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ANNUAL

PROGRESS REPORT

UCI SPECS Program on Prostate Cancer

Year 4

July 1, 2008 - June 30, 2009

SPECS

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

SPECS

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

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

Dear Colleague,

We are pleased to provide you with a copy of the annual progress report for the UCI SPECS consortium project on the development of gene signatures of prostate cancer. The report is taken directly from the FY04 progress report provided to the NIH in May of 2009 as part of the required noncompetitive annual reporting review process. The SPECS participants have amassed considerable new information about gene expression of prostate cancer at the time of first diagnosis including profiles for predicting the aggressive nature of new cases, the diagnosis of prostate cancer, the risk of subsequent surgery leading to positive surgical margins, and the alteration of gene expression with age. Indeed since the writing of the annual report preparation of manuscripts for publication on the subjects of diagnosis and prognosis have been drafted and we are looking forward to the coming year as a period of increased productivity.

Yours sincerely,

Dan Mercola
July 1, 2009

This report covers the period July 1, 2008 through May 1, 2009, year four of the UCI SPECS program.

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.

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

Introduction. Here the progress for year four 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 report, 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 utilizing their RNA multiplex PCR technology for application to patient FFPE blocks. Althea will base 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 nonrelapsed 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 has focused on the development of a diagnostic test 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 2009.

2. The UCI SPECS program is cooperating with the UCI SETsquared program, a translational initiative between UCI, UCSD, and seven universities in 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). Twenty four biopsies have been received and three have been used for RNA preparation which has proved to provide ample RNA for array analysis. We are in the process of carrying out expression analysis of these and all forthcoming samples. Clinical annotation including all necessary values for calculation of the Kattan nomogram values will be provided. The prediction of outcome based on the nomograms will be used to test the predictions based on our prognostic classifier (see **Aim 1,** below). Ultimately clinical outcome data will be compared to these predictions as a prospective validation of the prognostic classifier. 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 uch–screen answering facility and on-line transmission of results for evaluation by the survey developers (Viocare Thechnologies Inc.). 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.** An interim evaluation of the correlations of dietary factors and BMI will be carried out this summer and a power analysis of whether a meaningful correlation with gene expression will be carried out.

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 data base and involved the correlation of BMI with clinical parameters of outcome and severity of the prostate cancer. Three manuscripts have been prepared for submission in June 2009. The titles are summarized in **Part E. Publications, submitted manuscripts**.

5. The annual one day face-to-face UCI SPECS meeting was held on January 17, 2009 at the Aliso Creek Resort in Laguna Beach and was attended by most participating PIs of the UCI SPECS prostate project (apologies received from David Duggan, TGEN). A CD is being prepared for circulation. This was followed by a one day workshop on prostate cancer with guest speakers from across the country and open to the UCI research community and SPECS participants (the program is included in the **Appendix** to this report). The meeting was attended by 65 people and enjoyed wide appreciation. The event was sponsored by generous support from Aperio Biotechnology Inc., Althea Technologies Inc., NuGen Inc., the Affymetrix Corporation and the UCI Cho Family Comprehensive Cancer Center.

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 in relapsed (aggressive) and nonrelapsed (indolent, good prognosis) prostate cancer. This aim is largely complete. Several additional gene profiles have been developed with potential for use in diagnosis and prediction of cases at risk for development of positive surgical margins when treated by open prostatectomy based on characteristics of gene expression of tissue available at the time of diagnosis. In all cases the profiles were derived by application of multiple linear regression analysis of array data. The approach provides estimates of gene expression for specific cell types present in most samples: tumor epithelial cells, tumor adjacent stroma cells, and epithelial cells of benign prostate hyperplasia. The profiles may be summarized as follows.

1. Prognostic profiles. Two profiles based on either tumor epithelial cells or stroma cells have been derived. 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 nonrelapsed and relapsed (chemical relapse, PSA > 0.2) of $p < 0.01$. 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. (2). The tumor gene profile consists of 28 genes and the stroma profile consists of 22 genes. These genes and the 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. 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. This result has been exploited to define genes that are significantly differentially expressed at the time of diagnosis in cases that eventually exhibit positive surgical margins (see margin profile below).

