Final Progress Report for Research Projects Funded by Health Research Grants

Instructions: Please complete all of the items as instructed. Do not delete instructions. Do not leave any items blank; responses must be provided for all items. If your response to an item is "None", please specify "None" as your response. "Not applicable" is not an acceptable response for any of the items. There is no limit to the length of your response to any question. Responses should be single-spaced, no smaller than 12-point type. The report **must be completed using MS Word**. Submitted reports must be Word documents; they should not be converted to pdf format. Questions? Contact Health Research Program staff at 717-783-2548.

1. **Grantee Institution:** The Trustees of the University of Pennsylvania

2. **Reporting Period (start and end date of grant award period):** 1/1/2010-12/31/2013

3. **Grant Contact Person (First Name, M.I., Last Name, Degrees):** Gearline R. Robinson-Hall, BSF

4. **Grant Contact Person's Telephone Number:** 215-746-6821

5. **Grant SAP Number:** 4100050912

6. **Project Number and Title of Research Project:** 8-Genomic Profiling for Molecular Diagnostics of Prostate Cancer

7. **Start and End Date of Research Project:** 01/01/2010-12/31/2013

8. **Name of Principal Investigator for the Research Project:** Timothy Rebbeck, PhD

9. **Research Project Expenses**.

   9(A) Please provide the total amount of health research grant funds spent on this project for the entire duration of the grant, including indirect costs and any interest earned that was spent:

   $746,976.78

   9(B) Provide the last names (include first initial if multiple individuals with the same last name are listed) of **all** persons who worked on this research project and were supported with health research funds. Include position titles (Principal Investigator, Graduate Assistant, Post-doctoral Fellow, etc.), percent of effort on project and total health research funds expended for the position. For multiple year projects, if percent of effort varied from year to year, report in the % of Effort column the effort by year 1, 2, 3, etc. of the project (x% Yr 1; z% Yr 2-3).

Last Name, First Name	Position Title	% of Effort on Project	Cost
Rebbeck	PI	2%Yr 3; 22%Yr4	$79,975.74
Baldwin	PI	5%Yr1	$9,127.84
Zeigler	Res.Asst.Professor	36%Yr4	$40,333.91
Zhou	Data Analyst	33%Yr3; 27%Yr4	$65,998.83
Buas	Educ.Fellowship	79%Yr2	$9,987.15

9(C) Provide the names of **all** persons who worked on this research project, but who *were not* supported with health research funds. Include position titles (Research Assistant, Administrative Assistant, etc.) and percent of effort on project. For multiple year projects, if percent of effort varied from year to year, report in the % of Effort column the effort by year 1, 2, 3, etc. of the project (x% Yr 1; z% Yr 2-3).

Last Name, First Name	Position Title	% of Effort on Project
None		

9(D) Provide a list of **all** scientific equipment purchased as part of this research grant, a short description of the value (benefit) derived by the institution from this equipment, and the cost of the equipment.

Type of Scientific Equipment	Value Derived	Cost
Sequenom Massarray Analyzer	Increased the capacity for DNA processing and analysis	$241,500

10. Co-funding of Research Project during Health Research Grant Award Period. Did this research project receive funding from any other source during the project period when it was supported by the health research grant?

Yes_____ No____X_____

If yes, please indicate the source and amount of other funds:

11. Leveraging of Additional Funds

11(A) As a result of the health research funds provided for this research project, were you able to apply for and/or obtain funding from other sources to continue or expand the research?

Yes____X_____ No_____

If yes, please list the applications submitted (column A), the funding agency (National Institutes of Health—NIH, or other source in column B), the month and year when the

application was submitted (column C), and the amount of funds requested (column D). If you have received a notice that the grant will be funded, please indicate the amount of funds to be awarded (column E). If the grant was not funded, insert "not funded" in column E.

Do not include funding from your own institution or from CURE (tobacco settlement funds). Do not include grants submitted prior to the start date of the grant as shown in Question 2. If you list grants submitted within 1-6 months of the start date of this grant, add a statement below the table indicating how the data/results from this project were used to secure that grant.

A. Title of research project on grant application	B. Funding agency (check those that apply)	C. Month and Year Submitted	D. Amount of funds requested:	E. Amount of funds to be awarded:
Social and Biological Effects on Prostate Cancer Disparities	☐NIH ☒ Other federal (specify: CDMRP/ DOD) ☐ Nonfederal source (specify:_)	6/2013	$1,223,408	$TBD
SPORE in Prostate Cancer	☒NIH ☐ Other federal (specify:___) ☐ Nonfederal source (specify:_)	5/2014	$TBD	$TBD

11(B) Are you planning to apply for additional funding in the future to continue or expand the research?

Yes____X_____ No_____

If yes, please describe your plans:

There will be many opportunities to build on the present research through NIH R01 and other related grants. In addition to a SPORE project (listed above), we will also submit this project as an R01 grant to NIH.

