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14. ABSTRACT This annual report for the Physician Research Training Award focuses on progress in the genetic analysis of circulating hormone refractory prostate cancer micrometastases. As metastatic tissue is often inaccessible in advanced prostate cancer patients, analysis of circulating tumor cells may provide understanding of the biology of hormone refractory prostate cancer as well as chemotherapy resistance. Oligonucleotide array comparative genomic hybridization allows the assessment of genetic changes that may occur in the process of metastasis and chemotherapy resistance. Genomic profiling using this technology goes beyond cell counting, and circumvents technical complexities related to working with RNA. Reproducible genomic alterations are observed A functional collagen adhesion matrix (CAM) assay was used to enrich CTCs from prostate cancer patients' blood. CAM ingestion and epithelial immuno-staining identified CTCs, which were genotyped using oligonucleotide array comparative genomic hybridization. The highest CTC counts were observed in men with metastatic hormone refractory prostate cancer compared to castration sensitive prostate cancer. Copy number profiles for CRPC CTCs were similar to paired solid tumor DNA, and distinct from corresponding DNA from the residual CAM-depleted blood. CAM CTC enrichment may allow cellular and genetic analyses in prostate cancer.					
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CV- Jonathan Rosenberg, MD	

Introduction

This project focuses on the genetic analysis of circulating hormone refractory prostate cancer micrometastases with the goal of identifying mechanisms of chemotherapy resistance. Hormone refractory prostate cancer (HRPC) metastatic tissue is difficult to obtain for research, as most metastatic sites are not conducive to biopsy. However, circulating tumor cells (CTC's) have been found in high numbers in patients with metastatic HRPC. CTC's represent an untapped resource for studying the genetics of metastatic HRPC. These cells are easily accessible in the peripheral blood. The purpose of this research is to detect genetic alterations that occur during the development of chemotherapy resistance, to give insight into the mechanisms behind this resistance, and determine potential therapeutic strategies to combat it. To accomplish this, we have refined the techniques needed to isolate CTCs and genomic DNA from those cells, amplify the DNA if necessary, and evaluate genomic alterations using oligonucleotide comparative genomic hybridization (oCGH). Frequently used CTC isolation technologies (e.g. Veridex) do not allow for highly efficient interrogation of DNA because the viable CTCs are not recovered in sufficient purity. We sought to use a technique that would allow us to go beyond CTC enumeration. Our results using the Vitatex technology to capture living CTCs suggest that this approach is feasible and cost efficient, and can be used to study genomic alterations in CTCs. At the end of this reporting period, Dr. Rosenberg, the PI, moved institutions to the Dana Farber Cancer Institute.

Tasks 1. Isolate circulating tumor cells from patients with metastatic HRPC using Vitatex technology

Most prior studies involving CTCs in prostate cancer patients have been enumeration studies or gene expression studies. Gene expression is dependent on RNA extraction procedures and on environment. Therefore, the disparate published results may be related to minor differences in RNA isolation techniques and the environment of the cells prior to and after isolation. Expression profiles of CTCs may share only limited concordance with cells from the primary tumor and significant variation within and between patients is expected. Genomic profiling will go beyond cell counting, and circumvent technical complexities related to working with RNA.

Task 1 has been completed as of month 42. This is summarized in the manuscript that is attached. Briefly, peripheral blood for CTC isolation with the Vitatex technology was collected from 47 prostate cancer patients (Task 1a and 1b), including 34 patients with castration resistant prostate cancer. DNA for array CGH was able to be prepared from 12 of 13 samples (Task 1c). One specimen required amplification, but sufficient DNA was recovered the other 11 for direct analysis.

Task 2. Isolate circulating tumor cells from patients with metastatic HRPC using USC/Caltech's Microfilter Device (Months 26-42)

We were unable to come to a final agreement to collaborate with the USC/Caltech group, so no progress was made on this front, and will not be continued in the future.

Tasks 3. Define immunohistochemical profile from CTC's isolated in Task 1 and 2 (months 24-44)

Task 3 has been completed as of month 42 (see attached manuscript). Working with collaborators at the State University of New York, Stonybrook, we were able to label tumor cells by their ability to ingest red fluorescent CAM fragments. These cells were identified by uptake of fluorescent CAM and co-staining with green fluorescein-conjugated anti-CK antibodies for epithelial cells to distinguish them from the circulating epithelial-like cells that were often observed in blood samples of healthy individuals. Cells labeled by ingested CAM and stained with anti-CK antibodies (CAM+Epi+) were scored as CTCs, whereas CD45+ cells were scored as contaminating normal circulating cells.

Task 4. Obtain primary tumor specimens from patients in task 1 and 2 (months 18-40)

Task 4 has been completed as of month 42. Matched CTC and FFPE specimens from two patients were accessible for this project. One of the CRPC cases had primary and metastatic tissue and a second case had primary tumor material available for research purposes.

Task 5. Compare oligonucleotide CGH profiles of isolated CTC's in tasks 1 and 2 to profiles from reference metastatic hormone refractory prostate tissue and primary tumor specimens from Task 4. (26-48)

Task 5 completed month 42. The percentage of the genome that was aberrant was greater in the CTC fraction versus the mononuclear cell fraction for the 3 matched cases studied, displaying the difference without making any assumptions of the underlying statistical distribution. Copy number profiles of these matched CTC-primary/metastatic cases were compared and found to be similar based on the Kappa score approaching one. Cancer related genes were identified when focusing on genes altered in 50% or more of the CTCs. Loss of the POTE15 gene was frequently detected in the CTCs. Consistently, differential expression of POTE related genes has been observed in prostate cancer. The GSTT1 locus was frequently deleted in the CTCs. GST enzymes function in the detoxification of electrophilic compounds, including carcinogens, therapeutic drugs, environmental toxins and products of oxidative stress. Therefore, loss of GSTT1 could make a cell more susceptible to malignant transformation, and less likely to respond to therapeutic intervention. GSTT1 expression has been reported to be significantly lower in CRPC tumors as compared to untreated primary tumors. Loss of the SUSD4 gene was frequent in the CTC samples. Although the function of SUSD4 is not known, the expression of SUSD4 was significantly decreased in metastatic tumors compared to primary prostate tumors and normal prostate tissue. An ADAM5-like protein and cyritestin (part of the ADAM family of proteins) were frequently gained in the CTCs. ADAM enzymes cleave extracellular portions of transmembrane proteins. In breast cancer, ADAM10 was found to activate the HER2 receptor, thus promoting tumor growth. The sample size is too small to draw conclusions about the genes altered, although these preliminary findings are provocative. High kappa scores were observed between CTCs and metastatic tumor specimens suggesting a high degree of concordance between the tumor and circulating cell DNA profiles.

Task 7

Educational Development

Dr. Rosenberg met regularly with Dr. Small prior to leaving UCSF to discuss research and clinical trial design, as well as with Dr. Paris to discuss progress on CTC isolation and characterization. Dr. Rosenberg has been named to the CALGB Genitourinary Oncology Core Committee, and is involved in the decision-making for the new and ongoing clinical trials and translational research of the Committee.

Key Research Accomplishments:

- Demonstration that reproducible genomic changes can be observed in CTCs using the Vitatex isolation technology.

Reportable Outcomes:

1. Paris PL, Kobayashi Y, Zhao Q, Zeng W, Sridharan S, Fan T, Adler HL, Yera ER, Zarrabi MH, Zucker S, Simko J, Chen WT, Rosenberg J. Functional phenotyping and genotyping of circulating tumor cells from patients with castration resistant prostate cancer. Cancer Letters;In Press, Corrected Proof.

Conclusions:

We have demonstrated that the Vitatex technology can be used to isolate CTCs for genomic analysis. High quality DNA is able to be isolated from these cells. oCGH using CTC DNA isolated by the Vitatex system suggests that recurrent genomic alterations are present in CTCs. The Agilent array technology is high resolution allowing the identification of specific genes that may be altered in metastatic and chemotherapy refractory HRPC. The CGH data suggests that the CTC's are genetically concordant with paired HRPC solid tissue specimens previously collected.



