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ANNUAL

PROGRESS REPORT

UCI SPECS Program on Prostate Cancer

Year 5

July 1, 2009 - June 30, 2010

SPECS

"Strategic Partners for the Evaluation of Predictive Signatures of Prostate Cancer"

SPECS

A multi-institution research study sponsored by the National Cancer Institute

UCI SPECS Program

"Strategic Partners for the Evaluation of Predictive Signatures of Prostate Cancer"

Annual Report - 2010

(Adapted from the annual report provided to The National Cancer Institute, NIH)

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The report is available at the UCI SPECS web site:
http://www.pathology.uci.edu/faculty/mercola/UCISPECSHome.html

"Strategic Partners for the Evaluation of Predictive Signatures of Prostate Cancer"

This report covers the period 7/1/09 through 6/30/10, year five, the final funding period of the UCI SPECS program.

Section A. Specific Aims (*verbatim of year 1 progress report)*.

1. ***Aim 1 (Z. Jia, J., M. McClelland, D. Mercola Y. Wang,)***. Bioinformatics methods will be used to confirm and refine the currently identified biomarkers by comparing their predicted expression intensities against those of independent cases.
2. ***Aim 2 (D. Duggan and J. Trent**).*** Aim 2 has been extended to define SNP-RNA expression correlations that distinguish aggressive prostate cancer from indolent prostate cancer and normal tissue (revised aim as described in the year one progress report.
3. ***Aim 3. (T. Ahlering, P. Carpenter, S. Krajewski, D. Ornstein, and J. Reed***).*** Custom tissue microarrays are being developed to test the prognostic value of antibodies to the same candidate markers of progression as defined in **Aim 1**.
4. ***Aim 4. (T. Ahlering, P. Carpenter. T. Downs, Z. Jia, C. Kane, C. Lee, M. McClelland, D. Mercola, and J. Wang-Rodriguez,)***. Successfully evaluated genes in the previous aims will be validated in an observational prospective clinical trial using 500 newly recruited prostatectomy cases to test the prognostic predictions based on antibody and PCR results.

* D. Ornstein moved to Texas in September of 2008 and resigned from the program.
** J. Trent, original site PI succeeded by D. Duggan in 2008
*** J. Reed, original site PI succeeded by S. Krajewski in 2008

Section B. Studies and Results (Progress Report by Aims).

Introduction. Here the progress for the fifth and final year of the UCI SPECS program is summarized according to the four Specific Aims of the program stated above. In addition several new developments not specifically included among the original aims which further develop the translational emphasis of the NIH SPECS program are summarized here.

1. As noted in the previous progress reports, In December of 2007 Proveri Inc., the virtual start-up biotechnology company that holds a world-wide exclusive license from the University of California on the biomarker IP developed during the predecessor "Director's Challenge" project and improvements of the current project, provided a sublicense to Althea Biotechnology Inc.(http://www.altheatech.com) of San Diego for the development of a *prognostic* assay (http://altheadx.com/prostate-cancer-prognostic-assay.php) utilizing their RNA multiplex PCR technology for application to patient FFPE blocks. Althea based their biomarkers on updated biomarkers identified by the UCI SPECS project (see **Aim 1**, below). Several lists of genes that are differentially expressed between (i) the stroma adjacent to prostate tumor and stroma remote from the tumor site from the same cases and (ii) between tumor tissues of non-relapsed prostate cancer patients and relapsed cancer patients have been provided. In both cases the lists are for two major cell types found in prostate cancer, epithelial tumor cells and stroma cells. During 2008, Althea also commenced development of a diagnostic test (http://altheadx.com/prostate-cancer-diagnostic-assay.php) by using genes from (i) to develop a multiplex PCR assay that distinguishes between stroma of tumor-bearing and non tumor prostate samples. The multiplex assay is applicable to FFPE patient biopsy material. The assay does not require the presence of tumor cells but is based on the differential expression of genes in the stroma adjacent to tumor sites. Such a test may be applied to "equivocal" biopsy samples that have insufficient diagnostic tumor material or contain high grade prostate intraepithelial neoplasia (HGPIN) or atypical site of acinar proliferation (ASAP). Of the million biopsies carried out in the U.S. every year approximately 20% fits into these categories. Althea reports excellent results for their technical validation studies and plans to employ their test as a reference lab in the AltheaDx CLIA lab by the end of 2010.

A prospective validation of the diagnostic classifier was proposed as a grant application to the NCI Early Detection Research Network which received a priority/impact score of 22 and has been recommended by Council in June 2010 for funding.

2. The UCI SPECS program is cooperating with the UCI SETsquared program, a translational initiative between UCI, UCSD, and seven universities in the south of England. This is a phased program that aims to establish collaborations between UC and UK universities. Phase I is designed to establish proof of principle and is funded by the UK-based arm of the SETsquared program. We are working with Dr. Hardev Pandha of Surrey University to obtain fresh frozen research biopsies of UK prostate cancer patients to determine whether biopsy material can be reliably analyzed on pangenomic UI33 plus 2 Affymetrix arrays and used for assessment of outcome of disease (see profiles developed in **Aim 1**, below). 46 biopsies have been received and 23 have been used for expression analysis using U133 Plus 2.0 arrays. The

histological diagnosis of these cases is being compared to that predicted based on the SPECS Diagnostic Classifier profile (12, 14, 16).
This program is supported by a $40,000 grant from the U.K. The use of biopsy material extends the validation of SPECS-derived classifier to clinically relevant material.

3. The "piggy back" dietary study that was initiated in April of 2006 at UCSD and extended to the UCIMC later in the year has progressed. The goal of this study is to determine whether diet-associated factors including BMI correlate with the differential expression of prognostic genes. As noted in the progress report for **year 3**, this study involves the collection of blood for the assessment of diet–related factors and the collection of the results of a self-administered computer-based survey of dietary intake by providing to patients a laptop computer with a touch–screen answering facility and on-line transmission of results for evaluation by the survey developers (Viocare Inc., Washington, http://www.viocare.com/vioffq.aspx). The UCIMC portion of the study is supported by a subcontract from Dr. G. Saxe, UCSD. The number of patients, samples and questionnaire results acquired to date is summarized in **Table 7**. Over 300 patients have been recruited. The questionnaire results are analyzed by an algorithm developed by Dr. Saxe and Viocare which provides estimates of over 200 dietary factors. The current goal is to correlate these factors with clinical values such as BMI, Gleason, grade, TMN stage, and to develop separate support to extend this work to gene expression analysis in order to define the impact of diet on gene expression and outcome.

4. A Ph.D. student, Jacqueline Major, associated with the Saxe study at UCSD has completed her dissertation in June of 2009 on "Body Mass Index and Prostate Cancer Progression". The study was entirely based on the analysis of the SPECS prostate project database and involved the correlation of BMI with clinical parameters of outcome and severity of the prostate cancer.

5. The UCI SPECS program hosted the SPECS PI meeting at the Beckman Conference Center on the UCI campus on January 17 and 18. The annual meeting was followed by a one day local meeting among all site PIs and key personnel of the UCI SPECS program at the Beckman Center. A CD of all presentations was prepared and circulated to attendees. This year's event was sponsored by generous support from Aperio Biotechnolgy Inc., Althea Technologies Inc., NuGen Inc., the Affymetrix Corporation and the UCI Cho Family Comprehensive Cancer Center.

6. An SBIR phase I grant was awarded to Proveri Inc. (http://www.provericorp.com), founded for translational development, in partnership with Vala Sciences Inc. (http://www.valasciences.com) of San Diego to the development of an antibody biomarker panel of 5-7 antibodies that may be applied to prostate cancer biopsies to identify cases at high risk of relapse following prostatectomy. Phase I has been completed and showed feasibility of no-background staining of prostate FFPE tissue with stroma specific biomarkers derived from the UCI SPECS program. The results were provided in support of a pending application for phase II.

7. During the period 2009-2010 14 manuscripts supported in whole or part by the UCI SPECS program have been published (listed on page 24 in the **Appendix**).

8. During the final funding period the host institute for Dr. M. McClelland, Dr. Yipeng Wang, and Ms. Huazhen Yao, the Sidney Kimmel Cancer Center in San Diego, ceased operations in June, 2009. All but Dr. McClelland were direct employees of UCI. Dr. McClelland was accepted as a Visiting Professor at UCI and as the Scientific Director of the Vaccine Research Institute of San Diego where he moved his laboratory. Dr. McClelland then hosted Dr. Wang and Ms. Yao. The neighboring Sanford-Burnham Institute acquired the former SKCC building and agreed to continue to host Ms. Sawyers in her current office.