12. Future of Research Project. What are the future plans for this research project?

The research conducted here will be extended to build nomograms for clinical use using the biomarkers developed and studied here as well as an extended set of biomarkers that will be studied in ongoing research.

13. New Investigator Training and Development. Did students participate in project supported internships or graduate or post-graduate training for at least one semester or one summer?

Yes____X_____ No_____

If yes, how many students? Please specify in the tables below:

	Undergraduate	Masters	Pre-doc	Post-doc
Male			1	2
Female				
Unknown				
Total			**1**	**2**

	Undergraduate	Masters	Pre-doc	Post-doc
Hispanic				
Non-Hispanic			1	2
Unknown				
Total			**1**	**2**

	Undergraduate	Masters	Pre-doc	Post-doc
White			1	1
Black				1
Asian				
Other				
Unknown				
Total			**1**	**2**

14. Recruitment of Out-of–State Researchers. Did you bring researchers into Pennsylvania to carry out this research project?

Yes_____ No____X_____

If yes, please list the name and degree of each researcher and his/her previous affiliation:

15. Impact on Research Capacity and Quality. Did the health research project enhance the quality and/or capacity of research at your institution?

Yes____X_____ No_____

If yes, describe how improvements in infrastructure, the addition of new investigators, and other resources have led to more and better research.

The requirement to collect and study prostate tumor tissue was a new endeavor at the

University of Pennsylvania. The research conducted here provided the necessary infrastructure to develop the pathology, laboratory, and biomarker resources needed to undertake prostate tumor-based research.

16. Collaboration, business and community involvement.

16(A) Did the health research funds lead to collaboration with research partners outside of your institution (e.g., entire university, entire hospital system)?

Yes_____ No____X_____

If yes, please describe the collaborations:

16(B) Did the research project result in commercial development of any research products?

Yes_____ No____X_____

If yes, please describe commercial development activities that resulted from the research project:

16(C) Did the research lead to new involvement with the community?

Yes____X_____ No_____

If yes, please describe involvement with community groups that resulted from the research project:

During the course of this research, we expanded our interest in cancer health disparities research. At the same time, the PI began to develop cancer outreach and diversity initiatives via the Abramson Cancer Center. The research undertaken and funded by this project served as the main paradigm for taking biomarker-based and risk prediction research to the community, as well as expanding diversity in research to African American populations in the Penn catchment area.

17. Progress in Achieving Research Goals, Objectives and Aims.

List the project goals, objectives and specific aims (as contained in the grant agreement). Summarize the progress made in achieving these goals, objectives and aims <u>for the period that the project was funded (i.e., from project start date through end date).</u> Indicate whether or not each goal/objective/aim was achieved; if something was not achieved, note the reasons why. Describe the methods used. If changes were made to the research goals/objectives/aims, methods, design or timeline since the original grant application was submitted, please describe the changes. <u>Provide detailed results of the project.</u> Include

evidence of the data that was generated and analyzed, and provide tables, graphs, and figures of the data. List published abstracts, poster presentations and scientific meeting presentations at the end of the summary of progress; peer-reviewed publications should be listed under item 20.

This response should be a DETAILED report of the methods and findings. It is not sufficient to state that the work was completed. Insufficient information may result in an unfavorable performance review, which may jeopardize future funding. If research findings are pending publication you must still include enough detail for the expert peer reviewers to evaluate the progress during the course of the project.

Health research grants funded under the Tobacco Settlement Act will be evaluated via a performance review by an expert panel of researchers and clinicians who will assess project work using this Final Progress Report, all project Annual Reports and the project's strategic plan. After the final performance review of each project is complete, approximately 12-16 months after the end of the grant, this Final Progress Report, as well as the Final Performance Review Report containing the comments of the expert review panel, and the grantee's written response to the Final Performance Review Report, will be posted on the CURE Web site.

There is no limit to the length of your response. Responses must be single-spaced below, no smaller than 12-point type. If you cut and paste text from a publication, be sure symbols print properly, e.g., the Greek symbol for alpha (α) and beta (ß) should not print as boxes (□) and include the appropriate citation(s). DO NOT DELETE THESE INSTRUCTIONS.

During the funding covered under this research project, we continued the collection and processing of samples for biomarker studies. In the prior year, we finalized our validation studies of formalin-fixed paraffin-embedded (FFPE) tissues, and demonstrated that RNA integrity was optimized at 10 um thickness.

a) Test Samples: We have collected a sample of 41 test FFPE tissues for use in the development of study assays. These test samples were selected to represent the same kind of tissues that will be studied in the formal evaluation of markers (see below). Archived FFPE tissue blocks were retrieved for ten slow and ten rapid progression cases, and the prostate tissue was sampled by cutting multiple 10μ sections of tumor or adjacent normal tissue. DNA was extracted using Qiagen, Agencourt, and Ambion kits. Optimal extraction was achieved using the Qiagen kit, which was selected for use in all future extractions. Automated extraction using the qiaCube robot was performed.