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Functional phenotyping and genotyping of circulating tumor cells from patients with castration resistant prostate cancer

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ABSTRACT

Circulating tumor cells (CTCs) hold promise for studying advanced prostate cancer. A functional collagen adhesion matrix (CAM) assay was used to enrich CTCs from prostate cancer patients' blood. CAM ingestion and epithelial immuno-staining identified CTCs, which were genotyped using oligonucleotide array comparative genomic hybridization. The highest CTC counts were observed in men with metastatic castration resistant prostate cancer (CRPC) compared to castration sensitive prostate cancer. Copy number profiles for CRPC CTCs were similar to paired solid tumor DNA, and distinct from corresponding DNA from the residual CAM-depleted blood. CAM CTC enrichment may allow cellular and genetic analyses in prostate cancer.

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1. Introduction

Prostate cancer is the second leading cause of male cancer related deaths in the United States, accounting for approximately 30,000 deaths annually [1]. Ultimately, resistance to androgen deprivation therapy and chemotherapy are the underlying causes of mortality in patients with advanced prostate cancer and the mechanisms of this resistance are still not understood. A major constraint in studying resistance mechanisms in advanced prostate cancer has been the limited accessibility to metastatic tissue, because biopsy of metastatic prostate cancer is often difficult and impractical, and the yield of cancer cells is low.

In most primary epithelial tumors, a rare subset of malignant epithelial cells emigrates to the bloodstream,

and proliferates at distant sites [2,3]. Analysis of circulating tumor cells potentially allows for increased understanding of how cancer metastasizes with subsequent clinical application of this knowledge [4,5]. The majority of CTC detection methods available to date are based on enrichment of the tumor cells from blood using antibodies against epithelial surface antigens, as no cancer-specific markers exist yet [4,5]. Current methods including immuno-affinity and density gradient centrifugation enrichment procedures isolate cell populations that require further characterization of viability and metastatic propensity because the majority of tumor cells in the circulation are dead or dying [3,6,7]. Furthermore, current antibody-based CTC detection in prostate cancer has thus far only demonstrated clinical utility as a prognostic tool for classifying patients into high- and low-risk categories [8–11]. An alternative cell enrichment approach of isolating viable tumor cells from blood is required for biomarker discovery and development for more effective future clinical applications.

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The proclivity of a tumor cell to invade collagenous matrices is one of the hallmarks of metastasis [12,13]. We reasoned that an enrichment step based on invadopodia function, subcellular structures involved in cancer invasion into collagenous matrices [14–16], would serve to separate metastatic cells from all other cell types found in blood. Here we applied this functional cell separation method using a collagen adhesion matrix (CAM), modified as previously described [17,18], to enrich viable tumor cells from the blood of prostate cancer patients. In this study, we performed cellular analysis using fluorescence microscopy to identify CTCs that exhibit high avidity for and invasiveness into the extracellular matrix. Circulating cells that ingest fluorescently labeled CAM fragments (CAM+) are verified to be tumor cells by immuno-staining with common cytokeratin (CK) epithelial markers (Epi+) [5]. These epithelial markers are low or lacking in circulating leukocytes. CAM-enriched cells are viable and devoid of normal leukocytes [19–21] or apoptotic and necrotic cells [6,22,23] that may react with antibody staining non-specifically. In the present study, we hypothesize that certain CTCs exhibiting high avidity for, and invasiveness into the extracellular matrix, represent cancer cells disseminated into blood of prostate cancer patients. And, these cells are better indicators for metastasis and hormone responsiveness than the cells isolated using antibodies against epithelial surface antigens alone.

In addition, we examined the genomic profiles of CTCs enriched by CAM from blood of patients with CRPC by using oligonucleotide (oligo) array comparative genomic hybridization (aCGH). aCGH is a valuable tool for identifying clinically significant DNA copy number changes in tumor genomes, particularly in prostate cancer, which can then be examined for associations with clinical parameters [24–26]. Genomic gains and losses often coincide with genes crucial for tumor progression, and genomic loci with aberrant copy number can lead to better clinical diagnostics and prognostication [27,28]. Recently, oligo aCGH was shown to yield high resolution gene copy number mapping information of solid tumors [29,30]. This facilitates the identification of candidate genes mapping to a locus of altered dosage. Using aCGH, we previously found a significant concordance between the copy number changes in primary prostate tumors and unmatched metastatic tumors [31]. Also, copy number changes of matched primary prostate tumor and hormone naïve lymph node metastasis are almost identical, suggesting that this method could be used for detecting genomic biomarkers with associated metastatic phenotypes [32]. Based on these observations, we hypothesize that a subset of copy number aberrations representative of a primary tumor and its metastatic lesions can be identified in CTCs, and thereby potentially extending the clinical utility of such a biomarker.

2. Materials and methods

2.1. Patients and healthy blood donors

Twenty-one patients with metastatic CRPC, 13 patients with castration sensitive localized or metastatic prostate

cancer (CSPC) prior to androgen deprivation, and 20 healthy male subjects were recruited for blood donation at the Stony Brook University Medical Center and the Veteran Administration Medical Center at Northport, NY. Similarly, another 13 patients with progressive metastatic CRPC and two healthy male individuals at the UCSF Urologic Oncology Clinic underwent blood sampling were recruited after written, informed IRB approved consent was obtained.

2.2. Blood sample preparation

Between 14 and 20 ml (median = 16 ml) of peripheral blood were collected in Vacutainer™ tubes (Becton Dickinson, Franklin Lakes, NJ; green top) with lithium heparin as the anticoagulant. Blood samples were delivered to the laboratory at room temperature within one to four hours from collection, and processed immediately.

Aliquots of 3 ml of whole blood were subjected to one of the two CAM-initiated rare cell enrichment methods. *CAM fractionation*: Blood samples were transferred into a CAM-coated tube (Vita-Cap™, Vitatex Inc., Stony Brook, NY) and incubated for 3 h at 37 °C while rotating at 10 rpm to imitate blood flow and maximize the contact between cells and the CAM substrate. The tubes were washed with 37 °C phosphate buffered saline (PBS) media to remove non-adherent cells. Tumor cells were eluted from the tube using collagenase and either used directly for microscopy, or DNA of CAM-bound cells was extracted for oligo aCGH. *Mono-nucleated cell (MNC) – CAM fractionation*: Blood samples were subjected to Ficoll density gradient centrifugation to obtain the MNC. MNC were then seeded onto a 16-well chamber slide coated with CAM containing fluorescently labeled type I collagen (Vita-Assay™, Vitatex Inc., Stony Brook, NY) for 12 h and the non-adherent cells were washed away with PBS to generate the remaining red fluorescent CAM-labeled cells.

2.3. Cell spiking experiments

To determine the effectiveness of CAM rare cell enrichment methods, model experiments were conducted in which 3–3000 PC3 tumor cells (as pre-determined by flow cytometry), labeled with PKH67 green fluorescence (Sigma, St. Louis, MO) were spiked into 3 ml samples of blood from healthy donors. The human prostate cancer cell line, PC3, was purchased from American Type Culture Collection (ATCC, Rockville, MD). The spiked fluorescent cells were recovered by both CAM and MNC-CAM methods and evaluated with fluorescence microscopy. In parallel, tumor cells were recovered by immunomagnetic cell separation using the Dynal Collection™ Epithelial Enrich system (Invitrogen, Carlsbad, CA) for additional comparison.

2.4. Tumor cell enrichment and CAM ingestion labeling

To determine the invasive phenotype of CTCs, MNC from 0.5 ml whole blood aliquots were seeded onto one well of a red fluorescent CAM-coated 16-well chamber slide (Vita-Assay™, Vitatex Inc., Stony Brook, NY). Cells were incubated for 12–18 h to label the captured cells *in vitro*. This step labels tumor cells by making use of their ability

to ingest red fluorescent CAM fragments. Cells were then fixed with 3.5% paraformaldehyde/PBS, permeabilized with 0.1% Triton X-100, and subsequently immuno-stained using a mixture of green fluorescent antibodies against pan-CK (cytokeratins 1, 4, 5, 6, 8, 10, 13, 18, 19: clones C11 and others, Sigma, St. Louis, MO), epithelial cell adhesion molecule (EpCAM, clone Ber-Ep4, DakoCytomation, Carpinteria, CA), and epithelial surface antigen (ESA, clone B29.1, Biomed, Foster City, CA) for analysis by digital fluorescence microscopy imaging. In addition, CAM-enriched cells were stained with an anti-CD45 antibody (clone T29/33, DakoCytomation, Carpinteria, CA) and followed by red color alkaline-phosphatase–anti-alkaline-phosphatase (APAAP) conjugated secondary antibodies (DakoCytomation, Carpinteria, CA) for internal control of cells with common leukocyte markers and normalization of fluorescence signal. Thus, CTCs were identified as circulating cells that ingested CAM and were stained with anti-epithelial antibodies (CAM+Epi+). In a CRPC patient, 89% of CAM+Epi+ cells were also positive for prostate specific antigen (PSA), suggesting a possibility to identify prostate CTCs as CAM+PSA+ cells. A Nikon E-400 inverted fluorescence microscope equipped with a Microfire digital camera system and Image Pro Plus software were used to examine and analyse the image results.