9. At the end of the current funding period a no cost extension was requested and approved and will be used to maintain significant SPECS resources as noted below. All subcontracts to participating sites were terminated during or before the final funding period.

10. It should be noted that the Sidney Kimmel Cancer Center (SKCC), the host institute of Michael McClelland and SPECS participants Anne Sawyers and Huazhen Yao, ceased operation in June of 2009 owing to financial constraints. Michael McClelland accepted appointments at UCI as a visiting Professor with space assignment and grant development privileges and as the Scientific Director of the Vaccine Research Institute of San Diego (VRISD). In order for VRISD to accommodate the McClelland group, the institute rented larger space coincidentally in a former SKCC building. The remaining SKCC buildings were taken over by the Sanford-Burnham Institute for Medical Research (SBIMR), which hired Ms. Sawyers part time and provides and continues to provide office space for Ms. Sawyers. Ms Sawyers acquired all samples listed for SKCC largely from the Kaiser Permanente Hospital of San Diego which are listed in the Appendix as "SKCC/KPH" for continuity with previous years' reports.

Specific Aim 1. Bioinformatics Identification and technical Validation of expression biomarkers using Independent test sets of prostate cancer cases. (Z. Jia, M. McClelland, D. Mercola, and Y. Wang). Aim 1 is focused on the technical and experimental validation of candidate genes that have been identified as differentially expressed between conditions of clinical significance for the diagnosis, prognosis, and analysis of prostate cancer. Three profiles have been

developed. Several manuscripts on the methods and applications have been submitted published ((1, 5, 13, 14)) during this final funding period (**Section E.**) and several are in preparation. The main findings are summarized as follows.

1. Multigene profiles for the determination of cell-type composition using expression arrays of any source for prostate cancer ((13)). An approach particular to the UCI SPECS program is the determination of cell-type specific gene expression for the four major cell types occurring in prostatectomy specimens of tumor: prostate tumor epithelial cells, the stroma cell compartment, the normal epithelial cells of benign prostate hyperplasia (BPH), and the normal epithelial cells of dilated cystic glands. The proof of principle was first demonstrated in Stuart et al., 2004 (10). Multigene profiles that are characteristic of tumor cells and of the stroma compartment now have been defined. These expression values are independent of the grade, stage, and outcome of the disease and therefore may be applied to any independent set of RNA expression values to determine the cell-type composition of the sample. The results have been validated on independent data sets where the cell type composition is known from pathologists' estimates. The method was then applied to independent data sets of unknown composition that are publically available and presented as array results of "prostate cancer". Our analysis shows that the tumor composition varies enormously from 10% to 80% with the stroma component varying in reciprocal fashion. These results indicate that available data sets are very heterogeneous. Thus, results from these data sets for the identification of biomarkers of aggressive tumors or indolent tumors or other states are likely to be variable among authors and not reproducible. This result validates the growing suspicion from several labs, notably Chinnaiyan and Rubin and coworkers (8, 11) that a major obstacle in the development of biomarkers for prostate cancer is cell-type heterogeneity. LCM does not provide a practical compensation since expression by multiple cell types for a large series of samples requires the preparation of an impractical large number of samples. Our methods provide a means of determining biomarkers with cell-type specific values as illustrated here.

2. Diagnostic profiles. The definitive clinical diagnosis of prostate cancer requires obtaining a biopsy containing tumor (2). Over one million biopsy procedures are carried out in the U.S. every year. About 50-100,000 are ambiguous owing the absence of frank tumor but the presence of PIN (prostate intraepithelial neoplasia), ASAP (atypical small acinar proliferation) or other formation leading to the common comment of "highly suspicious of adenocarcinoma". Such cases are referred to repeat biopsy in, typically, 3-12 months. During this period the patient receives no guidance and any tumor may progress. Moreover, about 50% of such cases will prove to be adenocarcinoma.

We developed a 126 gene classifier that accurately (average accuracy 96%) detects the "presence-of-tumor" using gene expression values of stroma alone. The profile was derived by comparing gene expression of tumor-adjacent stroma to that of normal volunteer biopsies obtained at UCI. The classifier was tested on over 300 independent samples including validation using manually microdissected tumor-adjacent stroma free of histological recognizable tumor cells. The basis set of genes, the starting list of several thousand genes, has been provided to Althea and is the source of their diagnostic 30-gene multiplex PCR assay. A manuscript describing our classifier has been submitted for publication (2). A prospective clinical trial using biopsy material to be obtained at UCI and at Northwestern University has been proposed to the Early Detection Research Network which was recommended for funding in the June 2010 Council meeting.

3. Prognostic profiles. Two profiles based on either tumor epithelial cells or stroma cells have been derived (3, 15) In each case initial gene profiles were derived from application of the multiple linear regression to our U133A Affymetrix data set of 142 cases measured on 156 arrays with mean clinical follow-up of 39 months. Genes were selected with a probability of differential expression between non-relapsed and relapsed (chemical relapse, PSA > 0.2) of $\boldsymbol{p} < 0.01$. Validation was achieved by repeating the process on newly derived U133plus2 Affymetrix array data set of 91 cases on 135 arrays. Genes with one or more probe sets with $p < 0.01$ that exhibited the same direction of change in relapsed cases were considered validated. These genes were further tested in an independent case set of 79 cases described by Stephenson et al. (9). The tumor gene profile consists of 28 genes and the stroma profile consists of 22 genes. These genes as well as individual results from each data set have been provided to Althea Technologies by sublicense for development as a multiplex PCR predictive assay using patient FFPE biopsy or prostatectomy tissue specimens and this test is under active development for commercialization. Selected genes are being used to identify antibodies for further validation using the SPECS TMA (see **Aim 3**). In addition to the potential of these profiles for use in prognosis, the results indicate that tumor stroma contains considerable differential expression with prognostic value. Finally, these studies revealed that cases with positive surgical margins need to be removed from the analysis. A manuscript is in preparation.

4. Methods. Several technical papers on enhanced methods of analysis have been published or submitted during this final funding period (3, 4, 6, 7, 13, 14) These include an improved "bimodal" form of multiple linear regression analysis (3) and methods to utilize the Kattan nomogram as a control population for a clinical trial of a new protocol of adjuvant chemotherapy of prostate cancer completed at UCI and submitted for publication (7).

Specific Aim 2. (D. Duggan and J. Trent*). The goal of these studies is to identify SNP (single nucleotide polymorphism) variations and to determine whether particular SNPs correlate with gene expression changes. The potential significance of this study is that SNP sequence maybe determined for any patient from somatic cells such as

blood cells or buccal smears. Thus SNP changes that are found to correlate with predictive expression changes may provide a much more versatile predictive assay. Moreover this information may provide an understanding of the basis of the differential expression changes in terms of the properties of location of the correlated SNP.

The key experimental step of this aim is to identify an approximately equal number of samples that contain tumor with (i) a known outcome of relapse within three years or (ii) remain progression free for five years. This is a limiting step because only 65% of collected samples contain tumor. Among the tumor bearing samples only a minority contain sufficient tumor to support sufficient RNA preparation for expression analysis and have sufficient nontumor tissue for the preparation of DNA which is required for the SNP analyses. A total of 74 cases have been used for the complete analysis made up from non-tumor portions of 44 non-relapsed cases by 60 months and 30 relapsed cases by 36 months. The experimental work at TGen was completed last year and the consolidated data transmitted in the fall of 2009. Owing to time constraints on Dr. Duggan's time, analysis of the data was not carried out at TGen and all array data was provided to UCI in the fall of 2009. Thus by mutual agreement, TGen was not a budgeted site during this final funding year. The Illumina data was provided to UCI last fall. Therefore the experimental side of this aim is complete.
The analysis of this very extensive data requires the use of multiprocessor supercomputers and will be carried out on a collaborative basis with Michael McClelland who has the appropriate computer.

Specific Aim 3. Tissue microarray (TMA) development. (T. Ahlering, T. Beach, P. Carpenter, S. Krajewski, and J. Reed, J. Rogers, J. Wang Rodriquez). The goal of this aim to fabricate prostate cancer TMAs in order to (1) validate newly identified RNA biomarkers from **Aim 1**, (2) to validate cell-type specific expression on the protein level, and (3) to identify antibody reagents for prognostic and diagnostic assay development. The fabrication phase of this aim is complete.