b) Analysis Samples: We will use the Study of Clinical Outcomes, Risk and Ethnicity (SCORE) study at Penn for the evaluation of the proposed markers. The SCORE study is a prospective, longitudinal cohort of CaP cases ascertained at Penn since 1995. All participants donate a biosample containing genomic DNA, complete a standardized questionnaire at the time of their clinic visit, and provide access to medical records and follow-up. As of this submission, 1,460 CaP cases have formalin-fixed paraffin embedded (FFPE) tissue with longitudinal follow up. A

description of the case set available to this study is presented in Table 1. In addition to the FFPE biosamples, approximately 190 fresh-frozen CaP tissues are available for study, and the collection of fresh tissues continues through a protocol established by Drs. Rebbeck and Feldman.

Using the optimized protocol, we obtained the following biosample yields from the tissues studied:

Sample ID	# sections	ng/ul	A260	260/280	Constant	total (ug)
1N_R	8	166.65	4.166	2.09	40	3.7
1T_R	8	248.28	6.207	2.1	40	5.6
2N_R	6	245.33	6.133	2.11	40	5.5
2T_R	3	1269.03	31.726	2.12	40	28.6
1N_D	8	171.45	3.429	1.93	50	5.6
1T_D	8	228.15	4.563	1.94	50	7.4
2N_D	6	301.81	6.036	1.94	50	9.8
2T_D	3	1101.66	22.033	1.97	50	35.8
RNA: elute 27 ul > 25 ul > 22.5 ul post spec/bioanalyzer						
DNA: elute 40 ul > 35 ul > 32.5 ul post spec/bioanalyzer						

Using these samples, we generated data on a series of biomarkers, as outlined below.

These data demonstrate the robust nature of methylation across laboratory replications, and variability across loci for specific laboratory conditions.

Sequenom oncocarta, somatic mutations

For the analysis of somatic mutations, we have generated preliminary data for both specific somatic genome changes and fusion translocations. For somatic genetic analysis, we have defined a panel of changes that have been identified through literature searches. We have undertaken assay development using the test FFPE prostate samples described above. DNA from the test samples described above was sent to Affymetrix for OncoScan microarray assays. This DNA was determined to have insufficient mass for the assay, so new extractions are now underway to supplement the samples.

The protocol used for these data generation is as follows:

* download CpG annotations for Illumina whole-genome analysis (call technical support/etc)

* locate published primers/amplicons in the literature

published primers are typically for methylation-specific PCR (or sequenom-epityper)
eg. primers are specific for bisulfite-converted methylated DNA

* download sequence files> http://www.genenames.org/

mRNA (REFSEQ) AND genomic (ensembl) sequence encompassing mRNA
ENSEMBL> export data +/- 10kb> raw sequence (TEXT)
OR UCSC genome browser for genomic seq>

* BLAST mRNA/genomic to identify promoter location within genomic DNA /etc
OR for Illumina CpG IDs> BLAST source sequence field (excel doc) VS genomic DNA
be aware of transcript variants, alternative start sites, etc
http://blast.ncbi.nlm.nih.gov/Blast.cgi?PAGE_TYPE=BlastSearch&PROG_DEF=blastn&BLAST_PROG_DEF=megaBlast&BLAST_SPEC=blast2seq

* SAVE genomic sequence surrounding transcriptional start site (TSS), eg –5kb > + 5kb

* CONVERT genomic sequence to FASTA format if not
http://searchlauncher.bcm.tmc.edu/seq-util/readseq.html

* CONVERT sequence to bisulfite-converted (methylated / C>T + strand)>
http://biq-analyzer.bioinf.mpi-inf.mpg.de/tools/BiConverter/index.php

* run published primers through primer3 to verify amplicons (use BS-converted genomic seq)
http://frodo.wi.mit.edu/primer3/

* check epipanel for suitable prevalidated EpiTyper primer sequences
SequenomStandardEpiPanel.PDF (download from sequenom website)

* verify epipanel sequences/amplicons through primer3>
compare locations of published sets VS epipanel amplicons>
primer3 + BLAT http://genome.ucsc.edu/cgi-bin/hgBlat?command=start

* select suitable epipanel primers for ordering (IDT)>
amplicons 100-200+nt
targeting specific regions known to be differentially methylated (literature,if available)
ALWAYS REMEMBER primer adapter sequences> T7 promoter/etc (!)