2.5. Cell culture

Blood samples were subjected to Ficoll density gradient centrifugation to obtain the mono-nucleated cells (MNC). The MNC from 0.5 ml whole blood aliquots were seeded onto one well of CAM-coated 96-well microtiter plate (Vita-Assay™, Vitatex Inc., Stony Brook, NY) for 12 h and the non-adherent cells were washed away by removing media and replacing with fresh media. Cells were cultured with CCC media (1:1 mixture of Dulbecco's modified Eagle's medium and RPMI1640 medium supplemented with 10% calf serum, 10% Nu-serum, 2 mM L-glutamine, 1 unit/ml penicillin, and 10 µg/ml streptomycin). In five of eight cases of patients with metastatic CRPC, tumor cells from blood were successfully cultured for 10 days and developed approximately 80 cellular colonies of epithelioid morphology per 1 ml of blood. Judging from approximately 72 cells per colony, the average proliferation rate of CTCs captured by CAM is 40 h.

2.6. DNA extraction and whole genome amplification

After performing the CAM tumor cell enrichment, DNA was extracted using the Wizard DNA Purification Kit (Promega, Madison, WI). The manufacturer's whole blood protocol was followed for the CAM– fraction (white blood cells, WBC) and the tissue culture cell protocol was followed for the CAM+ fraction (CTCs). The DNA quality was visualized by gel chromatography and quantified by a Nanodrop UV–Vis spectrophotometer (ThermoScientific, Wilmington, DE). 500 ng of high molecular weight DNA was used for oligo aCGH.

Whole genome amplification of DNA isolated from CAM-enriched cells with yields less than 500 ng was carried out using the GenomePlex Whole Genome Amplifica-

tion Kit (Sigma, St. Louis, MO). 100 ng of genomic DNA was fragmented and then amplified according to the manufacturer's instructions. Amplified products were then column purified using the QIAquick Purification Kit (Qiagen, Valencia, CA), as per the manufacturer's protocol. Whole genome amplification was necessary for one of the nine patient's CTC DNA as the yield was less than 500 ng. Whole genome amplification, when necessary, does not introduce unacceptable copy number artifacts as determined using aCGH [33].

2.7. Oligonucleotide aCGH (oligo aCGH)

Oligo aCGH experiments were performed with Agilent's 244 K oligonucleotide arrays using 500 ng genomic DNA according to Agilent's protocols. Commercial male DNA obtained from blood of healthy donors (Promega, single lot), served as reference DNA for all oligo aCGH hybridizations. Agilent's Feature Extraction software was used to extract feature level data (e.g., signal intensities). All oligo aCGH data was analysed through the assistance of the UCSF Helen Diller Family Comprehensive Cancer Center's Biostatistics Core. We have previously optimized copy number assessment algorithms for high density oligonucleotide arrays and have found that a combination of circular binary segmentation (CBS) [34] and median absolute deviation (MAD) yield the best results (unpublished data). The array CGH data was segmented using CBS to translate experimental intensity measurements into regions of equal copy number. To enumerate genomic aberrations, the experimental variability (sample MAD) of each aCGH profile was estimated by taking the scaled MAD of the difference between the observed and segmented values. A clone was declared gained or lost if its absolute segmented value exceeded the sample MAD for a given profile. Copy number profiles for CTCs of prostate cancer patients were compared to each other and when appropriate to that of their matched primary, metastatic tumor(s) and/or white blood cells.

3. Results

3.1. Enrichment of rare prostate tumor cells from whole blood by CAM

The efficiency of isolating prostate tumor cells using CAM was determined by spiking a specified number of fluorescently labeled tumor cells into whole blood and quantifying their recovery rate using fluorescence microscopy (Fig. 1). Approximately 1–1000 PC3 prostate cancer cells (estimated using flow cytometry) were spiked into 1 ml of whole blood derived from a healthy donor and subjected to either a one-step CAM enrichment (Fig. 1A, CAM) or a two-step MNC-CAM enrichment in which whole blood was pre-processed using Ficoll density gradient centrifugation (Fig. 1A, MNC-CAM). PC3 cells in blood are CAM avid and five times larger than the circulating non-tumor cells such as white blood cells (Fig. 1A, double arrows indicating tumor cells and single arrows WBCs). Efficient recovery of fluorescently labeled PC3 cells was observed (Fig. 1B, CAM: 50, 26–73 [% mean, range]; MNC-CAM: 81, 58–100), even at the lowest concentration of ten spiked tumor cells. Recovery rates were higher in MNC-CAM than CAM enrichment methods, but were comparable under both conditions (Fig. 1B, $r^2 = 0.99$), suggesting a potential steric hindrance of red blood cells in the tumor cell-CAM contact. In comparison with anti-epithelial antibody purification, the efficiency of CAM recovery of tumor cells was nearly a hundred times higher than using Dynal Collection™ Epithelial Enrich. Only a 0.27% ($N = 12$) recovery rate was observed when 1000 PC3 cells were spiked and recovered using an anti-epithelial antibody method (Fig. 1B). Surpris-

