle and that ectopic expression in a PPAR γ KO cell line induces differentiation through regulation of a unique set of genes to be studied here. It is expected that the study of these genes will provide insights into the normal metabolic infrastructure regulating prostate cellular differentiation and function (10). The function of PPAR γ in other tissues is demonstrated by the mechanism of action of PPAR γ agonists (TZDs). TZDs restore insulin sensitivity and glucose homeostasis through an increase in intramuscular adipocytes, resulting in reduced muscular lipotoxicity and inflammation (15). When we treated mice with a TZD, we also found an increase in FABP4-positive intramuscular adipocytes within the prostate (Figure 3a) as well as an expansion of the PPAR γ 2⁺/AR⁺ smooth muscle (Figure 3b). Importantly, TZD-induced expression of FA metabolism genes correlated with significantly increased expression of AR and probasin (400-fold) in mice (10), suggesting a potential metabolic link to epithelial differentiation. Unlike human prostate where PPAR γ 2 was not readily detectable except in the smooth muscle (Figure 3d), in mouse prostate PPAR γ 2 is also sporadically expressed in epithelia (Figure 3c). The loss of FA metabolism in prostate-specific PPAR γ KO animals potentially explains their autophagic phenotype (24). Moreover, a PPAR γ KO mouse prostate epithelial cell line rescued with PPAR γ 2 demonstrated a significant (non-overlapping) increase in CK14⁺ basal cells in culture (Figure 3f) and in tissue regeneration experiments (10) as well as a corresponding significant (non-overlapping) increase in AR expression and responsiveness (Figure 3g). Rescue with PPAR γ 1 isoform did not

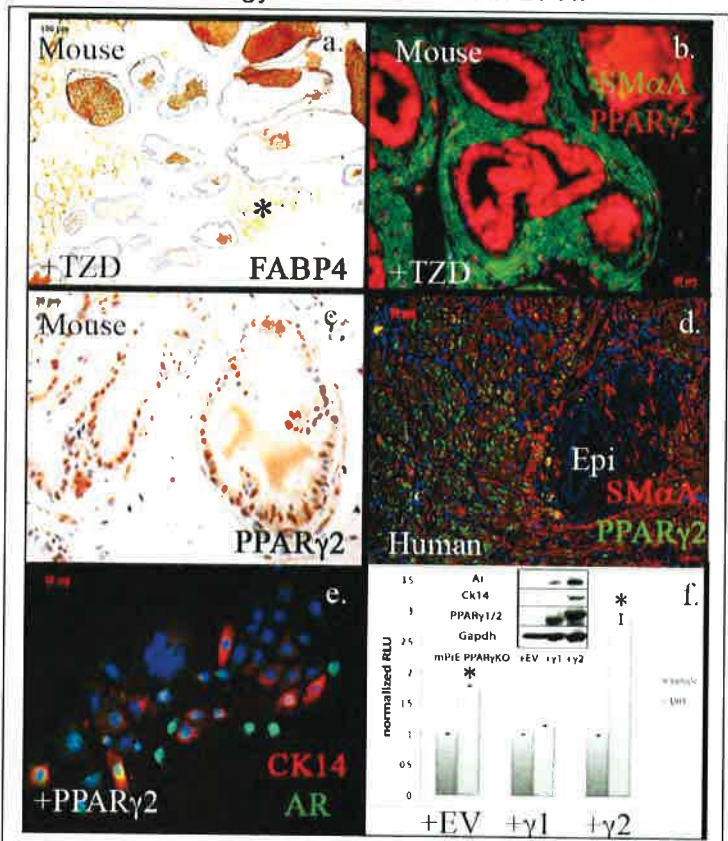


Figure 3. PPAR γ 2 regulates prostate epithelial differentiation through metabolic control of FA metabolism. (a) Intramuscular adipocytes are increased in TZD-treated mouse prostates. PPAR γ 2 is expressed in smooth muscle (b) and some epithelia (c) of mice, but only in smooth muscle of human prostate (d). Ectopic PPAR γ 2 expression increases basal cell numbers and luminal cell AR expression and responsiveness (e,f).

increase basal cells or AR responsiveness, suggesting an isoform- specific regulation of prostate differentiation by PPAR γ 2. These data suggest that PPAR γ 2 potentially regulates epithelial differentiation through a paracrine mechanism. Microarray and qPCR validation of prostate epithelial cells expressing ectopic PPAR γ 2 identified a number of genes involved in FA and glucose metabolism (10). These genes included SCD1 and CD36, a well-characterized FA desaturase and long chain FA transporter, respectively. Both SCD1 and CD36 are implicated in obesity, insulin resistance and inflammation (25, 26) and are expressed in specific prostate tissues (Figure 2c,d), which may represent target lipid metabolic cell types that govern normal prostatic differentiation and immunomodulation (modeled in Figure 5a). Although SCD1 and CD36 are highly expressed in basal cells and smooth muscle, respectively (Figure 3c&d), their roles in prostate differentiation have never been tested. Aim 1 will test the role of stromal PPAR γ 2/CD36 in driving epithelial differentiation. Our data from PPAR γ -rescued cells showed that decreased SCD1 expression in PPAR γ 1-rescued cells was associated with decreased differentiation and increased FA saturation (10), suggesting a potential connection between the saturation of FAs and prostate differentiation. Notably, polyunsaturated fatty acids (PUFAs) are active participants in partitioning FAs towards oxidation (27) and are putative ligands of PPAR γ (28). With consultant Dr. [REDACTED] (see attached letter), the role of SCD1 in prostate differentiation (Aim 2) will be determined.

Obesity is linked to inflammation through cellular lipotoxicity and results in insulin insensitivity in multiple tissues (25). While etiological factors driving inflammation are tissue-specific, a high ω -6: ω -3 fatty acid ratio as well as chemokine and eicosanoid production are generally pro-inflammatory, which can be reversed by ω -3 polyunsaturated fatty acid supplementation (29). Prostates from numerous mouse models of obesity and diabetes displayed increased inflammation (Figure 5h). Under chronic HFD treatment, most PPAR γ -regulated genes were downregulated in prostates; however, CD36 expression was increased (10). CD36 is a multifunctional scavenger receptor originally referred to as fatty acid translocase (FAT) that regulates the

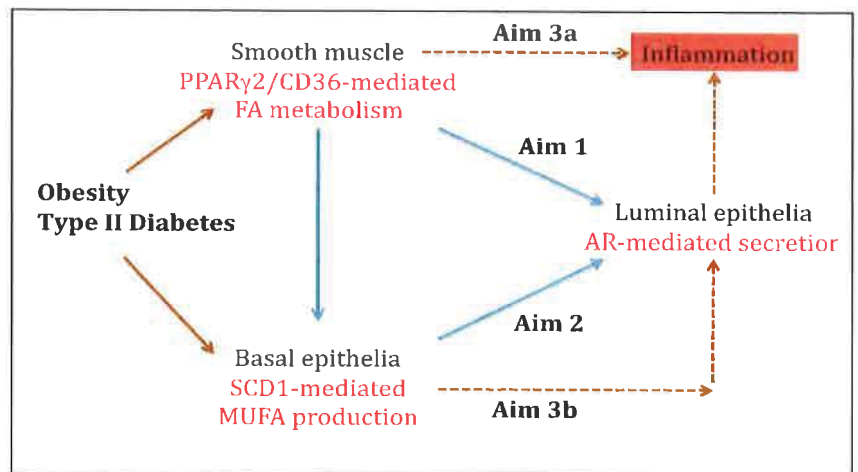


Figure 4. Hypothetical tissue-specific metabolic activities mediating prostate differentiation. Based on their respective metabolic genes expressed and the role of these genes in other tissues affected by systemic metabolic disorders, the smooth muscle and basal cells are potential targets of obesity and T2D. Preliminary evidence demonstrates a role for PPAR γ 2 in increasing basal and luminal cell differentiation. The **hypothesis** will be tested that stromal PPAR γ 2-mediated FA (FA) metabolism regulates luminal differentiation either directly (**Aim 1**) or indirectly through basal cell monounsaturated fatty acid (MUFA) metabolism (**Aim 2**). The role of stromal and basal metabolic genes in regulating high fat diet-induced inflammation will be tested in **Aim 3**.

Models for studying lipodomics, differentiation and inflammation in prostate: In order to dissect the potential cellular interactions governing prostatic differentiation, we have developed novel *in vitro* 3D co-culture and *in vivo* tissue recombination models. First, in collaboration with Dr. [REDACTED] (consultant, [REDACTED])

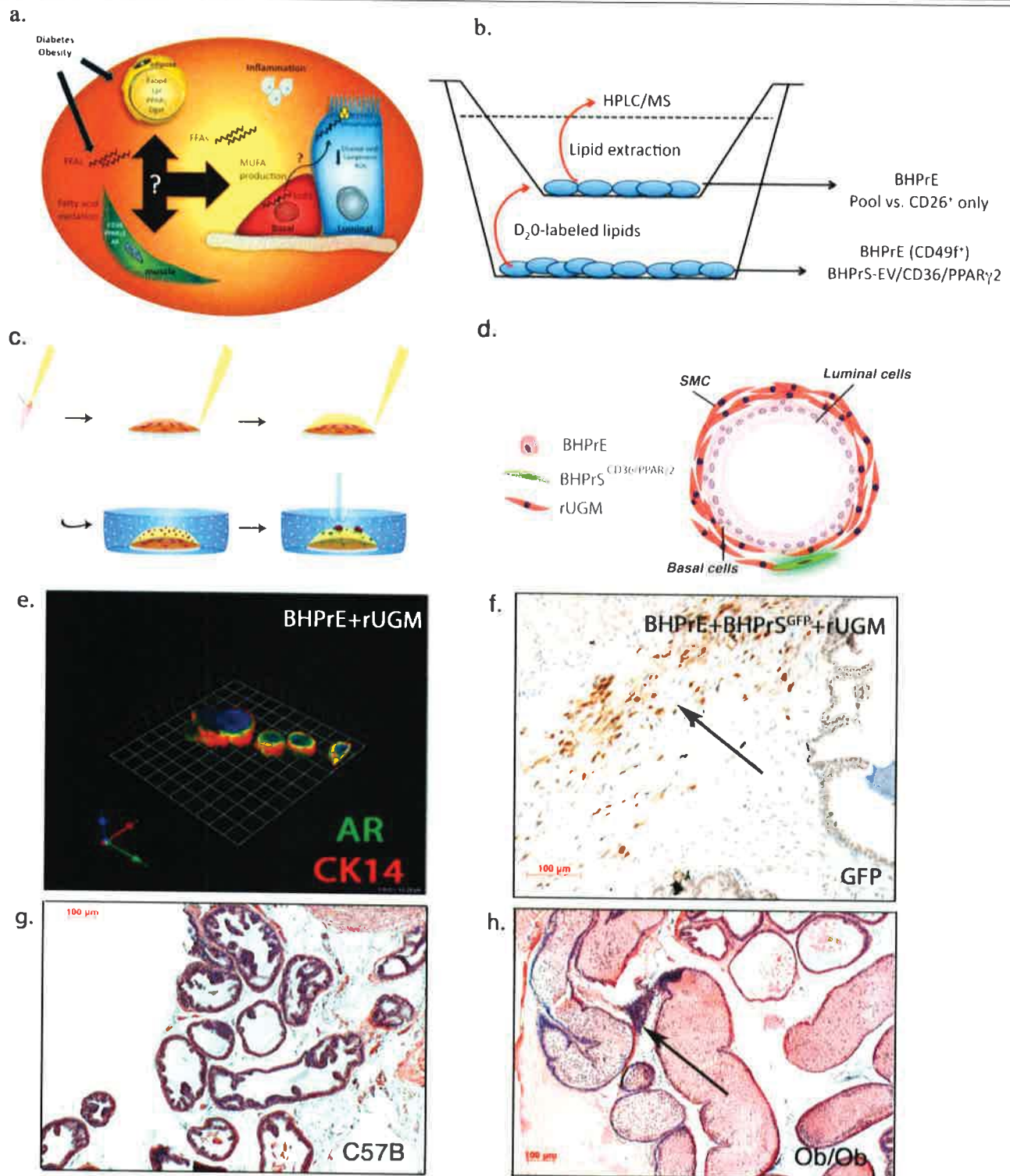


Figure 5. Models of tissue interaction and obesity-induced inflammation. (a) Potential effects of diabetes/obesity through FA metabolism in adipose, smooth muscle and/or basal cells driving inflammation in BPH. (b) Diagram of metabolic tracing experiment using co-culture of modified stroma or basal cells to drive differentiation in luminal epithelia. (c) Diagram and (d) preliminary results of 3D co-culture model of epithelial differentiation. (e) Diagram and (f) preliminary results of tissue recombination experiment with modified human stromal cells (arrow indicates GFP-labeled cells). Widespread inflammation observed in 6 month obese animal prostates (arrow, **h**) vs. 6 month C57B control (**g**).

University) we have modified a 3D co-culture model (35) using human prostate epithelia (BHPRE) and inductive urogenital mesenchyme in matrix (modeled in Figure 5c). We have shown that these basal-like human prostate epithelial cells will organize and differentiate *in vitro* with nuclear AR expression in interior luminal cells surrounded by CK14⁺ basal cells (Figure 5d) and that PPAR γ 2 expression is required for differentiation in AR⁺ acini (data not shown). These data are being prepared for publication and represent a major advance for the study of prostate differentiation, similar to what has been achieved in breast research (36). This *in vitro* co-culture model will be used in Aim 1 and also for metabolic tracing studies in Aim 2. I have developed an *in vivo* model of tissue recombination to study stromal factors on epithelial differentiation using a human prostate stromal cell line (BHPRS) expressing GFP for tracking ((37), Figures 5e,f). Failure to identify stromal factors mediating epithelial differentiation has necessitated the use of inductive fetal urogenital mesenchyme for tissue regeneration experiments for 40 years (11, 38). It is expected that enhancing FA metabolism through genetic manipulation of the undifferentiated human prostate stromal cell line (BHPRS) will facilitate epithelial differentiation (Aim 1). Finally, we have also screened various transgenic and dietary mouse models of obesity and diabetes where we observed hyperplasia and inflammation (Figure 5e vs. 5f), which will serve as background for the analysis of CD36-mediated FA metabolism in prostate inflammation in Aim 3. **In summary**, we have developed and co-opted several useful *in vitro* and animal models to test the **hypothesis** that *PPAR γ 2-regulated fatty acid metabolic genes regulate prostatic differentiation and inflammation*. Affirmation of this hypothesis will fundamentally alter how the prostate is viewed in the context of systemic metabolic disease and provide basic molecular mechanisms for therapeutic intervention. Notably, Dr. Hayward also has a repository of BPH tissue that can be analyzed for alterations to PPAR γ 2 and related pathways and correlated with clinical and lipidomic profiles.

Specific Aim 1: Determine how stromal fatty acid metabolism mediates prostate epithelial differentiation.

Rationale: Preliminary data demonstrate that PPAR γ 2 expression increases FA metabolism and decreases glucose metabolism (10), resulting in increased percentage of basal epithelium and increased differentiation of luminal epithelium (Figures 3e,f). PPAR γ 2 and its downstream gene CD36 are highly enriched in smooth muscle (Figures 2d, 4b) and facilitate FA uptake and oxidation for function (30). We also showed that TZD treatment of animals resulted in smooth muscle and adipocyte expansion (Figure 3b), which correlated with increased FA metabolic genes and AR/probasin expression (Figure 3f) (10). Finally, in a pool of basal-like human prostate epithelial cells (BHPRE), induction of AR expression was dependent on expression of PPAR γ 2. It should be noted that PPAR γ 2 expression is enriched in smooth muscle *in vivo* (Figure 4d) and may only be expressed in epithelia in culture due to the absence of smooth muscle. These data suggest that paracrine FA metabolism may drive epithelial differentiation *in vivo*. To test the hypothesis that stromal FA metabolism drives epithelial differentiation, BHPRS human prostate stromal cells (37) will be manipulated to overexpress PPAR γ 2 or CD36 and 3D co-culture and tissue recombination/xenografting experiments will verify impact on epithelial lipid metabolism and differentiation. Notably, BHPRS are undifferentiated fibroblasts that express low levels of both genes and do not induce differentiation of BHPRE in tissue regeneration xenografting experiments.

1.1 Selection of BHPRE and BHPRS cell line derivatives: The BHPRE cell line in culture is a pool of human prostate epithelial cells with a majority CK14⁺/p63⁺ basal-like cells (39). The human prostate stromal BHPRS cell line was derived from the same patient as the BHPRE epithelial cell line and has been previously modified with other target genes for use in tissue recombination (37). To determine the direct or indirect effect of stromal FA metabolism on luminal differentiation, co-cultures will be performed with the total pool of basal and luminal cells or FACS-isolated luminal cells only (see Figure 4). FACS-mediated isolation of luminal cells using an established cell surface marker, CD26 (luminal cell-specific) (40) will be performed. Our laboratory has extensive experience in FACS-based cell sorting and stable cell line generation and have already used a lentiviral approach to genetically modify BHPRS cells to express GFP and genes of interest, optimizing conditions for tissue recombination/xenografting (Figure 5e, (37)). Furthermore, we have PPAR γ 2 lentivirus in stock (10) and CD36 lentivirus will be obtained from Dr. Ahmed. FA uptake and oxidation will be measured by incubating cells with labeled palmitate and profiling lipids and ¹³CO₂ production as described (41).

1.2 Influence of stromal fatty acid metabolism on prostate epithelial differentiation: BHPRS cells overexpressing PPAR γ 2 or CD36 will be co-cultured together or in separate chambers with BHPRE cells and assessment of basal (CK14, p63, SCD1, CD49f) and luminal (AR, PSA, NKX3.1, CK18, CD26) differentiation as well as lipogenesis (activation of FASN, ACC, mTOR, AKT), oxidative stress (dihydroethidium staining of reactive oxygen species), flow cytometry-mediated assessment of basal to luminal ratio (CD26⁺:CD49f⁺) and

androgen responsive element (ARE-) or PSA-driven luciferase reporters will be performed. Co-cultures with various BHPoS-BHPoS combinations, plus or minus drugs that modulate FA metabolism, will be performed in 2D and 3D to determine the effects of direct and/or indirect paracrine effects on luminal epithelial differentiation. As a positive control for induction of epithelial differentiation, fetal mesenchyme (rUGM) will be used as demonstrated previously (Figure 5d).

1.2a Influence of stromal CD36 and PPAR γ 2 on epithelial differentiation *in vitro*: Using 2D (combined or separated chambers) and 3D co-cultures in matrix models (Figure 5b&c), the effects of increased stromal FA metabolism on epithelial differentiation *in vitro* will be determined as described above. It is expected that isolation of CD26⁺ luminal cells from the BHPoS pool will induce lipogenesis and oxidative stress similar to that observed in our mouse prostate epithelial cells (10) due to absence of paracrine FA metabolism. Lipogenesis and oxidative stress of isolated CD26⁺ cells will be measured after co-culture with BHPoS cell line derivatives. Furthermore, the total pool of BHPoS cells will be recombined co-cultured with BHPoS cell lines to determine whether stromal fatty acid metabolism signals through basal cells to regulate luminal cell differentiation. Using this *in vitro* compartmentalized tissue model will help to determine the direct effect of stromal FA metabolism genes and drugs on luminal cell lipid metabolism and differentiation and whether this effect is mediated by basal cells. Finally, pre-treatment of BHPoS cell lines or fetal rUGM with drugs targeting FA metabolism will identify a specific metabolic node mediating CD36- or PPAR γ 2driven paracrine epithelial differentiation including TOFA, C75 (lipogenesis inhibitors), or etomoxir (FA oxidation inhibitor); alternatively, FA oxidation activators like metformin and pioglitazone will be used to induce epithelial differentiation through activation of stromal FA metabolism (as long as the stromal cell has the appropriate receptors). Drug effects will be monitored by lipidomics analysis, AR expression/function and CD26 positivity.

