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INTRODUCTION

Goals, Background and Relevance:

The **goal** of the proposal is to bring together a multidisciplinary, multi-institutional team of basic, translational, and clinical investigators to submit a **consortium proposal,** due June 12, 2002, that addresses the involvements of estrogens and antiestrogens in the genesis, prevention and treatment of prostate cancer (PCa).

The **overarching theme** of the consortium is to initiate and sustain a multiinstitutional, comprehensive, interdisciplinary, collaborative effort to gain an in-depth understanding of the mechanism of action of estrogen/antiestrogen or estrogen-related agents in PCa development and to then use the information to design, synthesize and/or utilize PCa-specific new generation of estrogen-related therapeutics for the treatment and prevention of this form of cancer.

Despite the historical use of estrogen in the treatment of PCa little is known concerning the direct biological effects they have on the prostate, what role they may play in carcinogenesis of the gland and what mechanisms mediate the therapeutic effects of these hormones/antihormones on PCa. Therapies for advanced prostate treatment are limited and effective chemopreventive strategies for PCa are lacking. The consortium projects collectively offer a comprehensive and unique approach to address these needs. Projects are selected based on their high relevance to PCa treatment and prevention, creativities in applying the state-of-the-art technologies, experience and the willingness of the investigator to work in a synergistic manner, and short translational time-frame for bringing the end products to the clinics. Three consortium projects are focused on mechanism by which estrogens may play a role in prostatic carcinogenesis. These include studies seeking to 1) identifying genetic polymorphisms of CYP1B1 that contribute to PCa risk and race-based disparity in incidence, 2) epigenetic mechanisms for silencing ER-β, during prostate carcinogenesis, and 3) estrogen-mediated oxidative DNA damage and its prevention by antioxidants. Results from these studies may yield new diagnostic genetic tests for PCa, identify high-risk individuals and populations, and for formulating novel preventative/intervention strategies. The other three projects are directly aimed at the treatment of advanced PCa. One is concerned with the synthesis, efficacy testing and defining mechanisms of action of a new class of estrogen-based therapeutic agents. Another is a comprehensive genetic and biological investigation of the herbal-based phytoestrogen formulation, PC-SPES, commonly used as alternative medicine by PCa patients, and the last is a clinical Phase II trial to test the efficacy of DES and Faslodex in the treatment of androgen-independent PCa. **All of these studies are highly relevant for the formulation of science-based strategies for PCa treatment and prevention.**

BODY

a. The Statement of Work of the Consortium Building Grant contains the following approved Tasks:

Task 1. First 6 week following notification of Development Award
Hire an Administrative Assistant. Set up tele-communcation contract with a telephone company. Find out the most convenient time for biweekly conference calls. Call investigators and advisors to find out the best time for the first meeting. Make arrangement for the first meeting. Set up a list of discussion topics for the first 4 conference calls and prioritize them. Organize the first two conference calls to set agenda for the meeting.

Task 2. Between Week 8-10 following notification of Development Award
Convene the first meeting as a group. Strategize and finalize proposals' outlines. Seek additional collaboration if needed. Set up tight time lines for deliverables in subsequent conference calls. Have investigators present an outline of their proposal and seek input from advisors. Foster additional collaboration among investigators.

Task 3. Week 11-20
Organize conference calls on a biweekly basis to check on progress of proposal preparation. Organize an intra-net through UMass for confidential communication. Organize a second meeting to take place approximately 4 weeks prior to grant submission. During the second meeting finalize the proposals and seek additional input from investigators and advisors. Set target date for final individual proposal submission.