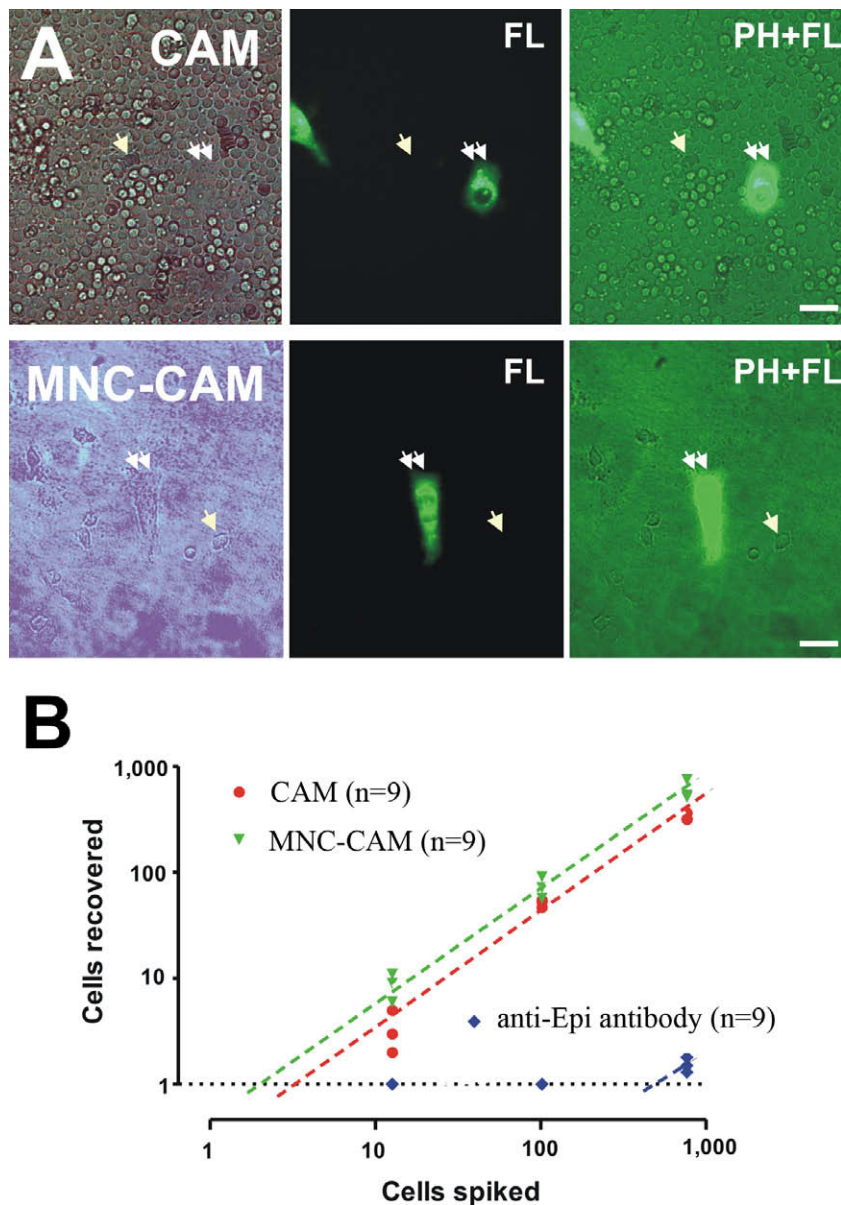


Fig. 1. Enrichment of prostate tumor cells from blood by CAM cell separation methods: (A) approximately 1–1000 fluorescently labeled PC3 cells were spiked into 1 ml of whole blood derived from a healthy donor, and recovered by either one-step CAM enrichment or two-step MNC-CAM enrichment assays. Double arrows indicate recovered tumor cells. Single arrows show co-isolated white blood cells. PH denotes phase contrast microscopy and FL is fluorescence microscopy. Photographs labeled PH + FL indicate the super-imposed image of the same field. Bar = 10 μ m and (B) comparison of the yields of PC3 carcinoma cells isolated from blood using the CAM or MNC-CAM protocols and using the Dynal CELLction™ Epithelial Enrich Kit. Fluorescently labeled PC3 cells were spiked into blood samples from healthy donors, followed by one of the cell separation protocols. Cell enumeration was done with FL microscopy.

ingly, when cells derived from different tumor cell lines were used to perform epithelial cell capture by others using other devices and methods, [6,11] 100% of the spiked tumor cells were recovered. This apparent discrepancy between our antibody-captured cell result and previous antibody-based methods [6,11] could be explained by: (a) a possible down regulation of targeted epithelial surface antigen in the PC3 cells used in our study; (b) sophisticated devices and procedures used in previous antibody-based methods. Nevertheless, the CAM-initiated tumor cell enrichment performs well for whole blood and pre-processed MNC fraction, and both CAM methods achieve a high recovery rate similar to the recent CTC-chip epithelial antibody method [11].

3.2. Viability and proliferative propensity of cancer cells enriched by CAM

To evaluate the metastatic propensity of the cell capture from CRPC patients, we conducted a series of experiments in which MNC-CAM fractions were tested for cell viability, proliferative potential and immunoreactivity with anti-CK antibodies (Fig. 2) [35]. We verified the viability of tumor and “normal” cells from blood of CRPC patients, pre- and post-CAM enrichment, by using Molecular Probes LIVE/DEAD Viability Kit #4 (Invitrogen, Carlsbad, CA: live cells stain green fluorescence with calcein AM and dead cells stain red fluorescence with ethidium homodimer-1). Prior to CAM enrichment, less than 9% of cells in blood samples were via-

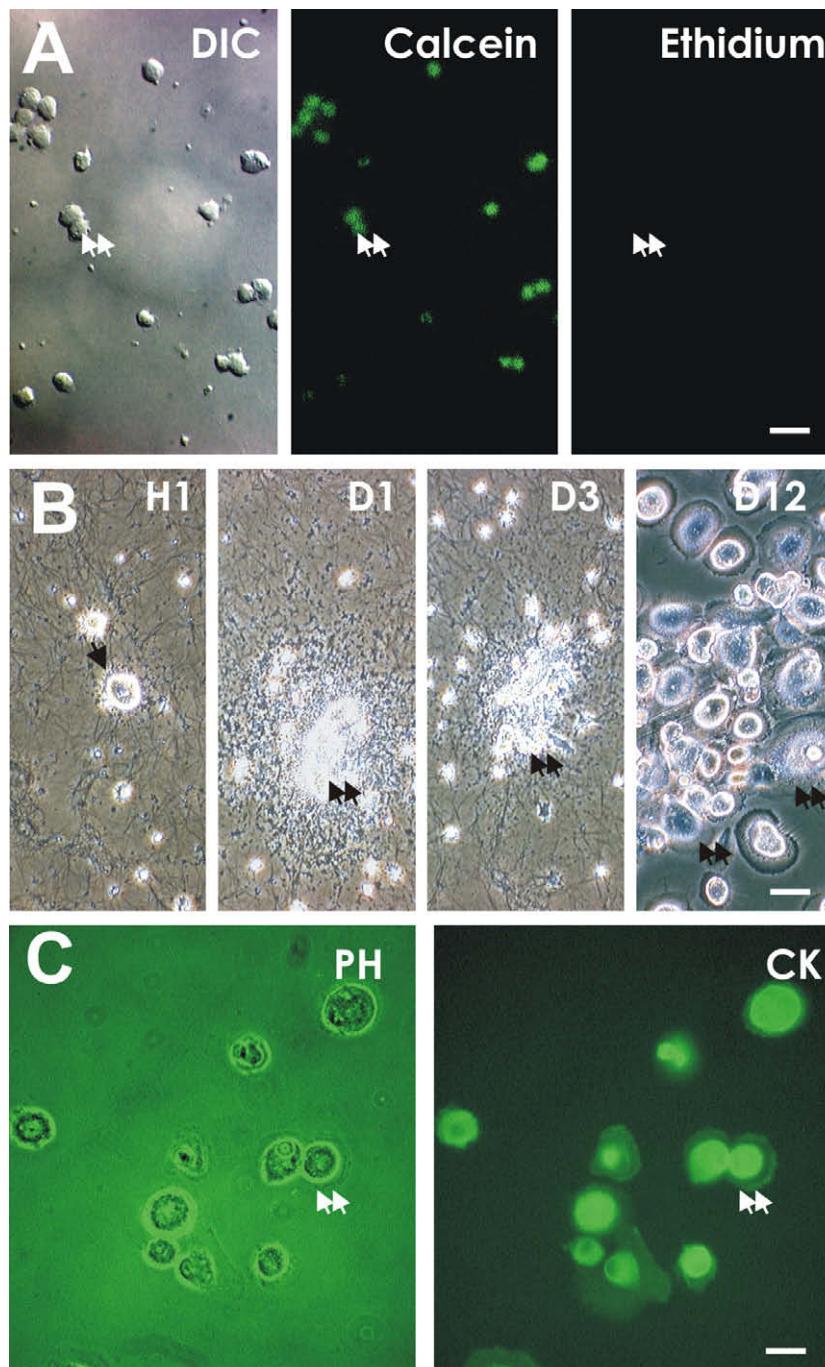


Fig. 2. Viability, proliferative properties and CK immuno-reactivity of CAM-enriched cells from CRPC patients: (A) live green fluorescent cells and lack of dead red fluorescent cells in fractions post-CAM enrichment. Bar = 60 μ m; (B) CAM-enriched cells isolated from CRPC patients were cultured on the fibrous CAM scaffold for one hour (H1), one day (D1), three days (D3) and 12 days (D12). Live cells were photographed under phase contrast (PH) microscopy. Tumor cells grew as time increased; from H1 to D3, cells were seen as solitary (arrows) and afterward became clustered epithelioid cells (double arrows). Bar = 60 μ m and (C) cells cultured for 12 days shown under PH microscopy (double arrow) and fluorescence microscopy for staining with antibodies against pan-cytokeratins (CK, double arrow). Bar = 30 μ m.

ble (results not shown). However, post-CAM enrichment, greater than 99% of cells was viable (Fig. 2A). Overall, cell viability increased greater than 10-fold after CAM enrichment.