The composition of the UCI SPECS TMA is summarized in **Table 2.** The TMA consists of **15 TMA blocks** and therefore the whole TMA is represented on 15 slides. The TMA consists of **443** unique prostate cancer cases drawn from archived cases of UCI, nearly all of which are over 5 years old with substantial clinical annotation. **107** cases are present in duplicate or **540** tumor cores. There are **4** blocks of control normal prostate tissue largely from the rapid autopsy program of the Sun Health Research Institute. There are **228** cores of control normal prostate tissue. In addition, selected stroma from these cases are represented in two blocks devoted to benign prostate hyperplasia and normal stroma. A unique feature of the TMA is the addition of cores taken from tumor cases of pure tumor stroma, BPH, dilated cystic glands, and – where available – prostate intraepithelial neoplasia (PIN). Thus the complete TMA contains **2562 cores**. Slides of all tumor cases were vetted by two pathologists (J. Wang-Rodriquez and P. Carpenter) who provided uniform assessment of Gleason Scores to bring them in line with revised definitions. Fields for "punching" were selected by the two pathologists who color coded glass slides of the individual case slides which were then used in over-lay to identify regions for punching by Dr. Krajewski. The pathologists vetted H & E slides of the final TMA blocks for confirmation.

Clinical data of all tumor cases was retrieved from UCI medical center and the associated Long Beach Veterans Affairs Medical Center consisting of diagnosis, date of surgery, pre-operation PSA value, all available post-operation PSA values, pathology TMN stage, surgical margin status, relapse status, post-operative treatment, and many other variables which have been added to the UCI SPECS database together with TMA coordinates. An ascertainment file providing the completeness of retrieval of clinical data has been formed.
Two digital image libraries of TMA have been developed. The first is based on the Aperio scanning technology. The library consists of high resolution digital images of the H & E stained TMA, a Masson's trichrome stained image and immunohistochemical labeled (IHC) images for **29 antibodies** as summarized in **Table 3.** The antibodies have been chosen to correspond to one or three groups: reference antibodies for the identification of particular cell types such as tumor cells (*e.g.* antiAMACR), prostate epithelium (*e.g.* antiPSMA aka antiFLOH1, and antiPSA); antibodies for the validation of the Diagnostic Classifier (*e.g.* antiFFHLI, antiBUB3, antiCAV1) and antibodies for the validation of prognostic profile. These digital images are available via a web-based portal to Aperio server of the Sanford-Burnham Medical Research Institute. A UCI SPECS user log-in information page on access is available at https://stanscope.burnham.org/ or https://stanscope/login.php. Users may view all cores of all TMA slides for all 29 antibodies that have been digitized (as noted in **Table 3**) at high resolution via a zoom facility. The Aperio system provides for a variety of advanced analytical studies via electronic demarcation (electronic pencil) selection of areas of interest or the use of Aperio software for the selection of classes of antibody labeling features. One Aperio feature has been specifically written to correspond to classical "immunoscores" where pathologists score tumor labeling by estimating intensity on a 3 point scale and estimate the percent of tumor cells labeled. The immunoscore equals the product of the two and runs from 0 – 300. We have compared visual immunoscores for antiBclB (provided by Maryla Krajewska and S. Krajewski) with the Aperio digitized values of the same cores with a correlation coefficient of 0.89. The validation indicates that the library of antibody labeled images may be validly examined by using digitized values and may be a major tool for the validation of UCI SPECS-generated diagnostic and prognostic profiles (see **D. Plans**, below).

The second image library is based on immunofluorescence labeled images using a subset of the antibodies **of Table 3** corresponding to genes of the UCI SPECS diagnostic classifier. This library was formed as part of the phase I SBIR contract grant to Proveri Inc. and Vala Sciences Inc. (see **F. Project Generated Resources,** below). The images were developed by the high throughput proprietary scanning histocytometer fluorescence microscope and software developed by Jeffrey Price, CEO, Vala Sciences, Inc. Most images are of conventional FFPE histology slides of one to five prostate cancer cases. The technology resolves multiple colors of multiple antibody (multiplex) labeled sections, provide large dynamic range compared to IHC or to RNA array data, and is being used to develop a panel of antibodies for diagnosis of "presence-of-tumor" based on the labeling of stroma. This work has been invited for application of phase II for the high throughput screening of antibodies corresponding to the Diagnostic Classifier for full development of a multiplex test using the UCI SPECS TMA as a screening platform (see **D. Plans**, below).
In summary, Aim 3 on the development of a prostate cancer TMA is substantially completed. Antibody reagents and tools have been developed for the validation of the UCI SPECS gene signatures and for their translation to antibody-based clinical tests.

Specific Aim 4. Prognostic test of predictive gene profiles (T. Ahlering, P. Carpenter. T. Downs, Z. Jia, C. Kane, C. Lee, M. McClelland, D. Mercola, and J. Wang-Rodriguez). The goal of this aim is to recruit new prostate cancer cases and utilize their fresh surgical specimens and biopsies to assess the accuracy of the current predictive gene profile derived in **Aim 1.** Cases for this study were recruited in four centers: NWU, UCI Medical Center, UCSD (SDVA and Thornton Hospitals), and SKCC (Kaiser Permanent Hospital, San Diego). Recruitment commenced in September 2005 and continued through June 30. 2010 (as noted below, a separately funded program for maintaining recruitment at UCI for cases that also agree to be part of the Diet questionnaire will continue). Nearly 3300 cases have been recruited, about 2000 from the three major sites in California (UCIMC, UCSD, and KPH-San Diego) with 1923 cases recruited at the NWU site (**Table 4**). The average age of the cases is approximately 28 months.

It was the original plan of the UCI SPECS program to assess the accuracy of the predicative profile by carrying out array-based expression analysis. The use of arrays would also provide and enormous increase in the amount of expression data which could be used to extend the predictive profile and test several other profiles. Similarly, an extended variety of clinical data such as family history, drug history, major medical conditions, etc. would be collected to create an enhanced resource where gene expression could be correlated with clinical variables especially those that may identify patients at increased or decreased risk for poor outcome or response to treatment. These plans were reduced to developing the tissue and database resources for this test owing to budget cuts introduced as the time of the original award. The oldest cohort from the first year of recruitment (from Sept. 2005) is a group of 401 cases currently with an average follow-up of 45 months. The prognostic profile predicts the risk of biochemical failure within 36 months of prostatectomy or the probability of remaining progression free at 60 months. Thus the older cohort is useful for a one-way test of the accuracy for the prediction of high risk now and will be useful for a complete test in 15 months. The clinical database contains all the variables of the Case Report Forms, results of the pathology reports for examination of the prostatectomy tissue, subsequent treatment histories, as well as post surgery PSA values and other variables. The Data Dictionary contains over 250 fields. The SPECS prospective tissue resource and database is, therefore, maturing into a valuable resource.

In order to complete the proposed analysis, follow-on funding has been requested in two pending applications. First is a response to an RFA from the NCI Early Detection Research Network (EDRN) which received a score of 22 and has been recommended for funding. The second is an RC4 application which received a score of 33 (13 percentile) and is pending Council Action in September 2010. It appears likely that the resources developed here will be utilized for the intended purpose.

Specific Aim 4-B (A. Ahlering, D. Mercola, S. Saxe). Diet SPECS study. Patients being recruited for the prostate cancer prospective study are being consented to participate in the "piggy back" SPECS diet survey study (see **Introduction**) as of December 2007 at UCSD and as of April 2008 at UCI. To date 378 subjects have also agreed to participate in the diet study (**Table 7**). 247 have completed the questionnaire as inpatients using a study lap top computer with a touch screen response system for completing the questionnaire. 244 have provided additional blood to be used for dietary metabolite measurements and validation of the questionnaire. 135 are scheduled for home completion. The completed questionnaires are electronically transmitted to Viocare Inc. of Washington State and analyzed using an algorithm devised at Fred Hutchinson Cancer Center as modified in collaboration with Gordon Saxe. The algorithm provides 222 values about diet including caloric intake and dietary intake related to fat, cholesterol, protein, vitamins, minerals, and many others for each patient. Examples of the questionnaire-derived diet variables are illustrated in **Table 8**. The accuracy of representative values such as serum β-carotene may be checked by comparison to blood values measured for selected patients. To date, 150 blood samples have been provided to UCSD where Dr. Saxe's funded study supports the analyses by the UCSD General Clinical Research Center. The validation study of the observed values compared to the

questionnaire deduced values is in progress. The validation will provide error values to be assigned to all 222 deduced values based on the error observed for the validation of the selected serum measurements.