1.2b Influence of stromal CD36 and PPAR γ 2 on epithelial differentiation *in vivo*: Until now, inductive fetal urogenital mesenchyme has been the only stromal tissue capable of inducing epithelial differentiation in tissue regeneration experiments (39), which was also used to induce epithelial differentiation in 3D co-culture (Figure 3e). Using CD36- or PPAR γ 2-expressing BHPoS cells, we will test whether stromal cells with high FA metabolism are similarly capable of inducing differentiation of BHPoS cells (pool or CD26⁺ only). Notably, the aforementioned inhibitors of FA metabolism (TOFA, etomoxir, C75) have also been used effectively in animals and, pending these *in vitro* results, could be used to measure inhibition of prostate differentiation *in vivo*. Alternatively, we have recently shown that a TZD increases FA oxidation and prostate differentiation (10). Analysis of differentiation in these *in vivo* experiments will comprise immunohistochemistry/qPCR for PPAR γ 2, CD36, CK14/18, p63, AR and PSA for epithelial differentiation as well as sm- α -actin for stromal differentiation

| Stromal Cell line | Reporter Epithelium | Expected co-culture and TR outcomes | +TOFA/etomoxir/C75 | +metformin/TZD |
|-----------------------|---|---|--|---|
| BHPoS-Ctrl | BHPoS (CD49f ⁺ /CD26 ⁺ or CD26 ⁺ only) | basal ⁺⁺⁺ /luminal ⁺ Luminal Lipogenesis ⁺⁺ | basal ⁺ /luminal ⁻ Luminal Lipogenesis ⁺⁺⁺ | basal ⁺⁺⁺ /luminal ⁺⁺⁺ Luminal Lipogenesis ⁺⁺ |
| BHPoS-CD36 | BHPoS (CD49f ⁺ /CD26 ⁺ or CD26 ⁺ only) | basal ⁺⁺ /luminal ⁺⁺ Luminal Lipogenesis ⁻ | basal ⁺ /luminal ⁻ Luminal Lipogenesis ⁺⁺⁺ | basal ⁺⁺⁺ /luminal ⁺⁺⁺ Luminal Lipogenesis ⁻ |
| BHPoS-PPAR γ 2 | BHPoS (CD49f ⁺ /CD26 ⁺ or CD26 ⁺ only) | basal ⁺⁺ /luminal ⁺⁺ Luminal Lipogenesis ⁻ | basal ⁺ /luminal ⁻ Luminal Lipogenesis ⁺⁺⁺ | basal ⁺⁺⁺ /luminal ⁺⁺⁺ Luminal Lipogenesis ⁻ |
| Fetal rUGM | BHPoS (CD49f ⁺ /CD26 ⁺ or CD26 ⁺ only) | basal ⁺⁺ /luminal ⁺⁺ Luminal Lipogenesis ⁻ | basal ⁺ /luminal ⁻ Luminal Lipogenesis ⁺⁺⁺ | basal ⁺⁺⁺ /luminal ⁺⁺⁺ Luminal Lipogenesis ⁻ |

Table 1. Expected Outcomes for Aim 1

Expected outcomes, potential problems, and alternative approaches: Over-expression of PPAR γ 2 or CD36 in BHPoS should drive AR expression and responsiveness, possibly through induction of basal cell differentiation (tested in Aim 2). If stromal FA metabolism directly impacts epithelial differentiation through a paracrine signal, this proposal will have modified the historic view that peptide growth factors (42, 43) drive paracrine epithelial differentiation. Alternatively, FA metabolism may drive differentiation of BHPoS fibroblasts into AR-positive smooth muscle, which may then drive paracrine differentiation through a different mechanism

(perhaps even peptide growth factors), which could be further tested. Alternative cell lines to BHP_{PrS} (44) and BHP_{PrE} (39) are available. Inhibition of PPAR γ 2/CD36-mediated epithelial differentiation by TOFA, etomoxir or C75 will depend what aspect of FA metabolism is normally driven by prostate stroma; however, it is estimated that TOFA (ACC/SCD1 inhibitor) will only have an effect in the presence of basal cells, which express SCD1 and harbor neutral lipids under normal conditions (see Figure 2a and Aim 2). However, if the differentiation of smooth muscle and its paracrine effect on epithelial differentiation are dependent on FA oxidation (vs. uptake/production/modification), then the CPT1 α inhibitor etomoxir should inhibit stromal CD36/PPAR γ 2-driven epithelial differentiation. As a control, differentiation of BHP_{PrE} cells in 3D by inductive fetal mesenchyme (Figure 5d) pre-treated with FA metabolism inhibitors will be used to negatively impact differentiation.

FACS isolated CD26⁺ luminal cells may not survive as a cell line in the absence of basal cells. mPrE-PPAR γ KO prostate epithelial cells, which only contain 5% basal cells (10), or FACS-isolated fresh human luminal cells, which we have access to weekly, could be substituted. If stromal FA metabolism genes are not driving the observed increase in epithelial differentiation, microarray data from PPAR γ 2-rescued cells identified other pathways implicated in prostate epithelial function including increased cholesterol and phospholipid metabolism and decreased glucose and glutamine metabolism, which could be substituted into the established model systems. As an alternative *in vivo* approach to this aim, smooth muscle-specific knockout of PPAR γ (45) could be co-opted for studying prostate epithelial differentiation. Failure of stromal FA metabolism to drive epithelial differentiation does not preclude that basal cell SCD1 expression drives luminal differentiation (Aim 2) or that obesity-induced CD36 or SCD1 expression drives prostatic inflammation (Aim 3).

Specific Aim 2: Determine how monounsaturated fatty acid metabolism mediates luminal differentiation.

Rationale: A key metabolic difference between PPAR γ 1 and PPAR γ 2 re-expression in mouse PPAR γ knockout prostatic epithelium was that PPAR γ 2-induced epithelial differentiation was correlated with an increase in basal cells and SCD1. PPAR γ 1 re-expression decreased differentiation and significantly increased FA saturation (10). PPAR γ agonists have been shown to induce SCD1 (46), a FA desaturase expressed in specific tissues. In skin, SCD1 is expressed in undifferentiated cells and maintains sebocyte development and skin lipid composition through production of monounsaturated fatty acids (MUFAs) (47). SCD1 expression is induced by saturated FAs, insulin, carbohydrates and cholesterol (25). SCD1 is also expressed in prostate basal cells (Figure 3c), which is correlated with neutral lipid storage (Figure 3a). It remains unclear whether SCD1-mediated MUFA production is directly related to luminal differentiation. Given its support of suprabasal cell differentiation in other tissues, it is postulated that SCD1 expression in prostate basal cells provides a lipid metabolic mechanism for the loss of luminal differentiation in prostates of animals with defective basal cells (48). Preliminary data show that although SCD1 is involved in *de novo* lipogenesis and is significantly increased by PPAR γ 2 expression in basal cells, the cumulative effect on the pool of basal (20% of total population) and luminal cells was to reduce *de novo* lipogenesis and lipotoxicity (10). We also show that neutral lipid storage in basal cells may be lost in BPH (Figure 3a&b). This is perhaps related to a loss of SCD1 or acquired insulin insensitivity in T2D, which would be an intriguing follow-up to this study. Experimental proof of the hypothesis that basal cell lipogenesis regulates luminal cell differentiation has vast implications for the target cell population of systemic metabolic dysfunction in prostate (Figure 4), suggesting that basal cell lipogenic dysfunction (Figure 3b) may be an etiological factor in BPH.

2.1 Cell line selection: SCD1 will be over-expressed or knocked down in CD49⁺ BHP_{PrE} cells, using constructs from Dr. Ntambi (see letter of support). CD26⁺ BHP_{PrE} cells will be used as a reporter cell line.

2.2 Influence of SCD1-mediated MUFA production on luminal epithelial differentiation *in vitro* and *in vivo*: Similar to the stromal-epithelial co-culture and tissue recombination experiments in Aim 1, basal-luminal interactions will be tested using CD49⁺ BHP_{PrE}-Ctrl/SCD1/SCD1^{shRNA} cell lines and CD26⁺ BHP_{PrE} luminal cells to test induction of AR expression and responsiveness. An established SCD1 inhibitor, TOFA (49), will be added as a negative control. We showed that TZD treatment increased SCD1 expression and prostate differentiation *in vivo* (10), which has precedent in other tissues (50). ROS (flow cytometry) and lipogenesis (Western blot, lipidomics) of luminal cells will also be measured.

2.3 Metabolic tracing studies of fatty acid metabolites in basal-luminal interactions: In order to identify lipid metabolites of basal cell SCD1-mediated lipogenesis or stromal CD36/PPAR γ 2-mediated FA metabolism driving luminal differentiation, metabolic tracing studies will be performed using deuterium-labeled lipids incubated in either BHP_{PrS} or BHP_{PrE} cell lines according to protocols established by consultant Hachey ((51); see letter of collaboration). Tracer enrichment in samples collected from BHP_{PrS} or BHP_{PrE} derivatives,

conditioned media or the target luminal epithelial monolayer in 3D co-culture will be analyzed by tandem LC-LC/MS lipidomic analysis to determine how PPAR γ 2, CD36 or SCD1-driven fatty acid metabolism affects luminal lipid profiles and differentiation (Figures 5b&c). Induction of AR expression in BHPRE cells through increasing serum levels (5% to 10%) is dependent on PPAR γ 2 (manuscript in preparation), which may regulate processing of lipids for luminal differentiation through induction of SCD1 expression. High serum induction of AR will serve as a positive control for SCD1 knockdown experiments. Addition of deuterium-labeled MUFAs to 5% serum will also be tested directly on luminal reporter cells to determine effects on differentiation.

2.4 Influence of global SCD1 knockout on prostate differentiation: We showed previously that TZD treatment of mice induced prostate differentiation markers as well as SCD1 (10), suggesting a possible mechanism through either basal cell FA metabolism or adipocytes, which also express SCD1 (52). To determine whether TZDs drive prostate differentiation through SCD1, global SCD1^{-/-} mice ((53), provided by Dr. Ntambi) will be treated with TZDs and markers of prostate differentiation will be assessed as before (10).

Expected outcomes, potential problems, and alternative approaches: The goal of this aim is to determine how MUFA production by basal cells drives luminal cell differentiation and whether paracrine signaling from PPAR γ 2⁺/CD36⁺ stroma mediates the basal-luminal interaction. The ability of SCD1 expression in basal cells to drive luminal differentiation would not preclude that stromal FA metabolism drives luminal differentiation (Aim 1) or that obesity-driven CD36 drives prostatic inflammation (Aim 3). Because of the established role of FAs in mediating prostate health and differentiation (54), MUFAs may act as a lipid source for luminal cell secretory function (see Figure 5a). Therefore, metabolic tracing is expected identify increased deuterium labeling of lipids in conditioned media or luminal reporter cells. As demonstrated in Figure 3a, human prostates harbor neutral lipid droplets in the epithelium, particularly in the basal layer, which may be due to SCD1 expression (Figure 3c). BPH is strongly associated with insulin insensitivity (6, 55) and insulin activates lipogenesis through genes like SCD1 (25). Preliminary mass spectrometry analysis with Dr. Hachey (Figure 2) and oil red O staining of neutral lipids and lipoproteins in human normal vs. BPH tissue (Figure 3a vs. b) demonstrate a decrease in neutral lipids, which may normally be anti-inflammatory through maintenance of proper lipid composition.

Fatty acid uptake by stromal CD36 or PPAR γ 2 is expected to provide a lipid source for differentiation of basal (increased CK14, p63, SCD1) and luminal (increased AR/PSA) cells. If technical difficulties with enrichment or identification of deuterium-labeled lipids are encountered in co-culture, radioisotopic labeling of MUFA biosynthesis with C13-labeled acetate/palmitate is an established alternative protocol (56) and will at least facilitate the correlation of CD36, PPAR γ 2 or SCD1-mediated FA uptake and lipogenesis with paracrine epithelial differentiation. If SCD1 ablation fails to abrogate TZD-induced increases in prostate differentiation due to confounding systemic metabolic perturbations, floxed animals are available (47) to be crossed with CK14-Cre^{ERT2} mice for basal cell specific ablation. Other lipogenic genes including acetyl-coa carboxylase, fatty acid synthase or ATP citrate lyase could be substituted for SCD1.

Specific Aim 3: Determine how CD36-mediated fatty acid metabolism in vivo mediates differentiation and obesity-induced prostatic inflammation.

Rationale: Except for adipose and liver, most tissues acquire essential FAs through dietary means instead of *de novo* synthesis. Cellular uptake of these FAs is mediated by transporters including CD36, a widely expressed importer of cholesterol, lipoproteins and LCFAs (57). CD36 is commonly mutated in humans and is correlated with insulin resistance (32) and plasma levels of soluble CD36 are correlated with inflammation in T2D (58). However, ablation of CD36 also protects animals from insulin insensitivity and inflammation due to a decrease in inflammatory cytokine production (IFN- γ , MCP-1 and TNF- α), suggesting that CD36 deficiency results in an anti-inflammatory state (26, 57). We demonstrated that prostate smooth muscle and luminal epithelia express CD36 (Figure 2h). However, we also found that CD36 increased under chronic HFD treatment vs. other PPAR γ -regulated genes (10), suggesting, as in other tissues, that CD36 may mediate the pro-inflammatory phenotype in prostates of HFD-treated animals (Figure 5h).

Influence of global CD36 knockout on obesity-induced prostatic inflammation: To determine how obesity-induced CD36 modulates a pro-inflammatory environment, prostates from HFD-treated control and CD36-ablated animals from Dr. [REDACTED] will be examined for inflammatory cytokine production (see rationale) and inflammatory infiltrates will be identified and quantified using flow cytometry (Core Service) and IHC. This will determine whether the inflammation observed in Figure 3h is similar to the characterized T cell infiltration in human BPH (59) or to the CD36-mediated macrophage infiltration observed in adipose of obese animals (26), which will resolve whether HFD-treated animals are a true model of human BPH. We will also identify changes

in FA composition of phospholipids, diglycerides and triglycerides and pro-inflammatory eicosanoid production (core services) to determine whether systemic obesity modulates local lipid metabolism and cytokine/eicosanoid production in prostate and whether this modulation is correlated with inflammation.

Expected outcomes, potential problems, and alternative approaches: It is expected that ablation of CD36 will inhibit obesity-induced prostate inflammation due to decreased uptake of LCFA and conversion of arachidonic acid to pro-inflammatory eicosanoids (34, 60). However, a metabolic switch to other FA transporters like LPL (33) or increased glucose oxidation through decreased PDK4 may compensate for decreased FA uptake, similar to our observation in PPAR γ -ablated cells (10). Without any compensation by other FA transporters and under normal dietary conditions, it is expected that ablation of CD36 would cause a decrease in AR expression and activity due to decreased LCFA, lipoprotein and cholesterol uptake. A potential problem with CD36 knockout animals is the confounding effect of a global knockout on systemic metabolism. While providing insight into the effects of systemic metabolism on prostate differentiation and inflammation, the direct actions of these genes could be studied using prostate-specific ablation. Dr. [REDACTED] laboratory has probasin-Cre^{ERT2} and Ck14-Cre^{ERT2} animals and Dr. [REDACTED] has floxed CD36 animals, which could be used to target CD36 ablation to specific prostate epithelial layers at the adult stage. If ablation of CD36 fails to mollify either differentiation under normal dietary conditions or obesity-induced prostatic inflammation, other targets aberrantly regulated by excess fat/cholesterol and implicated in inflammation like SCD1 (through Dr. [REDACTED] (47, 53)) or PPAR γ (17) could also be examined. Furthermore, a model of prostatic inflammation through CD36-driven prostaglandin production would provide a mechanistic basis for the premise that COX-2 inhibitors could be therapeutic for BPH patients (61), which could be tested in obese animals.

In summary, if it is true that basal cells are fed FA metabolites by stroma and are the predominant insulin target tissue in prostate, mediating luminal differentiation through SCD1-induced lipogenesis of MUFAs, then this proposal will have changed the paradigm of therapy by switching from targeting smooth muscle contractility to targeting fatty acid metabolism. Future experiments will focus on molecular mechanisms of inflammation and hyperplasia due to obesity-induced defects in smooth muscle fatty acid oxidation and basal cell lipogenesis, for which existing drugs including metformin or TZDs could prove effective.

VERTEBRATE ANIMALS

1. Provide a detailed description of the proposed use of the animals in the work outlined in the Research Design and Methods section. Identify the species, strains, ages, sex, and number of animals to be used in the proposed work.

For Aims 1 and 2, 36 nude male mice and 18 pregnant rats will be used. For Aims 2 and 3, 357 transgenic and control C57B male mice will be used. As noted in the text, numbers are necessarily best estimates at this point and are based on power calculations shown in Tables 1 and 3. However, the precise number of such combinations is unknown at this point since this is to a large extent dependant upon variables that will not be clear until the experiments are underway. All final animal numbers must be justified to the satisfaction of the ~~Vanderbilt University~~, and will likely be modified in a step-wise manner as experiments progress. All efforts will be made to refine experiments and reduce animal usage. A likely outcome, should this work succeed, is a reduction in the need for animal models as good mathematical and computer-based approaches come online.

2. Justify the use of animals, the choice of species, and the numbers to be used. If animals are in short supply, costly, or to be used in large numbers, provide an additional rationale for their selection and numbers.

The overall goal of this grant is to understand how stromal-epithelial interactions regulate prostate differentiation and immunomodulation through fatty acid metabolism. This is a critical issue in benign human prostate hyperplasia development, which cannot be simulated outside of a living animal. Where possible, cell culture experiments will be performed to address pertinent questions on pathways involved. However, stromal and epithelial cell interactions and inflammation will change the tissue architecture as the prostate disease progresses. Mouse models offer the greatest opportunity to test these questions in a realistic manner. A total of 36 nude mice and 18 pregnant rats are used because these experiments require an immunocompromised strain and significant rejection problems can sometimes be seen when human cells are grown in athymic mice. These mice have been shown to be excellent hosts for experiments of this type. A total of 357 C57B, CD36 null and SCD1 null mice will also be studied for their contribution to prostate differentiation and immunomodulation under high fat diet or TZD treatment. These mice will be provided by Drs. ~~Wang~~ and ~~Abumata~~ (see letters of support). Numbers used are based upon power calculations using the minimum numbers of animals to achieve statistical significance.

- 2.1 Animals used for tissue recombination experiments: Aims 1 and 2 involve the use of nude mouse hosts for recombination of human cell lines with inductive fetal mesenchyme. This will require the sacrifice of a total of 18 pregnant rats for harvesting of rUGM and 36 nude mice. See Table 2.
- 2.2 Animals used for transgenic experiments: Aims 2 and 3 involve the use of C57B (Control), SCD1- and CD36-ablated animals to study the effect of these genes on prostate differentiation and inflammation downstream of high fat diet-induced obesity or TZD treatment. A total of 18 mice per group at 3 time points requires that 51 animals for each of 7 groups be used. See Table 3.