2. Diagnostic Profiles. Multiple linear regression was used to define genes with significant differential expression between tumor cases and paired nontumor tissues of the same cases using our U133A data set. These genes are the basis for the development of the multiplex PCR diagnostic assay by Althea. Additional refinement has been carried out at UCI. A modified profiles based on stroma cells has been derived by application of multiple linear regression analysis to a case set consisting of the U133A array-measured cases with tumor content <15% in order to examine cases dominated by tumor-adjacent stroma. The series was supplemented by 17 normal volunteer biopsies cases. Multiple linear regression analysis was applied to define genes with a differential expression between normal and tumor-bearing cases of $p < 0.01$. The resulting genes were filtered to remove all genes with an expression coefficient in tumor cells > 20% of the expression coefficient for stroma cells. The resulting 17 genes were refined by application of PAM which utilizes ten-fold cross validation to all remaining cases of the U133A data set which includes 22 nontumor cases. The tumor and nontumor status was specified in this training step. The resultant classifier included all 17 genes. The classifier was tested on multiple independent data sets consisting of (i) the SPECS U133plus2 case set, (ii) the Stephenson cases set, (iii) 17 normal volunteer biopsy cases, and (iv) 12 rapid autopsy normal prostate specimens. In all tests an accuracy of 96-100% was obtained. This classifier is of potential use in providing a stroma-based diagnosis of patient biopsies read by pathologists with an equivocal diagnosis owing to a lack of sufficient histological recognizable tumor cells. Moreover, the study indicates that tumor-adjacent stroma contains many more genes than previously thought that exhibit expression changes owing to interactions with tumor or the tumor microenvironment. These results are being prepared for publication.

3. Prediction of risk of the condition of surgical positive margins following open prostatectomy. Multiple linear regression analysis of cases with known positive surgical margins compared to negative surgical margins reveals 230 significantly differentially expressed genes. These cases are derived from prostate cancer cases which eventually underwent open surgical prostatectomy at one of four collection sites in San Diego County California between 2000 and 2004. These genes are being refined exactly as for the classifier with the exception that no cell specific criterion is being applied as a filter. It is likely that a predictive classifier will be developed that may be used in a logistic regression model to calculate the risk of the condition of surgical positive margin status following open prostatectomy. Since the actual development of surgical positive margins may be related to the trait of tumor cells to attain peripheral localization in the prostate gland, it appears that one or more genes in such a classifier may be functional in favoring peripheral localization or in the origination of a tumor in a peripheral site that subsequently challenges surgical technique. Separate studies with samples obtained following robotic surgery (see Aim 4) will be required to learn whether these observations are relevant to robotic surgery.

Specific Aim 2. (D. Duggan and J. Trent). The goal of these studies is to identify SNP 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 a blood cells or buccal smears. Thus SNP changes that are found to correlate with predictive expression changes may provide to a much more versatile predictive assay. Moreover this information may provide an understanding of the basis of the of the differential expression changes in terms of the properties of location of the correlated SNP.

New samples have been prepared for this analysis. All samples are prepared from cases that have U133plus2 array data. All samples are from nontumor portions of these cases and all samples cases are

provided from known relapsed or nonrelpased cases which are provided in approximately equal numbers in order to maximize the statistical power. To date 60 cases have been provided. To date approximately 40 Illumina SNP chips have been run. Dr. Duggan is attempting to complete the SNP chip runs by June 30, 2009. After that, an interim analysis will be carried out with Arthur Jia, our SPECS biostatistician.

Aim 3. Tissue microarray development. (T. Ahlering, T. Beach, P. Carpenter, S. Krajewski, and J. Reed, J. Rogers, J. Wang Rodgriquez). The goal of this aim to fabricate prostate cancer TMAs to (1) validate newly identified biomarkers, (2) to validate cell-type specific express on the protein level, and (3) to identify antibody reagents for prognostic assay development. The fabrication phase of this aim is complete. To date **747** cases have been arrayed. The array includes normal tissue controls and standard cell lines as reference material. In addition, many cases are represented by multiple cores containing specific cell types such as tumor adjacent stroma, BPH, remote stroma, PIN, etc. In all there are over 2500 cores on the arrays. The composition of the arrays is summarized in **Table 2**.

Current work is focused on analysis of antibody labeling. Previously two antibody biomarkers were characterized on the prototype arrays (Krajewski 2007;Krajewska 2008). These studies were carried out with manual estimates of antibody labeling intensities. This is inadequate for analysis of the large arrays and planned assessment of multiple antibodies. We are seeking to determine whether the digitization functions of the current Aperio software can be validated by correlation with a visually estimated data set for anti-BCL-B. Simultaneously several antibodies suggested by the profiles and classifiers or Aim 1 are being investigated. Finally, collaboration with Dr. J. Price, CEO and President of Vala Sciences Inc., is being developed. This group has developed and marked robotic scanning microspectrofluorimeters and has developed extensive software packages for the high throughput analysis of TMAs (www.valasciences.com). We have developed a joint program for high throughput screening of diagnosis and prognostic antibodies with the goal in mind of validating at the protein level the SPECS results and the goal of developing panels of antibodies for clinical tests. An SBIR application has been submitted for support of this project.