The goal of the enhanced diet-supplemented database is to (i) correlated known and new dietary factors with clinical risk factors such as pre-operative PSA, grade, stage, nomogram, and other risk factors to identify dietary factors of risk and (ii) to complete gene expression analysis of selected cases for correlation of gene changes associated with particular dietary factors. Follow-on funding for the completion of these goals is being sought such as the RC4 program (see **Aim 4-A**) to complete these studies. A pending RC4 grant has received a impact/priority score of 33 (13 percentile) and further funding decisions await the Counsel meeting in September 2010.

In summary, 3297 subjects have been consented and tissue collected by the four tissue-collecting sites of the UCI SPECS program since September 2005 (**Table 4**). Individual subject clinical annotation has been accumulated in the SPECS database for three sites (UCIMC, UCSD, SKCC/KPH). The database dictionary consists of over 250 variables. In addition, 245 cases included the information of the completed diet study questionnaire. This aim was impacted by the revision of the budgets for the SPECS program when it was determined at NCI level to accommodate six SPECS programs compared to the originally envisaged four programs. Therefore, no expression analysis has been carried out on these cases as indicated in prior progress reports. However the value of the tissue and database resource increases with age as the outcome of the consented subjects becomes better defined and the collected material constitutes a major resource for the validation and extension of the multigene signatures defined here as well as for other NIH investigators. Follow-on funding has been sought through an application to the Early Detection Research network (EDRN RFA 09-017; 1 U01 CA152738-01). The application seeks funding for completion of the expression analysis as well as for a prospective study to validate the stroma-based Diagnostic Classifier using biopsy material to be collected at UCIMC and at the NWU SPORE and analyzed in the UCI CLIA Molecular Genetics laboratory. This application has received an impact/priority score of 22 in May 2010 and has been selected for funding by Counsel (NGA pending approval of JIT submissions). The probable funding via the EDRN is testimony to the promise and worth of the resources created in **Aim 4**. ■

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Section C. Significance.

1. Translation. The goals of these studies remain the development of a multigene signatures for diagnosis, prognosis and definition of cell type distribution in samples of prostate cancer used for expression analysis. The gene signatures have been developed. The intellectual property has been provided to the University of California for patent protection (see **Intellectual Property** below). A world-wide exclusive license from the Regents of the University of California to Proveri Inc., developed by D. Mercola and M. McClelland to assist translation, has been updated and a sublicense from Proveri Inc. in the field of RNA provided to Althea Technologies Inc. who are applying their technology in the use of multiplex PCR to develop clinical tests applicable to formalin-fixed paraffin-embedded (FFPE) tissue blocks of patient material. Two tests, diagnosis and prognosis, are in advanced stages of development as described in the Althea websites ((http://www.altheadx.com/prostate-tumor-presence-assay.php and http://altheadx.com/prostate-cancer-prognostic-assay.php).

A second translational approach is to exploit our knowledge of gene expression changes correlated with prognosis and diagnosis to define antibodies that may be applied to patient material. A 679-case TMA has been created. This may be used for high through-put screen of antibodies for the validation that proteins corresponding to the signature genes are indeed expressed in the predicted cells type and predicted tumor risk group. In addition validated antibodies have potential as diagnostic or prognostic reagents. Proveri Inc. together with Vala Sciences Inc. is exploiting this potential. Vala Sciences Inc. has developed automated scanning fluorescence microscopy equipment and techniques for the rapid scanning and digitization of TMAs. Our goal is to identify antibodies corresponding to the gene products of the diagnostic and prognostic profiles that can be used in a panel of 5-7 antibodies each for application to FFPE patient material as diagnostic and prognostic tests. This work was funded by a phase I SBIR grant from NCI to Proveri Inc. during this final funding period. The results were presented to NCI and an invitation to apply for Phase II SBIR is expected. The TMA is a key resource for this program.

2. Resources. Resources are summarized in **Section F. Project-Generated Resources** and include the TMA, intellectual property, nucleic acid microarray data, and the clinical database. The significance of these resources is (i) their availability to other NIH-supported investigations, (ii) as resources for the generation of funding of follow-on studies that extend the goals of the SPECS program, (iii) and in facilitating continued translational efforts through Proveri Inc. and facilitating the current collaborations with AltheaDx Inc. and Vala Sciences, Inc.

3. Bioinformatics Methods. A variety of new methods for the analysis of gene expression has been developed and is summarized in **E. Publications.**

Section D. Plans.

1. The no-cost extension and resources maintenance. The final funding period of the UCI SPECS program ends 6-30-10. Approval has been obtained to continue the program on a no-cost extension basis to 6-10-11. This period is badly needed to maintain resources and to prepare for the use of the resources in approved but not yet funded NIH studies such as the EDRN project (see **F. Project-Generated Resources**). In addition this period is necessary for renewal of regulatory approvals at UCI and participating sites of maintenance of HIPPA-containing databases and tissue resources. In addition physical maintenance of the tissue resources at four sites by maintenance of liquid nitrogen storage and regular examination is required. During this period the tissue at UCSD, VRI-SD, NWU SPORE, and the UCIMC will be consolidated by move to D. Mercola's lab at UCIMC. This will allow for over sight by one person and for simpler alarm arrangements in the cases of temperature control failure. In the meantime, alarm arrangements at UCSD, VIR and UCIMC will extended for telephone alert of the laboratory manager of the D. Mercola lab, UCI. The database will be made current by updating with all available clinical data through 6-30-10 and will be audited for ascertainment of the fields of the data dictionary.

2. Continued limited UCI SPECS recruitment and database development: The Diet Study. It is planned to continue patient recruitment by informed consent at one site, the UCI Medical Center, and to continue administration of the electronic diet questionnaire together with clinical data follow-up and annotation. One current UCI SPECS clinical coordinator, Mark Riola, will carry out this activity. The work will be supported by a continuing subcontract from the parent grant of Dr. Gordon Saxe at UCSD. The clinical coordinator will consent patients, bank postprostatectomy tissue,

blood, and urine and maintain clinical records exactly as per the UCI SPECS protocols. This project will increase the number of cases with diet variables data. Since questionnaire responses, the larger numbers are important. The diet variable are to be correlated with clinical variables and, eventually, with gene expression values for the same cases. Gene expression measurements will require that devoted funded for these measurements be identified. This study is planned for funding (probably ca. 10-1-10) and depends upon continued maintenance of several resources of the UCI SPECS study such as the regulatory affairs requirements, tissue storage facilities, and maintenance of the database.

3. Experimental validation and extension of the Diagnostic Classifier. The next step in the development of the Diagnostic Classifier is a prospective clinical trial. This has been proposed in our application to the EDRN program of the NCI ("The Prostate Cancer Tumor Microenvironment Exhibits Differentially Expressed Genes Useful for Diagnosis "(EDRN RFA 09-017; 1 U01 CA152738-01)). This application has been recommended for funding by Counsel and therefore appears likely to start sometime in the fall of 2010. The program is a 5-year UO1 consortium project with Dr. Chung Lee of the NWU Prostate Spore. Dr. Lee will investigate the functional basis for the stroma-specific expression changes used to define our 114-gene Diagnostic Classifier. The results of the EDRN project will be analyzed in a blinded protocol in cooperation with an established EDRN validation unit. That is, this UO1 program has three participating sites. A NCI Counsel-approved budget of $ 4.56 million has been recommended. This program depends upon the availability of the UCI SPECS tissue resources, database and clinical coordinator program and therefore the maintenance of these resources.

Althea Biotechnologies Inc. has requested samples for advanced validation of their diagnostic and prognostic signatures based on UCI SPECS results. This is a critical step in validating their signatures and facilitating eventual FDA approval. The Althea signatures are composed of 30 genes that are to be measured on patients FFPE biopsy material. Althea requests to validation these signatures using UCI SPECS banked frozen tissue of known outcome. Cooperation in this program requires that the UCI SPECS tissue resource be maintained and staffed sufficiently to prepare portions of tissue and provide the material to Althea and for the project to be staffed sufficiently to provide database files.