In addition, CAM-captured cells were readily cultured on the fibrous CAM scaffold *in vitro* (Fig. 2B). Tumor cells isolated by CAM from blood of CRPC patients were solitary and became epithelioid, producing colonies

of CK+ epithelial cells from 1 week to 3 months (Fig. 2C). In contrast to CTCs captured by anti-epithelial antibody systems observed previously, [6,11] we have noticed no morphological feature that distinguishes CTCs from other circulating cells enriched by CAM, i.e., CTCs were seen to be heterogeneous in size and shape (Figs. 2A and 3A). However, CTCs cultured for more than 7 days exhibited epithelioid morphology (Fig. 2B

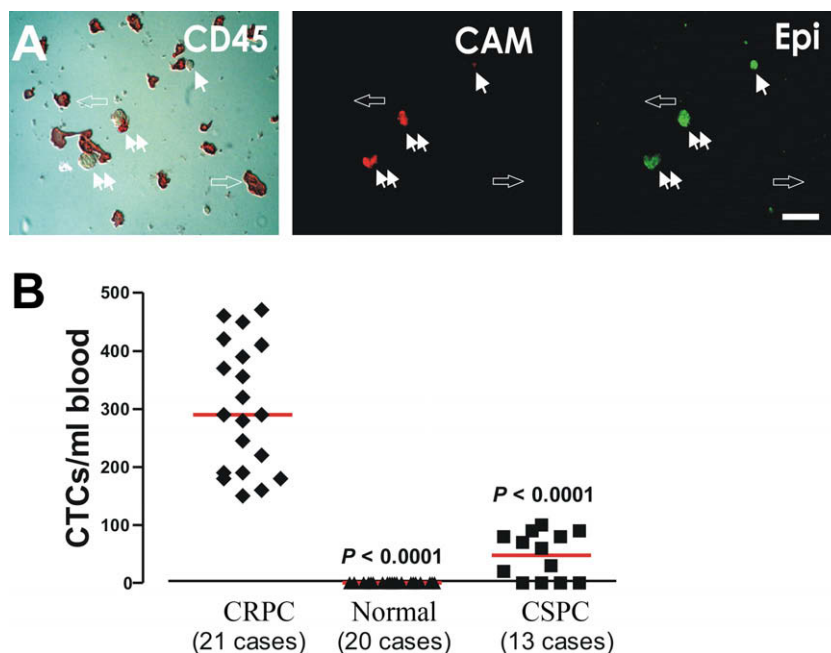


Fig. 3. CTC characterization based on the expression of CK epithelial lineage antigens and cellular uptake of CAM: (A) detection of CD45–CAM+Epi+ cells (double arrows) as CTCs in a patient with metastatic CRPC. Circulating epithelial cells are CD45–CAM–Epi+ (single arrows), and leukocytes are CD45+CAM– (open arrows). Bar = 20 μ m and (B) correlation between the number of CTCs in one ml of blood for 21 patients with metastatic CRPC, 13 CSPC and 20 healthy donors. Cells were counted manually under differential contrast interference/epifluorescence microscopy. The bars represent the median values in each subgroup of blood samples.

and C) that was distinct from the remaining hematopoietic cells. We thus concluded that CAM enriches cells from blood of CRPC patients that are viable and exhibit tumor progenitor propensity.

3.3. Characterization of CTCs isolated by CAM from CRPC patients

Having optimized the MNC–CAM enrichment with controlled quantities of prostate tumor cells, we tested its capacity to capture CTCs from whole blood samples donated by prostate cancer patients. A total of 34 samples from 34 patients with prostate cancer including metastatic CRPC ($n = 21$) and CSPC ($n = 13$) were studied. The average volume of blood analysed was 3.5 ml per sample (range, 2.2–6.5 ml), and aliquots of 0.5 ml per well of a 16-well chamber slide were stained with a specific antibody mixture. We also examined samples from 20 healthy male individuals (3.0 ± 0.5 ml [mean $\pm$ s.d.] of blood per subject) as controls.

CTCs captured from blood samples of cancer patients were identified using the invasive phenotype of cancerous cells, i.e., labeling tumor cells by their ability to ingest red fluorescent CAM fragments (see Methods and methods for detailed procedures); cells were identified by uptake of fluorescent CAM and co-staining with green fluorescein-conjugated anti-CK antibodies for epithelial cells (Fig. 3A, double arrows) to distinguish them from the circulating epithelial-like cells (Fig. 3A, single arrows) that were often observed in blood samples of healthy individuals. Cells labeled by ingested CAM and stained with anti-CK antibodies (CAM+Epi+) were scored as CTCs, whereas CD45+ cells were scored as contaminating normal circulating cells.

CTCs were identified in 21 of 21 (100%) patients with metastatic CRPC and in nine of 13 patients with CSPC. The number of CTCs isolated ranged from 150 to 740 per ml for CRPC (322 ± 143 [mean $\pm$ s.d.] CTCs per ml) and 0–100 (48 ± 40) for CSPC patients (Fig. 3B). None of the 20 healthy subjects had any identifiable CTCs; two BPH patients had no CTCs. We calculated the sensitivity (100% for CRPC and 69% for CSPC) and specificity (100%) of the CAM-initiated CTC detection for prostate cancer. We also evaluated the reproducibility of CTC capture using split samples and showed high experimental reproducibility ($r^2 = 0.98$, $n = 5$). Thus, the CAM cell separation platform provides a highly enriched population of CTCs enabling the identification of CTCs in subjects with metastatic and clinically localized prostate cancers and warrants further clinical study.

3.4. Genetic analysis of CRPC CTCs isolated by CAM

To determine whether CAM-captured cells are suitable for subsequent genetic analyses, we performed genomic copy number profiling of blood samples from thirteen patients with CRPC. The DNA extraction procedure was performed on the CAM fraction (CTC sample) and the MNC fraction from cells left unbound by CAM (WBCs). DNA was isolated from the CAM fraction for 12 of the 13 patients. The yield of one of the twelve samples (CTC3) was too low to perform oligo aCGH, so it was amplified (aCTC3). All DNAs were evaluated by gel chromatography (Fig. 4A), except CTC1 which only had enough DNA for oligo aCGH. Three of the CTC DNAs were very degraded (CTC3, 5, 10) as evident by the presence of a DNA smear and the lack of a high molecular weight band (Fig. 4A).

Oligo aCGH was performed for all CTC DNAs and a subset ($N = 3$) of matching WBC DNAs. It should be noted that all of the nine CTC samples with high molecular weight DNA performed well on the microarrays, by exhibiting good fluorescence signal to noise ratios. The three degraded CTC samples (CTC3, 5, 10) were subjected to an oligo aCGH protocol for degraded samples (Agilent part #5190–0419), but still did not pass Agilent's quality control metrics. To evaluate the reproducibility of the Agilent data, the average standard deviation of the $\log_2$ ratios for 1000 randomly dispersed replicate probes on an array was calculated. For the nine CTCs with acceptable oligo aCGH, the average standard deviation was 0.02.

Matched CTC and WBC DNAs for three cases were profiled by oligo aCGH and compared. The percentage of the genome that was aberrant was greater in the CTC fraction versus the MNC fraction for the three matched cases studied, displaying the difference without making any assumptions of the underlying statistical distribution (Fig. 4B). One of the CRPC cases (CTC13) had primary and metastatic tissue and a second case (CTC8) had primary tumor material available for research purposes. Copy number profiles of these matched CTC–primary/metastatic cases were compared and found to be similar (Table 1). A Kappa score approaching the value one suggests a high degree of similarity. Oligo aCGH data for all tumor samples are available in [Supplementary data](#).

The summary of copy number changes for each locus across the nine CTC samples is displayed in Fig. 5. A detailed list of these alterations and the probes are supplied in [Supplementary data](#). Noteworthy, recurrent

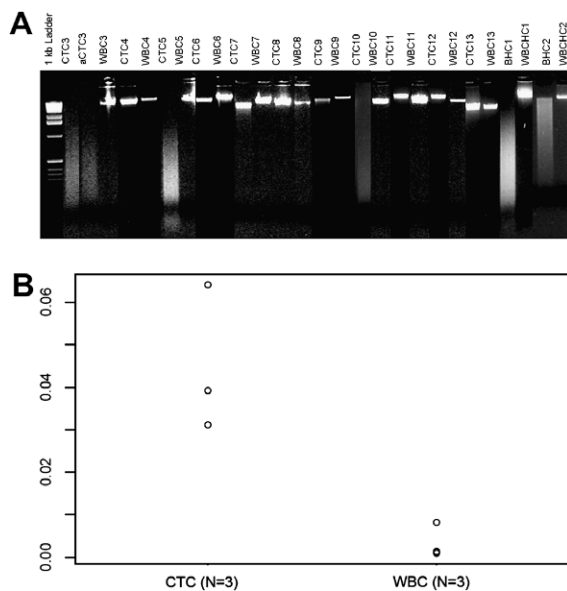


Fig. 4. (A) DNA quality visualized by gel chromatography. 100–200 ng of DNA from cells isolated with the Vitatex one-step VitaCap tubes was loaded into each gel lane. The CAM+ fraction from 11 patients is paired with the matching CAM– fraction (WBC). aCTC represents the amplified sample. Two healthy controls were included, with the CAM+ fractions denoted as BHC1 and BHC2, and their matching CAM– fractions as WBHC1 and WBHC2. A 1 kb ladder was run for molecular sizing. The smears for the CAM+ fraction in the healthy controls, suggests that patients three, five and ten may not have had CTCs and (B) comparison of copy number changes in matched CTCs and WBCs. The fraction of the genome that is altered is plotted on the y-axis.