Specific Aim 4. Prognostic test of predicative 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 fresh surgical specimens and biopsies to assess outcome using the current predictive gene profile and to prospectively compare the predicted outcome to observed outcome during year five and as a follow-on long term project. Cases for this study are being recruited in four centers: NWU, UCI, UCSD (SDVA and Thornton Hospitals), and SKCC (Kaiser Permanent Hospital, San Diego). In addition, plans are underway to add the UCI-associated hospital in Long Beach, LBVA. The total number of cases recruited over the past year and from the inception of the study is summarized in **Table 4** and associated Demographic, Grading, and Staging data is summarized in **Tables 5 and 6.** Over 1800 frozen tissues have been obtained many with plasma and/or urine. The original goal is to validate selected biomarkers by PCR. Should array costs continue to decrease it may be possible to carryout complete pangenomic expression analysis. In view of the number and diversity of classifiers developed here (**Aim 1**), the broader range of data collection by use of arrays is highly desirable.

Specific Aim 4-B (A. Ahlering, D. Mercola, G. Saxe). Diet SPECS study. Patients being recruited for the prostate cancer prospective 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 153 casers, up from 27 cases in last years report, have been consented of which 132 have had blood drawn and provided to the NIH-sponsored General Clinical Research Centers of USCD and UCi (**Table 7**). In addition 111 patients have completed the computerized questionnaire (**Table 7**). An interim analysis of the association of diet with outcome and with gene expression is planned for the coming year. A longer range goal of this study is to utilize the present observational study as a proof of principle that sample acquisition and data base resources are available for the development of a potential phase II trial in which relapsed patients may be offered participation in a randomized intervention trial to test the efficacy of diet and life style change to modify the subsequent course of disease. This initiative will require the development of a new proposal for follow-on funding to the SPECS study.■

References cited in progress report (Section B) and Table 1 (Appendix)..

1. Stuart, R.O., et al., *In silico dissection of cell-type-associated patterns of gene expression in prostate cancer.* Proc Natl Acad Sci U S A, 2004. **101**(2): p. 615-20.
2. Bibikova, M., et al., *Expression signatures that correlated with Gleason score and relapse in prostate cancer.* Genomics, 2007. **89**(6): p. 666-72.

3. Stephenson, A.J., et al., *Integration of gene expression profiling and clinical variables to predict prostate carcinoma recurrence after radical prostatectomy.* Cancer, 2005. **104**(2): p. 290-8.
4. Singh, D., et al., *Gene expression correlates of clinical prostate cancer behavior.* Cancer Cell, 2002. **1**(2): p. 203-9.
5. Yu, Y.P., et al., *Gene expression alterations in prostate cancer predicting tumor aggression and preceding development of malignancy.* J Clin Oncol, 2004. **22**(14): p. 2790-9.
6. LaTulippe, E., et al., *Comprehensive gene expression analysis of prostate cancer reveals distinct transcriptional programs associated with metastatic disease.* Cancer Res, 2002. **62**(15): p. 4499-506.

C. Significance.

The goal of these studies remain the development of a multigene profile that identifies at the time of diagnosis, prostate cancer patients with poor prognosis and good prognosis. In addition gene signatures useful for diagnosis and possibly the prediction of risk for the condition of positive surgical margins following surgery have been developed. The results have attracted the interest of Althea Technologies who is actively carrying out development of clinically applicable tests. This may help achieve our translational goals of providing clinical tests for guidance in treatment and enhanced treatment choices for improved results.

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A 747-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 cell type and predicted tumor risk group. In addition validation antibodies have potential as diagnostic or prognostic reagents. We have attracted the interest of Vala Sciences in helping with this translational step. Vala has both TMA screening expertise and anti body kit manufacturing and marketing experience. Thus, this project is now in a position to carry out R and D for the production of antibody panels for diagnosis or prognosis. These reagents will very likely be based on different genes from those of the Althea tests since the Althea technology exploits RNA that is preserved in FFPE tissue which is rare while antibodies exploit genes with protein products. Thus the two technologies are complementary. And the two approaches provide equally significant results.

Pangenomic expression data has been collected on 60 cases archived from the "Director's Challenge" program and most of these cases have also been profiled on the Illumina million SNP chip. This analysis will continue and when suitable numbers are available, SNP alterations that correlate with expression changes will be determined. Particular SNPs can be assessed from any tissue such as buccal smears or white blood cells. Thus the definition of SNPs that significantly correlate with expression that itself has predictive value may provide a somatic cell based test for determining the probability of outcome following a diagnosis of prostate cancer.

