Progress Report
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We have established a specimen bank at the Nevada Cancer Institute that has accrued approximately 1500 patient specimens with aliquots of serum, plasma and DNA (leukocytes from the buffy coats). This specimen bank is linked to the electronic medical record and has already been used to study coagulation parameters in patients with PC (prostate cancer). We have found that there is a good correlation between the clinical staging and elevated d-dimers. There is a slightly lower correlation with the F1.2 (an activation fragment of prothrombin). The presence of phospholipid vesicles capable of supporting coagulation were elevated in most patients but there was little stratification in the levels when compared to the clinical status.

We have begun to study the efficacy of monitoring CTC (circulating tumor cells) in assessing the stage of disease in patients with PC. In our initial studies on over 120 prostate cancer patients we compared the CTC with the concomitant classical markers used for prostate cancer, such as PSA and the rate of doubling of the PSA. The more recent data on longitudinal studies of the CTC in prostate cancer patients suggests that the CTC numbers change in concert with other markers such as PSA. These parameters are not always concordant and we are correlating the overall clinical status with the CTC.

Other studies have begun to elaborate which of the patients having CTC have folic acid binding receptors. We have been able to show that we can identify folate receptor positive prostate cancer cells in the CellSearch™ circulating tumor cell assays. We are examining whether this labeling of circulating tumor cells can be used to identify patients who will respond to folate conjugated chemotherapeutic reagents.

Dr. James Tung from Stanford University has joined NVCI. He comes with 10 years of experience in flow cytometric cell sorting. We have acquired a new iCyte cell sorter. This sorter is capable of rapid isolation of labeled cells. In addition, we have acquired a rapid scanning camera to analyze large number of cells for the presence of small numbers of cells labeled with with multiple fluorescent tags. Also, we have acquired a laser capture microscope that is being used to isolate selected circulating tumor cells for specific nucleic acid analysis.

Dr. Oscar Goodman M.D. Ph.D. has joined us from Sloan Kettering in New York and is working with us to characterize circulating tumor cells. He has attempted to establish cell cultures from patient blood specimens in which we have found

high levels of circulating tumor cells. We have not been successful at propagating the cells at the present time but will continue this on more enriched populations of CTC.

We have been trying to identify the populations of putative pluripotential stem cells from a variety of tumors. Dr. Yupo Ma has found a very early developmental marker called SALL4. He has characterized the roll of this zinc finger transcription factor in early development. He and his coworkers have been using the antibodies to SALL4 to identify stem cells in several types of cancers. We are now looking at whether we can identify stem cell markers on the circulating tumor cells. In addition to using the SALL4 we have included the polycomb marker BMI-1, another early stem cell marker, in our studies. We have been able to amplify the RNA present in the serum for these two markers in patients with prostate cancer and patients with breast cancer. We are now examining whether the levels of these stem cell marker RNAs can be used to develop a blood test. We have particularly focused on serum collected from patients with prostate cancer and breast cancer and are preparing to examine sera from melanoma patients. We are correlating the results with the patient's clinical course.

In collaboration with Dr. Gil Mor and colleagues in the Department of Obstetrics and Gynecology at Yale University, Dr. Ward has continued to define an effective panel of biomarker proteins in blood for the detection of early stage ovarian cancer. In 2005, this group of investigators reported (Mor et al. PNAS 102, 7677, 2005) that a set of four proteins (leptin, prolactin, osteopontin and IGF-1 insulin-like growth factor-1) could distinguish between the blood of healthy women and those with ovarian cancer. This test had a sensitivity of 95% and a specificity of 95% in a blinded study of 255 individuals. In 2007, the group identified macrophage inhibitory factor one (MIF-1) that was also differentially expressed in the blood of healthy women and women with ovarian cancer (Agarwal, R et al. Am. J. Obst. Gynecol. 196, 348, 2007). By adding CA-125, the only currently approved biomarker for ovarian cancer, to the previous five markers a blood screening test was developed that had a 99.7% sensitivity and a 97.5% specificity in distinguished ovarian cancer patients from healthy controls in a blind study involving 562 women (Visintin et al. Clinical Cancer Research. 2007 In press). This blood test is by far the most accurate yet devised for the early detection of ovarian cancer.

We are asking for a no cost date extension of the DOE project to 01/31/09. Sufficient funds will remain to fund additional research for this period. During this next year (2009) we hope to;

(a) continue assessing the use of CTC in PC patients receiving therapy in longitudinal studies and submit for publication
(b) further isolate and characterize PC CTC submit extend folate marker studies on CTC and submit for publication

(c) expand studies on stem cell markers (SALL4 and BMI-1) on the CTC and on serum from patients with cancer (PC, Breast cancer and melanoma).
(d) Increase the enrollment of patients into the NVCI specimen bank and establish a bank of normal (no cancer history) subjects.
(e) Collect additional benign ovarian disease serum samples and expand repository sample size for clinical trial studies.