4. SBIR Translational program. As noted, a Phase I SBIR between Proveri Inc. and Vala Sciences was completed 3/30/10 entitled "Tissue Imaging for Prostate Cancer Prognosis and Diagnosis" (NCI Contract #HHSN261200900055C, W. Lernhardt, PI). The project is devoted to the development of clinical assays using panel of 5-7 antibodies where the antibody targets are drawn from the 88 prognostic UCI SPECS gene signature or from the 114 gene Diagnostic Classifier. In phase II the UCI SPECS TMA will be used to screen selected antibodies in order to define which antibodies will be validated on a statistical number of samples to specifically label prostate cancer according to risk (prognosis) or specifically label tumor-adjacent stroma and to optimize a protocol for the simultaneously labeling (multiplex) of patient biopsy tissue sections. The results of this program have been presented to and favorably received by the NCI Program Director Dr. Greg Evans and colleagues and invitation to apply for Phase II is expected. Continuation of this translational program depends upon the continued maintenance and availability of UCI SPECS resources especially the clinical database corresponding to the TMA and access to and preparation of TMA slides.

5. Follow-on grant development. Several applications are pending for the development of UCI SPECS topics:

NIH - RC4 ssubmitted 3/15/10 entitled "Retrospective and Prospective Validation of Prognostic Classifiers for Prostate Cancer". Has received an impact/priority score of 33 (13 percentile).
CDMRP - PC101793 submitted 6/9/10 to the DOD entitled "High Throughput Biomarker and Diagnostic/Predictive Synthesis on Formalin Fixed Paraffin Embedded (FFPE) Tissue Sections".

A response to the NCI SPECS II RFA has been submitted entitled "Prospective Assessment of the Accuracy of a Multi-gene Signature for Prognosis of Prostate Cancer by Selection of Patients for a Phase III Randomized Trial of Adjuvant Chemotherapy".

A SBIR Phase II application is likely to be invited for our completed phase I SBIR project "Tissue Imaging for Prostate Cancer Prognosis and Diagnosis".

All of these projects depend upon the maintenance and availability of the UCI SPECS resources. The plans of the no cost extension period are aimed at achieving this.

Section E. Publications from July 1, 2009 (see also total publications supported by the UCI SPECS program, Appendix, page 9).

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Wang Y, Xia XQ, Jia Z, Sawyers A, Yao H, Wang-Rodriquez J, McClelland* M. and **Mercola* D.** *In silico* estimates of tissue components in surgical samples based on expression profiling data. *Cancer Res;* 70(16); July 27, 2010. PMID: 20663908 (*contributed equally).

Cheng, C., Bettahi, I., Cruz-Fisher, M., Pal, S., Jain, P., Jia, Z., Holmgren, J., Harandi, A. and de la Maza, L. Effective vaccination against Chlamydia trachomatis using the major outer membrane protein adjuvanted with CpG oligodeoxynucleotide coupled to the nontoxic B subunit of cholera toxin. Vaccine, 27:6239-6246 (2009). PMID: 19686693.

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Published Abstracts.

Farah B. Rahmatpanah, Zhenyu, Jia, Jessica E. Alspaugh, Tatsuya Azumi, Eileen Adamson, Becky Pio, Frank E. Jones and Dan **Mercola**. Transcription regulation by HER2 in Breast Cancer Cell Lines. Advances in Breast Cancer Research: Genetics, Biology, and Clinical Applications AACR, San Diego , October 13-16, 2009.

Zhenyu Jia, Yipeng Wang, Anne Sawyers, Huazhen Yao, Steve Goodison, Michael McClelland, and Dan **Mercola** "The Prostate Cancer Microenvironment Exhibits Numerous Differential Gene Expression Changes that May be Used for Diagnosis in the Absence of Tumor Cells", AACR EROTC meeting on Prostate Cancer Microenvironment, Versailles, France. Minisymposium oral presentation. October 22, 2009.

Zhenyu Jia, Yipeng Wang, Anne Sawyers, Huazhen Yao, Steve Goodison, Michael McClelland, and Dan **Mercola.** Diagnosis of prostate cancer without tumor cells using differentially expressed genes in the tumor microenvironment. *Proceedings of the American Association for Cancer research.101st AACR Annual Meeting*, April 17-21, 2010 in Washington, DC. Poster Session Abstract # 5184

NIH SPECS related meetings.

1, NIH/NCI programmatic meeting, T. Lively, D. Mercola, hosts, Annual SPECS PI meeting, University of California at Irvine, January 17-18, 2010.

As invited lectures:

August 12, 2009, D. Mercola, "Prostate Cancer Microenvironment Biomarkers for Diagnosis and Prognosis", CHI meeting, Christine Lingham, org., Washington, D.C., August 10-11, 2009.

October 22, 2009, D. Mercola, "The Prostate Cancer Microenvironment Exhibits Numerous Differential Gene Expression Changes that May be Used for Diagnosis in the Absence of Tumor Cells", AACR EROTC meeting on Prostate Cancer Microenvironment, Versailles, France.

April 26, 2010, D. Mercola, CHI meeting, Christine Lingham and Gregory Weiss, organizers, "Diagnosis of Prostate Cancer Using Differentially Expressed Genes in Stroma", University of California.

June 2, 2010, D. Mercola, Annual Meeting of the American Urology Association, podium presentation, "Diagnosis of Prostate Cancer Using Differentially Expressed Genes in Stroma".

Section F. Project-Generated Resources.

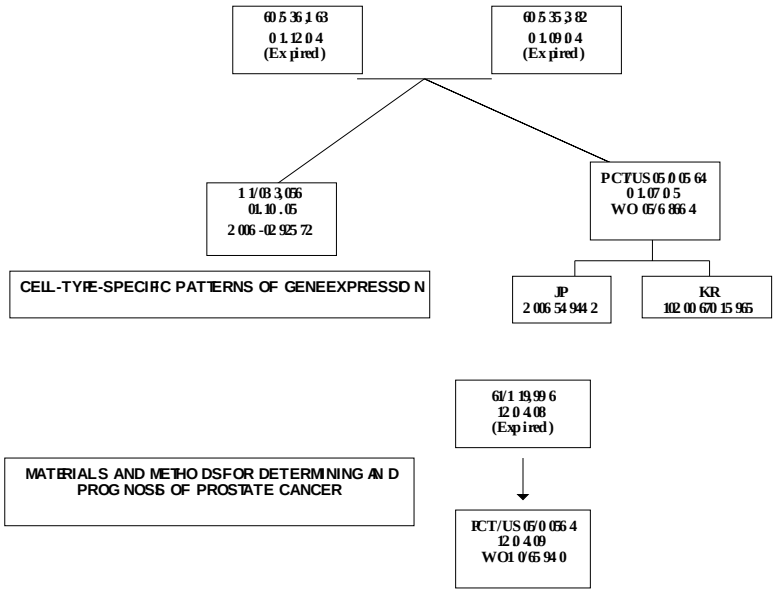
1. Prostate Tissue Bank. The SPECS generated specimen bank based consisting of 3297 cases recruited by informed consent is summarized in **Tables 4-6** (**Appendix**). The associated database have been assembled on the UCI SPECS servers for all cases except those recruited at the NWU SPORE which are archived locally as summarized in **Table 4 (Appendix)**.

