Final Summary Report for DOE Grant #DOE-FG02-06ER64280
entitled
"*Longitudinal Bank for Serum, Plasma and DNA for Detection of Biomarkers*"

PI: *Dr. David C. Ward*
Deputy Director
Nevada Cancer Institute

Summary Report (as of 01/31/09):

With the support of this DOE appropriation, NVCI has established a biorepository for serum, plasma, DNA and urine specimens. Over 2,500 patients have been consented which is over 90% of all new patients seen at NVCI. The specimens have been coded, centrifuged, aliquoted and frozen at -80°C in a rapid manner so that they are all processed in less than 1 hour from the acquisition. There are over 28,000 aliquoted, coded tubes in our inventory. Specimens from 200 control volunteer subjects without any history of cancer also have been banked. The patient specimens are encoded and the demographics and therapeutic treatments are linked to the Oncore software. This computer program catalogues the specimens and provides a rapid conduit between the biorepository and the NVCI electronic medical record. The coding and software were chosen to be interactive with the National Cancer Institute's CaBIG network thus facilitating interaction and interchanges of information and specimens with a large number of cancer centers across the country.

The present inventory has specimens from: 185 lung cancer cases, 160 breast cancer cases, 550 prostate cancer (PC) cases, 300 hematological malignancy cases, 135 colon and GI cancer cases plus a variety of miscellaneous types of cancer. There are 250 urine samples from PC cases and 260 frozen specimens from bone marrow biopsies. There were over 150 cases of PC where we have obtained samples over a longitudinal course beginning at the initiation and during therapy with a variety of treatments (including hormonal, chemotherapy and radiation therapy).

In addition to the standard tests used to monitor PC, we have used the analysis and quantitation of circulating tumor cells (CTCs) to assess the effectiveness of therapies. CTCs have been recently accepted by the US FDA as a prognostic tool in advanced PC. We have analyzed 886 CTC samples on cancer patients. However, a number of questions remain regarding the use of this test. The optimal clinical cut-off has not been well defined. Also, the predictive value of CTC in the setting of low burden advanced PC has not been evaluated. CTCs were enumerated in blood samples from 100 patients with castration resistant PC. The CTC were compared with the clinicopathologic characteristics and conventional biomarkers such as PSA and LDH. Patients receiving ongoing oncologic follow-up for 26 months and overall survival statistics were documented. Fifty of the patients were alive at the end of the study. CTC counts correlate well with overall survival ($p=0.001$) and are correlated with other biomarkers. Threshold analysis identified 4CTC/7.5ml of blood (compared to the approved value of

5.0) as an optimal cut-off value with respect to survival outcomes as well as being predictive of metastatic disease. Univariate analysis confirmed a tight relationship between the cut-off value for CTC and the other biomarkers. Multivariate analysis with bootstrap sampling validation identified LDH ($p=0.002$) and CTC ($p=0.001$) as having independent prognostic significance.

We have also studied whether miR-141, reported to be elevated in the plasma of PC patients, is correlated with the CTC, PSA and LDH. In this study, 14 patients were evaluated over their treatment period. The levels of miR-141 in plasma were measured using qRT-PCR and a comparison of the change in miR-141, CTC, LDH, and PSA levels and the velocity of change between these biomarkers was determined to be 0.89 ($p<0.001$), CTC velocity vs. miR-141 velocity was 0.80 ($p=0.001$), and LDH velocity vs. miR-141velocity was 0.70 ($p=0.005$). These studies showed strong correlation of miR-141 velocities vs. PSA, CTC and LDH velocities suggesting that plasma miR-141 level has a significant prognostic potential. The changes in miR-141 correlated well with the clinical outcome during the period of observation.

There is a strong association between hypercoaguability, thrombosis and cancer. We had previously shown that the D-dimer (a plasmin degradation product formed from the cleavage of cross-linked fibrin which indicates that thrombosis and fibrinolysis have occurred in vivo) is elevated in PC patients. We have examined whether there is a relationship between the D-dimer levels, CTC and PSA and whether these parameters are concordant when measured over a period of time during therapy for refractory PC. Concomitant quantitative determinations of plasma D-dimer levels, CTC and serum PSA were serially obtained on 24 patients with refractory PC and statistical analysis was preformed by classifying patients according to increasing (positive velocity) or decreasing (negative velocity) of the CTC or PSA levels over time using analysis of variance (ANOVA). The CTC counts ranged from 0 to 4732/7.5 ml blood, the d-dimers ranged from 125 to15,608 ng/ml and the PSA ranged from 0.2 to 9051 ng/ml. A significant correlation was identified between CTC velocity and each of PSA and D-dimer velocities ($p<0.001$ and $p=0.018$, respectively). Similarly, a significant correlation was found between the D-dimer and the PSA velocities ($p<0.001$). Classification analysis was performed by classifying patients according to increasing (positive velocity) or decreasing (negative velocity) D-dimer, CTC, or PSA levels over time using ANOVA.