* IF NO epipanel primer sets available for gene of interest> use EpiDesigner for primer design
http://www.epidesigner.com/
1-5kb input sequence surrounding TSS / 5-8 primer polyX / 4-6+ primer non-CpGs Cs / 5-15+ amplicon CpGs / 100-250bp amplicon / T-reaction only
SAVE primers to database file> DETAILED option

* CHECK primers/amplicons in RSEQMETH to determine suitability before ordering
save amplicon sequence to text file (from database file)> load into RseqMeth (R)
avoid amplicons with multiple overlapping/duplicated mass fragments/etc

Amplification Optimization
-reaction volume> 5ul-20ul-50ul
-Taq 0.8-2U/reaction

-magnesium 1-4mM
-template 1-2ul of 20ul EZ-elution
-primers 200nM
-dNTPs 200uM
-annealing temperature> 1 specific product + maximal amplification

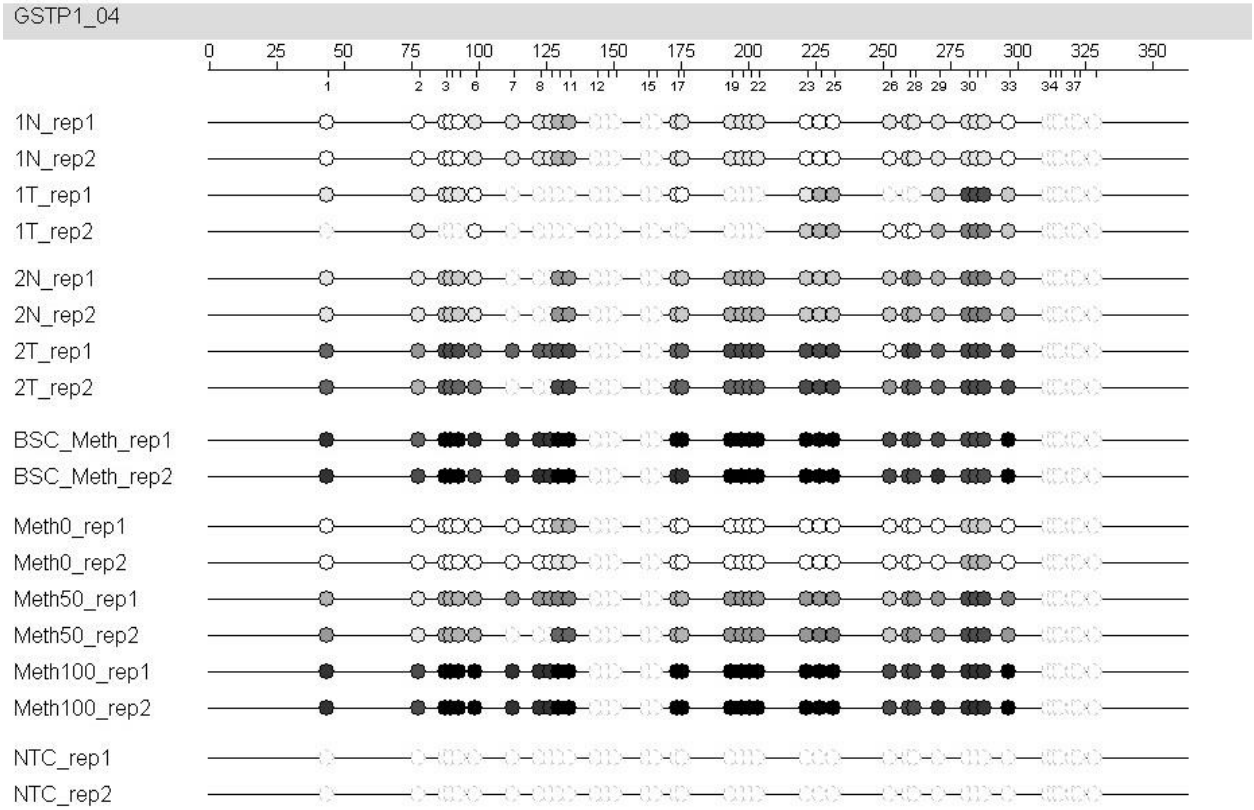
-prepare large batch of bisulfite-converted FFPE DNA for PCR optimization testing
-perform gradient PCR (~58-64 deg annealing) x4 (eg 58-60-62-64)
-adjust other variables as needed
-200uM dNTPs, 200nM primers, ~1U Taq/20ul, 2ul DNA (of 1:2 dilution of 20ul EZ-elution)

-prepare large batch of bisulfite-converted methylation standards (0-25-50-75-100%)
-run PCR>watch for biased amplification (more product from 0% than 100% me)
-perform EpiTyper test run with these standards for each amplicon
-eliminate amplicons for which major bias is evident (0-25-50-75-100%)

Genome Methylation

EpiTyper Panel data were generated to evaluate methylation patterns. In order to evaluate methylation profiles in prostate tumors, we have developed a panel of loci for targeted evaluation. The panel was identified using literature searches that include previously reported regions of altered methylation as well as those reported to be differentially methylated in prostate tissue. These steps included identification of potential me-CpG sites represented on Illumina whole-genome methylation microarrays, previously published primers/amplicons for methylation-specific PCR, and sites represented in catalog Sequenom-EpiTyper assays. Cross-checking the targeted panel for suitable prevalidated EpiTyper primer sequences was undertaken, and primers were selected that produce amplicons of 100-200nt targeting specific regions for evaluating differential methylation. If no existing primer sets were available for the locus of interest, we used EpiDesigner (http://www.epidesigner.com/) to create custom assays.