Table 1

Metric comparing copy number changes in matched primary, metastatic and circulating tumor cells.

Sample comparison	Kappa score
Met13 versus CTC13	0.84
CTC13 versus RP13	0.90
MET13 versus RP13	0.91
CTC8 versus RP8	0.81

changes were detected. Some cancer related genes mapped to the loci aberrant in 50% or more of the CTC samples. These included POTE15 (protein expressed in prostate, ovary, testis, and placenta 15), similar to ADAM5 (disintegrin and metalloprotease domain 5), cyritestin protein, SUSD4 (sushi domain containing four) and GSTT1 (glutathione S-transferase theta 1). Considering the high number of captured viable CTCs, the CAM CTC enrichment method provides a powerful opportunity for CTC-based genomic analyses.

4. Discussion

We have applied a CAM based functional cell separation method to obtain sufficient quantity and quality of viable CTCs for cellular analyses and genomic profiling of prostate cancer. The CAM method of tumor cell enrichment is distinctive among current CTC enrichment technologies [4,5] in that it readily isolates viable tumor cells on a platform enabling further cellular and molecular analyses as shown in this report. Cell culture plates or tubes coated with col-

lagen-based CAM scaffolds allow blood cells to be washed out, resulting in a 10^6 -fold enrichment, which is the highest cell enrichment among existing technologies. Viable CTCs concentrated on CAM scaffolds are readily extracted with DNA and RNA buffers for molecular analyses of cancer metastasis or treated with collagenases to make into cell suspension for cellular assays such as flow cytometry and fluorescence microscopy.

Currently, no consensus has been reached on which methods should be best to detect CTCs in epithelial cancer patients. Traditionally, it was believed that epithelial cancers spread primarily through the circulation and the presence of epithelial cells in blood or bone marrow could be an indicator of metastatic cancer cells. Cells in blood captured by antibodies against epithelial surface antigens augmented with sophisticated devices have been a popular means to detect CTCs [4–7]. However, some research has shown that epithelial cells may be present in blood of healthy populations, either as non-specific epithelial antigen binding to leukocytes, or as benign epithelial cells in circulation [4,5]. Additionally, recent work investigating the role of epithelial mesenchymal transition in cancer metastasis has found a heterogeneous downregulation of epithelial surface antigens in invasive epithelial tumor cells [5,19–21], thus reliance on a few standard epithelial markers alone for identification of tumor cells may not be reliable. Furthermore, current detection techniques do not distinguish viable cells from non-viable cells. The life-span of a CTC is limited by anoikis [3–7,22,23], thus a proportion of epithelial-recovered CTCs may represent dead or dying cells which may not contribute greatly to cancer spread.

To address these concerns, we evaluated the feasibility of using a CAM-initiated CTC assay that enriches cells based on their invasive properties in addition to their expression of epithelial cell markers. We reason that detection of these *invasive* CTCs may offer an alternative method to assess metastatic progression that may be of prognostic value. In this exploratory study, we found that CRPC patients have 322 ± 143 [mean $\pm$ s.d.] CTCs per ml of blood, a number that is three times higher than was reported using a sensitive anti-epithelial antibody CTC-chip technology [11]. We also found that there are 48 ± 40 [mean $\pm$ s.d.] CTCs per ml of blood in CSPC patients. Whether this increase in CTC detection is a result of increased production or release of tumor cells into blood, or decreased CTC death or clearance remains to be determined. Regardless, these findings suggest a clinical utility for quantifying invasive CTCs in monitoring cancer progression.

Copy number profiling DNA from blood yields good fluorescence signal to noise ratios and thereby improves the confidence of determining which loci are altered. This is important when sufficient DNA is not available for replicate experiments and monetary constraints. Also, DNA copy number changes in the blood are less variable day to day, as opposed to RNA. The DNA from the bound fraction for the healthy controls was always degraded (Fig. 4A; BHC1, BHC2). The DNAs from the CAM fractions were not always high molecular weight gel bands (Fig. 3; CTC3, 5, 10) suggesting that these specimens did not con-

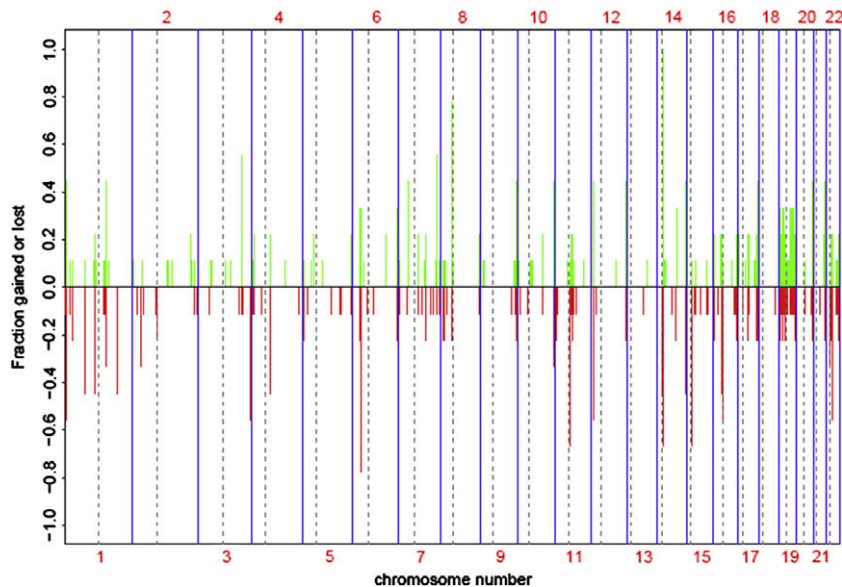


Fig. 5. CRPC CTC copy number changes. Copy number aberrations for the nine CTCs with high mw DNA (see Fig. 4A) and that passed Agilent's quality control metrics is shown versus the chromosomal position. Gains are shown in green and deletions in red. Recurrent changes between patients were observed and may represent loci associated with CRPC.

tain sufficiently high number of CTCs. Therefore, we focused on the remaining CTC samples for analysis.

Although a common concern with assessing copy number profiles of CTCs is that WBCs may contaminate the sample (i.e., the CAM fraction in our approach), this should not interfere significantly here. First, aCGH of tumor samples has been shown to allow up to 60% contaminating normal DNA while still providing accurate copy number information for the tumor [36]. Secondly, our cellular analysis shows that CAM-enriched cells are greater than 99% viable, proliferate into CK+ cell colonies, and exhibit ability to degrade and ingest collagenous matrices, characteristics of the tumor invasive phenotype [15,16]. Although WBCs possess germline changes, the CTCs should contain additional tumor acquired alterations. The fraction of the genome that was altered was always greater for the CTC fraction when compared to matched WBC sets (Fig. 4B), providing further evidence that we are indeed isolating tumor cells from the blood. Recurrent changes in the matched CTC and MNC cell fractions could be related to either varying levels of WBC contamination in the CAM fraction and/or to germline changes which could be informative. Recently, germline copy number variants (CNVs) have been identified, and may contribute to a significant proportion of normal genetic variation in humans [37,38]. Since these CNVs often overlap genes, they may impact gene expression and cause or confer risk to complex diseases such as cancer. Changes seen in only one fraction are believed to be either unique to the CTC or the matching WBCs.

Our prior work suggests that primary prostate and metastatic lesions possess similar copy number changes [31,32]. In line with this observation, the matched primary and metastatic tumors for CTC13 show such concordance (Table 1). This observation was extended to the CTCs,

which showed similar copy number changes to their matched primary and/or metastatic tumor DNAs (Table 1). This provides additional evidence that the CTCs are indeed being isolated with this CAM based approach.