3. Provide information on the veterinary care of the animals involved.

~~Vanderbilt University (VU)~~ and the Veteran's Administration ~~Tennessee Valley~~ Healthcare System ~~(VATVHS)~~ maintain a joint Animal Welfare Assurance. A single Institutional Animal Care and Use Committee serves both ~~VU and VATVHS~~. ~~Vanderbilt University~~ has been an AAALAC International accredited institution since 1967. The most recent site visit was conducted in 2008. The Division of Animal Care is responsible for the veterinary care of all animals housed at ~~Vanderbilt University~~. ~~James Wallace~~, DVM, ~~DAABIA~~ serves as the Assistant Vice Chancellor for Research, the Director of the Division of Animal Care and the Attending Veterinarian. Four additional veterinarians provide day-to-day care for the animals housed at ~~VU~~. The Division of Animal Care includes 6 veterinary technicians, 1 Animal Shipping Coordinator, 1 Environmental Enrichment Coordinator, 1 Operations Manager, 4 Facility Managers, 4 Floor Supervisors and 35 Animal Care Technicians.

Mice are housed in micro-isolator caging. Approximately 99% of all cages are on ventilated rack systems with contact bedding (Bed O'Cob) and nesting material (CareFresh or Nestlet). Ventilated cages are changed every other week. Animal Care Technicians enter the rooms daily and perform health checks twice a day. Animals identified with health concerns are tagged for evaluation by the veterinary technician. The veterinary technicians perform initial examinations, treatments, and give recommendations. In addition, veterinary

technicians check every animal room daily. Veterinary technicians will contact veterinarians to check the daily active case list.

A sentinel health-monitoring program is in place and utilizes CD-1 sentinels exposed to soiled bedding from animals on the rack. One cage of two sentinels is placed on each rack of 140 cages. The sentinels are tested monthly for parasitology, and quarterly for serology of common murine viruses and bacteria.

4. Describe the procedures for ensuring that discomfort, distress, pain, and injury will be limited to that which is unavoidable in the conduct of scientifically sound research. Describe the use of analgesic, anesthetic, and tranquilizing drugs and/or comfortable restraining devices, where appropriate, to minimize discomfort, distress, pain, and injury.

Prior to surgery, mice will receive buprenex for analgesia. Surgery will be performed under general anesthesia and analgesics (ketofen) will be provided for 48 hrs following surgery as deemed appropriate by the ~~Vanderbilt~~ IACUC and veterinary staff. Animals undergoing surgery will be weighed prior to surgery and their weight will be checked regularly. Animals that are apparently not in good health (as determined by activity, feeding, posture, grooming, respiration rate and/or body weight) will be the subject of examination and professional advice by veterinary staff. Such animals will be treated on a case-by-case basis. All animals (tissue recombination hosts and transgenic mice) will be weighed at the initiation of the experiment and twice a month thereafter. If there is any reason based upon the appearance of an animal that its weight has decreased, weighing can be performed at any time. Animals which have lost more than 15% of their starting body weight will be euthanized. Minor infections will be treated by antibiotics per veterinary advice. Any animal deemed to be suffering will be euthanized.

5. Describe any method of euthanasia to be used and the reasons for its selection. State whether this method is consistent with the recommendations of the Panel on Euthanasia of the American Veterinary Medical Association. If not, present a justification for not following the recommendations.

Mice and rats are sacrificed by cervical dislocation under isoflourane anesthesia. This is consistent with the Panel on Euthanasia of the American Veterinary Medical Association and is also approved as an SOP by the ~~Vanderbilt~~ IACUC.

All experiments performed are consistent with the Animal Welfare Act and subject to approval by the ~~Vanderbilt~~ IACUC.

Tables for Proposed Experiments.

Statistical analysis plans and power analyses were provided by the ~~Vanderbilt~~ Biostatistics core. As the need arises, the core will be further consulted to confirm the validity of data sets and to further refine animal use. The statistical models here are analysis of variance. In all experiments, we will use a sample size of 12 mice per group. We will be able to detect differences between groups that are equivalent to 1.2 to 1.6 with 80% power depending on how many pair wise comparisons will be made within each experiment. Because of early termination (multiple time points with separate scoring of endpoints) designed in this experiment, the expected sample size is reduced compared to a conventional 1-stage design if there is no treatment effect. Each sampling unit (mouse) will contribute more than one observation, namely, 2 sites (left and right kidneys) and 3 replicates (grafts) within each site. Thus, we will fit mixed-effects models treating the treatment groups as fixed and mice and grafts as random effects. An initial sample size of 12 per group should provide appropriate data coverage as multiple observations per sample will add more information. When quantitating outcomes such as immunoreactivity, an important primary outcome variable of the experiments for this aim is the number of stained cells per unit area. If we expect the proportion of the stained cells to be above 10%, we will use high power fields containing on the order of 100 cells; we will use lower power fields as the expected proportion of the stained cells decreases so that the observed number of stained cells is at least 10. Thus, when we expect less than 1 % of the cells to be stained, we plan to use fields containing >1,000 total cells. We will describe the

numbers/proportions of the stained cells for each genotype numerically and graphically. To stabilize the variance, we will apply a square root transformation to the number of stained cells, which theoretically follows a Poisson distribution. Comparisons between genotypes will be made using simple two-sample t-tests. If the normality assumption is still violated after the transformation or if the average number of stained cells is less than 5, we will apply nonparametric methods such as Wilcoxon rank sum tests to compare the two groups. As we will consider a number of markers, multiplicity should be controlled. We will use the Holm step-down tests to adjust the critical values. The overall type I error rate for the series of experiments will be controlled at 5%. If the number of stained cells follows a Poisson distribution, the standard deviation of the square root transformed variables will be approximately 0.5 no matter what the original average is. Assuming independence between two genotypes, we can compute the standard deviation of the difference to be 0.7. Table 1 summarizes the differences we can detect with 90% power; for this computation, comparison-wise type I error rate is set at 0.005, and we assume that the average count of the reference group is 10 and 20. Table 2 summarizes the numbers of mice and rats needed for tissue recombination/xenografting experiments in Aims 1 and 2.

| Table 1: Detectable difference with 90% power for tissue recombination experiments | | | | |
|---|------|------|------|------|
| Sample size per group | 8 | 12 | 16 | 20 |
| Reference group mean = 10 | 23.4 | 19.7 | 18.0 | 17.0 |
| Reference group mean = 20 | 37.7 | 33.1 | 30.8 | 29.5 |

| Table 2: Numbers of Mice and Rats for tissue recombination experiments in Aims 1 and 2: | | | | | |
|---|---|-----------------------------------|-----------------------------------|---------------------|--|
| Groups | Cell lines | Number of Tissue Recombinants | Number of pregnant rats for UGM * | Number of nude Mice | Time points of tissue collection after surgery (2, 4 and 6 months) |
| 1 | BHPrS-Ctrl | 12/timed point | 1/timed point | 2 mice/timed point | 3 timed points |
| 2 | BHPrS-CD36 | 12/timed point | 1/timed point | 2 mice/timed point | 3 timed points |
| 3 | BHPrS-PPARg2 | 12/timed point | 1/timed point | 2 mice/timed point | 3 timed points |
| 4 | BHPrE ^{CD49f} -Ctrl | 12/timed point | 1/timed point | 2 mice/timed point | 3 timed points |
| 5 | BHPrE ^{CD49f} -SCD1 | 12/timed point | 1/timed point | 2 mice/timed point | 3 timed points |
| 6 | BHPrE ^{CD49f} -SCD1 ^{shRNA} | 12/timed point | 1/timed point | 2 mice/timed point | 3 timed points |
| Totals: | | 36 nude mice and 18 pregnant rats | | | |
| * We typically isolate between 12-16 embryos/pregnant rat. | | | | | |

Sample Size and Power Analysis: The ~~Vanderbilt~~ University Biostatistics ~~Core~~ charges a fee for service. The primary objectives of the Core are: 1) To provide study design and review all laboratory, and animal studies including feasibility assessment, power analysis and sample size estimation; 2) To collaborate in projects data analysis, interpretation of results, and the writing of final study reports and manuscripts.

| | | | | | |
|--|------------|------------|------------|------------|--|
| Table 3. | | | | | The results reported above are also applicable to down or up regulation of gene X. First, we seek to demonstrate decreased X expression. Secondly, we look for changes resulting from this altered expression. As above, 12 mice per group allow good power in detecting biologically meaningful changes in X expression, as well as small to moderate changes in prostatic differentiation. |
| Detectable Treatment Differences as a Function of cv (12 mice per group, 80% power) | | | | | |
| cv | .2 | .3 | .4 | .5 | |
| Detectable Difference | 19% | 26% | 33% | 38% | |

Statistical Analysis Plan: To detect effects of altered SCD1 or CD36 expression, we consider pairwise comparison of altered SCD1 and CD36 in control groups vs. TZD or high fat diet fed for each time point. Two sample t-tests (possibly after data transformation) are used to compare mean expression levels. Measures evaluated include tissue volume, proliferation, inflammation, gene expression, and histology. The discussion is based upon 12 mice per group (3 time points). *Sample Size and Power Analysis:* This reduction is deemed to be the minimum required to produce a downstream biological effect. Because gene X expression has not been quantified in this model system, we conservatively allow the co-efficient of variation (cv) to range from 0.4 to 1.0 in calculating the power of the hypothesis test. This test for detecting a difference in mean α expression between treatment groups has a 0.05 level of significance. We seek to detect prostate changes resulting from SCD1 or CD36 reduction. These changes include changes in tissue volume, morphology, inflammation, lipid alterations and reduced/increased gene X expression. Twelve mice per group allow detection of small to moderate changes in volume and expression. Table 3 shows the amount of change needed to achieve 80% power for cv values ranging from 20% to 50%. If the cv is 30%, we have 80% power to detect a 26% reduction in expression in a downstream product (at the 0.05 level of significance). Table 4 shows the number of transgenic and control animals needed for the experiments proposed.

| Table 4: Aims 2 and 3: CD36- and SCD1-ablated male mice fed chow, high fat diet or TZD | | | | | |
|---|----------------|----------------------------|--------------------------------|-----------------------------|------------------------------|
| Group | Number of mice | Experimental groups | Time points 3,6 and 12 months* | Histology (paraffin/frozen) | FACS/lipidomics/ RNA/Protein |
| 1 | 51 | C57B - chow | 3 timed points | 6/timed point | 12/timed point |
| 2 | 51 | C57B - HFD | 3 timed points | 6/timed point | 12/timed point |
| 3 | 51 | C57B - TZD | 3 timed points | 6/timed point | 12/timed point |
| 4 | 51 | SCD1 ^{-/-} - chow | 3 timed points | 6/timed point | 12/timed point |
| 5 | 51 | SCD1 ^{-/-} - TZD | 3 timed points | 6/timed point | 12/timed point |
| 6 | 51 | CD36 ^{-/-} - chow | 3 timed points | 6/timed point | 12/timed point |
| 7 | 51 | CD36 ^{-/-} - HFD | 3 timed points | 6/timed point | 12/timed point |
| Total males | 357 | | | | |
| 12 month time point will be performed only if necessary | | | | | |

RESOURCE SHARING PLAN

Data Storage: Data generated during my studies will be stored and maintained in REDCap, a secure, web-based application for building and managing databases.

Data and Model Sharing: I will adhere to the NIH policies regarding data and resource sharing. As set forth in my proposal, I expect to generate genetically manipulated prostate stromal and basal epithelial cell lines, which will represent valuable scientific tools for the prostate community. Cell lines will be maintained in liquid nitrogen, free of contamination with pathogens and/or other microorganisms. Following thorough characterization and peer-reviewed publication, data and models will be shared with investigators at academic institutions upon request.

PROGRAM CONTACT:

TRACY RANKIN

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**SUMMARY STATEMENT
(Privileged Communication)**

Release Date: 12/11/2012

Principal Investigator

Application Number: 1 K01 DK098277-01

STRAND, DOUGLAS WILLIAM PHD

Applicant Organization: VANDERBILT UNIVERSITY MED CTR

Review Group: DDK-D

Kidney, Urologic and Hematologic Diseases D Subcommittee

Meeting Date: 10/20/2012

RFA/PA: PAR12-020

Council: JAN 2013

PCC: KTR UTR

Requested Start: 01/01/2013

Project Title: Mechanisms of Fatty Acid Metabolism in Prostate Differentiation and Disease**SRG Action:** Impact Score: 44**Next Steps:** Visit http://grants.nih.gov/grants/next_steps.htm**Human Subjects:** 10-No human subjects involved**Animal Subjects:** 30-Vertebrate animals involved - no SRG concerns noted

| Project Year | Direct Costs Requested | Estimated Total Cost |
|-----------------|---------------------------|-------------------------|
| 1 | 72,883 | 78,714 |
| 2 | 75,105 | 81,113 |
| 3 | 98,994 | 106,914 |
| 4 | 100,473 | 108,511 |
| 5 | 101,983 | 110,142 |
| TOTAL | 449,438 | 485,393 |

ADMINISTRATIVE BUDGET NOTE: The budget shown is the requested budget and has not been adjusted to reflect any recommendations made by reviewers. If an award is planned, the costs will be calculated by Institute grants management staff based on the recommendations outlined below in the COMMITTEE BUDGET RECOMMENDATIONS section.

ADMINISTRATIVE NOTE

SCIENTIFIC REVIEW OFFICER'S NOTES

RESUME AND SUMMARY OF DISCUSSION: This application was submitted in response to the Program Announcement PAR-12-020: NIDDK Mentored Research Scientist Development Award (K01). The candidate proposes to examine whether alterations in fatty acid metabolism affect prostatic differentiation and immunomodulation. Strengths of the application include the novelty of the proposed research; the experience of the candidate in the areas of research; the excellent team of mentors and collaborators; and the outstanding environment for the proposed research and training. Despite these strengths, there are some weaknesses which reduce the enthusiasm for this application. The publication record of the applicant is modest. The career development plan is underdeveloped; justification for new training and plans for evaluating the candidate's progress are not described. The research plan, although feasible and supported by preliminary data, lacks cohesiveness and clarity. The rationale for and relevance of some experimental approaches is uncertain. Finally, whereas the institutional support to the candidate appears strong, the candidate's effort and protected time are not addressed. Overall, this application is within very good to good range.

DESCRIPTION (provided by applicant): The overall goals of this proposal are to determine whether alterations in fatty acid metabolism affect prostatic differentiation and immunomodulation and to develop my technical and professional abilities to become an independent investigator. My professional goal is to acquire a position as a faculty member at a top-tier academic institution capable of providing a fully supportive research environment for my pursuit of the molecular links between systemic metabolic stress and chronic urogenital dysfunction. I will continue to foster strong interdisciplinary relationships with epidemiologists, diabetologists and molecular biologists to capitalize on the novel models of stromal-epithelial interactions developed in our laboratory. To achieve these goals, I will enroll in research training activities provided by the NIH MMPC/NIDDK, the [REDACTED] University [REDACTED] Medical Center Biomedical Research and Training office and the [REDACTED] Institute for [REDACTED] Training and Research. These include meetings on mouse and experimental models for metabolic research, grantsmanship, biostatistics and proteomics analysis. The [REDACTED] Diabetes and Research Training Center also provides funding opportunities, meetings and seminars in conjunction with its [REDACTED] Enrichment, Training and Outreach Program, with which I will continue to be actively involved.

Additional career development mechanisms will include instructive seminars at national meetings, mentored guidance in grant and manuscript preparation, and academic job interviewing. An advisory committee will evaluate the completion of both my scientific and career development milestones and facilitate my transition to an independent investigator. Benign prostatic hyperplasia and associated lower urinary tract symptoms (BPH/LUTS) are a severe physical and financial burden, which, given their association with metabolic dysfunction, will continue to grow in the number affected. Moreover, clinical management of BPH/LUTS has reached limitations in efficacy, predominantly due to a lack of understanding of basic prostatic metabolism. Therefore, increased focus on the fundamental molecular mechanisms governing prostatic differentiation and immunomodulation is needed in order to identify new targets for preventing benign growth and inflammation in obese and diabetic patients. Based on our preliminary studies, I hypothesize that fatty acid metabolism is a key mediator of the differentiation program driven by stromal-epithelial interactions and that chronic obesity and type II diabetes disrupt the normal metabolic hierarchy governing differentiation and immunomodulation. The specific aims of this study are as follows: Aim 1: Determine how stromal fatty acid metabolism mediates prostate epithelial differentiation. Aim Two: Determine how monounsaturated fatty acid metabolism mediates luminal differentiation. Aim Three: Determine how CD36-mediated fatty acid uptake in vivo mediates differentiation and obesity-induced prostatic inflammation.

PUBLIC HEALTH RELEVANCE: The mechanisms responsible for development of benign prostatic hyperplasia (BPH) remain unclear despite recent strong epidemiological correlations with metabolic syndrome and type II diabetes. It is anticipated that the results of this proposal will provide a molecular rationale for targeting systemic or local fatty acid metabolism to reduce prostatic hyperplasia and inflammation. Successful completion of this proposal will address deficiencies in current therapeutic

modalities for BPH by deepening our understanding of the basic metabolic machinery mediating prostatic differentiation and immunomodulation.

CRITIQUES

(Note: The critiques below were prepared by the reviewers assigned to this application. These commentaries and criterion scores do not necessarily reflect the position of the authors at the close of the group discussion, nor the final majority opinion of the group, although reviewers are asked to amend their critiques if their position changed during the discussion. The resume and other initial sections of the summary statement are the authoritative representation of the final outcome of group discussion. If there is any discrepancy between the peer reviewers' commentaries and the priority/impact score on the face page of this summary statement, the priority/impact score should be considered the most accurate representation of the final outcome of the group discussion.)

CRITIQUE 1:

Candidate: 3

Career Development Plan/Career Goals /Plan to Provide Mentoring: 2

Research Plan: 3

Mentor(s), Co-Mentor(s), Consultant(s), Collaborator(s): 1

Environment / Commitment to the Candidate: 2

Overall description: In this K01 application, the candidate proposes to develop his technical and professional skills with the goal of becoming an independent investigator with a faculty position at a top-tier academic institution. This goal will be achieved through investigation of the mechanism of fatty acid metabolism in prostate differentiation and disease. The candidate proposes that prostate epithelial development is mediated by paracrine signals from the stromal and basal compartments involving metabolites of fatty acid metabolism. He proposes that this mechanism helps to explain the strong epidemiological correlation of metabolism syndrome, diabetes and benign prostate hyperplasia (BPH). He proposes to test the hypothesis that fatty acid metabolism is a key mediator of the differentiation program driven by stromal-epithelial interactions and that chronic obesity and type II diabetes disrupt the normal metabolic hierarchy governing differentiation and immunomodulation. Three specific aims are proposed; (1) to determine how stromal fatty acid metabolism mediates prostate epithelial differentiation; (2) to determine how monounsaturated fatty acid metabolism mediates luminal differentiation; (3) to determine how CD36-mediated fatty acid uptake in vivo mediates differentiation and obesity-induced prostatic inflammation.