Patients are being recruited for prospective testing. In addition, certain dietary features are being determined by questionnaire and blood analysis. Patients of this cohort that relapse but do not seek immediate hormonal or radiation therapy may be offered a diet and life style intervention trial. In particular, the over use of radical prostatectomy may be reduced at considerably decreased morbidity, anguish, and expense.

D. Plans.

Aim 1 as originally proposed is complete. The IP generated has been provided to the technology transfer office of the University of California at San Diego in accordance with the licensing agreement with UCSD and certain gene lists have been provided to our translational partner, Althea, in accordance with an exclusive sublicense to Althea from Proveri Inc. We will work with Althea to facilitate their R and D for diagnostic and prognostic tests. Regular meetings with Joseph Monforte, CSO, Althea, Michael McClelland, Waldemar Lernhardt and D. Mercola occur. As noted, the first test for reference laboratory use in the Althea CLIA lab is due by December 2009.

Aim 2 is scheduled for an interim correlation of expression and SNP data in the summer of 2009. The rate limiting factor is the completion of the DNA analysis on Illumina arrays by David Duggan. This analysis will inform us of whether a limited correlation should be pursued on lfewer genes than available on the arrays or extended samples need to be prepared and run. Since power improves with sample number, we will also continue to screen banked material for suitable samples.

Aim 3 is now in an analytical phase. Two plans are being pursued. First, we have a collaboration with Vala to develop a high throughput screening of antibodies on the TMAs. This plan is being pursued.

Second, antibodies corresponding to promising predictive genes such as BUD3, are being explored with Stan Krajewski following procedures that we have used extensively in the past. This phase will be facilitated by a method of digitization of the arrays that correlates with visual scoring. The current Aperio digitization software options need further improvement for practical use and these modifications are being developed in cooperation with Aperio Technologies directly. Both manual evaluation and improved software are being pursued together.

Aim 4 has progressed to the point where sufficient patients have been accrued for evaluation of risk of outcome by our current classifier. We will use PCR methods to make these assessments. FFPE blocks of the same cases will be used in collaboration with Althea to simultaneously test their multiplex PCR predictive assay.. The validation of this assay is behind that of the diagnostic test but should be ready for prognostic assessment at the end of the year.

E. Publications from July 1, 2008.

Jia, Zhenyu, Tang, Sha, Mercola, Dan, and Xu, Shizhong. Detection of Quantitative Trait Associated Genes Using Cluster Analysis. *In* Evolutionary Computation, Machine Learning, and Data Mining in Bioinformatics, Proceeding of the 6th European Conference, EvoBIO 2008, LNCS 4973, pp. 83–94, 2008.Naples, Italy, March 2008, Elena Marchiori and Jason H. Moorte, Eds., Springer-Verlag, Germany. (available at http://www.springerlink.com/content/jqu7258505064p2r/).

Maryla Krajewska, Shinichi Kitada, Jane N. Winter, Daina Variakojis, Alan Lichtenstein, Dayong Zhai, Michael Cuddy, Xianshu Huang, Frederic Luciano, Cheryl H. Baker, Hoguen Kim6, Eunah Shin7, Susan Kennedy, Allen H. Olson, Andrzej Badzio, Jacek Jassem, Ivo Meinhold-Heerlein, Michael J. Duffy, Aaron D. Schimmer, Ming Tsao3, Ewan Brown, Anne Sawyers, Michael Andreeff1, Dan Mercola, Stan Krajewski and John C. Reed. Bcl-B Expression in Human Epithelial and Nonepithelial Malignancies Clinical Cancer Research 14, 3011-3021,

James A. Koziol, Anne Feng, Zhenyu Jia, Yipeng Wang, Michael McClelland, and Dan Mercola. The Wisdom of the Commons: Ensemble Tree Classifiers for Prostate Cancer Prognosis. *Bioinformatics.* 2008; 25: 1460-2059 (Electronic)

Zhang Q, Helfand BT, Jang TL, Zhu LJ, Chen L, Yang XJ, Kozlowski J, Smith N, Kundu SD, Yang G, Raji AA, Javonovic B, Pins M, Lindholm P, Guo Y, Catalona WJ, Lee C. Nuclear factor-kappaB-mediated transforming growth factor-beta-induced expression of vimentin is an independent predictor of biochemical recurrence after radical prostatectomy. Clin Cancer Res. 2009 May 15;15(10):3557-67. Epub 2009 May 15. PubMed PMID: 19447876.