2. Prostate Cancer Tissue Microarray (TMA). The UCI SPECS Tissue Microarray consists of 443 tumor cases, 232 normal prostates, 29 normal control human tissues, duplications, and core of selected cell types (BPH, stroma, PIN, etc.) from prostate cancer cases totaling 2562 cores (**Table 2, Appendix**). Clinical annotation has been acquired for most tumor cases which have >10 y clinical follow-up and organized in the UCI SPECS data in association with TMA core addresses. The entire array is represented in 15 blocks. Twenty five antibodies have been optimized and applied to selected blocks by immunohistochemistry (IHC) and high resolution digitized images formed (**Table 3, Appendix**). A web-based portal for access and analysis of the images and their corresponding digitized values has been developed which is archived on the Aperio server at the Sanford-Burnham Institute for Medical Research. Aperio "virtual TMA" as well as custom software developed by Aperio in collaboration with the SBIMR may be applied for analysis. All original case blocks of the TMA have been retained in the custody of A. Sawyers, a full-time UCI employee and UCI SPECS clinical coordinator in a locked office assigned to Ms. Sawyers at the SBIMR. I

3. Intellectual Property Resources.

A. Patent Development. During the final funding period patent protection activity has continued in cooperation with the Regents of the University of California through the technology transfer office at UCSD, at the UCI Office of Technology Alliances, and with the license holder of the IP, Proveri Inc. The progress on patent development during the final funded year is related to previous development in the accompanying block diagram of **Figure 1** and **Table 1.** Briefly, Progress has been made in both of two families of patents consisting of four pending patents. The first family is based on "cell-type specific patterns of gene expression" and comprises one non-provisional filing in the U.S. and two national phase filings in Japan and Korea. The second family is based on "Material and Methods for Determining and Prognosis of Prostate Cancer" and comprises a pending PCT application that will enter the national phase in 2011. Specific activities during the final funding period are indicated by the bolded dates. It is recognized that NIH may have certain rights. The fabrication of the UCI SPECS tissue microarray together with its novel features has been formally disclosed to the UCI OTA as a matter of *proforma* diligence however the substantial amount of prior art surrounding this technology including published TMA for prostate cancer make patenting unlikely in spite of the novel features developed for the validation of cell type specific gene expression (**Aim 3)** of this project.

Figure 1. Evolution of two Families of Patent Filings



1B. I.P. Disclosures to the UCI Office of Technology Alliances and iEdison Registration. As noted in **Figure 1** and **Table 1,** patent protection preceded the start of the UCI SPECS program and was based on initial results of the NCI "Director's Challenge" program disclosed to UCSD in accordance with the data sharing agreement (aka "The Inter-Institutional Agreement") of the "Director's Challenge" program. All supporting material for subsequent patent work has been provided to Megan Patel, LLB of the UCI office of Technology Alliances who has confirmed by agreement with Donna Shaw of the UCSD IPTT office that UCI will be continuing originator of patent development and will liaise with Proveri Inc. in the translational efforts of Proveri. Disclosures have been made by completion and submission of UCI Disclosure of Invention forms and accompanied by relevant publications or submitted articles. As a matter of working relations for translation, Ms. Patel cooperates with Waldemar Lernhardt, CEO, Proveri Inc. in patent strategy. In turn Waldemar Lernhardt consults with James Mullen III, Ph.D., a patent expert at Morrison and Forester Inc, San Diego, to develop PTO responses and claims preparation.

iEdison filings to iEdison.gov by UC corresponding to disclosures to UC are required to be completed within 2 months of disclosure and the EIR numbers are listed in **Table 1.**

4. Nucleic Acid Microarray Resources and Data Sharing. Four microarray platforms have been utilized during the course of the UCI SPECS project. Data acquisition was completed this year. 284 samples from 200 subjects have been applied to Affymetrix expression analysis arrays using U133A or U133 plus 2.0 arrays. These data have been deposited in public databases with accession numbers as noted in **Table 9 (Appendix**). In addition, 99 samples used for expression analysis have been applied to Affymetrix Exon arrays. These data are being analyzed to determine with alternate splice variants specific to risk categories that may be recognized as summarized in **Aim 1** above. Finally, 74 of the same samples as used for expression analysis have been used to derive nontumor DNA that has been applied to Illumina Inc. "million probe set" SNP microarrays. These samples are half from non-relapse and half from relapsed prostate cancer cases in order to identify SNPs associated with outcome status. All array data sets have stored on the UCI SPECS server. The data is being analyzed to identify SNP of prostate cancer that correlate with risk of poor outcome as summarized in **Aim 2** above.

TABLE 1. PATENT FILINGS FOR CELL-TYPE-SPECIFIC PATTERNS OF GENE EXPRESSION
iEdison EIR # 2560101-03-0010. & 0577501-09-0017

Application No.	Country	Filing Date	Status
60/535,382	US	01.09.04	Expired and converted
60/536,163	US	01.12.04	Expired and converted
11/033,056	US	01.10.05	Pending, next Office Action expected **September 2010**
PCT/US05/00564	PCT	01.07.05	Entered National Phase
2006549442	JP	01.07.05	**Office Action received, response due September 15, 2010 – translation**
1020067015965	KR	01.07.05	**Request for examination filed January 4, 2010**

PATENT FILING FOR MATERIALS AND METHODS FOR DETERMINING AND PROGNOSIS OF PROSTATE CANER
iEdison # 05077501-09-0098

Application No.	Country	Filing Date	Status
61/119,996	US	12.04.08	Expired and converted
PCT/US05/00564	PCT	12.04.09	**Published, National Phase deadline is June 4, 2011**

5. The UCI SPECS Database Resource. The SPECS project clinical data is stored in the relational database Microsoft SQL Server 2005 (MSSQL). The MSSQL database is on a Windows 2003 server that is within the UCI local area network (LAN) and physically maintained in a refrigerated and secure room of the UCI HSIS group on the UCI campus in Beck Hall adjacent to the building of the Mercola labs (Medical Sciences 1). Custom SPECS software applications enable end users to interface with the MSSQL database to perform data entry and retrieve information. Separate applications are used for data entry and reporting. Data entry by the Tumor Bank Manager, Ms. Sawyers, is done using entry forms within Microsoft Access. Microsoft Access is configured to be a front-end application that connects to the back-end MSSQL database. Information retrieval is done through a web application visible only within the UCI LAN. The web application is written in Pylons, a web application framework using the programming language Python. Remote users outside the UCI LAN can use the Microsoft Access application by using Microsoft Remote Desktop Services. Remote users can access the web application using the UCI Web VPN.

For disaster preparedness, a second server is in to which backup occurs nightly. The back-up server is located at the Vaccine Research Institute in a locked server room under the local supervision of Dr. M. McClelland in accordance with the disaster preparedness plan of the UCI SPECS protocol.

The servers are managed remotely by Manuel Sutton, the UCI SPECS database manager.

Content summary. The data dictionary for the database contains over **580** items covering annotation for **5468 patients** in **29** relational tables on MS Access forms. **2574** records contain post prostatectomy survival data. For example, as of June 30, 2010 among the frozen tissues, those with post-operative PSA records greater than n years are: 1054 with $n \geq 3$ yrs., 633 with $n \geq 5$ yrs.; 299 with $n \geq 7$ yrs; and 146 with $n \geq 9$ yrs. This information is invaluable in planning experiments to meet EDRN and other pending projects (see **D. Plans**). Considerable clinical data retrieval efforts since January 2010 have been carried out to provide records for a fully updated database by the end of the funding period. These are to be entered into the database during the NCE period to complete what was obtained during the finding period. This effort will greatly support the Planned projects (**D. Plans**).

6. Grants. During the final funding period additional grant support that specifically supports translation of SPECS-developed results.

6-1. SBIR NIH SBIR, (Waldemar Lernhardt, Ph.D., CEO, Proveri Inc. – PI) 9/30/09 – 3/30/10.
Phase I,(Phase II, pending)
"Tissue Imaging for Prostate Cancer Prognosis and Diagnosis"**.**
NCI SBIR Contract HHSN261200900055C.

This project is focused on the development of methods for the automated identification and analysis of antibody binding to tumor and tumor adjacent stroma of prostate cancer and the application of these methods to tissue microarrays of prostate cancer. The SBIR project utilizes antibodies corresponding to genes identified in the UCI SPECS program as correlated with diagnosis and is therefore a follow-on application of the UCI SPECS results.

6-2. 2UO1 CA152738-01 (Mercola – PI) 5-year; scheduled for funding, fall 2010
NIH/NCI Early Detection Research Network (EDRN) , NCI; RFA-CA-09-017;
"**The Prostate Cancer Tumor Microenvironment Exhibits Differentially Expressed Genes Useful for Diagnosis**"
The goal of this project is to develop a tissue resource and apply the tissue resource to a prospective clinical trial of the UCI SPECS Diagnositic Classifier for the diagnosis of prostate cancer using patient fresh frozen biopsy material for suspected prostate cancer cases where the initial biopsy used in the prospective study is ambiguous and a second biopsy is scheduled. The study will be evaluated by comparison of the predicton made for the first biopsy to the clinical results observed for the second biopsy. Early detection will be evaluated by comparison of the time of the first biopsy to the average time of second biopsies, usually 3 – 12 months. The functional role of the genes of the Diagnositic Classifier will be investigated by testing the hypothesis that paracrine factors of the tumor alter gene expression of tumor-adjacent but not distant stroma *via* the wnt and TGFberta1 regulated pathways.