Task 4. Two weeks before submission date
Receive all applications and contract agreements. Put together final application for submission

Task 5. After submission of the Grant Proposal
Continue conference calls monthly to foster continue collaboration. Prepare for animal approval and human subject protocols. Look into a commercial hosting site for website for the consortium. Update research progress among investigators.

b. Key Accomplishments

Work accomplished under Task 1:

Once we received the announcement of the award, I drafted an advertisement to hire an Administrative Assistant to facilitate planning of meetings and establishment of all communication among the investigators. We checked out price-structures of tele-communcation contracts with several telephone companies and signed a contract with one. We then used emails and phone calls to establish the date of our first meeting with the investigators and the advisors. The meeting date was set on March 15-16, at Boston Logan Airport Hilton Hotel for one and a half days. Conferences calls and emails were then used to set the agenda for the meeting. Each project leader was asked to prepare hard copies of specific aims of their projects and a 20 minute presentation of their project to the group.

Work accomplished under Task2:

On March 15 and 16, all principal investigators and their key collaborators, as well as the external advisors convened at the Hilton Boston Logan Airport Hotel. All project leaders handed out copies of their specific aims and appended materials and gave a 20 minutes presentation on their planned project. A question and answer period followed each presentation. The external advisors were specifically asked to evaluate each project, comment on the strengths and weaknesses. The entire group then makes friendly suggestions for modification. Specific aims of each projects were modified in an attempt to adhere to the overarching theme and maximize synergize. Weak aims, personnel and projects were eliminated. The group

hammered out the final outlines for each proposal and identified areas and additional collaboration needed for each project. Timelines were set so that the first draft of the proposal were due in six weeks. One external advisor and two project leaders were assigned to read the first draft of a proposal and provide feedback. All first drafts were to be reviewed by the statistical cores to provide statistical power to the projects. Tight time lines for deliverables were enforced through subsequent conference calls with external advisors and investigators. Dr. Massimo Loda of Dana-Faber Cancer Institute was recruited as an additional external advisor to the consortium planning grant.

Work accomplished under Task 3:

Conference calls on a biweekly basis were arranged to check on progress of proposal preparation. The Principal Investigator approached the University of Massachusetts to set up a plan for confidential communication among investigator for future data sharing. A meeting was set up between the Principal Investigator and the Director of Research at University of Massachusetts to obtain a special agreement for cost sharing if the proposal was funded. The amount of cost sharing amounts to 33% of the entire application. The Principal Investigator and her Administrative Assistant were diligently in obtaining all the contract agreements, intellectual property sharing agreements, and other regulatory paper work among all school. The Administrative Assistant also obtained all supportive documents from Project Leaders and converted them to PDF files. Due to time constrain, the originally planned second meeting as a group was aborted. Instead, frequent communications via email and conference calls took its place. Most of the first drafts of proposal were received one month before the submission date and sent out for review and statistical analyses evaluation. The comments, suggestions and feedbacks were returned to project leaders. There were considerable amount of collaborative work being done during this period to support each others' proposals. Synergisms among the various projects had received very high priority during this re-writing and finalizing grant writing period.

Work accomplished under Task 3:

Two weeks before the submission date, all proposals and their appended materials were received on time. The materials were converted into PDF files, a process that was found to be highly time-consuming. The Principal Investigator and the Administrative Assistant worked vigorously on the overall part of the proposals and attempted to tie all the loose ends of the final drafts. The majority of the final proposal received professional editing. Signatures from all participants and institutions were collected. All budgets and budget justifications were checked by University of Massachusetts Sponsored Research Office before final signed off. The final proposal was sent out on time to reach DOD on June 12, 2002.

Work accomplished under Task 5:

After submission of the grant proposal, emails and conference calls were used to foster continue collaboration. Project leaders were urged to prepare documents for animal usage approval and human subject protocol approvals.