Published Abstracts.

Yipeng Wang, Zhenyu Jia, Michael McClelland, and Dan Mercola. In silico estimates of tissue percentage improve cross-validation of potential relapse biomarkers in prostate cancer and adjacent stroma. In: Proceedings of the 99th Annual Meeting of the American Association for Cancer Research; 2008 Apr 12-16; San Diego, CA. Philadelphia (PA): AACR; 2008. (Abstract no. 999).

Darren R. Tyson, Junichi Inokuchi, Julia D. Wulfkuhle, Zhenyu Jia, Alice Lau, Seiji Naito, Emanuel F. Petricoin, Dan Mercola, David K. Ornstein. Reduced annexin A1 expression contributes to prostate cancer by activation of Akt and induction of IL-6. In: Proceedings of the 99th Annual Meeting of the American Association for Cancer Research; 2008 Apr 12-16; San Diego, CA. Philadelphia (PA): AACR; 2008. (Abstract no. 2227).

Maryla Krajewska, Jane N. Winter, Daina Variakojis, Alan Lichtenstein, Dayong Zhai, Michael Cuddy, Xianshu Huang, Frederic Luciano, Cheryl H. Baker, Hoguen Kim, Eunah Shin, Susan Kennedy, Allen H. Olson, Andrzej Badzio, Jacek Jassem, Ivo Meinhold-Heerlein, Michael J. Duffy, Aaron D. Schimmer,

Ming Tsao, Ewan Brown, Dan Mercola, Stan Krajewski, John C. Reed. Bcl-B expression in human epithelial and non-epithelial malignancies. In: Proceedings of the 99th Annual Meeting of the American Association for Cancer Research; 2008 Apr 12-16; San Diego, CA. Philadelphia (PA): AACR; 2008 (Abstract no.2180).

Zhenyu Jia, Yipeng Wang, James Koziol, Yipeng Wang, Michael McClelland, and Dan Mercola. A New Bi-Model Classifier for Predicting Outcome of Prostate Cancer Patients. In Joint Statistical Meeting, Denver, Colorado, August 8-11, poster, session 242, Section on Health Policy Statistics, Section on Statistical Computing, Section on Statistics in Epidemiology, WNAR, Tuesday, August 5

Yipeng Wang, ZhenYu Jia, Xiao-Qin Xia, HuaZhen Yao, Anne Sawyer, Jessica Wang-Rodriguez, Steve Goodison, Dan Mercola, Michael McClelland. Improved identification of RNA prognostic biomarkers for prostate cancer using in silico tissue percentage estimates Proceedings of the 100th Annual Meeting of the American Association for Cancer Research; 2009, April 18-22, 2009; Denver, CO, abstract 1627.

Zhenyu Jia, Yipeng Wang, James A. Koziol, Anne Sawyer, Huazhen Yao, Michael McClelland, Dan Mercola. A bi-model classifier that allows RNA expression in mixed tissues to be used in prostate cancer prognosis Proceedings of the 100th Annual Meeting of the American Association for Cancer Research; 2009, April 18-22, 2009; Denver, CO, abstract #1630.

Submitted manuscripts from thesis of Jacqueline Majors.

Major, JM., Klonoff-Cohen, H., Macera, C., Mercola, D., Pierce, J.P., Saltzstein, S., Kattan, M., Slymen, D.J., "Associations of obesity with clinicopathologic characteristics and 10-year nomogram in RP patients," 2009.

Major, JM., Klonoff-Cohen, H., Macera, C., Mercola, D., Pierce, J.P., Saltzstein, S., Slymen, D.J., "Association of obesity with early presenting, aggressive prostate cancer," 2009.

Major, JM., Klonoff-Cohen, H., Macera, C., Mercola, D., Pierce, J.P., Saltzstein, S., Sawyers, A., Slymen, D.J., "Association of obesity with gene expressions involved in Wnt signaling pathway," 2009.

NIH SPECS related meetings.

1, NIH-sponsored annual SPECS PI meeting. Vanderbilt University, Nashville, TN, December 2, 2009. D. Mercola, Y. Wang, Z Jia, Waldemar Lernhardt, Joseph Monforte, Jessica Wang-Rodriquez.

As invited lectures:

July 22, 2008; "Toward the development of tests for the prediction of outcome for the diagnosis of prostate cancer". Kaiser Permanente Prostate Cancer Evaluation Support Group Meeting, 4647 Zion Ave, San Diego, CA, Barbara Barker, host.