1. Candidate:

Strengths

- The applicant Dr. [REDACTED] is into his fifth year of post-doctoral fellowship experience. He completed his PhD degree work with Dr. [REDACTED] in 2007 where he worked on the roles of fibroblast growth factor and transforming growth factor beta in prostate stroma epithelial interactions. He joined Dr. [REDACTED] lab in 2007.
- Dr. [REDACTED] has first authored or co-authored six research papers, 4 reviews and a book chapter.
- He received awards both during his graduate studies and during his post-doctoral fellowship. As a Post-Doc, he has been successful in obtaining intramural and extramural funding.
- Dr. [REDACTED] letters from his mentoring committee and his personal statement indicate that he is committed to becoming an independent investigator. His record indicates that he has the potential to achieve this goal.

Weaknesses:

- The candidate's publication record after 11 years of combined graduate work and post-doctoral fellowship of two first authored publications could be considered modest.

2. Career Development Plan/ Career Goals and Objectives

Strengths

- The applicant's career goals are consistent with the objective of becoming an independent investigator.
- The scope of the development plan will likely lead to the necessary scientific development to enable the applicant to reach his stated goal.
- Based on the applicant's prior training and experience, this project will build on previous training and experience and will contribute to a successful career of scientific independence.
- A mentoring committee is in place and will meet every six months to monitor the applicant's progress.
- The development plan will contribute to the success of the career objectives.

Weaknesses

- None noted

3. Research Plan

Strengths

- The proposed project will provide experience in a number of state-of-the art techniques that will serve the applicant well as he pursues his career goals. The research plan will result in training in mass spectrometry, lipid biology, and stable isotopic tracer analysis. The candidate will also gain training in the creation of lipodomic and proteomic tools to characterize/analyze the metabolic profiles of normal prostate, BPH and prostate inflammation.
- In addition to training already received in tissue recombination models, this project will add training in 3D co-culturing techniques and creation of transgenic animals.
- In Aim 3, the candidate will determine how CD36-mediated fatty acid metabolism *in vivo* mediates differentiation and obesity-induced prostatic inflammation. The studies will employ CD36 knockout animals treated with HFD (high fat diet) and the prostates will be examined for inflammatory cytokines. These studies are relatively straightforward given that the knockout animals are available.
- The applicant has addressed potential problems and provided alternative approaches.

Weaknesses:

- A feasible mechanism of how FA metabolism regulates epithelia differentiation is not presented. It is not clear what signaling pathways might be involved. Based on Fig 4 the role of inflammation is not apparent and whether the candidate believes this might be involved. Based on preliminary data (primarily Figure 3), the applicant concludes that PPAR γ 2 regulates epithelial differentiation through a paracrine mechanism. However, the only data in support of this are IHC, which suggest that PPAR γ 2 may not be strongly expressed in human prostate epithelium. On the other hand, CD36 a downstream mediator of PPAR γ 2 action is expressed in epithelium. Thus, a direct effect of PPAR γ 2 in the epithelia compartment cannot be eliminated.
- In Aim 1 the applicant will co-culture stroma cells (BHP γ S) over expressing PPAR γ 2 or CD36 cells with epithelia cells (BHP γ E) with and without drugs that modulate FA metabolism. Fetal mesenchyme (rUGM) will be a positive control. Appropriate markers of epithelia differentiation will be assessed. A concern with the experimental design is that it is not clear that BHP γ E cells do not express PPAR γ 2 or CD36 at some level. If they do express these factors it will be difficult to determine paracrine effects. It is not clear why PPAR γ 2 KO epithelial cells are not used for these studies.
- In Aim 2, the candidate will determine how monounsaturated fatty acid metabolism mediates luminal differentiation. Studies in this aim focus on the role of SCD1 and production of MUFAs

in luminal differentiation. While the studies as described will implicate SCD1 in epithelia differentiation, it is not clear how the role of MUFAs will be established except by inference.

4. Mentor, Consultants, Collaborators

Strengths

- Dr. [REDACTED] the mentor, is a professor of Urologic Surgery and Cancer Biology and director of the [REDACTED] Benign Urologic Disease Center. Dr. [REDACTED] is an experienced investigator in prostate biology, including prostate cancer and benign prostatic diseases. His experience and his accomplishments make him eminently qualified to mentor the candidate. Dr. [REDACTED] letter outlines the role and guidance that he will provide the candidate. He has assembled a mentoring committee that will help to oversee the candidate's development and to ensure that the candidate's training is appropriate and of high quality. Dr. [REDACTED] has trained graduate students, clinicians and post-doctoral fellows. He is the recipient of grant awards from NCI, NIDDK and the DoD Prostate Cancer Program and is currently supported by two R01 grants and an U01 grant. Dr. [REDACTED] letter addresses the potential of the candidate and indicates that the ideas driving this proposal are the candidate's. Dr. [REDACTED] expressed the strong opinion that the candidate has the potential for a successful career in the proposed area of research.
- Dr. [REDACTED] a member of the candidate's mentoring committee, is a professor and holds the [REDACTED] William L. Bary Chair in Urologic Surgery. He is also the director of the Urologic Research Program, Department of Urologic Surgery. Dr. [REDACTED] is a leader in prostate research and has made many significant contributions to our understanding of the cell and molecular biology of the prostate. He is a leading expert in the development of transgenic animal models of prostate diseases. He is currently supported by multiple grants from NCI and the DoD Prostate Cancer Program. He is eminently qualified to serve on the mentoring committee and to mentor the candidate. He will bring important experience and expertise to the candidate's training program.
- Dr. [REDACTED] the other member of the mentoring committee is a professor of Pharmacology and Biochemistry and director of the Mass Spectrometry Core facility at [REDACTED]. He has experience in lipid biology, mass spectrometry and isotopic tracer research. His research expertise is in the area of metabolism and quantitative analysis of biomolecules (sterols, lipids, endogenous metabolites, drugs and proteins) using stable isotopic tracers. In addition, he has expertise in compartmental analysis of complex physiologic systems using stable isotope metabolic tracers. Dr. [REDACTED] is involved in other mentoring programs at the university and is committed to developing the technical skills and academic professionalism of junior faculty members and post-doctoral fellows.
- This is an outstanding committee of mentors that has developed a more than adequate plan for the career development of the candidate.

Weaknesses

- None noted

5. Environment and Institutional Commitment

Strengths

- The academic environment at the [REDACTED] Medical Center and the Department of Urologic Surgery is outstanding. The Chair of the department provided a letter supporting this K01 application and Dr. [REDACTED] transition to an independent investigator.
- The Chair indicates commitment of space and resources to this proposal. The support outlined in the Chair's letter is adequate to ensure the success of this proposed project and the candidate's transition.
- The institution appears committed to Dr. [REDACTED] transition to independence regardless of the outcome of this application.

STRAND D

- While the environment and institutional commitment statement did not address the effort that the candidate will devote to the research, it is clear that the institution is committed to the candidate's success.

Weaknesses

- None noted

Protection of Human Subjects:

Not Applicable

Vertebrate Animals:

Adequately addressed

Training in the Responsible Conduct of Research:

Adequately addressed

Resource Sharing Plans:

Adequately addressed

Budget and Period of Support:

Recommend as Requested

CRITIQUE 2:

Candidate: 2

Career Development Plan/Career Goals /Plan to Provide Mentoring: 1

Research Plan: 2

Mentor(s), Co-Mentor(s), Consultant(s), Collaborator(s): 1

Environment /Commitment to the Candidate: 1

Overall Impact: This is an innovative application from a solid candidate who proposed to test the research hypothesis that fatty acid metabolism mediates prostate differentiation and immunomodulation. Strengths of the application include the experience of the candidate in the proposed area of research, prostate biology and BPH/LUTS. The proposed research is feasible, supported by strong preliminary data and will involve *in vitro* 3D co-culture and tissue regeneration models as well as genetically engineered mouse models. The research environment at the Vanderbilt University is ideally suited for the proposed research. The career development plan is well-described. Mentors, consultants and collaborators are well qualified to provide guidance for the candidate to conduct the proposed studies. However, it is not clear whether the expression levels of PPAR γ 2 and its down-stream genes CD36 and SCD1 are altered in human BTH specimens. This concern is relatively minor because the proposed study will have significant impact on our understanding of prostate biology.

1. Candidate:**Strengths**

- Dr. [REDACTED] is a well-trained young investigator and is ideally suited for the proposed research. Letters of recommendation from well-established investigators all indicated the high potential for Dr. [REDACTED] to become an independent investigator. He is not only academically strong but also has leadership ability.
- Dr. [REDACTED] listed 7 peer-reviewed publications, with him being the first or co-first author on 3 of them. Dr. [REDACTED] received a postdoctoral fellowship from DOD, a T32 postdoctoral fellowship, and an NIH loan repayment program grant.

- Dr. Strand has demonstrated a strong commitment for a career in Urological Research through his training in this field.

Weaknesses

- None noted

2. Career Development Plan/Career Goals & Objectives**Strengths**

- The career development plan for Dr. Strand is well developed. Dr. Strand will have opportunities and guidance from his mentor, Dr. Simon Hayward, to develop his own ideas about benign urological disease. He will be trained to develop and write about his own hypothesis and to organize research projects and budgets.
- Plans for enhancing the candidate's grant writing skills through didactic course work are adequately described.
- The candidate has organized a scientific career development advisory committee to evaluate, monitor and guide his research and career development progress.

Weaknesses

- None noted

3. Research Plan:**Strengths**

- The proposed research is potentially important because its success will provide new insights into the nutritional regulation of stromal-epithelial interactions and differentiation in the prostate, which may be involved in hyperplasia and inflammation in BPH/LUTS.
- The idea of linking fatty acid metabolism to stromal-epithelial interactions and prostate differentiation is highly innovative and supported by preliminary data.
- The candidate will use cutting edge research technologies, including 3D co-culture and tissue recombination techniques as well as HPLC/MS-based metabolic tracing, to dissect the mechanisms by which stromal fatty acid and monounsaturated fatty acid influence prostatic differentiation. These studies will not only reveal new insights into prostate biology but also provide an opportunity for the candidate to develop a vigorous independent research program and to obtain strong training in conducting research projects.
- The candidate has addressed potential pitfalls and alternatives.

Weaknesses

- Alterations of fatty acid metabolism in BPH pathogenesis are not clear.

4. Mentor(s), Co-Mentor(s), Consultant(s), Collaborator(s):**Strengths**

- Dr. Simon Hayward will be the primary mentor for Dr. Strand. He is a well-established investigator in Urological Research and has made significant contributions, particularly in stromal-epithelial interactions in the prostate.
- Co-mentors, Dr. Robert Martini and Dr. David Hachey, have extensive experience in research and in mentoring junior investigators. They will provide expertise in transgenic mice and in LC-MS for the proposed research project.
- The consultant for Dr. Strand will provide expertise in fatty acid metabolism and 3D co-culture methods.

Weaknesses

- None noted

5. Environment and Institutional Commitment to the Candidate:**Strengths**

- The Department of Urologic Surgery at Vanderbilt is a top ranked Urology Department in the US and provides outstanding research environment for the proposed research. The department is also committed to promoting Dr. Strand within 2 years to a faculty position as an independent investigator, regardless of the outcome of this K01 application.
- The overall research and training environment at the Vanderbilt University is outstanding.

Weaknesses

- None noted

Protections for Human Subjects:

Not Applicable (No Human Subjects)

Vertebrate Animals:

Acceptable

- No concern.

Biohazards:

Not Applicable (No Biohazards)

Training in the Responsible Conduct of Research:

Acceptable

Comments on Format (Required):

- Formal training at Vanderbilt in a one-day symposium.

Comments on Subject Matter (Required):

- Appropriate.

Comments on Faculty Participation (Required; not applicable for mid- and senior-career awards):

- The training is conducted by several professors in different and relevant disciplines.

Comments on Duration (Required):

- The symposium on Responsible Conduct of Research is one day long.

Comments on Frequency (Required):

- Completed a day-long symposium on Responsible Conduct of Research in 2007 and will complete a refresh course in 2012.

Select Agents:

Not Applicable (No Select Agents)

Resource Sharing Plans:

Acceptable

Budget and Period of Support:

Recommend as Requested

CRITIQUE 3:

Candidate: 3

Career Development Plan/Career Goals /Plan to Provide Mentoring: 5

Research Plan: 5

Mentor(s), Co-Mentor(s), Consultant(s), Collaborator(s): 2

Environment / Commitment to the Candidate: 2

Overall Impact: Modest publication record and has already garnered a DOD postdoctoral award as well as local pilot funding. The career development plan does not clearly identify gaps in training and plans to fill them, local seminars or opportunities to present his work, national meetings or milestones for progress. The research topic is novel, but the plan is somewhat difficult to follow – particularly Aim 1. Aim 3 seems unrelated to the overall theme of the project. The mentorship team is strong and supportive and covers all aspects of the proposed research. The institutional commitment is strong, but the letter omits protected research time.

1. Candidate:

Strengths

- This is a well trained and motivated young investigator who has published now seven original journal articles in the field of prostate cancer and BPH. With the acceptance of his most recent article this is a total of 3 first author publications.
- He has now been at [REDACTED] for 5 years with 5 original publications from that period – the most recent is a first author publication. He has authored or co-authored 4 reviews.
- The candidate has received DOD postdoctoral funding as well as pilot funding from [REDACTED]

Weaknesses

- One first author publication in a 5 year postdoctoral period is a bit modest.

2. Career Development Plan/Career Goals & Objectives (K24 Plan to Provide Mentoring):

Strengths

- The candidate's career objective is to obtain a tenure track position in the area of urogenital disease research.
- The mentorship committee chosen is excellent and should be able to provide needed advice on all aspects of the proposed project

Weaknesses

- The plan is lacking in description of: 1. Technical coursework or workshops that might enhance the candidate's training, 2. Local seminars or opportunities to present data, 3. National meetings to be attended.
- The plan should probably explicitly describe perceived deficiencies and strategies to gain the needed training.
- Milestones for professional development should be presented.

3. Research Plan:

Strengths

- The research question whether lipid metabolism in the prostate and stromal–epithelial interactions influences development of BPH is innovative and potentially very interesting.
- The research plan is relevant to the applicant's desire to study the interaction between prostate epithelium and stroma.

Weaknesses

- Overall the background and underpinnings of the project are presented in a very confusing manner. For instance, Figure 3 which is the basis of many of the proposed experiments is very poorly labeled and difficult to interpret. For instance, what does the graph in Figure 3F represent? What is the inset – is it the Figure 3G referred to in the text? Figure 5 is even more

confusing and poorly explained in the Figure legend. What is the *in vivo* model referred to in the text?

- Aim 1: It is not clear exactly what the hypothesis is – does the applicant believe that a fatty acid or fatty acid metabolite is the paracrine mediator between stroma and epithelium? Is this explicitly stated? How relevant the model system is if PPAR gamma or CD36 must be overexpressed to see an effect? It is not completely clear what the readouts for these experiments will be – is it numbers of basal vs. luminal cells?
- Aim 2: The hypothesis that MUFA metabolism in basal cells regulates differentiation in luminal cells is novel and this aim is better developed and the experiments described are logical.
- Aim 3: The hypothesis that CD36 mediated fatty acid uptake in the prostate epithelium mediates obesity induced prostate inflammation is interesting, but it seems unrelated to the other two aims and the stromal- epithelial hypothesis.
- A project timeline should be added to the plan.
- The detailed statistical analysis in the vertebrate animal section while worthwhile seems to be a way around the page limits.
- A minor point - There is mention of getting fresh human prostate cells in the project, but there is no description of how this will happen anywhere in the proposal.

4. Mentor(s), Co-Mentor(s), Consultant(s), Collaborator(s):

Strengths

- The mentor is well published with more than adequate grant funding to support the candidate.
- The additional members of the mentorship committee are well placed to assist him with proteomic and fatty acid analysis, animal models of obesity and metabolism and *in vitro* 3D models.

Weaknesses

- Minor concern that Dr. [redacted] is at [redacted] – how will the critical 3D models be accomplished? Will the candidate obtain some onsite training there?

5. Environment and Institutional Commitment to the Candidate:

Strengths

- Very strong research environment in the Dept of Urology. Impressive history of publication for basic science and clinical trainees.
- Outstanding resources with the group apparently poised to submit a center grant application.
- Letter from Dr. [redacted] is quite supportive with promise of lab and office space. There is a promise to promote the candidate to a faculty position regardless of the outcome of the K application.

Weaknesses

- Protected time is not explicitly mentioned in the Institutional letter.

Protections for Human Subjects:

Not Applicable (No Human Subjects)

Data and Safety Monitoring Plan (Applicable for Clinical Trials Only):

Vertebrate Animals:

Acceptable

- Detailed description of why animals are being used, the numbers used, the vet care and euthanasia

Biohazards:

Not Applicable (No Biohazards)

Training in the Responsible Conduct of Research:

Comments on Format (Required):

- Day long symposium and CITI course refresher

Comments on Subject Matter (Required):

- Covered in detail

Comments on Faculty Participation (Required; not applicable for mid- and senior-career awards):

- Names of involved faculty included

Comments on Duration (Required):

- Included

Comments on Frequency (Required):

- Once in 2007 and again in 2012.

Select Agents:

Not Applicable (No Select Agents)

Resource Sharing Plans:

Acceptable

- Will adhere to NIH policy

Budget and Period of Support:

Recommend as Requested

THE FOLLOWING RESUME SECTIONS WERE PREPARED BY THE SCIENTIFIC REVIEW OFFICER TO SUMMARIZE THE OUTCOME OF DISCUSSIONS OF THE REVIEW COMMITTEE ON THE FOLLOWING ISSUES:

VERTEBRATE ANIMALS (Resume): ACCEPTABLE

SCIENTIFIC REVIEW OFFICER'S NOTES:

The plans outlined in the application to obtain training in the responsible conduct of research are adequate to satisfy this requirement.

COMMITTEE BUDGET RECOMMENDATIONS: The budget was recommended as requested. Recommended direct cost levels are estimated and are subject to further adjustment based on the Institute's standard budget calculation practices.

NIH has modified its policy regarding the receipt of resubmissions (amended applications). See Guide Notice NOT-OD-10-080 at <http://grants.nih.gov/grants/guide/notice-files/NOT-OD-10-080.html>.

The impact/priority score is calculated after discussion of an application by averaging the overall scores (1-9) given by all voting reviewers on the committee and multiplying by 10. The

criterion scores are submitted prior to the meeting by the individual reviewers assigned to an application, and are not discussed specifically at the review meeting or calculated into the overall impact score. For details on the review process, see http://grants.nih.gov/grants/peer_review_process.htm#scoring.

MEETING ROSTER

**Kidney, Urologic and Hematologic Diseases D Subcommittee
National Institute of Diabetes and Digestive and Kidney Diseases Initial Review Group
NATIONAL INSTITUTE OF DIABETES AND DIGESTIVE AND KIDNEY DISEASES
DDK-D 1**

October 23, 2012 - October 25, 2012

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Consultants are required to absent themselves from the room during the review of any application if their presence would constitute or appear to constitute a conflict of interest.