6-3. CANCER PROJECT OF WASHINGTON D.C. (Gordon Saxe – PI; D. Mercola UCI site PI) 9/1/08-8//31/11.
"**Identification of gene transcript levels that correlated with diet of newly diagnosed prostate cancer patients**"

UCI SPECS consented patients are also recruited by informed consent to complete an electronic diet questionnaire at the time of diagnosis of prostate cancer and to provide blood used for analysis of analytes that confirm the veracity of diet survey results. The diet questionnaire results are analyzed by the Viorcare Inc./Fred Hutch Cancer Research Institute algorithm to determine over 250 dietary values which are then correlated with gene expression as measured by Affymetrix U133 plus 2.0 arrays of fresh frozen prostate cancer tissue of the same patients collected by the UCI SPECS project. ■

Appendix

(Tables cited in parts B - F of the progress report)

Table 1. Data Sets utilized for the Identification &Validation of Biomarkers of Prostate Cancer

Data Sets	Array platform	Targets[d]	Relapse (total)	Non-Relapse (total)	Time to Relapse data available?	preOP-PSA	Gleason	TNM stage		Ref.
1[a,b]	U133A2	22,283	85	57	yes	yes	yes	yes	yes	1
2[a]	Illumina	511	25	84	partial (only for relapse samples)	no	yes	yes	no	2
3[c]	U133A	22,283	37	42	no	yes	yes	yes	no	3
4	U95Av2	12,626	8	13	no	no	no	no	no	4
5	U95Av2,B,C	37,891	23	25	yes	yes	yes	yes	no	5
6	U95Av2	12,626	9	14	no	yes	yes	yes	no	6

[a] Contains data on tissue percentages.

[b] These data sets contain information on follow-up time. Relapse was defined as PSA reaches detectable level after prostatectomy within the first four years. All non-relapse cases were cases followed-up over two years and showed no sign of relapse.

[c] These data sets contain information on follow-up time. Relapse was defined as three consecutive PSA increases >0.1ng/ml within the first four years. All non-relapse cases were cases followed-up over two years and showed no sign of relapse.

[d] Number of target transcripts represented on the array.

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Table 2: Summary of the Composition of the Prostate Cancer Tissue Microarray

Array	Institution	Tissues	Number of cases	Total CA Case (unique)	Core Cell Type		
					Cell Lines	Biopsies	per array
ARR83	SKCC	CA+PIN; BPH; Normal; Cell Lines	28	28 cases	24	0	196
ARR84	SKCC	CA+PIN; BPH; Normal; Cell Lines	20	20 cases	0	0	117
subtotal	SKCC		48	48 cases	24	0	313
Array 94	UCI	CA+PIN; Cell Lines	52	48 cases	14	4	220
Array 95	UCI	CA+PIN;	58	58 cases	0	0	220
Array 96	UCI	BPH; Normal/Stroma; Cell Lines	(45)	0 cases	2	0	220
Array 97	UCI	BPH; Normal/Stroma; Cell Lines	(44)	0 cases	2	0	216
Array 98	UCI	CA+PIN; BPH; Normal	20	20 cases			
subtotal	UCI		130 (219)	126 cases	18	4	876
ARR100	LB-VA	CA+PIN; Cell Lines	96	96 cases	4	0	242
ARR101	LB-VA	CA+PIN; Cell Lines	41	41 cases	4	0	222
subtotal	LB-VA		137	137 cases	8	0	464
ARR102	UCI-Robotic	CA+PIN; Cell Lines	86	86 cases	4	0	220
ARR104	UCI-Robotic	CA+PIN; Cell Lines	50	46 cases	4	4	112
subtotal	UCIRobotic		136	132 cases	8	4	332
TOTALS			451 (540)	443 cases	58	8	1985
				Normal form PCa	Rapid Autopsy		
ARR103	UCI/SHRI	Variety of Human Normal Tissues + prostate	55	29	19	5	5	102
ARR105	UCI/SHRI	Normal Prostate from 24h Autopsies	58	45	8 (13)	4	0	173
ARR106	UCI/SHRI	Normal Prostate from 24h Autopsies	57	51	1 (6)	4	0	142
ARR107	UCI/SHRI	Normal Prostate from 24h Autopsies	58	47	1 (1)	4	0	160
subtotal	UCI/SHRI		228	172	37 (62)	17	5	577
TOTAL			679 (768)	615	37 (62)	17	5	2562
PROJECT TOTAL				443 Prostate CA		58	8	2562
				172 Normal Prostate				
				37 Autopsy cases				
				15 TMA Blocks				
				2562 Cores				

Table 3. Antibodies Applied and Digital Images formed of the Prostate Cancer Tissue Microarray

Standardization Antibody against:	Type	Antibody source	Array ID#	Virtual Slide	Virtual TMA Block on STIC/SBMRI
PSA	MAB	DAKO	TMA# 83-84; 94-98; 100-103	yes	TMA# 83-84; 94-98; 100-103
Prostate-Acid Phosphatase	Rb polyclonal	Sigma# P56641	TMA# 83-84; 94-98; 100-103	yes	TMA# 83-84; 94-98; 100-103
E-Cadhedrin	MAB	BD#610181	TMA# 83-84; 94-98; 100-103	yes	TMA# 83-84; 94-98; 100-103
Beta-Catenin	MAB	BD Transduction Lab; #610154	TMA# 83-84; 94-98; 100-103	yes	TMA# 83-84; 94-98; 100-103
AMACR	Rb-monoclonal	DAKO#M3616	TMA# 83-84; 94-98; 100-103	yes	TMA# 83-84; 94-98; 100-103
BclB	Rb-poly	BR-49/BIMR	TMA# 83-84	yes	TMA# 83-84
BCL2	Rb-poly	AR-01/BIMR	TMA# 83-84; 94-98; 100-103	yes	TMA# 83-84; 94-98; 100-103
BAX	Rb-poly	AR-02BIMR	TMA# 83-84; 94-98; 100-103	yes	TMA# 83-84; 94-98; 100-103
HMWC	MAB		TMA# 83-84	yes	TMA# 83-84
SFRP1	Rb polyclonal	Novus; NB600-499	TMA #83-84; TMA 94-98	yes	TMA #83-84; TMA 94-98
FRZD7	Rb polyclonal/Aff	GenWay 18-141-10554	TMA #83-84; TMA 94-98	yes	TMA #83-84; TMA 94-98
IL-6	Mouse monoclonal	GenWay 20-663-4809	TMA #83-84; TMA 94-98	yes	TMA #83-84; TMA 94-98
DDR1	Rb polyconal	Collaboration-China	TMA# 83-84; 94-98; 100-103	yes	TMA# 83-84; 94-98; 100-103
Hif1-alpha	MAB	Novus Cat#NB100-123	TMA #83-84; TMA 94-98	yes	TMA #83-84; TMA 94-98
TR3/Nur77	MAB	R& D System: Cat#2ZH1648H	TMA #83-84; TMA 94-98	yes	TMA #83-84; TMA 94-98
AR (androgen receptor)	Rb polyconal	N-20 ;#sc-816; Santa Cruz	TMA #83-84; TMA 94-98	yes	TMA #83-84; TMA 94-98
Caspase 14	Rb polyconal	AR-76/SBMRI	TMA #83-84; TMA 94-98	yes	TMA #83-84; TMA 94-98
PSA	MAB	DAKO	TMA# 83-84; 94-98; 100-103	yes	TMA# 83-84; 94-98; 100-103
CAV1	Rb-MAB	Epitomics; 1249	TMA #83-84; TMA 94-98	yes	TMA #83-84; TMA 94-98
FOLH1	Rb polyconal	Sigma; HPA010593	TMA #83-84; TMA 94-98	yes	TMA #83-84; TMA 94-98
BUB3	Rb polyconal	Sigma; B7811	TMA #83-84; TMA 94-98	yes	TMA #83-84; TMA 94-98
Cyclin D2	MAB	Sigma; C7339	TMA #83-84; TMA 94-98	yes	TMA #83-84; TMA 94-98
HOX13		????	TMA #83-84; TMA 94-98	yes	TMA #83-84; TMA 94-98 yes
FHL1	MAB	Abgent; AT2048a	TMA #83-84; TMA 94-98	yes	TMA #83-84; TMA 94-98
CLU5	Rb polyconal	Sigma; HPA002185	TMA #83-84; TMA 94-98	yes	TMA #83-84; TMA 94-98
p63	MAB		TMA #83-84; TMA 94-98	yes	TMA #83-84; TMA 94-98
Livin	Rb polyconal	SBMRI	TMA #83-84; TMA 94-98	yes	TMA #83-84; TMA 94-98
Bcl-G	Rb polyconal	SBMRI	TMA #83-84; TMA 94-98	yes	TMA #83-84; TMA 94-98