Concordant changes in D-dimers, CTC and PSA measurements are detected in patients with refractory PC. However, although levels of D-dimers correlate with changes in CTC numbers over time, they show a much stronger correlation with serum PSA levels, possibly reflecting a greater influence of overall tumor burden fluctuations compared to intravascular tumor cells on cancer related coagulopathies. These observations will lead to additional studies on whether there is any correlation between CTC, D-dimers, plasma microparticles and thrombosis.

We and others have found that the polycomb gene BMI-1 is expressed in PC cells and in breast cancer cells. We have attempted to correlate the clinical course with RT-PCR determinations of BMI-1 RNA in plasma and with ELISA assays for BMI-1 in serum and

plasma. Neither of these assays has shown a consistent correlation with CTC, PSA or the clinical assessments. Because we still feel that BMI-1 plays a key role in the malignant phenotype of PC, we have designed lentivirus vectors to knock down the level of BMI-1 that are conditionally expressed under control of a neomycin sensitive promoter. We are examining whether the in vitro kinetics of PC-3 cell growth is altered by BMI-1 knockdown and whether *in vivo* tumor growth of PC-3 cells (in a mouse xenograft model) are controlled by BMI-1 expression.

Abstracts and Papers in Press or in Submission that were supported by DOE Funding:

Goodman OB, Fink LM, Symanowski JT, Wong DA, Ward DC, Vogelzang NJ. **Longitudinal analysis of circulating tumor cell (CTC) counts in assessment of treatment efficacy.** *Abstract #5169, ASCO 2008.*

Goodman OB, Fink LM, Symanowski JT, Wong B, Pomerantz D, Ma Y, Grobaski B, Broome D, Ward DC, Vogelzang NJ. **Utility of circulating tumor cells as a prostate cancer biomarker: patient clinical characteristics and biomarker cluster analysis.** *AACR Meeting Abstracts 2008:997.*

Khoury JD, Adcock DM, Tiefenbacher S. Symanowski JT, Goodman OB, Ma Y, Ward DC, Tung J, Grobaski B, Vogelzang NJ, Fink LM. **Changes in Circulating Tumor Cells over Time Correlate with D-Dimer and Serum Prostate-Specific Antigen Levels in Patients with Refractory Prostate Cancer.** *AACR Meeting Poster Abstract 2009.*

Gonzales JC, Fink LM, Goodman OB, Symanowski JT, Ma Y, Grobaski B, Broome D, Vogelzang NJ, Ward DC. **Comparison of Circulating MicroRNA with Velocity of Circulating Tumor Cells, LDH and PSA for Determining Treatment Response in Metastatic Prostate Cancer Patients.** *[submitted Clinical Cancer Research March 2009]*

Gonzales JC, Fink LM, Goodman OB, Symanowski JT, Ma Y, Grobaski B, Broome D, Vogelzang NJ, Ward DC. **Comparison of Circulating MicroRNA with Velocity of Circulating Tumor Cells and PSA for Determining Progression of Prostate Cancer.** *AACR Meeting Poster Abstract 2009.*

Goodman OB Jr, Fink LM, Symanowski JT, Wong B, Grobaski B, Pomerantz D, Ma Y, Ward DC, Vogelzang NJ. **Circulating tumor cells in patients with castration-resistant prostate cancer baseline values and correlation with prognostic factors.** *Cancer Epidemiol Biomarkers Prev. 2009 Jun;18(6):1904-13.*

Chan F, Goodman OB, Fink LM, Vogelzang NJ, Pomerantz D, Grobaski B, Khoury J. **Dramatically Elevated Circulating Tumor Cell Numbers in a Patient with Small Cell Neuroendocrine Carcinoma of the Prostate.** *Archives of Pathology, 2009 In Press*