AUA Office of Research Early Career Investigators Workshop

Mock Study Section Grant Application

“Improving Diagnosis of Congenital Genitourinary Anomalies”

A funded R01 application and its review (PI redacted)

RESEARCH STRATEGY:

1) **Significance:** The fragility of the embryonic development of the urogenital tract, gonads and external genitalia is reflected in the high prevalence of disorders of urogenital (GU) tract of development in newborn humans ranging in severity from isolated urogenital abnormalities to complete sex reversal. Cryptorchidism (failure of testis descent into the scrotum) is found in 2-3% of full-term males (1). Hypospadias or defects in the growth and closure of the external genitalia affect nearly 1 in 125 live male births(15). Sexual ambiguity (genital phenotypes that are not clearly male or female) occurs in about 1 of 2000 to 4500 babies (19). Although these are among the most common birth defects, the molecular basis underlying the etiology of congenital GU defects is surprisingly poorly understood (20, 21). Fetal exposure to environmental toxicants may cause some GU birth defects (20, 22). Point mutations in a small subset of genes (reviewed in (20),(21)) can adversely affect human urogenital tract development. Nevertheless, these known and suspected causes do not account for the high prevalence of GU birth defects. Many urogenital birth defects are associated with major congenital malformations or multiple minor anomalies (Online database of Mendelian Inheritance in Man (<http://www.ncbi.nlm.nih.gov/sites/entrez?db=omim>)), which suggests a causative chromosomal abnormality. However, routine cytogenetic karyotype analysis of children with GU defects reveals low rates of structural defects associated with disorders of sexual development (30, 31). *This study focuses on defining the causes of urogenital birth defects using a novel microarray comparative genomic hybridization approach.*

1) **Innovation: Microdeletion and Microduplication Syndromes as a Cause of Urogenital Birth Defects:** Some syndromes such as mental retardation, birth defects, developmental delay and autism are caused by specific submicroscopic chromosomal rearrangements(1, 35). The development of comparative genomic hybridization (aCGH) into a microarray format allows the identification and diagnosis of cryptic deletions or duplications of genomic regions that were once invisible using traditional cytogenetic methods, including karyotype analysis and fluorescence in situ hybridization (FISH)(1). Subtle rearrangements can occur in regions flanked by low-copy repeats and likely result from non-allelic homologous recombination between different copies of repeats during meiosis. Submicroscopic imbalances cause copy number variants (CNV) or changes of DNA segments which can influence gene expression levels by disrupting genes or regulatory sequences, creating fusion genes or altering gene dosage. Submicroscopic structural chromosomal defects cause microdeletion and microduplication syndromes (36-40) or confer risk of complex disorders (41, 42).

An example of a well-known cryptic chromosomal rearrangement involved in the etiology of a human reproductive disorder is the Y chromosome microdeletion, a cause of human male infertility. Indeed, today there are numerous recognized genomic syndromes associated with birth defects, mental retardation and other syndromes. Many additional candidate regions associated with genomic disease are under investigation. Based on this, we tested the hypothesis that submicroscopic chromosomal alterations, too small to be detected by routine cytogenetic methods, may exist in patients with human disorders of urogenital development. This approach has not been widely used and we were among the first to use this method for GU defects.

2) **Rationale for This Approach:** Changes in dosage or structure of genes within the affected DNA segments can result in haploinsufficiency or altered transcription profiles, which may disturb the intricate fine-tuned network of genes controlling the human urogenital development. Importantly, examples of gene dosage alterations have been identified for factors controlling mammalian sex development. Deletion of the sex-determining gene *Wnt4* causes masculinization of XX mouse pups, whereas its duplication and overexpression in humans leads to XY sex reversal(46, 47). Duplications of large segments of DNA encompassing *DAX1* or *SOX9* cause sex reversal in humans (48, 49). These findings together with those presented in the **Progress Report** strengthen the emerging concept that GU differentiation, sex determination and differentiation are gene dosage sensitive at multiple steps of their pathways. In addition to dosage effects, imbalances may lead to disruption of regulatory sequences that control the expression of neighboring genes; thus, a gene related to GU development may lie adjacent to the microdeletion or -duplication.

In the previous funding period, we studied probands presenting with hypospadias, cryptorchidism and ambiguous genitalia (among the most common congenital urogenital defects seen in pediatric urology clinics). We then compared the resolution of detection of cryptic abnormalities by microarray-based CGH screening with results from the routinely used karyotype. We further analyzed the contribution of these structural anomalies to the observed GU phenotypes by studying their association with the urogenital traits, as well as their inheritance and their recurrence. We re-capitulated the birth defect in several mouse models of candidate gene duplication or deletion. ***For the first time, findings revealed the presence of frequent microdeletions and microduplications in the genome of children born with urogenital disorders and established de novo germline rearrangements as significant risk factors for developmental defects of the human urogenital tract. Our work revealed unrecognized gene defects underlying these birth defects.***

II) APPROACH: Progress Report (September 15, 2007- December 1, 2011)

Previous Aim #1: Define the Genetic Consequence of Abnormal Meiotic Recombination for Children With Congenital Genitourinary Defects. We will test the hypothesis that Chromosome Microarray Analysis (CMA) can improve our ability to detect of subtle chromosomal defects in children born with genitourinary defects. **This Aim is completed.**

A) De Novo Copy Number Variants (CNV) Are Associated With Congenital Genitourinary Defects:

We screened 116 children born with idiopathic GU defects using a clinically validated array-based comparative genomic hybridization platform (CMA). 8951 controls without urogenital defects were compared with our cohort of affected patients. Clinically relevant imbalances were found in 21.5 % of the analyzed patients. Most anomalies (74.2%) evaded detection by the routinely ordered karyotype and were scattered across the genome in gene-enriched subtelomeric loci. Among these defects, confirmed de novo duplication and deletion events were noted on 1p36.33, 9p24.3 and 19q12-q13.11 for ambiguous genitalia, 10p14 and Xq28 for cryptorchidism and 12p13 and 16p11.2 for hypospadias. These variants were significantly associated with genitourinary defects ($P=6.08 \times 10^{-12}$). The causality of defects observed in 5p15.3, 9p24.3, 22q12.1 and Xq28 was supported by the presence of overlapping de novo chromosomal rearrangement unrelated patients. In addition to known gonad determining genes including SRY and DMRT1, novel candidate genes such as FGFR2, KANK1, ADCY2 and ZEB2 were identified. These results should change the diagnostic strategy used by urologists for GU birth defects. Identification of germline rearrangements for GU birth defects will impact genetic counseling and contribute to the elucidation of the molecular mechanisms underlying the pathogenesis of human development.

Tannour-Louet, et al., Identification of De Novo Copy Number Variants Associated with Human Disorders of Sexual Development. Published, PLOS ONE(16).

Previous Aim #2: Perform Genome-Wide Comparative Genomic Hybridization Microarray Analysis of Children with Genitourinary Defects: Summary of Candidate Gene Analysis (This aim is COMPLETED; 5 genes affecting GU development in humans have been validated)

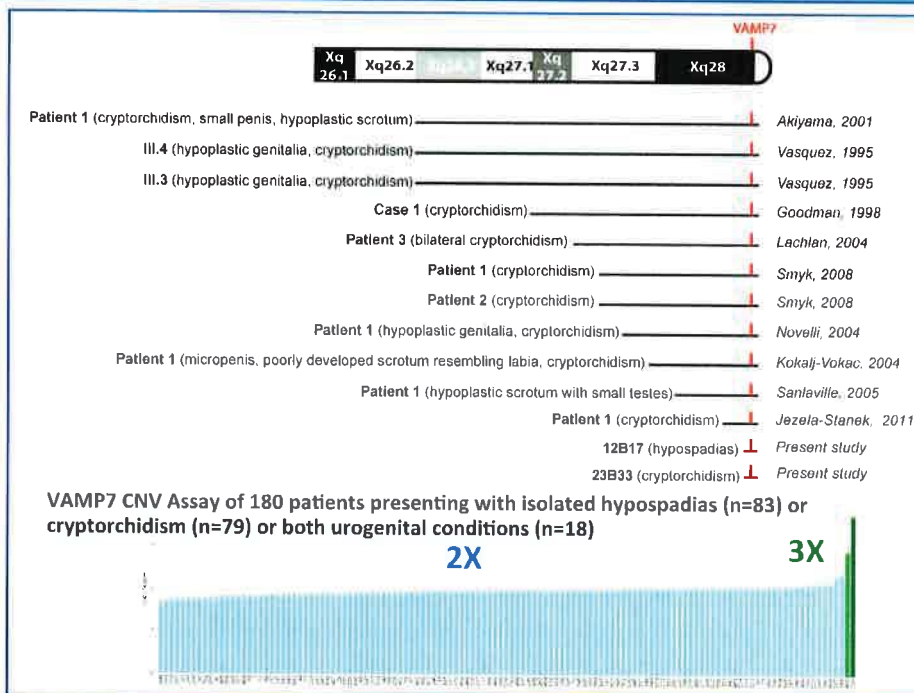


Figure 1 Identification of a terminal Xq28 gain encompassing VAMP7 in 46,XY children presenting with disorders of masculinization of the urogenital tract. CGH microarray analysis showed a copy number gain in two unrelated patients with idiopathic cryptorchidism or hypospadias. A locus specific RP11-479B17 probe FISH confirmed the CGH array finding while RP11-815E21 at Xq22.3 showed a normal copy number. A UCSC genome browser view (May 2006 Human Assembly) indicates that the only gene encompassed by the RP11-479B17 probe is vesicle-associated membrane protein 7 (*Vamp7*) (Not shown). (Upper Panel) Literature review of patients with Xq28 duplication encompassing VAMP7 with defective virilization of the male reproductive tract (3-10)(Lower Panel) Enrichment by CNV Taqman qPCR of the VAMP7 copy gain in a cohort of unrelated children with idiopathic cryptorchid or hypospadias. 8951 unaffected controls had 2 copies of VAMP7. **Two hypospadias and two cryptorchid children had VAMP7 duplication out of 182 total patients tested.**

I Increased Gene Copy Number of VAMP7 Alters Androgen Action and Disrupts Human Male Urogenital Development and Function: Androgen action is essential for male phenotypic development. We identified four unrelated patients with idiopathic cryptorchidism and/or hypospadias with a de novo microduplication of Xq28 encompassing the VAMP7 gene (Figure 1). This structural variant encompassed vesicle-associated membrane protein 7 gene (VAMP7) that encodes a member of SNARE family. SNAREs are small cytoplasmatically-orientated membrane-associated proteins that drive membrane fusion events in the

secretory and endocytic pathways(10). A literature review revealed the presence of additional patients with Xq28 terminal duplications including VAMP7 who presented with syndromic cryptorchidism, hypospadias and/or micropenis among their clinical features (**Figure 1, upper panel**). Accordingly, we hypothesized that a gene within Xq28, VAMP7, plays a role in human disorders of male urogenital development. 8951 controls tested did not have the VAMP7 microduplication($p<0.009$)(16). Quantitative PCR (qPCR) analysis of VAMP7 copy number in the genome of an additional cohort of 180 patients presenting with isolated hypospadias (n=83) or cryptorchidism (n=79) or both urogenital conditions (n=18) identified one additional case of isolated inguinal cryptorchidism and one patient with glandular hypospadias and chordee, both bearing a VAMP7 gain (**Figure 1, lower panel**) for a total of 4 patients with VAMP7 duplications (2 cryptorchid, 2 hypospadias).

A) VAMP7 Protein Was Detected in Cytoplasmic Lysates From Human Fetal Testis and Ovary: VAMP7 mRNA levels were detected throughout the adult human male genital tract including testis, epididymis, seminal vesicles, prostate and penile tissue. In human adult testis with histologically normal spermatogenesis, a punctuated granular pattern of VAMP7 staining was seen in Sertoli cells, as well as in the germ cells. Leydig cells also displayed VAMP7 positive immunoreactive cytoplasmic staining. Expression was present in adult human corpora cavernosa and the androgen-dependent penile spines of the preputial epithelium. Fetal mouse testes (gestational day 16.5) and developing genital tubercle positively stained for VAMP7. Immunostaining of murine testis at adult stages revealed the presence of the VAMP7(not shown). VAMP7 mRNA and protein are noticeably present in the human and murine urogenital tract during development as well as in adulthood.

B) Increased Gene Dosage of VAMP7 Results in Defects of Testicular Descent and External Genitalia Morphogenesis in Mice: Transgenic mice overexpressing VAMP7 variably displayed unilateral and bilateral cryptorchidism and degrees of hypospadias, accompanied with focal spermatogenic defects, reduced sperm motility and subfertility(**Figure 2A-F**). Hormone levels, testis, epididymis, seminal vesicle, penile weights were normal. Elevated levels of VAMP7 significantly reduced androgen receptor (AR) activity by trapping the AR in the endosomal compartment despite the presence of its ligand. Increased VAMP7 expression drastically enhanced estrogen receptor alpha (ER α) transcriptional activity. While *in vivo* transcriptional activity of androgen receptor was decreased in the testes of VAMP7 mice, expression of estrogen-responsive genes including ATF3, a gene implicated in human hypospadias, were up-regulated in the testis and penile tissues of mutant mice (**Figure 3,4**).

C) VAMP7 Over-Expression Enhances Estrogen Receptor (ER) Transcriptional Activity: Since a balance between androgen and estrogen receptor action, as opposed to androgens alone, is required for male reproductive development, we investigated the impact of VAMP7 on the estrogen receptor signaling pathway. Using our transgenic animals overexpressing VAMP7, we analyzed the relative expression of known genes involved in estrogen responsiveness. Of the 10 genes tested, we found increased mRNA levels of the activating transcription factor 3 (ATF3) gene whereas the expression of other key players in the estradiol pathway such as ERS1, ERS2, SRCs, RIP140 were unchanged (**Figure 4A,B** and not shown). Moreover, the protein levels of ATF3 were up-regulated *in vivo* in the VAMP7 transgenic animals. ***These observations are important because altered expression of ATF3 is a marker of hypospadias in humans*** ((50, 51)).

D) To Further Define The Role Of VAMP7 in ER Signaling, We Tested Its Functional Effect On A Classic Estrogen-Responsive Element Driving The Expression Of A Luciferase Reporter. A significant synergistic effect of VAMP7 was observed when cotransfected with ER in the presence of estradiol (**Figure 4C**). This result was consistent with our observations on gene expression and suggested that VAMP7

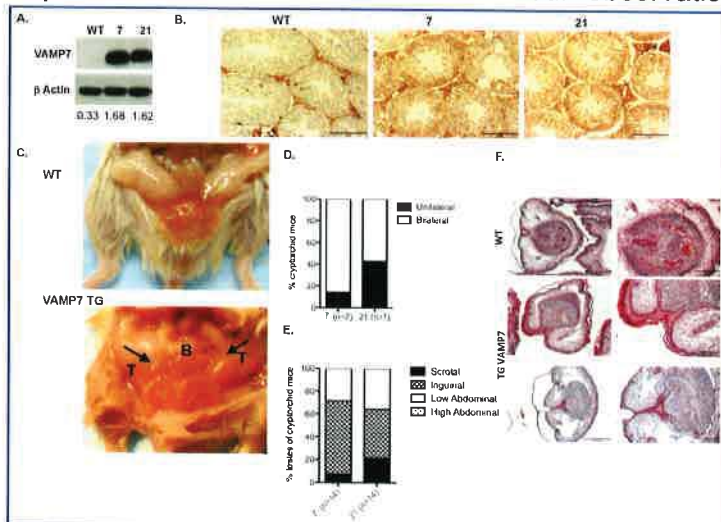


Figure 2 Mice with Elevated Levels of VAMP7 Displayed a Cryptorchid Phenotype and Defective External Genitalia. **A.** Western blot indicating overexpression in VAMP7 transgenic mice obtained after microinjection of BAC RP11-479B17. Testis protein levels in two independent strains (Tg7 and Tg21) were compared to those of wild type mice. **B.** Immunohistological staining of VAMP7 protein in the adult testis of wild type and transgenic mice to assess the tissue distribution of the transgene. **C.** Transgenic VAMP7 mice (VAMP7 TG) are cryptorchid (with an empty scrotum) as compared to wild type littermates (full scrotum). B: bladder; T: testis. Black arrows: testis. **D.** Percentage of the occurrence of unilateral and bilateral cryptorchidism in VAMP7 transgenic mice. **E.** Distribution of cryptorchid testes position in VAMP7 transgenic mice. **F.** Hematoxylin eosin staining of a cross section of the penis of transgenic VAMP7 mouse at gestational age E18.5. The urethra is still open on the ventral (lower) side of the penis(**Panel F, Preliminary**).

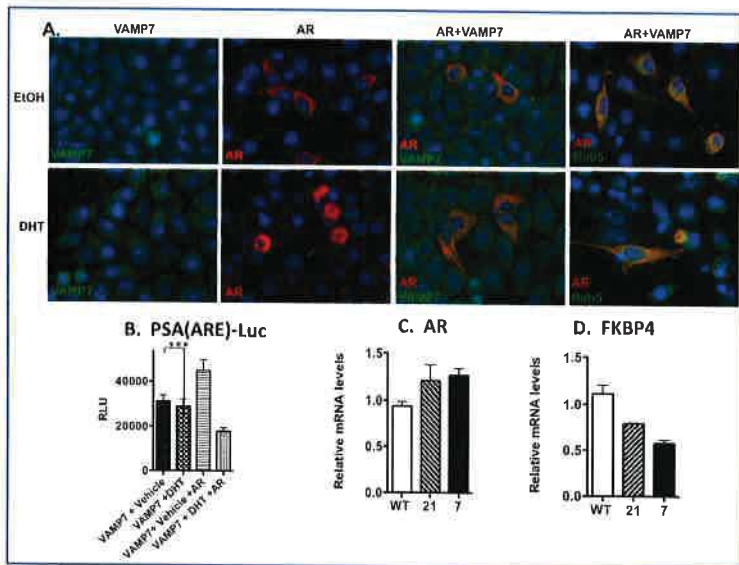


Figure 3 Elevated Levels of VAMP7 Perturb AR Ligand-Dependent Nuclear Translocation and Reduce Its Transcriptional Activity. **A.** HeLa cells were transfected with AR, VAMP7 or both. Upon stimulation with EtOH or DHT, localization of VAMP7, AR and Rab5 (endosome marker) were determined using immunofluorescence staining. **B.** Luciferase assays with VAMP7 and/or AR in HeLa cells in presence or absence of DHT (10^{-6} M). **C.** qRT-PCR analysis of AR and **D.** FKBP52 mRNA levels in testis of VAMP7 transgenic mice.

modulates ER action in the cells. To confirm this point, genetic invalidation of VAMP7 with siRNA was tested in a model cell line. As expected, the selective knock-down of VAMP7 significantly decreased the gene expression of ATF3 measured by real-time PCR confirming that VAMP7 is an important positive regulator of ER regulation of ATF3 gene expression. To substantiate these results, we used gene expression microarray technology to study the impact of VAMP7 gene dosage changes. In confirmation of our observations, Ingenuity Pathway analysis revealed that ER signaling was the third most significant pathway to be impaired in the absence of VAMP7. Thus, VAMP7 is a molecular determinant that appears to disrupt androgenic and estrogenic action in developing and adult genitourinary tissues and it should be defined as a critical factor for proper urogenital tract development.