Cancer related genes were identified when focusing on genes altered in 50% or more of the CTCs. Loss of the POTE15 gene was frequently detected in the CTCs. Consistently, differential expression of POTE related genes has been observed in prostate cancer [39]. The GSTT1 locus was frequently deleted in the CTCs. GST enzymes function in the detoxification of electrophilic compounds, including carcinogens, therapeutic drugs, environmental toxins and products of oxidative stress. Therefore, loss of GSTT1 could make a cell more susceptible to malignant transformation, and less likely to respond to therapeutic intervention. GSTT1 expression has been reported to be significantly lower in CRPC tumors as compared to untreated primary tumors [40]. Loss of the SUSD4 gene was frequent in the CTC samples. Although the function of SUSD4 is not known, the expression of SUSD4 was significantly decreased in metastatic tumors compared to primary prostate tumors and normal prostate tissue [41]. An ADAM5-like protein and cyritestin (part of the ADAM family of proteins) were frequently gained in the CTCs. ADAM enzymes cleave extracellular portions of transmembrane proteins. In breast cancer, ADAM10 was found to activate the HER2 receptor, thus promoting tumor growth [42]. The sample size is too small to draw conclusions about the genes altered, although these preliminary findings are provocative.

Tumor cells were demonstrated to be recovered from the circulation by CAM, which facilitated subsequent genomic analyses. This approach allows one to go beyond enumeration of CTCs and warrants further studies aimed at CTC genomic profiling for identification of potential bio-

markers. To the best of our knowledge this is the first time CTCs have been profiled by oligo aCGH. Since blood samples are easy to obtain, this report shows that CTCs may represent a largely untapped resource for studying metastatic prostate cancer. As castration resistance ultimately leads to death in metastatic CRPC, new tools such as this will give insight into hormone resistance in prostate cancer and will delineate important pathways that may be targeted therapeutically.

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Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at [doi:10.1016/j.canlet.2008.12.007](https://doi.org/10.1016/j.canlet.2008.12.007).

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2001-2008	Member, Association of Northern California Oncologists
2001-present	Member, American Society of Clinical Oncology

Editorial Boards:

Editorial Board Member

2009-	<u>Journal of Clinical Oncology</u>
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Ad Hoc Reviewer:

2004-present	<u>Cancer</u>
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2004-present	<u>Journal of Urology</u>
2005	<u>Urology</u>
2005-present	<u>Clinical Genitourinary Oncology</u>
2005-present	<u>Expert Review of Anticancer Therapy</u>
2006-present	<u>Journal of Clinical Oncology</u>
2007	<u>Biomedcentral</u>
2008	<u>JAMA</u>

Awards and Honors:

1988-1992	Angier B. Duke Scholarship, Duke University
1991	Faculty Scholar Award for Outstanding Undergraduate Scholarship, Duke University
1991	Howard Hughes Undergraduate Research Fellow, Duke University
1992	Horn Biology Prize, Duke University
1992	Phi Beta Kappa, Duke University
1992	Lublin Award for Most Outstanding Premedical Student, Duke University
1992-1994	Harvard Medical School MSTP Fellowship
1995-1996	Howard Hughes Medical Institute Medical Student Research Fellowship
2000	David E. Rogers Research Prize Finalist, Department of Medicine, Cornell University
2001-2002	Amgen Fellowship, UCSF Hematology/Oncology Division
2002	Best Poster Presentation, AACR Conference: The Role of Telomeres and Telomerase in Cancer
2003	Honorable Mention, Best Poster Presentation, UCSF Inter-SPORE Meeting
2003	ASCO/AACR Methods in Clinical Cancer Research Workshop
2009	Dunkin Donuts Rising Star Award, Dana Farber Cancer Institute

PART II: RESEARCH, TEACHING, AND CLINICAL CONTRIBUTIONS

A. Narrative report of Research, Teaching, and Clinical Contributions

As a clinical researcher, I have developed the kidney and bladder cancer clinical trials portfolio at the UCSF Comprehensive Cancer Center. I have served as the UCSF urologic oncology representative to the Mayo Clinic National Cancer Institute Phase II Consortium. I have written, accrued, and reported 3 National Cancer Institute-sponsored clinical trials in prostate and bladder cancer. At UCSF, I have developed several kidney and bladder cancer investigator-initiated trials, and participated in numerous industry-sponsored trials in kidney, bladder, and prostate cancer. I have chaired a Cancer and Leukemia Group B phase II study in advanced bladder cancer (CALGB 90207). I have developed the concept, written the protocol, and am the overall principal investigator of the upcoming Intergroup NCI-sponsored phase III bladder cancer study of standard gemcitabine-cisplatin chemotherapy with or without anti-angiogenic therapy using bevacizumab (CALGB 90601). This phase III placebo-controlled study is expected to begin accrual in the summer of 2009.

The availability of tissue for research in advanced prostate cancer has been a limiting factor in research on this disease. Circulating tumor cells are accessible to collection, are present in high numbers in patients with metastatic prostate cancer, and represent a potential source of new tissue for research. As a recipient of a Department of Defense Prostate Cancer Physician Research Training Award, I have collaborated with laboratory researchers to evaluate new technology to examine genomic changes in circulating prostate cancer cells to better understand the biology of hormone refractory prostate cancer. I am also a co-investigator on a UCSF institutional grant to evaluate recurrent genomic alterations in metastatic hormone refractory prostate cancer.

My interest in advanced prostate cancer has focused on salvage chemotherapy regimens for hormone refractory prostate cancer. My work in this area has helped define the field as it stands today. I have developed, opened, and completed a multi-institutional randomized phase II study of two chemotherapy regimens as second-line chemotherapy for hormone refractory prostate cancer. This was the first prospective report of chemotherapy in the chemotherapy-refractory population, and defined the natural history of these patients for the first time. In addition, I have built upon this work with a DOD Clinical Trial Development grant to develop a follow-up NCI-sponsored multi-institutional phase I/II study to identify better therapeutic options for patients with hormone refractory prostate cancer. This study has completed phase I, and the phase II study is ongoing. The regimen that I developed is being considered as for testing in an Intergroup phase III study.

As a teacher, I was asked to develop the curriculum for the clinical research block of the UCSF Hematology/Oncology fellowship program didactic core course. I recruited seven faculty from within the division as well as from other disciplines (regulatory affairs, medical ethics, biotechnology and pharmaceutical industry) to develop the curriculum and lecture series. I have also lectured to the medical residents and fellows about kidney, bladder, and prostate cancer for the last 3 years. In addition to formal teaching, I was actively involved in the education of fellows and residents in the UCSF Urologic Oncology clinic. I attended six weeks per year on the inpatient solid tumor oncology service and oncology consult service, where I led a team of fellows, residents, and students caring for hospitalized solid tumor patients. As part of my educational role, I lecture at continuing medical education courses about prostate, bladder, and kidney cancer, and both locally and nationally, as well as at symposia and conferences. In the UCSF Urologic Oncology practice, I coordinated the education of fellows and residents rotating through our clinic. At the Dana Farber Cancer Institute, I am the director for fellow education in the Lank Center for Genitourinary Oncology. I am responsible for ensuring that fellows receive a balanced and thorough education in genitourinary oncology.

Clinical cancer care is the foundation upon which all the rest of my work depends. Without the trust and willingness of patients to take risks to enter clinical trials, clinical cancer research cannot move forward. A cancer diagnosis is a devastating and life-altering event for a patient. The bond that often develops between a cancer patient, the clinical team, and the oncologist is very strong, and is built upon time spent with a patient educating them about their disease and treatment options. This education extends beyond the exam room, and is highlighted by my participation in the annual California Kidney Cancer Foundation annual Survivor's Day meeting. By participating as part of a multidisciplinary team of oncologists, medical oncologists, and

radiation oncologists, I strive to provide the best possible clinical care for my urologic cancer patients, integrating both standard as well as investigational therapies into their treatment.