October 2008. UCI Chao Familly Comprehensive Cancer Center retreat. Palm Springs, CA. The UCI SPECS prostate program and interactive prostate research development at UCI. Frank Meyskens, host.

F. Project-Generated Resources

1. Prostate Tissue Bank.

   The SPECS generated specimen bank based on over 1800 cases recruited by informed consent is summarized in **Tables 4-6** (**Appendix**). The associated data base and shadow charts (except NWU) have been assembled for all cases.

2. TMA

SPECS Tissue Microarray of 747 cases most of which have >10 y clinical follow-up as summarized in **Tables 2 and 3**. (**Appendix**) together with the associated data base.

3. Intellectual Property.

I. Gene signatures of the expression of four major cell types in prostate cancer tissue and the differential expression in high risk or aggressive cases have been obtained and provided in regular update reports to UC who has applied for a U.S. patent (Serial No. 11/033,056) and recently filed a response to Office Action ((15670-073001; UCSD Ref: SD2004-127;). It is recognized the NIH may have certain rights.

II. A data base of publicly available prostate cancer expression microarray studies consisting of 422 cases together with their associated clinical follow-up has been assembled with a variety of derived values such as the cell type composition for the four principal cell types of tumor (tumor epithelial cells, BPH epithelial cells, dilated cystic gland epithelium, and stroma cells). **Table 1**.

III. Provisional patent filing: January 9, 2009; Application no. 60/535,382, Fish and Richardson, PC, docket no. 15670-073P01; Title "Identification of biomarkers by in silico dissection". It is recognized that NIH may have certain rights.

IV. Disclousre-of-Invention filings to the Regents of the University of California care of Kevin Kennan, UCI Office of Technology Alliances. The following disclosures were filed in accordance with the SPECS data sharing agreement which has been agreed by all institutions participating in the UCI SPECS program. 1. June 9, 2009, disclosure of methods and genes identified as described for progress of **Aim 1,** above. 2. In preparation, disclosure of methods, samples, and cases utilized for the preparation of the 747 TMA. 3. In preparation, disclosure of methods, samples, and genes of classifiers for the diagnosis of prostate cancer from analysis RNA expression in patient biopsies and for the assessment of risk of the condition of positive surgical margins following open prostatectomy by analysis of RNA expression in patient biopsies.

Appendix

(Tables quoted in part B of the progress report)

Table 1. Data Sets Utilized for Identification and Validation of Biomarkers of Relapse of Prostate Cancer Following prostatectomy

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.

Ref. 1, [1]
Ref. 2, [2]
Ref. 3, [3]
Ref. 4, [4]
Ref. 5, [5]
Ref. 6, [6]

Table 2: UCI SPECS 747 Tissue Microarray (TMA) Development Status 5-1-09

Array	Institution	TMA Profile	Number of cases	Total CA Case	Core Numbers		
				Prostate	Cell Lines	Biopsies	per arra
ARR#83	SKCC			48 Cases			
ARR#83	SKCC	CA+PIN; BPH; Normal; Cell Lines	28	28 cases	24	0	196
ARR#84	SKCC	ines	20	20 cases	0	0	117
	UCI			109 cases			
Array 94	UCI	CA+PIN; Cell Lines	48	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			
	LB-VA			137 cases			
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
	UCI-Robotic			132cases			
ARR102	UCI-Robotic	CA+PIN; Cell Lines	86	86 cases	4	0	220
ARR104	UCI-Robotic	CA+PIN; Cell Lines	46	46 cases	4	4	112
SUBTOTALS			532	426 Prostate CA	58	8	198
ARR103	UCI/ Sun-Health	Variety of Human Normal Tissues + prostate		50 cases on : 19 autopsies&	5	5	102
				31 normal ate			
ARR105	Sun-Health	Normal Prostate from 24h Autopsies		54 cases	4	0	173
ARR106	Sun-Health	Normal Prostate from 24h Autopsies		53 cases	4	0	142
ARR107	Sun-Health	Normal Prostate from 24h Autopsies		58 cases	4	0	160
SUBTOTAL		Autopsy Cases	215	215 cases	17	5	577
PROJECT TOTAL			747	426 Prostate CA	75	13	256
				196 Normal Prostates			
				215 Normal Autopsy cases			
				15 TMA Blocks			
				2562 Cores			

Table 3. Optimized antibodies applied to SPECS TMA and used for Virtual Image Creation for remote viewing.