REPORTABLE OUTCOMES AND DELIVERBLES

1. A proposal of over 600 PDF pages was submitted on June 12, 2002.

Specific Aims and Objectives of the proposals were summarized below:

The consortium comprises of six research projects and one research core. Each is summarized as follows: **Dr. Bosland- (Project 1-PI)** will study the efficacies of dietary antioxidants to prevent PCa development in a sex hormone-, in particular estrogen-, oxidative stress relevant rat model of prostate carcinogenesis. With this model, proof-of-principle data will be generated to demonstrate that antioxidant activity is a major mechanism by which selenium, vitamin E, and lycopene protect against prostate cancer. The project will determine the magnitude of the protective activity of these antioxidants individually and in combination to provide efficacy information in support of the design of preventive clinical trials. These studies will provide the much-needed

rationale for the largest ongoing preventive clinical trial, the SELECT, in this country. **Dr Dahiya –(Project 2-PI)** will study polymorphism of the *cytochrome P450 1B1* (*CYP1B1*) gene whose enzyme catalyzes the conversion of estrogens to 4-OH catechol estrogen metabolites. These metabolites are highly carcinogeneic and several polymorphisms hyperactivate the enzyme. Using a large clinical specimen set with close to 50% African- American specimens, Project 2 seeks to establish specific CYP1B1 as risk factors of PCa and determine whether the high incidence of PCa in African-Americans could linked to a higher frequency of specific *CYP1B1* polymorphisms in this high-risk population. **Dr. Ho (Project 3-PI)**, together with Dr. Hanson and Dr. Chen, will test the hypothesis that a novel class of compounds with the unique of scaffold of 17α-phenylvinyl-estradial, termed APC-17s, possesses potent and specific anti-prostate cancer activity. The project will use a comprehensive drug discovery platform to identify lead compounds, characterize their activity and specificity, define their mode of action with regard to estrogenicity and "transcriptional fingerprints", generate structure activity relationship, re-derive additional improved compounds, and conduct pre-clinical testing on the best lead compounds. These studies may have significant payoffs as they may result in a new generation of therapeutics with higher efficacy and specificity for the treatment of PCa. **Dr Leav (Project 4-PI)** will examine whether down regulation of estrogen receptor- β (ER-β), which is abundantly expressed in the basal epithelial cells of human prostate, is a result of hypermethylation of the 5' sequence of the *ER-β*. Previous studies by Dr. Leav and Dr. Ho have demonstrated loss of ER-β expression correlates with PCa development and progression. These studies will unveil whether a major epigenetic mechanism, hypermethylation, is involved in development of prostate cancer both in humans and in the Noble rat model. These data may provide rationale for future clinical trials using improved demethylating agents for PCa prevention. Recently a novel herbal formulation, *PC-SPES* has emerged as a popular self-administered complementary alternative medicine taken by thousands of PCa patients. Early clinical trials demonstrate efficacy in advanced PCa patients. **Dr Nelson (Project 5-PI)** will test the hypothesis that the mechanism of action of PC-SPES can be characterized using comprehensive gene expression gene profiles and model systems that reflect *in vitro* and *in vivo* compound activity. Of significance, he will use a prostate-specific microarray fabricated in his laboratory to enhance the likelihood of identifying prostate-specific "transcriptional fingerprints" for the action of PC-SPES and those of its active compounds. The identified molecular pathways may serve as pre-clinical indicators of tumor or host response that can be used to facilitate the design of rational clinical trials. **Dr Taplin (Project 6-PI)** will conduct a randomized Phase II clinical trial to evaluate the efficacy of diethylstilbestrol, an estrogen, and Faslodex, a newly available antiestrogen in the treatment of androgen-independent PCa. As correlative studies, serum hormone and protein profiles will be studies with the goal of unveiling novel mechanisms of actions of the estrogen and the antiestrogen. In collaboration with Dr. Ho a novel proteomics serum profiling approach by MALDI-TOF and SELDI-TOF will be used to gain knowledge in the biology of advanced PCa and mechanism of therapeutic response and resistance. **Through the Biostatistical Core Dr Hsieh** will provide comprehensive expertise to all investigators on study design and data analysis.