II Methyl-CpG-binding Protein 2 (MECP2) Duplication is Associated with Cryptorchidism: During the course of our studies of genes on Xq28 associated with cryptorchidism, we became aware of patients with *MECP2* duplication syndrome

treated by Dr. Melissa Ramocki at Texas Children's Hospital. This is an X-linked neurodevelopmental disorder 100% penetrant in males(52). A subset of these males exhibited cryptorchidism (n=3), hypospadias (n=2) or both (n=2). To define the smallest region of overlap, a series of patients with duplication of Xq28 and *MECP2* were analyzed. The smallest region of overlap was ChrX: 152836642-153157753. This region encompasses *MECP2*, as well as *IRAK1* (interleukin-1 receptor-associated kinase 1) genes and microRNA718. Mice overexpressing *Mecp2* (*Mecp2* BAC Tg3 FVB) were generated and even mild levels of overexpression were detrimental(44). At one month of age, males showed unilateral inguinal cryptorchidism (Fig.5), again with abnormal spermatogenesis and spermatocyte arrest typical of cryptorchidism (not observed in the FVB controls). Thus, *MECP2* duplication identified by aCGH in humans can cause cryptorchidism in mice and humans. Analysis of mouse penile tissue for hypospadias is ongoing.

III WNT7B Duplication at 22q13: Three patients with hypospadias (2) or combined hypospadias and cryptorchidism showed duplicaton at 22q13. Interestingly, in 22q13 deletion syndrome, cryptorchidism is one of the phenotypic abnormalities (26). aCGH results showed duplication of *WNT7B* in these patients and a qPCR

assay of 32 patients confirmed these duplications(Fig 6). Two additional patients with hypospadias alone and with cryptorchidism are listed in the Decipher database. We will analyze *WNT7B* in the current year of funding. We purchased *Wnt7B* floxed mice and crossed them with *Sox-2-Cre* mice to target the *Wnt7B* deletion to urogenital tract. This strategy was used in the past to show that *Wnt7b* is essential for the

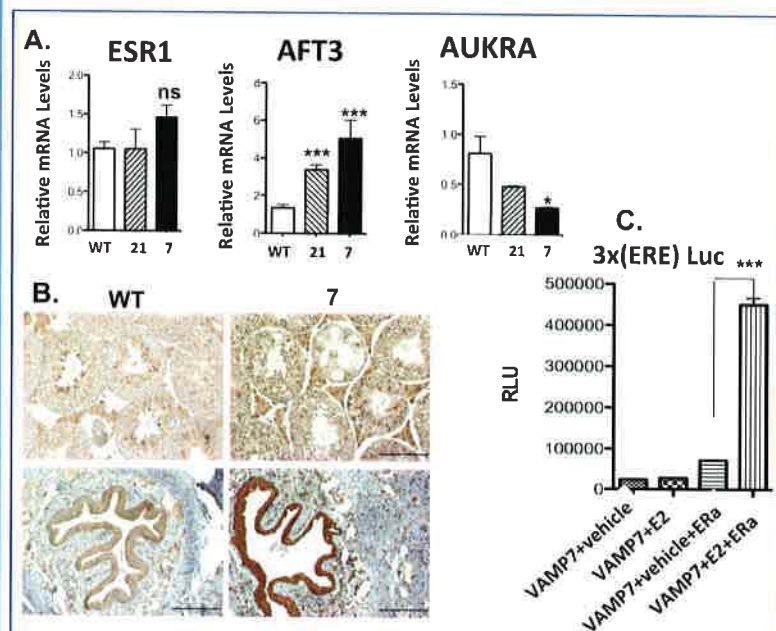


Figure 4 Vamp7 Overexpression Increases Estrogen Receptor Transcriptional Activity: **A.** Northern blot analysis shows that testicular extracts from *vamp7* transgenic over-expressing (lines 7, 21) and WT control mice show no difference in ERalpha expression, whereas ATF expression is increased and AUKA is decreased. **B.** Transgenic and control mouse testes (upper panels) and penile urethra (lower panels) immune-histochemistry shows the increase in ATF protein expression in line 7, 21 (not shown). **C.** Transient transfection assay using HELA cells expressing VAMP7, ER and an estrogen responsive element driving a luciferase reporter reveals a highly significant increase in ER transcriptional activity.

establishment of the cortico-medullary axis of the mammalian kidney through the regulation of cell cleavage planes within the collecting duct epithelium (28). These studies focused on kidney development. We are generating *Wnt7B* transgenic mice to confirm the role of this gene in the patient phenotype.

IV Candidate Gene *ZEB2* Deletion and Duplication in Cryptorchidism and Hypospadias: A FISH confirmed deletion at 2q22.11 was found by aCGH in child with cryptorchidism with hypospadias. Deletions in this region are associated with Mowat-Wilson syndrome which includes hypospadias and cryptorchidism as a part of the syndrome. Of the four genes deleted *KYNU*, *ARHGAP15*, *GTDC1*, *ZEB2*, our candidate is *ZEB2*. *ZEB2* (Zinc finger E-box binding homeobox) is a protein that interacts with a receptor-mediated, activated full-length SMAD. *Zeb2* is strongly expressed in the developing murine genital tract (<http://www.genepaint.org>). Knockout mice models of *Zeb2* presented reproductive system defects(53), <http://www.informatics.jax.org>. Moreover, *Zeb2* modulates Wnt signaling, a critical pathway for the development of the genital tract (54). Hence, *Zeb2* appears as a candidate involved in the male urogenital development. QPCR analysis of *ZEB2* copy number was performed on 90 patients. No other deleted patients were identified, but one patient had a duplication. Gene variants/ mutations were identified (Table 1) and the functional significance is under investigation.

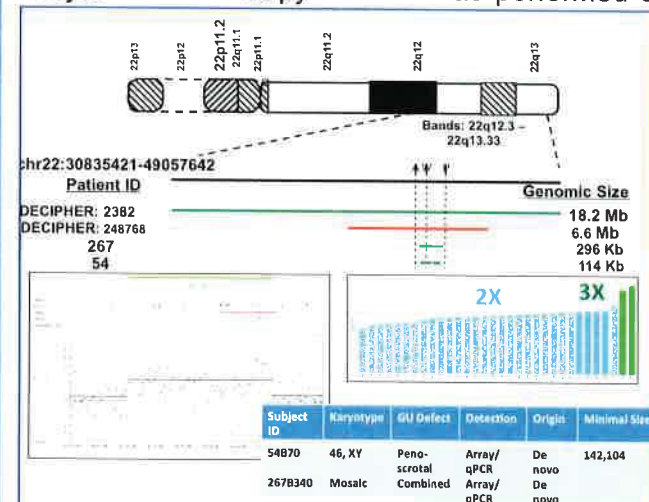


Figure 6 Minimal region within 22q12.3 - q13.33 associated with abnormal urethral development and testicular descent. Duplications detected in subjects with isolated hypospadias or combined hypospadias and cryptorchidism. (A) Schematic of structural variations (deletions and duplications) of chromosome 22. Patient 2382 (hypospadias) has a 18.2 Mb duplication and patient 248768 (vesicoureteral reflux) with a 6.6 Mb deletion were identified in the Database of Chromosomal Imbalance and Phenotype in Humans Using Ensembl Resource (DECIPHER). Patient 267 (penoscrotal hypospadias and left UDT) has a 296 Kb duplication and patient 54 (penoscrotal hypospadias) with a 114 Kb duplication were part of our cohort of subjects in the LMRRT. (B) Representative aCGH output of subject 267 indicates a duplication (mean log ratio 0.2646) at 22q13.31. (C) Subjects 267 and 54 have three copies at this loci as indicated by qPCR analysis. Blue is 2 copies, green is 3 copies of *WNT7B*.

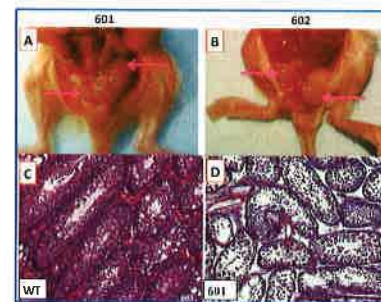


Figure 5 MECP2 Transgenic Mice Have Unilateral Cryptorchidism (Panels A,B, pink arrows): C). Wild-type (WT) testicular histology, D). Early spermatogenic arrest in cryptorchid testis (#601) in panel A.

V Chromosome 13q: A Susceptibility Locus for Epispadias: We found a microdeletion (*SUCLA2*) and microduplication (*PCDH9*) in region 13q in two brothers--one with epispadias and the other with hypospadias(Fig.7). *SUCLA2* is a mitochondrial enzyme expressed in several mouse and human tissues, including kidney and muscle. We found *SUCLA2* expression in the embryonic urethra, ureter and kidney. *SUCLA2* defects produce respiratory chain complex I disease-related mitochondria DNA depletion syndrome characterized by severe hypotonia and muscular atrophy in >30 subjects, one who also has hypospadias. Likewise, a defect in *TMEM70*, another mitochondrial enzyme, results in respiratory chain complex V disease with cardiomyopathy and hypospadias present in 50-100% of cases (55). The loss in *SUCLA2* was paternally inherited but the boys also inherited the SNPs 37Leu→Trp and 143Lys→Lys from their mother. Their normal sister did not inherit either the CNV or the SNPs. Mitochondrial nuclear genes may play an important role in the development of the GU tract.

VI Table 1 Summarizes the Genes Evaluated: This forward genetics approach allowed validation of 5 gene dosage changes causing human genitourinary defects.

APPROACH: EXPERIMENTAL PLAN: This PROGRESS REPORT provides the basis for our project:

✚ **Copy number variants are associated with genitourinary defects**

- ✚ **Clinical diagnosis of cryptorchidism, hypospadias (alone or in combination with cryptorchidism), gonadal dysgenesis and ambiguous genitalia can be improved with the use of clinically validated aCGH assays such as the CMA developed at Baylor College of Medicine**
- ✚ **Some variants in non-syndromic children are in syndromic regions associated with genitourinary birth defects**
- ✚ **Genome-wide aCGH can detect gene dosage (CNV) defects causing genitourinary birth defects**

Table 1 Summary of Candidate Genes Identified And Progress Towards Defining Causality

| Patient Phenotype (Birth Defect) | Chromosome location | #Patient Gain/ Loss | Human Syndromic Region with GU Defects? | Human Candidate Gene | Gene Expression During GU Development (Mouse) | Mouse Phenotype (Recapitulate Human Defect?) |
|---|---------------------|-------------------------------|---|---|---|---|
| Combined Cryptorchidism and Hypospadias | 2q22.11 | 1 Loss 1 Gain | Mowat-Wilson Hypospadias and cryptorchidism (2) | ZEB2 (Zinc finger E-box binding homeobox 2) | Genital tubercle and the developing murine urogenital tract (GUDMAP) | Zeb2 Targeted deletion- Reproductive defects |
| Cryptorchidism | 20q11.22 | 5 Gains | | Transcription factor E2F1 | Testis, genital tubercle, mesonephros, metanephros, lower urinary tract (GUDMAP) | E2f1 Transgenic Unilateral cryptorchidism Phenotype CONFIRMED |
| Ambiguous Genitalia Hypospadias Cryptorchidism | 5p15 5p15.2 | 1 Loss 1 Gain 1 Gain | Cri-du-Chat Hypospadias and cryptorchidism (11) | ADCY2 Adenylate cyclase 2 1-8 Genes 1-8 Genes | Testis, genital tubercle, mesonephros, metanephros, lower urinary tract(GUDMAP) | |
| Ambiguous Genitalia Cryptorchidism Combined Hypospadias | 9p23p24 | 5 Losses 1 Loss | Deletion 9p sex reversal syndrome (12, 13) | KANK1, DOCK8, DMRT KN motif and ankyrin repeat domain, dedicator of cytokinesis 8, doublesex and mab-3 related transcription factor genes | Embryonic Testis Cords (GUDMAP) | DMRT1 Targeted Deletion Gonadal Dysgenesis KANK1, DOCK8, DMRT Chromosome Engineering Ongoing |
| Ambiguous Genitalia | 10q26 | 1 Loss 1 Loss | 10q26 monosomy; genital anomalies (14) Monorchidism, cryptorchidism intersex (17) | FGFR2 (Fibroblast Growth Factor Receptor 2) 620 Genes | Metanephros (GUDMAP) | Fgfr2 Targeted deletion- Male to female sex reversal (18) Phenotype CONFIRMED |
| Ambiguous Genitalia Mixed Gonadal Dysgenesis | 22q11.2 | 1 Gain 1 Loss 1 Loss | 22q11.2 dup. synd. (23) DiGeorge Wilms tumor Smith-Lemli-Opitz Sex reversal (24) | 69 Genes 69 Genes 69 Genes | | <i>Sox10</i> gain of function- sex reversal(25) (<u>not in our minimal region</u>) |
| Combined Cryptorchidism - Hypospadias Hypospadias | 22q13 | 1 Gain 2 Gains | 22q13 deletion-syndrome cryptorchidism (26, 27) | WNT7B (Wingless-type MMTV integration site family member, 7B) | Mesonephros, metanephros, lower urinary tract, Epidermal and urethral epithelium of mouse genital tubercle (GUDMAP) | WNT7B transgenic to be created(28, 29) |
| Gonadal Dysgenesis | Yp11.31 | 4 Losses | Sex Reversal (32) | SRY (Sex determining region Y) 189 Genes 192 Genes 44 Genes 116 Genes | Genital ridge, early reproductive system (GUDMAP) | Sex Reversal 1. Transgenic(33, 34) 2. <i>Sry</i> Gene deletion (43) Phenotype CONFIRMED |
| Cryptorchidism Hypospadias Combined Cryptorchidism/ Hypopadias | Xq28 | 3 Gains 2 Gains 2 Gains | MECP2 Duplication | MECP2 Methyl-CpG-binding Protein 2 | | Unilateral cryptorchidism 1. <i>MECP2</i> Transgenic(44); Phenotype CONFIRMED |

2. *Mecp2* del. (45)

Table 2 Summary of Candidate Genes Identified And Progress Towards Defining Causality

| Patient Phenotype (Birth Defect) | Chromosome location | Patient# Gain/ Loss | Human Syndromic Region with GU Defects? | Human Candidate Gene | Gene Expression During GU Development (Mouse) | Mouse Phenotype |
|--|---------------------|------------------------|---|---|--|---|
| Cryptorchidism Hypospadias | Xq28 | 2 Gains 2 Gains | Xq28 Duplication Cryptorchidism Xq28 Deletion | VAMP7(SYBL-1) Synaptobrevin-like 1 | Testis, gubernaculum | Cryptorchidism 1. VAMP7(SYBL-1) Transgeneic Phenotype CONFIRMED 2. Targeted deletion |
| Hypospadias | 16q24.3 | 3 Gains | | TUBB3 β tubulin isotype III | Lower urinary tract, testis (GUDMAP) | |
| Combined Cryptorchidism Hypospadias | 2q36.2 16p11.2 | 1 Loss 1 Gain | | CUL3 (Cullin 3) KCTD13 (Ring) Sonic Hedgehog signaling | Genital tubercle (IHC) Genital tubercle (IHC) | |
| Bladder Exstrophy Micropenis/ Cryptorchidism Hydronephrosi s | 2p15-p16.1 | 5 Losses | 2.15-16.1 Microdeletion Syndrome (Autism) | OTX1 Orthodenticle homeobox 1 | Reproductive System (GUDMAP) | OTX1 deleted mouse embryos purchased from JAX |
| Epispadias Hypospadias | 13q14.2 | 1 Loss 2 Losses | Failure to thrive | SUCLA2 Succinate-CoA ligase, AD-forming β subunit | Bladder neck, urethra, (IHC) | Underdeveloped prepuce and absent preputial gland (KO) SUCLA2 deleted mice generated. |
| Bladder Exstrophy | 7p21.3 | 1 gain | | PHD Finger Protein 14 (PHF14) | Kidney, ureter, urethra, bladder (GUDMAP) | |
| Bladder Exstrophy Ambiguous Genitalia | 1p36 | 1 Loss | | 65 Genes | | |
| Hypospadias | 16p11 | 1 Loss | Cleft palate | 10 Genes | | |
| Cryptorchidism | 10p14 | 1 Gain | VACTERL syndrome, Imperforate Anus, Unilateral Renal Agenesis | 3 Genes | | |

****Additional regions of interest identified in Tannour-Louet, et al, 2010. (16)

- **Xq28--VAMP7 (SYBL-1):** Unilateral or bilateral cryptorchidism or hypospadias
- **Xq28--MECP2:** Cryptorchidism or hypospadias or both
- **Yq11—SRY:** Sex reversal (confirmed a known gene involved in sex determination)
- **E2F1:** Cryptorchidism

📌 **Genome-wide aCGH defined additional regions associated with genitourinary birth defects with candidate genes under analysis**

- **Ambiguous genitalia:** 9p23p24 (**KANK, DOCK8**), 10q26 (**FGFR2**), 22q11.2
- **Cryptorchidism and Hypospadias:** 2q22.11 (**ZEB2**), 5p15 (**ADCY**), 2q13 (**WNT7B**), 9p23p24 (**KANK, DOCK8**)
- **Hypospadias:** 5p15 (**ADCY2**), 22q13 (**WNT7B**), 16q24.3 (**TUBB3** β)
- **Bladder exstrophy:** 7p21.3 (**PHF14**), **HOXA1-11** (7p15.2), 11q14.1 (**DLG2**), 16p12.2 (**IGSF6, METTL9**)
- **Epispadias:** 2p13.1 (**NAT8**), 2q36.3 (**FBX036**), 6q15 (**RNGTT**), 13q14.2 (**SUCLA2**), 13q32.1 (**HS6ST3**), 20q11.21 (**SNTA1**), 20q13.13 (**PTGIS**), 8q22.1 (**UQCRB**) and 13q21.32 (**PCDH9**)

i) **Research Plan: Experimental Design and Overall Rationale:** The goal of this clinical research study is to enhance our ability to diagnose chromosome abnormalities present in children with congenital urogenital abnormalities. Throughout the history of clinical genetics, it has become obvious that our ability to diagnose genetic defects directly relates to the degree of resolution available to ask if chromosomal defects or genomic diseases are present. With each new technological advance, our ability to identify these defects has improved. For developmental defects, gene dosage anomalies, whether they result from microduplications or

microdeletions (our focus) or epigenetic modifications (altering expression levels), play a key role. The feasibility of successful outcomes with this approach is evident in our current funding period. **The five year goals of this project are to:**

- ✦ **Use genome wide aCGH to improve our ability to diagnose chromosome defects in children with congenital urogenital defects. Our goal is to identify previously unrecognized genetic "hot-spots" associated genomic diseases causing urogenital birth defects.**
- ✦ **Define new genes involved in normal genitourinary development and their mechanism of action.**
- ✦ **Develop novel mouse and *in vitro* models to define the identity and function of candidate genes in genitourinary development**

Aim #1: To reveal novel microdeletion and microduplication structural chromosome abnormalities in children with defects of the urogenital system and to use these findings for disease gene discovery

- ✦ **Hypothesis:** Subtle chromosome aberrations (<5-10 Mb) are associated with developmental defects of the genitourinary system.

I) **Perform Genome-Wide Comparative Genomic Hybridization Microarray Analysis of Children with Genitourinary Defects:** While in essence, we completed this aim in the previous funding period, our analysis of additional patients by aCGH continues to reveal new and previously unrecognized genetic "hot-spots" for urogenital birth defects. In this competitive renewal application, we propose to continue to perform aCGH on 160 additional patients and to add an additional, but rarer birth defect (Bladder Epispadias-Exstrophy-Complex) to our analysis for candidate gene discovery and validation.