Standardization Antibody against:	Type	Antibody source	Array ID#	Virtual slide	Virtual TMA Block
PSA	MAB	DAKO	TMA# 83-84; 94-97	yes	TMA# 83-84; 94-97
Prostate-Acid Phosphatase	Rb polyclonal	Sigma# P56641	TMA# 83-84; 94-97	yes	TMA# 83-84; 95
E-Cadhedrin	MAB	BD#610181	TMA# 83-84; 94-97	yes	TMA# 83-84; 94-97
Beta-Catenin	MAB	BD Transduction Lab; #610154	TMA# 83-84; 94-97	yes	TMA# 83-84; 94-97
AMACR	Rb-monoclonal	DAKO#M3616	TMA# 83-84; 94-97	yes	TMA# 83-84; 94-97
BclB	Rb-poly	BR-49/BIMR	TMA# 83-84	yes	TMA# 83-84
BCL2	Rb-poly	AR-01/BIMR	TMA#83; 94-97	yes	TMA# 83-84; 94-97
BAX	Rb-poly	AR-02BIMR	TMA#83; 94-97	yes	TMA# 83-84; 94-97
		OTHER SETS			
SFRP1	Rb polyclonal	Novus; NB600-499	TMA #83-84; TMA 94-97	yes	TMA# 83-84; 94-97
FRZD7	Rb polyclonal/Aff pure	GenWay 18-141-10554	TMA #83-84; TMA 94-97	yes	TMA# 83-84; 94-97
IL-6	Mouse monoclonal	GenWay 20-663-4809	TMA #83-84; TMA 94-97	yes	TMA# 83-84; 94-97
DDR1	Rb polyconal	Collaboration-China	TMA#83; 94-97	yes	No

Table 4. Summary of samples collected for prospective study during the current funding period (7/1/07 to date) and since the inception of the study (9-30-05).

Interval Summary of Consented SPECS Patients 5-1-08 to 1-30-09

Characteristic	SKCC (KPH)	NWU	UCSD/VA MC-SD	UCI
Consented Cases (418)	68	191	71	88
BPH	0	0	43	0
Prostate Cancer	68	191	28	91
Tissues Obtained (frozen)	176	738		91
Samples with Tumor				71(78%)
Samples without Tumor				20(22%)
Sample Review Pending				0
Mean Sample Tumor %				78%
Positive Surgical Margins		44		
Negative Surgical Margins				
Banked Plasma	68	155	52	77
Banked Urine	68	51	61 (postDRE)	6

Consented SPECS Prostatectomy Patients since inception of the study (9/30/05) to 1/30/09[1.]

	SKCC (KPH)	NWU[1]	UCSD/VAMC-SD	UCI
Consented (TOTAL 2368)[1.]	727	953	288	400
Mean Age	60.1	62.1	63(41-90)	62(40-80)
BPH		10	52	
Mean PSA (ng/ml)		unknown	7.3(<0.15-30.8)	
Prostate Cancer	727	939	236	
Mean PSA (ng/ml)		6.1(0.4-63.6)	7.13(0.01-77.8)	
Tissues Obtained (frozen)	175	3206	245	352
Samples with Tumor			65 (55%)	270(77%)
Samples without Tumor			115 (45%)	82(23%)
Sample Review Pending			65 40%	0
Mean Sample Tumor %			39%	76%
Positive Surgical Margins		324	51	
Banked Plasma	112	1790	245	268
Banked Urine	112	810	244 (7 postDRE)	178
Number/percent NED since surg	97			
Number/percent chemical relapse (PSA > 0.2 ng/ml)	28		9 (4%)	
Number/percent neg postop PSA		45%	196 (83%)	
Number/percent pos postop PSA (relapse and residual disease)		1.5%	26 (11%)	
Number pending PSA	85	53.5%	13 (6%)	

Table 5. Ethnicity of Consented Cases for Prospective Analysis (informative cases from inception to date)

Characteristic	UCSD/VA[1.] n=467 Consented Pts	UCSD/VA n=305 PCA	UCSD/VA n=162 BPH	UCI n=378 Consented PTs	NWU n=952 Consented Pts	SKCC n=197 consented Pts.
Mean age at enrollment	63.5	62.7	66.2	62	62.1	60.1(42-72)
Median age at enrollment	63 (41-90)	63 (41-84)	64 (44-90)	60(40-80)	61.4	60.0(42-72)
Ethnicity				378		79
African-American	54 (12%)	38 (12%)	16 (10%)	3(0.7%)	51(5.4%)	6(8%)
Asian/Pacific Islander	10 (2%)	10 (3%)	0	16(4.2%)	7(.7%)	1(1%)
Caucasian	358 (77%)	232 (76%)	126 (78%)	235(62%)	785(82.4%)	61(77%)
Filipino	12 (3%)	7 (2%)	5 (3%)	0	unknown	1(1%)
Native American	1 (<1%)	1 (<1%)	0	0	unknown	1(1%)
Hispanic	15 (3%)	9 (3%)	8 (5%)	1(0.2%)	17(1.8%)	10(13%)
Hawaiian	4 (1%)	1 (<1%)	1 (1%)	0	n/a	0
Other Ethnicity	5 (1%)	2 (1%)	4 (2%)	52(14%)	n/a	0
Not Reported/unknown	8 (2%)	6 (2%)	2 (1%)	71(19%)	92(9.8%)	112(67%)
subtotals	467	305	162	378	952	192
Total[1.]						1994[1.]