After assay design was complete, we optimized the amplification assays by testing a range of reaction volumes, Taq polymerase amounts (0.8-2U/reaction), magnesium (1-4mM), and input template DNA amounts. After establishing which PCR conditions would achieve maximal amplification for each targeted locus, we repeated PCR optimization using bisulfite-converted FFPE DNA as well as bisulfite-converted methylation standards (0-25-50-75-100%). We evaluated the assays for amplification (more product from 0% than 100% methylated), and performed EpiTyper test runs with these standards for each amplicon. Amplicons for which major bias is evident (0-25-50-75-100%) were eliminated.



microRNA profiling

Total RNA was extracted from FFPE slides of the same prostate test cases described above. Global microRNA (miRNA) profiling was conducted on Affymetrix miRNA GeneChips v3. Data were normalized by the Robust Multi-array Average algorithm in Partek Genomics Suite, and examined by principal components analysis (PCA, Figure 1). One outlier with poor array hybridization signal was excluded from further analysis. A paired T-test for tumor vs. normal expression levels was performed, and probes were identified with at least 1.5-fold differential expression signal and less than 5% false discovery rate adjusted for multiple testing. Of the resulting 175 probes, 111 showed statistically significant differences only in slow progression tumors, and 56 were only different in cases of rapid tumor progression. These candidate biomarkers will be used to develop a targeted panel of assays for miRNA expression in the larger cohort of samples now being retrieved.

Immunohistochemical markers for inflammatory infiltrate and evaluation.

The final set of markers being developed in this project are those relating to inflammation. All immunohistochemical stains will be performed with adequate positive and negative controls. The panel selected for T cell associated antigens will include CD3, CD4, CD8 and FoxP3 for T cells. In order to assist in the automated reading of the infiltrating T cells, basal cells of prostate glands will be highlighted using IHC stains 34Be12. Stained slides are scanned at low (40x), and high (400x) magnification using the Vectra imaging system (Vectra version 0.4, Cambridge Research and Instrumentation, Woburn, Mass.). A high-resolution image is generated for each

field of tumor to be scored and infiltrating lymphocytes counted by a machine detection system (InForm version 1.0.2, Caliper Life Sciences, Hopkinton, Mass.). Approximately ten representative tumor images will be scored for each case. Images are acquired using the multi-spectral imaging system and converted to optical density space, unmixed to separate spectral components, and annotated for ground truth (tumor, stroma, and background) by a pathologist. A tissue segmentation algorithm is then trained to identify areas of tumor and areas of stroma using the combined cytokeratin and hematoxylin information from the unmixed spectral images. Next, a cell segmentation algorithm is optimized for the task of identifying lymphocytes. This algorithm finds DAB positive lymphocytes within the tumor areas and stromal areas (peritumoral) and allows for counts in both spatially distinct compartments. The tissue and cell segmentation algorithms are applied to the entire set of tumor images in batch mode, producing separate output files for each core. These output files consist of a segmentation map demonstrating the tissue segmentation (tumor vs. stroma) and cell segmentation (regions identified as lymphocytes.) The output images are reviewed to eliminate images that do not show good tissue

Predictive Nomogram

We have completed the evaluation of biomarkers as predictors of risk and outcomes in prostate cancer cases. We have evaluated the accuracy and precision of two existing nomograms for risk of biochemical failure following prostate treatment in both Caucasian and AAM developed by Kattan and Stephenson. Both nomograms assign risks based on diagnostic PSA, Gleason sum, surgical margin status, and presence or absence of extracapsular extension, lymph node involvement, and seminal vesicle invasion. Neither model includes racial or ethnic ancestry as a covariate or a stratifying factor.