1) **Rationale:** This proposal focuses on improving our ability to diagnose and understand developmental disorders of the genitourinary tract on a molecular level. Despite strong evidence that there is a genetic component, the etiology of these malformations remains unknown. The **Progress Report** shows that aCGH can successfully identify gene dosage anomalies causing urogenital tract defect, an approach that we validated by the creation of novel mouse models. Thus, we will use aCGH to identify additional regions of microdeletions and microduplications to define the molecular basis of urological birth defects.

(a) **aCGH Analysis for Definition of CNVs Associated with Urogenital Defects:** The two microarrays to be used for disease discovery are commercially available from Affymetrix and from Nimblegen. Both platforms were successfully used for detection of chromosomal imbalances in tumors and mental retardation syndromes and in the **Progress Report**. Two platforms are used because the arrays vary in coverage. We validated arrays with samples from known fertile individuals with normal karyotype analyses, men with Y chromosome microdeletion and others with recognized chromosomal imbalances.

Array Characteristics: One of our routinely used arrays contains 720,000 probes on a single glass slide allowing a whole genome array with probes tiled through genic and intergenic regions with an average spacing of 6,000 bp. A 2.1 million-probe HD array with long oligonucleotide probes allows a 100 bp resolution with enhanced accuracy. Ultra-high resolution arrays permit detection of small deletions and amplifications, but this approach can also result in a detection of variants of unknown significance making data analysis challenging. Breakpoint mapping can be resolved to less than 100 bp intervals. (<http://www.nimblegen.com/products/cgh/index.html>). The array permits detection of homozygous and heterozygous deletions, single and multiple copy amplifications and unbalanced translocations. It will not reveal balanced translocations. The company provides the raw data, segmentation analysis, genome annotation and SignalMap software to visualize the data map. We use Nexus software for a data analysis. To date, we have aCGH data on well over 70 control controls for this analysis. Subject samples will be tested against gender matched controls and processed at Roche NimbleGen Service Lab Iceland. Data will be analyzed using two different analysis programs, Nexus-Copy-

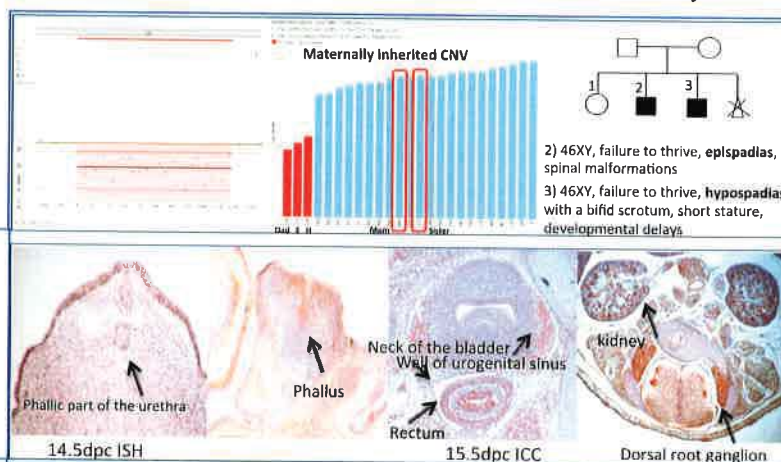


Figure 7 Succinate-CoA ligase, ADP-forming, b-subunit (SUCLA2) Paternally inherited Microdeletion is Present in Brothers with Epispadias and Hypospadias: aCGH analysis identified a 13q14.2 microdeletion which spanned SUCLA2. Both boys inherited the CNV from their mother and a SNP (L37W) from their father. Their unaffected sister does not have the SNP or the CNV. SUCLA2 is expressed in the developing penis, urethra, bladder neck and kidney.

Number software (BioDiscovery) and SignalMap (Roche). To confirm CNVs detected by aCGH, we will perform CNV-Taqman assays on at least 100 fertile controls and 100 racial/ethnicity/gender matched patients. TaqMan CNV reactions are performed in triplicate or quadruplicate using the FAM dye label-based assay and VIC dye label-based TaqMan CNV RNaseP for the internal controls. qPCR is performed with 20ng gDNA according to the manufacturer's protocol in an ABI 7900HT Fast Real-Time PCR System using the default universal cycling conditions. Relative quantitation analysis is done to estimate copy number for each sample by using the Copy Caller SoftwareV1.0. When candidate genes are identified, we will ask if the patients and controls have mutations. For PCR reaction 50ng of gDNA will be amplified using Phusion High-Fidelity PCR Master Mix with GC Buffer (NEB). After PCR, the purified products will be sequenced using Sanger sequencing (Genewiz). Data will be analyzed using Surveyor software (Softgenetics).

2) **Inclusion Criteria of Subjects for Analysis:**

(a) **Normal Male Controls:** Men who fathered a child with normal, genitourinary development, spermatogenesis, hypothalamic pituitary gonadal axis, ejaculatory function and fertility. They are examined by departmental urologists (Drs. Lipshultz, Khera). These control men are our semen donors from Dr. [REDACTED]'s clinical sperm bank. They have no urologic abnormalities, normal cycles, hormone levels and no karyotypic or known genetic anomalies.

(b) **Normal Females:** Phenotypically normal women who have naturally conceived a child. They have no urologic or gynecologic abnormalities, and no medical, karyotypic or known genetic anomalies.

(c) **Children with Gonadal Dysgenesis/Ambiguous Genitalia:** who do not have an endocrine-based defect underlying their abnormal development (either their own defect or their mother's during gestation), i.e., female or male pseudohermaphroditism. The children will be diagnosed with dysgenetic pseudohermaphroditism (mixed gonadal dysgenesis, testicular dysgenesis, gonadal dysgenesis) or true hermaphroditism (known causes are excluded).

(d) **Children/Men with Cryptorchidism:** who present with unilateral or bilateral cryptorchidism (abdominal or inguinal ring position will be the major groupings).

(e) **Children/Men with Hypospadias:** Conditions will range from simple distal hypospadias to midshaft to severe perineal forms of this defect. These three general urethral meatus positions will provide the basis for subcategories for patient classification.

(f) **Children with both Cryptorchidism and Hypospadias**

(g) **Parents of Children with GU Birth Defects:** To define *de novo* variants

(h) **Children with Bladder Epispadias-Exstrophy-Complex (BEEC):** These conditions include urological anomalies with different degrees of anterior midline defects that include epispadias (E) (the mildest phenotype with the urethral opening on the dorsum of the penis), bladder exstrophy (BE) (the bladder and related structures are open and located outside of the body), and cloacal exstrophy (CE) (the most severe with associated abnormalities of the bowel, urethra and bladder). These are rare conditions with incidences varying from 1:30,000 for BE; 1:100,000 for epispadias and 1:300,000 for CE. Anatomic classification is based upon the degree of extroversion of the bladder with grade 1 being most minimal to grade IV with the entire bladder extroverted with widely separated abdominal rectus muscles, imperforate anus, a wide separation of the symphysis and rotational displacement of the hips. Additional malformations are omphalocele, lipomenigocele or myelomenigocele, bilateral inguinal hernias and in boys cryptorchidism. Additional birth defects may be present(56). Most cases are sporadic, nonsyndromic, with a normal karyotype and unknown etiology. Rarely, an abnormal karyotype or genetic syndrome is found. Other malformations may be present.

• **Supportive Preliminary Data Showing Evidence of Gene Dosage Problems in BEEC:** DNA from 6-epispadias, 14-BE, and 3-CE patients were analyzed by aCGH using 720K NimbleGen arrays (Roche). Analysis was performed with Nexus Copy Number software (BioDiscovery) and SignalMap (Roche). Each suspected CNV was validated by FISH and qPCR as described in the **Progress Report**. Microduplications of Chromosome 7 were found in regions encompassing genes important in urinary tract development, such as HOXA1-11 (7p15.2) and a novel gene PHF14 (7p21.3). Additionally, several patients displayed a gain in 22q11.21 that correlates with a large duplication in the same region reported by (57-60) in BEEC patients. Microdeletions encompassing the developmental genes DLG2 (11q14.1) and NOTCH2 (1p12) were identified and are expressed during development in the GU system. All variants were *de novo* and not common in the general population. Studies of these genes and their expression profiles will help to correlate the clinical features of children with BEEC with their genetic makeup.

3) **Data Analysis:** Will be performed exactly as described in Tannour-Louet, et al., (16) and the **Progress Report**. We use commercially available software: SignalMap (Roche NimbleGen) and CopyCaller (Applied Biosystems) and NEXUS (BioDiscovery). We will analyze the contribution of the structural anomalies

to the observed GU phenotypes by studying their association with the genital traits, as well as their inheritance and their recurrence. We will use Fisher's exact test to define statistically significant associations,

Aim #2. To define the role of candidate genes in urogenital development through the use of novel *in vitro* and *in vivo* approaches

✦ **Hypothesis:** Gene dosage changes cause developmental defects of the urogenital tract

I) **How Do We Prioritize CNVs/Candidate Genes For Investigation?** We begin with selection of CNVs located in regions not found to occur commonly in the general population as documented in the public databases (<http://projects.tcag.ca/variation>) or based on the CMA clinical lab internal experience which includes analysis of over 26,000 individual patients. The CNVs identified by aCGH must be validated by fluorescent in situ hybridization (FISH) or QPCR(Fig.8). Evidence of causality (*de novo* changes not present in either parent, clustering of unrelated patients with GU birth defects at overlapping regions, significant association with GU defects when compared to subjects without GU birth defects (16) should be present. CNV overlapping genomic coordinates for a known genomic imbalance syndrome, or one containing known spermatogenesis genes or gene rich regions(61). The CNV is more likely to be pathogenic if it is a deletion, a homozygous deletion or an amplification with more than 1 copy gain(62). Of note, there are many examples where both a microdeletion and the reciprocal duplication syndromes cause a clinical phenotype. This will likely reflect the tight homeostatic control required for GU development if this occurs. We will use the strategy successfully employed by our group for gene discovery studies of genitourinary defects(16)

A) **Some regions overlap with known genetic syndromes with GU birth defects (Table 1):** For example, a large deletion of the subtelomeric cytoband 5p15 detected in patient 6 with genital ambiguity overlapped regions duplicated in patient 5 with hypospadias and patient 7 with cryptorchidism. This common region, which spanned the Cri-du-Chat syndrome locus, may include a master coordinator controlling sex determination, testis descent as well as external genitalia development. Hypospadias and cryptorchidism are among the malformations observed in the Cri-du-Chat syndrome suggesting a strong causative link of the 5p15 defect leading us to focus on *ADCY* as a candidate gene. *ADCY* shows a high and specific expression pattern in the testis as well as in the developing genital tract (<http://www.genepaint.org>). Moreover, the *Adcy2* protein regulates the intracellular levels of cyclic AMP, a crucial second messenger in major regulatory pathways involved in the biogenesis of the urogenital system such as Sonic Hedgehog signaling. Similarly the deletion 2q22 in patient 2 presenting with cryptorchidism and hypospadias was in the region associated with Mowat-Wilson syndrome (MWS). Among anomalies frequently observed in MWS are urogenital anomalies including hypospadias and undescended testis. MWS is a genetic condition caused by heterozygous mutations or deletions of *Zeb2* (zinc finger E-box binding homeobox 2 gene), a protein that interacts with a receptor-mediated, activated full-length SMAD. Targeted deletion of *Zeb2* in mice results in reproductive defects. Hence, *Zeb2* is a potential candidate involved in the male urogenital development. In general, duplications tend to result in a milder phenotype, although studies show that rates of deletion mediated by non-allelic homologous recombination are higher than that for duplications in the male germ line (35, 63).

B) **Gene expression studies define whether the candidate gene is expressed at the proper time during GU development:** Mouse and human data exist for many genes showing cell and organ specific expression during embryonic development and adulthood. For example, one strong candidate gene identified in this funding period, *KANK1*, deleted in five 9p23 ambiguous genitalia patients is highly expressed in the mouse embryonic urogenital tract (<http://www.genepaint.org>) and is able to physically interact and regulate the subcellular localization of beta catenin whose activation in normal XY mice disrupts the male program and results in male-to-female sex-reversal(27, 34). Genepaint (<http://www.genepaint.org>) and GUDMAP (<http://www.gudmap.org>) provide valuable resources in this regard. When the gene of interest has not been characterized during development, we prepared gender defined fetal tissue sections (whole mount embryos, genital ridge, wolffian/mullerian ducts, gonads, genital tubercle, etc.) for immunohistochemistry and in situ hybridization analysis of candidate gene expression. Serial sections are stained with the antibody or pre-immune serum (control), followed by routine methods for immunocytochemistry. Staining of sections of tissues

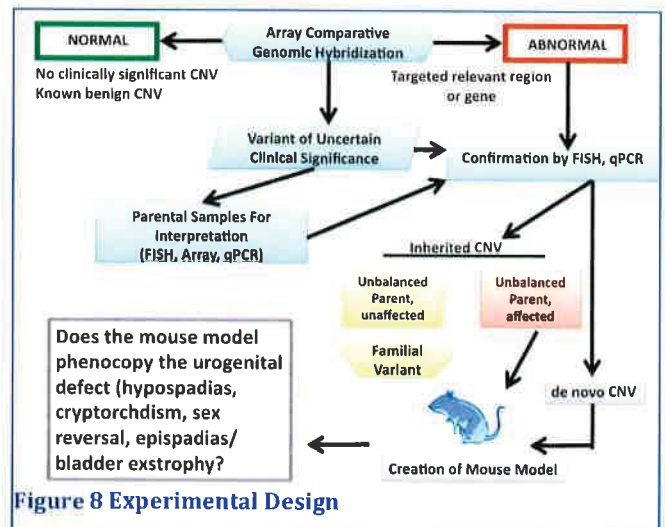


Figure 8 Experimental Design

known to express the gene of interest serve as positive controls. Candidate genes must be expressed during important developmental times of the urogenital tract, recognizing that a gene could be expressed in a different tissue (i.e. a hormone secreted by pituitary or hypothalamus) with a profound impact on GU development.

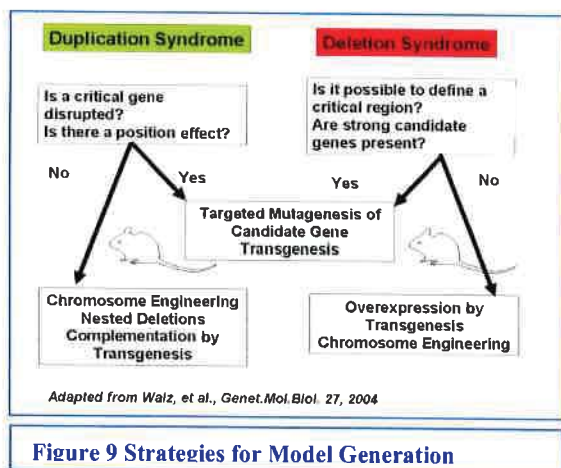
II) **In Vitro Studies of Gene Dosage Changes on Signaling Pathways:** We effectively use model cell lines, such as the NT2D1 cells, to provide important clues concerning the signaling pathways affected by gene dosage changes of candidate genes. We transfect the cell lines with an expression and control vectors for each gene candidate of interest. We use targeting and non-targeting (control) siRNA knockdown to examine the signaling pathway of the candidate gene. Each treatment is tested in triplicate, with a pool of siRNA constructs if required. Knockdown or overexpression of the candidate gene is confirmed by RT-PCR and statistical significance determined by Student's t-test or one-way analysis of variance depending upon sample or treatment number. An Affy Human U133 2.0 Plus Array will be used (as in **Progress Report**) to identify the signaling pathways affected by gene dosage changes resulting from over-expression or knockdown of each candidate gene. Genes with significantly altered expression levels observed in the expression array will be confirmed by RT-PCR. Ingenuity pathway analysis will be used to define key pathways impacted by the knockdown or overexpression of the candidate gene. Genes in the pathway are then interrogated to determine whether mouse models exist with urogenital defects or known genes in the developmental pathway.

III) **Generation and Analysis of Mouse Models That Mimic The Defects Thought To Underlie the Human Genitourinary Defects Defined Above:** During the last year of current funding (year 4), we will continue our phenotypic analysis of the mouse models generated/obtained (the 9p24.3 that causes XY male-to-female sex reversal (*Dock8*, *Kank1*), *Zeb2*, *Fgfr2*, *Wnt7b*, *Notch2*, *TUBB3* β , etc.). Analysis of some of these mouse models will continue beyond year 4. New mouse models will be generated based upon the additional findings of Aim #1. We will select region/gene(s) candidates from *de novo* CNVs identified that are within relatively small regions of microdeletion or microduplication.

A) **Cre-loxP Recombination Strategy:** Our first choice for the generation of these mouse models will induce defined chromosomal rearrangements in the mouse genome by engineering them in embryonic stem cells using the Cre-loxP site specific recombination system. However, the approach for the generation of chromosomal rearrangements is slightly different from that used for cell specific gene deletion strategies(64). This approach is currently being used for deletion of the 6 9p23 genes. For chromosome engineering, two sequential gene-targeting steps are necessary to prepare the endpoints for the selectable Cre-loxP recombination. The unique sites required for subcloning and introduction of *LoxP* sites will be defined on the basis of gene sequencing using primer extension reactions if the sequences are not yet determined or available from the genome project. Two genomic libraries are required, the 5' *hprt* and 3' *hprt* libraries containing in each vector backbone a single *loxP* site (Mutagenic Insertion and Chromosome Engineering Resource –MICER)(65). The basis of this approach is that each library contains half of the *Hprt* cassette in vector so that when reassembled, they can be selected for in culture due to their resistance to hypoxanthine, aminopterin thymidine when the *Hprt* gene is reconstructed after Cre recombination. It is possible to use coat color and other selectable markers to aid in this step. The strategy is shown in Figure 8 where the two serially inserted *loxP* sites are recombined by Cre recombinase with the reconstitution of *Hprt*. After selection in HAT, the region of the chromosome ("A") between the two-loxP sites will be lost. Because the insertion for both *loxP* sites is *Cis* the genomic part is deleted. If the endpoints are in different chromosomes, a balanced translocation occurs. If they are engineered in other orientations, then a duplication (same) or inversion (inverted) can be obtained(3). We will narrow the region that encompasses the candidate genes through the use of overlapping BAC clones spanning this deletion region and the generation of transgenic lines containing each. These mice will be bred to the deletion strain to restore part of the deletion with the BAC transgene.

1) **Alternative Approaches:** Other approaches to generation of mouse models can use nested deletions, targeted mutagenesis (if the gene is known) and transgenesis as described below and shown in **Figure 9**. Nevertheless, the nested deletion alternative may not fully reproduce the clinical spectrum observed and is not our first choice. If targeted mutagenesis is used, other genes in the region may have a modifying effect and this would necessitate a double knockout.