Proposed Research and methods: The experimental plan encompasses studies done on preneoplastic lesions (PIN) and various grades of PCa [Project 2 & 4), PIN and evolving PCa in testosterone plus estradiol-treated Noble rat model [Projects 1&4], the synthesis of estrogen –based compounds for potential PCa treatment and the evaluation of their mechanisms of action and, dose -response effectiveness [Project 3], the evaluation of genomic and biological effects of PC-SPES on PCa [Project 5] and a clinical trial comparing the efficacy of DES and Faslodex on patients with androgen-independent PCa will be studied [Project 6.] The statistical core will help investigators with study design, data entry and the statistical analysis of resulting data. **Methods** to be used includes: transcriptional profiling, proteomics, Real-time PCR, in situ hybridization, protein and enzymatic analysis, and PCR-RFLP, Laser capture microdissection, methylation specific-PCR, bisulfite genomic sequencing [Project 4] A Prostate derived c-DNA microarray platform and [Project 5].Clinical trials, proteomic and serum hormone analysis, toxicity testing. **Synergy:** There is considerable synergy among consortium members. Much collaboration was initiated when the consortium members met in Boston this past March. Drs Dahiya will be a coinvstigator on Dr Leav's project; he will aid Dr Leav in the design of primers for

his ER-β methylation studies in human and rat tissue. In turn Dr Leav will consult with Dr Dahiya on the grading of PCa. Dr Bosland will provide Dr Leav with specimens of Noble rat prostate for his studies. Dr Ho will be a co investigator on Dr Leavs project and will help interpret results and design primers. Dr Ho will work closely with Dr Hanson on the evaluation of estrogen –based compounds he has synthesized for PCa treatment. Dr Nelson will additionally evaluate the effects of candidate compounds from Dr Ho and Hanson project with his prostate-derived cDNA microarray platform. Dr Ho will aid Dr Nelson in determining PC-SPECS mechanism of action. Using his microarray, Dr Nelson will also interact with Dr Taplin to further define the effects of DES and Faslodex platform. Dr Ho will aid Dr Taplin in the use and application of Proteomic technology in her study.

Goals and End Products: A major broad goal will be to foster the development of a team of researchers who will continue to work together in the investigation of estrogen effects in the prostate. End products and measurable outcomes of the consortium projects include: 1) using sex-hormone-, in particular estrogen-induced animal model, the efficacies of three dietary antioxidants (vitamin E, selenium and lycopene), singularly or in combination, in PCa prevention will be evaluated, 2) the identification of key CYP1B1 polymorphisms that contribute to PCa risks and those associated with race-based PCa; 3) identification and characterization of a new class of novel anti-PCa compounds through synthesis of new compounds from a unique estrogen-like scaffold, mechanism-based screenings, structure-activity analyses, and transcriptional profiling; pre-clinical testing of lead candidates will be conducted on innovative *in vivo* models; 4) determining that hypermethylation is the likely mechanism of ER-β silencing which parallels the development of PCa; 5) the identification and isolation of the specific active compound(s) in PC-SPECS that accounts for anti-PCa activity through transcriptional profiling and HPLC-Mass Spectrometry identification of compounds; preclinical and clinical testing of lead compounds will also be performed; 6) the determination of DES and Faslodex as safe and effective treatments for androgen-independent PCa and advance our understanding of the mechanisms of the estrogenic/antiestrogenic actions through analyses of correlative data obtained on serum hormone levels and proteomic profiling, a highly innovative technology.

2. Outcome

The proposal received an overall score of 2.2 with a standard deviation of 0.26 and was not recommended for funding.

3. Other by products

To the best of the Principal Investigator's knowledge, two R01 based on activities and collaboration of this consortium building grant have been submitted to NIH for funding. Furthermore, several project leaders are in the process of re-organizing the stronger components of this consortium proposal into other applications for submission to NIH or DOD in the near future.

CONCLUSIONS

We have accomplished the majority of the work proposed under the Consortium Building grant. Although the Consortium proposal was not funded many productive lines of investigation and collaboration have been derived directly from this activity. It is expected that these continued research activities, initiated by the Consortium Building award, will significantly contribute to our understanding of how estrogens and antiestrogens affect prostate carcinogenesis, and the prevention and treatment of the disease.