Table 4A Samples Collected for Testing Gene Signatures During the Current Funding Period (2/1/09 to 1/31/10)

Interval Summary of Consented SPECS Patients 2-1-09 to 1-31-10

Characteristic	SKCC (KPH)[1.]	NWU	UCSD/VAM C-SD	UCI
Interval				
Consented Cases (641)	72	381	80	108
BPH	0	1	8	1 (PIN)
Prostate Cancer	66	370	72	107
Tissues Obtained (frozen)	74	1742	72	88 108-20 (no prostate)
Samples with Tumor			8	57
Samples without Tumor			21	31
Sample Review Pending			51	0
Mean Sample Tumor %			34.4%	65%
Positive Surgical Margins		380	19	13
Negative Surgical Margins			61	93
Banked Plasma	66	634	79	86
Banked Urine	66	80	72	0

1. As of June 2009 SKCC (The Sidney Kimmel Cancer Center) ceased operation and Dr. M. McCelland moved to the Vaccine Research Institute and Ms. A. Sawyers moved to the Sanford-Burnham Institute and continued to work on UCI SPECS subcontracts to collect samples at the Kaiser Permanente site in San Diego.

Table 4B. Samples Collected for Testing Gene Signatures from Inception of the Study to the Present (9-30-05 to 1/31/10).

Characteristic	SKCC (KPH)[1.]	NWU	UCSD/VAMC-SD	UCI
Consented (TOTAL 3297)	300	1923	570 (175 at Bx)	504
Mean Age	60.1	64.15	63	61.8 (40-80)
BPH		5	168	2(BPH)+3(PIN)
Mean PSA (ng/ml)		unknown	7.01	
Prostate Cancer	196	1744	402	499
Mean PSA (ng/ml)		5.9(0.27-)	6.72	4.3(0-74.5)
Tissues Obtained (frozen)	286	6022	324	467(499-32 no prostate)
Samples with Tumor			72 (22%)	339(73%)
Samples without Tumor			140 (43%)	128(27%)
Sample Review Pending			112 (35%)	0
Mean Sample Tumor %			40%	N/A
Positive Surgical Margins		1232	80	53(11%)
Banked Plasma	73	3361	373	386
Banked Urine	73	1021	447 (154 post-DRE)	182
Number/percent NED since surg	203		201	
Number/percent chemical relapse (PSA > 0.2 ng/ml)	25		14	7
Number/percent neg postop PSA		74.8%	201	
Number/percent pos postop PSA		12.6%	37	
Number pending PSA	50	18.35%	136	

1. As of June 2009 SKCC (The Sidney Kimmel Cancer Center) ceased operation and Dr. M. McCelland moved to the Vaccine Research Institute and Ms. A. Sawyers moved to the Sanford-Burnham Institute and continued to worked on UCI SPECS subcontracts to collect samples at the Kaiser Permanente site in San Diego.

Table 5. Ethnicity Distribution of Consented Cases for Prospective Analysis (inception to present)

Characteristic	UCSD/VA[1.] n=570 Consented Pts	UCSD/VA n=402 PCA	UCSD/VA n=168 BPH	UCI n=504 Consented PTs	NWU n=952 Consented Pts	SKCC n=286 consented Pts.
Mean age at enrollment	63.9	62.2	65.5	61.8	62.1	60.1(42-72)
Median age at enrollment	63 (41-90)	62 (41-84)	64 (44-90)	62	61.4	60.0(42-72)
Ethnicity	413 informative	331 informative	82 informative	430 informative	860 informative	172 informative
African-American	41 (7%)	33 (8%)	8 (5%)	3(0.6%)	51(5.4%)	14(8%)
Asian/Pacific Islander	13 (3%)	12 (3%)	1 (<1%)	27(5.4%)	7(.7%)	2(1.1%)
Caucasian	329 (58%)	263 (65%)	66 (39%)	354(70.2%)	785(82.4%)	132(77%)
Filipino	6 (1%)	6 (1%)	0	0	unknown	3(2%)
Native American	1 (<1%)	1 (<1%)	0	0	unknown	1(1%)
Hispanic	15 (3%)	11 (3%)	4 (2%)	6(1.2%)	17(1.8%)	20(12%)
Hawaiian	3 (<1%)	2 (<1%)	1 (<1%)	0	n/a	0
Other Ethnicity	5 (<1%)	3 (<1%)	2 (1%)	40(7.9%)	n/a	0
Not Reported/unknown	157 (28%)	71 (18%)	86 (51%)	74(14.7%)	92(9.8%)	112(39%)
subtotals	570	402	168	504	952	192
Total[1.]						2882

Table 6. Gleason Score Distribution and Stage Distribution for Consent Cases for Prospective Analysis (inception to present)

GLEASON	UCSD	NWU	UCI	SKCC/KPH
2+3=5	2	1	1	0
3+2=5	2	2	1	0
2+4=6	1	0	0	0
3+3=6	92	679	140	54
3+4=7	109	567	225	27
4+3=7	42	174	87	12
3+5=8	5	8	2	1
5+3=8	1	3	0	0
4+4=8	22	38	9	2
4+5=9	21	46	29	5
4+6=10	11	17	0	0
5+5=10	4	0	5	0
TOTALS	312 informative	1361 informative	499 informative	101 informative
No PCA on Path	6	243	5	0
Pathology Pending	15	na	0	32
	383	1780	504	133
STAGE				
pT0	3	5	1	0
pT2a	35	284	41	11
pT2b	10	36	2	8
pT2c	201	918	300	55
pT3a	32	188	3	7
pT3b	22	44	95	8
pt3(a+b)	Na	0	16	0
pT3c			1	
pT2	2	0	8	1
pT3	1	56	5	0
pT4	4	4	5	0
pT3R	na	0	1	0
TOTALS	310 informative	1535 informative	499 informative	90 informative
Channel TURP	3		na	1
Missing Path Stage	3	245	5	4
Pathology Pending	15		0	38
	331	1780	504	133

Table 7. Summary of Cases Consented for the Diet SPECS Cohort (inception to present)

Site	Start	Consented	Blood to GCRC	Questionnaire completed	Scheduled for home completion
UCSD	8/07	97	47	51	46
UCI	4/08	281	197	196	89
Totals		378	244	247	135

Table 8. Examples of Diet subjects dietary intakes based on an analyzed automated questionnaire

Variable	Units	Mean	Min	Max	Std Dev
Energy	kcal	1853.97	172.47	6630.72	852.01
Carbohydrate	g	196.93	30.00	557.59	94.02
Fat	g	69.90	11.51	262.32	37.49
Protein	g	73.66	12.84	178.20	31.13
Calcium	mg	866.66	147.98	2433.31	446.17
Genistein	mg	2.15	0.01	70.59	6.31
b-carotene	mcg	4300.54	174.76	33593.57	4186.64
Lycopene	mcg	5969.22	5.31	44410.00	5527.95
Vitamin D	mcg	5.19	0.57	19.23	3.38
Vitamin E	IU	15.46	3.33	182.10	16.82

Table 9. UCI SPECS samples applied to Nucleic Acid Microarrays and public access references.

Data	Platform	Number Subjects	Number Arrays	Tissue type distribution Tumor/Nontumor/Normal	GEO Accession Number
1	U133 Plus 2.0	P=87 B=18 A=13	108 27 13	68/40/0 0/0/27 0/0/13	GSE17951
2	U133A	P=82	136	65/71/0	GSE08218
	subtotal	**200**	**284**	**284**	
3	Exon arrays	P=99	99	59/408/0	none
4	Illumina SNP probe Array	P=74	74	0/74/0 (44 nonrelapsed cases; 30 relapse cases)	none
	TOTAL	**200**	**457**	**425**	

Abbreviations used: P, prostate cancer samples; B biopsy, frozen; A, Rapid Autopsy of the Sun Health Research Institute.

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