2) **Overexpression of Selected Genes By Transgenesis:** Transgenesis can be used to narrow down the critical chromosomal region and to produce overexpressing syndromes that are expected to complement the phenotype. This approach requires large genomic clones (YAC/BAC/Pac) that aid in the correct spatial and temporal expression after transgenesis. The Genetically Engineered Mouse Model Core at Baylor will produce all transgenic mouse models. Microinjected DNA encoding the gene of interest that integrates at random locations in the genome is to be used for genomic gain studies. The basic methodology involves the production of transgene founders through microinjection of DNA into the male pronucleus of a



fertilized mouse egg shortly after fertilization, followed by embryo transfer to a pseudopregnant recipient mouse. The quality of the transgene DNA has a major influence on transgenic production efficiency. Variation in the relative expression of the transgene is common and may confound the analysis. In addition, expression can be influenced by the position of integration and integration can result in inadvertent insertional mutagenesis. Accordingly, several independent founders will be developed from the same construct, expression levels assessed and the number of integration sites considered when the phenotypes of the transgenic lines are compared. Again, we will rely on this superb mouse core facility to generate these mouse models.

(a) **Caveats with Transgenesis:** Rearrangements may occur with increasing probability correlated with fragment

size. This caveat requires that we analyze the integrity of the inserted human transgene by STS assessment of loci spread throughout the YAC to ensure that there are no large deletions within the YAC. We will also look over 3-4 generations to characterize the YAC structure and ensure that intergenerational transmission does not cause rearrangements and that integration is stable. We will define the number of copies integrated by hybridization with specific probes. As a control, normal human DNA of a fertile male will be used. We will also use FISH to show that the YAC DNA is integrated as a single site and define the mouse chromosome of integration. Tissue specificity of expression will be examined. We predict a high level of tissue specific expression and will compare the human expression level with that of the endogenous mouse gene, if relevant. The advantage of the YAC approach for analysis of a genomic disorder by transgenesis is that large amounts of surrounding DNA are injected that can approximate the human situation. A potential caveat is that because dosage-sensitive genes can be influenced by strain background, different strains may have to be evaluated. This approach was used in the analysis of VAMP7.

B) Phenotypic Analysis of Mouse Models Generated:

1) **Histological:** The mice will be sacrificed during development and at 10-12 weeks (at least 10 per group) and the external urogenital system assessed and photographed. The animals are weighed prior to dissection and tissues individually weighed. The mice strains will be evaluated for the presence of cryptorchidism, partial or complete sex reversal, penis size, shape and placement of the urethral meatus, gubernaculum, oviducts, uterus, vagina, appearance of the testis, missing portions of the internal genital tract (vas deferens, epididymis, gonads, etc.) or urinary tract (urethra, ureter, etc.) and gonad weight. Sections of all tissues will be fixed and the histology evaluated in detail. If abnormalities are present, then further characterization will be undertaken. Controls will include wild-type littermates. Depending upon the type of gene deleted, spermatogenesis/ovarian function, the structure and presence/absence of specific tissue structures may or may not be normal. For example, a gene defect involved in hypospadias might have no effect on female urogenital development or may not affect testis or ovarian function.

2) **Endocrine Evaluation:** The hormonal status of all mice will be determined, including FSH (follicle stimulating hormone), LH (luteinizing hormone), testosterone, DHT, progesterone and estradiol. Adult (10-12 week old) mice (both mutant and wild-type) will be analyzed (Dr. Lamb supervises a large clinical andrology laboratory and is experienced with all of these analyses—at a micro scale for analysis of mouse serum). This assessment is important as endocrine abnormalities (biosynthesis, receptor action, metabolism) can affect the development of the external genitalia.

C) **Expected Results and Caveats:** This study will define new and previously unrecognized genomic defects associated with urogenital birth defects. Candidate genes will be defined and functionally validated. Perhaps the biggest difficulty that can be faced will be polymorphic regions of DNA that may give unclear results requiring extensive validation or large deletions/duplications encompassing many gene candidates.

D) **TIMELINE and Future Directions:** Completion of these studies will define a new etiology of birth defects of the human genital tract, providing a clear understanding of the genes required for normal genital development. The results of these studies will be far reaching. Although surgical correction has enhanced the lives of patients with GU birth defects, our ability to diagnose and understand the defects on a molecular level is deficient. The studies should answer important questions about the genetic basis of genitourinary development and the etiology of these common birth defects.



PROGRAM CONTACT:**DR. HEBERAH HASOOLY****301-594-6007****hr185f@nih.gov****SUMMARY STATEMENT**
(Privileged Communication)**Release Date:** **03/20/2012****Principal Investigator****Application Number:** **2 R01** **DK078121-05A1****LAMB, DOLORES JEAN PHD****Applicant Organization:** **BAYLOR COLLEGE OF MEDICINE****Review Group:** **UGPP****Urologic and Genitourinary Physiology and Pathology****Meeting Date:** **02/23/2012****Council:** **MAY 2012****Requested Start:** **07/01/2012****RFA/PA:** **PA11-250****PCC:** **KRR UGEN****Dual IC(s):** **HD****Project Title:** **Improving Diagnosis of Congenital Genitourinary Anomalies****SRG Action:** **Impact/Priority Score: 20 Percentile: 4****Human Subjects:** **30-Human subjects involved - Certified, no SRG concerns****Animal Subjects:** **30-Vertebrate animals involved - no SRG concerns noted****Gender:** **1A-Both genders, scientifically acceptable****Minority:** **1A-Minorities and non-minorities, scientifically acceptable****Children:** **1A-Both Children and Adults, scientifically acceptable****Clinical Research - not NIH-defined Phase III Trial**

| Project Year | Direct Costs Requested | Estimated Total Cost |
|-------------------------|-----------------------------------|---------------------------------|
| 5 | 250,000 | 391,250 |
| 6 | 250,000 | 391,250 |
| 7 | 250,000 | 391,250 |
| 8 | 250,000 | 391,250 |
| 9 | 250,000 | 391,250 |
| TOTAL | 1,250,000 | 1,956,250 |

ADMINISTRATIVE BUDGET NOTE: The budget shown is the requested budget and has not been adjusted to reflect any recommendations made by reviewers. If an award is planned, the costs will be calculated by Institute grants management staff based on the recommendations outlined below in the COMMITTEE BUDGET RECOMMENDATIONS section.

2R01 DK078121-05A1 Lamb, Dolores

RESUME AND SUMMARY OF DISCUSSION: The applicant remains focused on identifying specific gene defects causing congenital urogenital disorders and utilizes comparative genomic hybridization microarray technology to identify chromosomal alterations in afflicted children. The functional role of candidate genes is then evaluated through *in vitro* and *in vivo* studies, including the use of genetically modified murine models. Discussion was enthusiastic and no significant weaknesses were noted. The applicant was considered responsive to the previous critiques and productive during the prior period of support. The outstanding investigator, the identification of novel genes, translational potential of the studies, and environment were all considered significant strengths. The panel indicated the studies would advance the fields of urogenital development and pediatric urology.

DESCRIPTION (provided by applicant): A challenge for pediatric urology is the surgical treatment of birth defects of the lower urinary and genitourinary tract in boys. While genitourinary birth defects are among the most common diagnosed, the molecular basis of these defects is lacking. This application seeks support to continue our investigations into the genomic basis of these birth defects. We used state-of-the-art technology, comparative genomic hybridization microarray (aCGH), to identify submicroscopic gains or losses on chromosomes in children with congenital genitourinary defects. This work identified chromosomal regions where unrelated patients with the same birth defect exhibited de novo structural chromosomal duplications or deletions too small to be seen on a karyotype. A clinically validated aCGH identified structural chromosome defects in about 20% of children with genitourinary birth defects—an advance that can be used today by pediatric urologists. Thus our first hypothesis is proven: we improved the diagnosis of these children with aCGH. We seek to continue to identify genetic hotspots for congenital genitourinary defects. Importantly, the work identified a number of candidate genes for both normal human genitourinary development and birth defects. Based upon the current studies, we have a number of candidate genes for human urogenital tract development remaining to be analyzed and our aCGH studies continue to be informative and to suggest new areas for investigation. Accordingly, our specific aims remain similar to our currently funded proposal. We seek to identify the genetic and genomic defects that underlie these common birth defects. We will continue to show gene specific causality, beyond simple association with a birth defect, through generation of mouse models and definition of gene function during tissue morphogenesis. The long-term goal of this study is to improve the diagnosis of congenital genitourinary defects and to define the genetic basis for the failure of this key biologic process for children with birth defects of the urogenital tract. Although most urogenital tract defects are surgically corrected in children by the pediatric urologist, we seek to understand the cause of the birth defect and to know the likelihood whether any future children will be similarly afflicted. This proposal uses novel approaches to improve both our understanding of the molecular basis and to improve our ability to diagnose these common birth defects of the lower urinary and genitourinary tracts.

PUBLIC HEALTH RELEVANCE: Although most urogenital tract defects are surgically corrected in children by the pediatric urologist, we seek to understand the cause of the birth defect and to know the likelihood whether any future children will be similarly afflicted. This proposal uses novel approaches to improve both our understanding of the molecular basis and to improve our ability to diagnose these common birth defects of the lower urinary and genitourinary tracts.

CRITIQUE 1:

Significance: 2

Investigator(s): 1

Innovation: 1

Approach: 2
Environment: 2

Overall Impact: This is a resubmission of a competitive renewal from a highly productive lab that has made seminal discoveries in the genetics of urogenital abnormalities. The basic approach remains the same from the initial granting period where the investigator will perform high-resolution comparative genome hybridization microarray studies. Compared to classic karyotype analysis, this technique has yielded approximately twenty percent more genetic abnormalities. Based on the genetic deletions and/or gains from the comparative genome hybridization, the investigator has been confirming these findings with both in vivo genetically engineered mice and in vitro cellular studies to confirm indeed that deletions and/or gains in gene copy number can cause a clinical phenotype. The revised application, which was previously rated as outstanding, remains outstanding. The investigator has addressed the previous criticism, including modest productivity with a number of papers either under review or in progress. There is no question that further information gained from this investigation will allow more accurate clinical diagnosis, better genetic counseling for families, and further understanding of the mechanism for urogenital abnormalities. In summary, this is an outstanding revised competitive proposal with proof of concept, novel gene discovery and translational potential.

1. Significance:

Strengths

Congenital anomalies of the urinary tract are quite common. Presently, surgical treatment is the mainstay of therapy. Identifying abnormal genetics and hence mechanisms of abnormal development are a promising approach that may lead to both new preventative strategies and better diagnostic approaches to urologic diseases.

Weaknesses

None noted.

2. Investigator(s):

Strengths

This proposal is a competitive renewal with the initial funding period proving the concept that high-resolution comparative genome hybridization can identify genetic gains and losses in comparison to standard karyotype.

Weaknesses

The investigator and technician are the only two personal identify whereas surely postdoctoral fellows, graduate students and clinicians must be involved.

3. Innovation:

Strengths

High-resolution genetic studies remain innovative in that they have not been routinely used to detect urologic abnormalities.

Weaknesses

None noted.

4. Approach:**Strengths**

Proven capability to perform high-resolution comparative genome hybridization microarray studies.

Confirmatory mouse genetic studies to document phenotype.

In vitro confirmatory studies.

Identification of novel genes with both gain of function and loss of function.

Novel data that could directly impact genetic counseling of families.

Weaknesses

Relatively rare patient.

5. Environment:**Strengths**

Dr. Lamb's genetic laboratory and her clinical colleagues at Baylis are well positioned to perform the proposed experiments. The environment is excellent with no perceived weaknesses.

Weaknesses

None noted.

Protections for Human Subjects:

Acceptable Risks and/or Adequate Protections

Inclusion of Women, Minorities and Children:

G1A - Both Genders, Acceptable

M1A - Minority and Non-minority, Acceptable

C1A - Children and Adults, Acceptable

Vertebrate Animals:

Acceptable

Resubmission:

All critiques addressed.

Renewal:

This is a resubmission of a competitive renewal from a highly productive lab that has made seminal discoveries in the genetics of urogenital abnormalities. The basic approach remains

the same from the initial granting period where the investigator will perform high-resolution comparative genome hybridization microarray studies. Compared to classic karyotype analysis, this technique has yield approximately twenty percent more genetic abnormalities. Based on the genetic deletions and/or gains from the comparative genome hybridization, the investigator has been confirming these findings with both in vivo genetically engineered mice and in vitro cellular studies to confirm indeed that deletions and/or gains in our comp number can cause a clinical phenotype. The revised application, which was previously rated as outstanding, remains outstanding. The investigator has addressed the previous criticism, including modest productivity with a number of papers either under review or in progress. There is no question that further information gained from this investigation will allow more accurate clinical diagnosis, better genetic counseling for families, and further understanding of the mechanism for urogenital abnormalities.

Resource Sharing Plans:

Acceptable

Budget and Period of Support:

Recommend as Requested

CRITIQUE 2:

Significance: 3

Investigator(s): 1

Innovation: 1

Approach: 1

Environment: 1

Overall Impact: This proposal is a 2nd submission for this very experienced and very productive PI who has responded to the prior critiques. She has used the new method of comparative genomic hybridization to look for copy number variations in several common urologic conditions such as cryptorchidism and hypospadias that in the past were all deemed to have normal karyotypes when analyzed by conventional banding analysis. With the more advanced CGH, a higher proportion of these patients prove to have copy number variants than previously thought. The proposal features 2 specific aims. In aim 1, the PI proposes to use CGH to further characterize genomic hot spots where copy number variants can be identified. A strength of this proposal is that it starts with DNA isolated from clinical subjects with cryptorchidism, hypospadias, disorders of sexual differentiation, and now she will add in the exstrophy epispadias complex. As these chromosomal regions of copy variants are identified, it allows for the use of transgenic modeling in mice to try and recapitulate the clinical condition in the murine model (specific aim 2). There was substantial productivity on the preceding grant. While issues of the PIs productivity were mentioned in the prior review, it is critical to point out the detailed nature of this work and her commitment to publishing the findings in high impact journals. Carrying out the CGH studies with the human samples and then generating a transgenic overexpression or deletion is an elegant and thoughtful strategy that will not result in rapid publications.

1. Significance:

Strengths

The power of this proposal is that it starts with an analysis of human genomic DNA from the conditions in which she maps out these genetic hot spots.

Rather than merely identifying genetic anomalies, the PI then sets out on the more difficult task of demonstrating that these changes in gene structure actually result in the phenotype by recapitulating the condition in a transgenic mouse.

This approach can work especially well in the very rare disorders such as the exstrophy epispadias cloacal extrophy complex where it is highly unlikely one would ever be able to assemble a pedigree to do any kind of genetic analysis.

Such genetic information may well be useful in assessing the risk of recurrence of the birth defect in a subsequent pregnancy.

Weaknesses

Since many of these conditions are in effect syndromes, multiple hot spots will be identified which makes the analysis all the more challenging.

Translation to a clinical benefit for the patients in question is not seen at this time.

2. Investigator(s):

Strengths

Outstanding PI.

Long standing history of RO1 funding.

Productive and innovative.

Long history of working with clinicians to obtain samples from the phenotypes in question.

Weaknesses

None.

3. Innovation:

Strengths

Applies CGH to the study of diseases which in the past were not felt to have any underlying genetic etiology.

Approaches a disease such as cryptorchidism by starting with the patient's DNA and then working back from bed to bench (the challenge will come in going from bench back to the bedside)

Weaknesses

None.

4. Approach:

Strengths

Seeks to identify the genetic hot spots with CGH from an additional 160 patients with a new focus on the exstrophy epispadias cloacal exstrophy complex of disorders. This is a particularly high risk group of patients about whom more information is necessary.

Genetic overexpression or deletion of genes identified in AIM 1 to see if the clinical phenotype can be recapitulated in mice (Aim 2). This approach has been shown to work for the VAMP-7 whose transgenic overexpression lead to unilateral cryptorchidism.

Focus on copy number variants seems clinically relevant – so many studies point to no mutations in the gene structure (for example Ins3) in cryptorchidism. The problem in fact may be the copy number.

Detailed analysis of the reproductive phenotype outlined for any mice generated.

Weaknesses

As of now, no plan to see if there are therapeutic options for dealing with copy number mutations that could lessen the phenotype.

5. Environment:**Strengths**

Supportive environment for this proposal.

Clinical support in place.

Weaknesses

None.

Protections for Human Subjects:

Acceptable Risks and/or Adequate Protections

Inclusion of Women, Minorities and Children:

G1A - Both Genders, Acceptable

M1A - Minority and Non-minority, Acceptable

C1A - Children and Adults, Acceptable

Vertebrate Animals:

Acceptable

Renewal:

Very productive PI

Resubmission:

Acceptable

Budget and Period of Support:

Recommend as Requested

CRITIQUE 3:

Significance: 3

Investigator(s): 1

Innovation: 2

Approach: 2

Environment: 1

Overall Impact: This is a revised submission of a competitive renewal application that was well regarded in the first review. The proposal seeks to build on accomplishments made during the first funding cycle by applying comparative hybridization genomic methods to identify defects associated with urogenital malformations in newborns. They also continue efforts to identify the molecular mechanisms associated with manifestations of their identified gene defects using in vitro and in vivo (murine) models. The Investigative Team and Environment are clearly outstanding, and their genomic methods allow for identification of defects that other methods would miss. Their productivity seems adequate, and should increase naturally as a consequence of having already established a database of normal and disease genomic profiles to which their newly acquired data can be added and compared. The presence of this existing resource is an important factor in a grant design based heavily on discovery through its genomic arm. Identifying and validating the molecular mechanisms in vitro and in animal models is more time consuming, but also a valuable addition to their protocol. The relative rarity of the disorders included in their patient set somewhat limits the Significance, but the efforts to be "inclusive" in their sampling of observed defects seems to be an appropriate and natural way to expand and extend the work already accomplished.

Budget and Period of Support:

Recommend as Requested.

THE FOLLOWING RESUME SECTIONS WERE PREPARED BY THE SCIENTIFIC REVIEW OFFICER TO SUMMARIZE THE OUTCOME OF DISCUSSIONS OF THE REVIEW COMMITTEE ON THE FOLLOWING ISSUES:

PROTECTION OF HUMAN SUBJECTS (Resume): ACCEPTABLE

INCLUSION OF WOMEN PLAN (Resume): ACCEPTABLE

INCLUSION OF MINORITIES PLAN (Resume): ACCEPTABLE

INCLUSION OF CHILDREN PLAN (Resume): ACCEPTABLE

VERTEBRATE ANIMAL (Resume): ACCEPTABLE

COMMITTEE BUDGET RECOMMENDATIONS: The budget was recommended as requested.

NIH has modified its policy regarding the receipt of resubmissions (amended applications). See Guide Notice NOT-OD-10-080 at <http://grants.nih.gov/grants/guide/notice-files/NOT-OD-10-080.html>.

The impact/priority score is calculated after discussion of an application by averaging the overall scores (1-9) given by all voting reviewers on the committee and multiplying by 10. The criterion scores are submitted prior to the meeting by the individual reviewers assigned to an application, and are not discussed specifically at the review meeting or calculated into the overall impact score. For details on the review process, see http://grants.nih.gov/grants/peer_review_process.htm#scoring.

MEETING ROSTER

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Digestive, Kidney and Urological Systems Integrated Review Group
CENTER FOR SCIENTIFIC REVIEW
UGPP
February 23, 2012**

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* Temporary Member. For grant applications, temporary members may participate in the entire meeting or may review only selected applications as needed.

Consultants are required to absent themselves from the room during the review of any application if their presence would constitute or appear to constitute a conflict of interest.