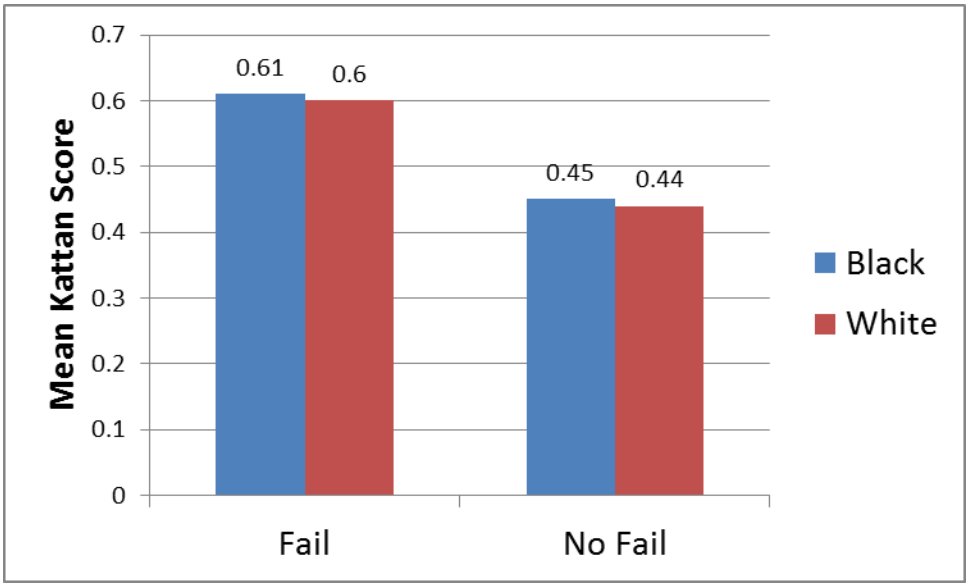


Figure 1: Mean Kattan Score by PSA Failure and Race

As shown in Figure 1, we have generated Kattan score data and correlated these data with race and clinical (PSA) failure. Among 109 African Americans and 937 European Americans, we find significant association betwee Kattan Score and probability of failure in both races ($p<0.001$). However, there was no difference in the prediction by race.

Using the Kattan nomogram as a base, we have begun todevelop an expanded nomogram that includes important new predictors of 5-year biochemical failure after prostate cancer treatment. We have begun to use the selected covariates and biomarkers with strong correlation with aggressive PCa disease, as shown in Table 1, to develop the new nomogram, as described in Kattan et, al.

Table 1: Selected biomarkers being generated in the SCORE and MADCaP databases

Biomarkers	Description	Assay*	Function
PCa-associated factors:			
TMPRSS2-ERG	Transcription factor gene fusion	FISH	Found in 36-78% samples; associated with aggressive disease
Ki 67	Tumor proliferation rate protein	IHC	Correlates with cancer-specific and overall survival
GSTP1	glutathione S-transferase P1 gene	qRT-PCR	Hypermethylated in 60-80% Pca; in serum, urine, biopsy tissue
OncoCarta (ProstaCarta)	A pre-designed panel for mutation profiling	qRT-PCR	Identify other potential biomarkers important in PCa
Prostate Specific proteins:			
MSMB	Prostate specific protein (10q11.2)	Microarray	Independent predictor of recurrence
PSMA	Cell surface membrane protein	IHC	Associated with PSA recurrence in high risk cohort
Androgen Pathway:			
AR	Intracellular receptor protein	IHC	Predictor of decreased biochemical recurrence-free survival
CYP3A gene	CY3A4*1B/ CY3A43*3 enzymes (7q21)	qRT-PCR	Associated with PCa occurrence and severity
SRD5A2	5-alpha reductase II	qRT-PCR	A49T, V89L variant correlates with extracaspular disease

Assays*: FISH-Flourescence In-Situ Hibridization; IHC- Immunohistochemistry; qRT-PCR- Quantitative reverse transcriptase polymerase chain reaction

Status**: Wet lab analysis of gene expression profile either ongoing or to be done in Dr. Rebbeck's lab in patient samples from the SCORE and MADCaP databases

These data provide the basis for a variety of additional studies outside the scope of this project. We will be able to generate a comprehensive model based on both clinical features and biomarkers based on the research undertaken in this project. In particular, we will be able to generate model results that are applicable to African American men. Aside from the present research, this area of research has not yet been done to date.

18. Extent of Clinical Activities Initiated and Completed. Items 18(A) and 18(B) should be completed for all research projects. If the project was restricted to secondary analysis of clinical data or data analysis of clinical research, then responses to 18(A) and 18(B) should be "No."

18(A) Did you initiate a study that involved the testing of treatment, prevention or diagnostic procedures on human subjects?

___X_Yes

_____No

18(B) Did you complete a study that involved the testing of treatment, prevention or diagnostic procedures on human subjects?

_____Yes

___X__No

If "Yes" to either 18(A) or 18(B), items 18(C) – (F) must also be completed. (Do NOT complete 18(C-F) if 18(A) and 18(B) are both "No.")

18(C) How many hospital and health care professionals were involved in the research project?

__3___Number of hospital and health care professionals involved in the research project

18(D) How many subjects were included in the study compared to targeted goals?

__1000____Number of subjects originally targeted to be included in the study
__1046____Number of subjects enrolled in the study

Note: Studies that fall dramatically short on recruitment are encouraged to provide the details of their recruitment efforts in Item 17, Progress in Achieving Research Goals, Objectives and Aims. For example, the number of eligible subjects approached, the number that refused to participate and the reasons for refusal. Without this information it is difficult to discern whether eligibility criteria were too restrictive or the study simply did not appeal to subjects.

18(E) How many subjects were enrolled in the study by gender, ethnicity and race?

Gender:
1046__Males
_____Females
_____Unknown

Ethnicity:
_____Latinos or Hispanics
_____Not Latinos or Hispanics
1046__Unknown

Race:
_____American Indian or Alaska Native
_____Asian
109___Blacks or African American
_____Native Hawaiian or Other Pacific Islander
937___White
_____Other, specify:_____
_____Unknown

18(F) Where was the research study conducted? (List the county where the research study was conducted. If the treatment, prevention and diagnostic tests were offered in more than one county, list all of the counties where the research study was conducted.)