Table 6. Gleason Score Distribution and Stage Distribution for Consented Cases for Prospective Analysis (informative cases from inception to date)

GLEASON	UCSD	NWU	UCI	SKCC 5/1/08-1/8/09
2+3=5	1	0	1	0
3+2=5	2	0	1	0
2+4=6	1	0	0	0
3+3=6	79	286	94	54
3+4=7	74	198	158	27
4+3=7	21	47	59	12
3+5=8	3	2	2	1
5+3=8	1	2	0	0
4+4=8	17	11	9	2
4+5=9	13	12	18	5
4+6=10	1	0	0	0
5+5=10	4	1	0	0
TOTALS	225	566	342	101
No PCA on Path	6	na	2	0
Pathology Pending	5	na	7	32
	236	566	352	133
STAGE				
pT0	2	2	2	0
pT2a	26	101	31	11
pT2b	8	1	0	8
pT2c	148	365	210	55
pT3a	17	60	67	7
pT3b	20	9	8	8
pt3(a+b)	Na	19	14	0
pT2	Na	na	2	1
pT3	Na	na	4	0
pT4	1	na	4	0
pT3R	na	na	1	0
TOTALS	222	557	343	90
Channel TURP	4		na	1
Missing Path Stage	5	8	1	4
Pathology Pending	5		7	38
	236	565	352	133

Table 7. Summary of cases consented for the observational diet SPECS study to date.

Site	Start	Consented	Blood to GCRC	Questionnaire completed	Scheduled for home completion
UCSD	8/07	60	45	33	12
UCI	4/08	133	134	95	38
Totals		193	179	128	50

The Microenvironment Adjacent to Prostate Cancer Exhibits Numerous Differential Expression Changes that Are Useful for Diagnosis without Tumor Cells

Abstract: for AACR meeting, October 2009, France, "Tumor microenvironment"

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The stroma around prostate cancer has long been recognized as functional in the growth and progression of prostate cancer (PCa). We have developed a linear model of prostate tissue that describes gene expression changes as a sum of contributions of four major cell types in tumor enriched samples including tumor cells, stroma cells, epithelial cells of BPH, and dilated cystic glands. When combined with knowledge of the cell type distribution as estimated by pathologists, the model provides estimates of gene expression for each cell type (1). By comparing the expression of stroma cells in low (<15%) tumor samples with normal volunteer biopsy samples, we derived 417 significant gene expression differences which were further filtered to remove genes with significant expression in tumor cells. The resulting 17 genes, which appeared to have high expression in stroma only when in the presence of tumor, were applied to a training set of 18 PCa cases and 17 noncancer tissues of the same cases all measured on U133plus2 Affymetrix arrays. The program Prediction Analysis of Microarray (PAM) yielded 97% accuracy for discriminating tumor cases vs. non tumor cases. The classifier was then tested on multiple independent prostate samples including 65 tumor cases measured on U133A publically available arrays (2), 79 published (3) tumor cases also measured on U133A, and 55 independent cases measured on U133plus2 arrays which yielded an accuracy of 96-100% for the three sets. To exclude performance that may be based on recognition of tumor cells, we tested the classifier on 9 additional independent normal volunteer biopsy cases and 7 normal rapid autopsy cases that were histologically confirmed to be tumor free which yielded 100% accuracy for both series. Thus a classifier based on tumor-adjacent stroma is highly accurate for discrimination of tumor and nontumor prostate tissue as tested on 199 independent tumor cases and 16 normal prostate cases without reference to tumor cell RNA expression. Many more gene expression changes appear to be specific for tumor-adjacent stroma than previously recognized and our differentially expressed genes point toward several functional pathways. A significant number of the million prostate biopsies in the U.S. per year have equivocal pathological readings, therefore, methods for augmenting diagnostic accuracy based on stroma may be helpful.

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