Philadelphia County

19. Human Embryonic Stem Cell Research. Item 19(A) should be completed for all research projects. If the research project involved human embryonic stem cells, items 19(B) and 19(C) must also be completed.

19(A) Did this project involve, in any capacity, human embryonic stem cells?
_____Yes
_X___ No

19(B) Were these stem cell lines NIH-approved lines that were derived outside of Pennsylvania?
_____Yes
_____ No

19(C) Please describe how this project involved human embryonic stem cells:

20. Articles Submitted to Peer-Reviewed Publications.

20(A) Identify all publications that resulted from the research performed during the funding period and that have been submitted to peer-reviewed publications. Do not list journal abstracts or presentations at professional meetings; abstract and meeting presentations should be listed at the end of item 17. **Include only those publications that acknowledge the Pennsylvania Department of Health as a funding source** (as required in the grant agreement). List the title of the journal article, the authors, the name of the peer-reviewed publication, the month and year when it was submitted, and the status of publication (submitted for publication, accepted for publication or published.). Submit an electronic copy of each publication or paper submitted for publication, listed in the table, in a PDF version 5.0.5 (or greater) format, 1,200 dpi. Filenames for each publication should include the number of the research project, the last name of the PI, and an abbreviated title of the publication. For example, if you submit two publications for Smith (PI for Project 01), one publication for Zhang (PI for Project 03), and one publication for Bates (PI for Project 04), the filenames would be:

Project 01 – Smith – Three cases of isolated
Project 01 – Smith – Investigation of NEB1 deletions
Project 03 – Zhang – Molecular profiling of aromatase
Project 04 – Bates – Neonatal intensive care

If the publication is not available electronically, provide 5 paper copies of the publication.

Note: The grant agreement requires that recipients acknowledge the Pennsylvania Department of Health funding in all publications. Please ensure that all publications listed acknowledge the Department of Health funding. If a publication does not acknowledge the funding from the Commonwealth, do not list the publication.

Title of Journal Article:	Authors:	Name of Peer-reviewed Publication:	Month and Year Submitted:	Publication Status (check appropriate box below):
1. None				☐Submitted ☐Accepted ☐Published

20(B) Based on this project, are you planning to submit articles to peer-reviewed publications in the future?

Yes____X_____ No_____

If yes, please describe your plans:

We are nearing the completion of a number of analyses that will result in manuscripts in the coming year.

21. Changes in Outcome, Impact and Effectiveness Attributable to the Research Project. Describe the outcome, impact, and effectiveness of the research project by summarizing its impact on the incidence of disease, death from disease, stage of disease at time of diagnosis, or other relevant measures of outcome, impact or effectiveness of the research project. If there were no changes, insert "None"; do not use "Not applicable." Responses must be single-spaced below, and no smaller than 12-point type. DO NOT DELETE THESE INSTRUCTIONS. There is no limit to the length of your response.

A proportion of prostate tumors in the population, particularly those identified by screening, may be clinically indolent. Treatments for CaP are not benign, and unnecessary treatment for CaP may have more adverse consequences than benefits. Therefore, identifying individuals who are most likely to have an adverse CaP outcome may optimize CaP screening and treatment. If this information is specifically addressed to AA populations, CaP disparities may also be ameliorated. While histopathological traits predict prognosis, our goal is to consider biological, environmental, neighborhood, and behavioral factors associated with CaP outcome differ by race. Prostate cancer disparities and the high rate of poor prognosis prostate cancer cases and AA men represent a critical public health problem. We have initiated a biomarker-based approach to evaluate whether individual factors can add predictive or prognostic information about prostate cancer outcomes. The simultaneous consideration of multilevel predictors in the context of a carefully designed epidemiologic study is innovative. CaP is one of the major cancer burdens in American men, and the ability to accurately determine which men will have a poor outcome is therefore highly relevant to the amelioration of prostate cancer disparities: knowledge of interactions of biology, environment, and behavior could be used to 1) identify individuals at risk of poor prognosis CaP for targeted screening or prevention modalities, and 2) to identify optimal treatment strategies.

- Unique Population: African American men are at highest risk for developing CaP and often present with advanced disease. The prediction of clinical outcomes through the use of the laboratory research and model building initiated here is critical in recommending appropriate treatment for CaP patients.
- Predictive Models: It is uncertain whether the current nomograms used to risk-stratify CaP patients for treatment recommendations truly apply to AA. To date, there has been limited evaluation of nomograms in AA. Despite increased disease recurrence and mortality trends among AA with CaP, current nomograms have been suboptimal in predicting the subset of AA patients that harbor aggressive disease and at higher risk for disease recurrence. Therefore, this study has the potential to identify a novel nomogram that will significantly improve the suboptimal predictive capability of current nomograms in detecting disease aggressiveness and poorer outcomes in AA. The ability to identify a subset of AA who are likely to have unfavorable clinical outcomes will enable clinicians to more accurately risk-stratify their patients for appropriate treatment options.
- Incorporation of Biomarkers and other Novel Predictors: The addition of potentially informative risk factors and biomarkers may significantly improve the predictive capacity of these nomograms in estimating aggressive disease measured as 5-year biochemical disease survival. The study design will involve the incorporation of select biomarkers associated with aggressive CaP into existing nomograms to generate a novel nomogram with significantly improved prediction of 5-year biochemical disease survival among AA. We will also explicitly consider neighborhood factors (which may be a reflection of health care access or other demographic characteristics not captured by race), as well as comorbidities and related factors such as actuarial life expectancy, which may be important in predicting treatment choice and outcomes in AA men.

22. Major Discoveries, New Drugs, and New Approaches for Prevention Diagnosis and Treatment. Describe major discoveries, new drugs, and new approaches for prevention, diagnosis and treatment that are attributable to the completed research project. If there were no major discoveries, drugs or approaches, insert "None"; do not use "Not applicable." Responses must be single-spaced below, and no smaller than 12-point type. DO NOT DELETE THESE INSTRUCTIONS. There is no limit to the length of your response.

In an age of intensive efforts to screen and diagnose CaP, it is clear that we are identifying many tumors which do not represent a clinical threat. Over-diagnosis of CaP may result in the detection of tumors with low biological threat and the aggressive treatment of these tumors results in unnecessary morbidity and mortality. Therefore, the ability to identify those tumors that are most likely to have clinically significant consequences is critical to understanding who should be treated and when. The research undertaken here has discovered markers that have the potential to identify those men who require more aggressive treatment, and possibly screening (e.g., based on their place of residence and their biological profile). Similarly, the biomarker signatures identified here may provide information about optimal screening strategies (e.g., in men with specific biomarker profiles), development of novel preventive agents or treatments (e.g., those involving the telomere-androgen signaling axis), and treatment decision-making (e.g., improved active surveillance vs. definitive treatment choices based on biologically-based risk of aggressive disease). Finally, this

research will further define the populations at risk based both on race/ethnicity and neighborhood context. If it can be shown that neighborhood (e.g., social or macro-environment) is a better surrogate metric for the development of disparities than race, our ability to identify men of all races who are at increased aggressive CaP risk will be enhanced.

23. Inventions, Patents and Commercial Development Opportunities.

23(A) Were any inventions, which may be patentable or otherwise protectable under Title 35 of the United States Code, conceived or first actually reduced to practice in the performance of work under this health research grant? Yes_____ No___X_____

If "Yes" to 23(A), complete items a – g below for each invention. (Do NOT complete items a - g if 23(A) is "No.")

a. Title of Invention:

b. Name of Inventor(s):

c. Technical Description of Invention (describe nature, purpose, operation and physical, chemical, biological or electrical characteristics of the invention):

d. Was a patent filed for the invention conceived or first actually reduced to practice in the performance of work under this health research grant?
Yes_____ No____

If yes, indicate date patent was filed:

e. Was a patent issued for the invention conceived or first actually reduced to practice in the performance of work under this health research grant?
Yes_____ No____
If yes, indicate number of patent, title and date issued:
Patent number:
Title of patent:
Date issued:

f. Were any licenses granted for the patent obtained as a result of work performed under this health research grant? Yes_____ No____

If yes, how many licenses were granted?_____

g. Were any commercial development activities taken to develop the invention into a commercial product or service for manufacture or sale? Yes___ No____

If yes, describe the commercial development activities:

23(B) Based on the results of this project, are you planning to file for any licenses or patents,

or undertake any commercial development opportunities in the future?

Yes_____ No_____X_____

If yes, please describe your plans:

24. Key Investigator Qualifications. Briefly describe the education, research interests and experience and professional commitments of the Principal Investigator and all other key investigators. In place of narrative you may insert the NIH biosketch form here; however, please limit each biosketch to 1-2 pages. *For Nonformula grants only – include information for only those key investigators whose biosketches were not included in the original grant application.*

Dr. Rebbeck is Professor of Epidemiology, Senior Scholar in the CCEB, Director of the Center for Genetics and Complex Traits, and Associate Director for Population Science in the Abramson Cancer Center. Dr. Rebbeck's research focuses on the genetic and molecular epidemiology of cancer. He has directed multiple molecular epidemiologic studies and international consortia to identify and characterize genes that are candidates for involvement in cancer etiology, and to describe the relationship of allelic variation with biochemical or physiological traits, cancer occurrences, and cancer outcomes. His research also focuses on the roles of these factors on prostate cancer disparities and prostate cancer in Africa. Dr. Rebbeck's research uses a multidisciplinary approach that combines methods from epidemiology, statistics, molecular biology, and classical genetics.