B. Funding Information

Past Funding:

2005-2006	DOD PC050338 Clinical Trial Development Award Phase I/II trial of epothilone analog ixabepilone, mitoxantrone, and prednisone in hormone refractory prostate cancer patients previously treated with chemotherapy	Principal Investigator
2007-2008	UCSF Research Evaluation and Allocation Committee Identification and validation of genomic events associated with hormone resistance in metastatic prostate tumors	Co-Investigator

Current funding

2005- 2009	DOD PC041220 Physician Research Training Award: Gene expression analysis of circulating hormone refractory prostate cancer micrometastases.	Principal Investigator
2009- present	Dana Farber Cancer Institute Dunkin Donuts Award Personalized therapy for urothelial carcinoma	Principal Investigator

C. Report of Current Research Activities

Clinical Trials

Phase I/II study of sorafenib and RAD001 in patients with metastatic renal cell carcinoma	Principal Investigator
Phase I/II Trial of epothilone analog BMS247550 (ixabepilone), mitoxantrone, and prednisone in hormone refractory prostate cancer patients previously treated with chemotherapy	Principal Investigator
CALGB 90601: A randomized phase III study of gemcitabine, cisplatin, bevacizumab, or gemcitabine, cisplatin, placebo in patients with advanced transitional cell carcinoma	Principal Investigator
Phase II study of c-MET RTK inhibitor XL880 in patients with metastatic papillary renal cell carcinoma	Co-Investigator
A phase II study of GW786034 using a randomized discontinuation design in subjects with locally recurrent or metastatic clear-cell renal cell carcinoma	Co-Investigator

Randomized Phase II Trial of Maintenance SU011248 Versus Placebo Post Chemotherapy for Patients With Advanced Urothelial Carcinoma Co-Investigator

Translational Science

Analysis of genomic alterations in circulating hormone refractory prostate cancer cells Principal Investigator

Identification and validation of genomic events associated with hormone resistance in metastatic prostate tumors Co-Investigator

Molecular markers of responsiveness to RAD001 and sorafenib Principal Investigator

Personalized therapy for urothelial carcinoma Principal Investigator

D. Report of Teaching

1. Local contributions

a. Medical student, medical resident, and medical oncology fellow clinical teaching:

2003-2008	Oncology Fellowship Core Curriculum lectures on bladder cancer. Preparation time: 3 hours, Contact time: 2 hours.
2005	Family Medicine Board Review Course: Hematology Review. Preparation time: 6 hours. Contact time: 3 hours.
2005-2008	Oncology Fellowship Core Curriculum lectures on renal cancer. Preparation time: 3 hours, Contact time: 2 hours.
2005-2008	Lecturer, Residency Subspecialty Lectures on prostate, bladder, and kidney cancer, 50 residents. Preparation time: 10 hours. Contact time: 6 hours.
2006	Small group leader for Cancer: Bench to Bedside. Led series of tutorial sessions for 15 medical students. Preparation time: 4 hours. Contact time: 8 hours.
2007	UCSF Radiation Oncology Core Curriculum, "Treatment of Advanced Kidney Cancer." Lecture to 15 residents. Preparation time: 2 hours. Contact time: 2 hours

c. Local invited teaching presentations

2002-2003	Lecturer, San Francisco General Hospital Oncology Education Series. "Testicular Cancer Therapy." Students, housestaff, and staff. 75 attendee's per year.
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- 2003 UCSF Division of Hematology/Oncology, Grand Rounds, “Toxic telomeres: A novel anti-cancer therapeutic strategy”
- 2006 UCSF Cancer Center Annual Cancer Update, Invited Lecture, Squaw Valley, CA. “Kidney Cancer Update: A changing paradigm.”
- 2006 California Kidney Cancer Foundation, Seminar, San Francisco, CA. “New directions in advanced kidney cancer: the ARISER and VEG102616 studies”
- 2007 UCSF Cancer Center Annual Cancer Update, Invited Lecture, Squaw Valley, CA. “Have TKI’s changed outcomes in advanced renal cancer.”
- 2007 California Kidney Cancer Foundation, Seminar, San Francisco, CA. “New directions in advanced kidney cancer: mTOR inhibitors”
- 2007 UCSF-Stanford Joint Silverado Tumor Board, Seminar, Napa, CA. Multidisciplinary management of urologic cancers.
- 2008 UCSF Cancer Center Annual Cancer Update, Invited Lecture, Squaw Valley, CA. “New approaches to kidney cancer: Too many choices?”
- 2008 California Kidney Cancer Foundation, Seminar, San Francisco, CA. “The role of mTOR inhibitors and novel TKI’s in papillary kidney cancer.”
- 2008 Dana Farber/Harvard Cancer Center Patient Day, Panel Member, Cambridge, MA

d. Continuing Medical Education:

- 2005 UCSF Urology Postgraduate Course, Medical Oncology Lecturer
Presented review of current management strategies for bladder and kidney cancer patients. Preparation time: 8 hours. Contact time: 3 hours.
- 2006 UCSF Current Controversies in Urologic Oncology
Presented review of current management strategies of advanced bladder cancer. Preparation time: 8 hours. Contact time: 3 hours.
- 2007 UCSF Urology Postgraduate Course, Medical Oncology Lecturer
Presented review of current management strategies for bladder and kidney cancer patients. Preparation time: 8 hours. Contact time: 3 hours.
- 2007 Oncology Instructor, UCSF Internal Medicine Review Course,
Presented comprehensive review of medical oncology. Preparation time: 12 hours. Contact time: 3 hours.

e. Advisory and Supervisory Responsibilities in clinical or laboratory setting

- 2003-2008 Outpatient Urologic Oncology Clinic Attending, UCSF Comprehensive Cancer Center: Supervised interns, residents, and fellows seeing patients with urologic malignancies. Approximately 8 sessions/month, 350 hours per year.
- 2005-2008 Attending Physician, UCSF Inpatient Oncology Service and Consult Service. Supervise and teach oncology fellows and residents. 1-2 months per year.
- 2008- present Inpatient Oncology Attending. Dana Farber Cancer Institute. Supervise and teach a team of 3 house officers. 1 month per year.

- 2008- present Attending Physician, Lank Center for Genitourinary Oncology:
Supervise and teach oncology fellows. Approximately 4 hours/month,
50 hours/year.

f. Teaching Leadership

- 2006-2008 Director and lecturer, Clinical Research Module, UCSF Division of Hematology/Oncology Core Curriculum Lecture Series. Designed the curriculum, selected lecturers, and organized the sessions. Preparation time: 18 hours. Contact time: 4 hours/year.
- 2008-present Director of Fellow Education, Lank Center for Genitourinary Oncology. Ensure fellows receive a balanced and broad education in genitourinary oncology.

g. Advisees/Trainees

- 2003-2004 Jorge Garcia, MD, Associate Staff, Taussig Cancer Center, Cleveland Clinic
- 2005-2006 Amy M. Lin, MD, Assistant Clinical Professor, UCSF
- 2005-2006 Amir Goldkorn, MD, Assistant Professor, University of Southern California
- 2007 Rosendo So-Rosillo, MD, Private Practice, Mountain View, CA
- 2007-2008 Andrea Harzstark, MD, Assistant Clinical Professor, UCSF

2. Regional, national, or international contributions

a. Invited presentations

National:

- 2005 Cancer and Leukemia Group B, Summer Meeting Genitourinary Committee Plenary Presentation. "When Taxotere fails: Current status and future directions"
- 2005 ASCO/AACR Multidisciplinary Prostate Cancer Symposium, Orlando, FL. "Response to second-line taxane based therapy after 1st line epothilone B analog ixabepilone therapy in hormone refractory prostate cancer"
- 2006 National Kidney Foundation Meeting, Invited Lecture, Chicago, IL. "Novel treatments for advanced renal cell carcinoma"
- 2007 Cancer and Leukemia Group B, Summer Meeting Genitourinary Committee Plenary Presentation, Baltimore, MD. "Targeting angiogenesis in advanced bladder cancer: rationale for CALGB 90601"
- 2007 US Oncology Community Oncology Research Leadership Conference, Dallas, Tx. Invited Lecture. "Targeted therapy in advanced bladder cancer"

- 2007 Sixth International Kidney Cancer Symposium, Invited Lecture, Chicago, IL. "XL880: MET and VEGF receptor inhibition in papillary kidney cancer"
 - 2008 Seventh International Kidney Cancer Symposium, Invited Lecture, Chicago, IL. "Management of bone metastases in kidney cancer."
 - 2008 American Society of Clinical Oncology, Annual Meeting, Chicago, IL. Session Chair, "Kidney, Bladder, and Testis Cancer".
- International:
- 2004 Cancer and Leukemia Group B, Summer Meeting Genitourinary Committee Plenary Presentation, Montreal, Canada. "Advanced bladder cancer: State of the Art"
 - 2006 International Congress on Kidney and Bladder Cancer, Orlando, FL. Invited Lecture, "Targeting Angiogenesis in advanced bladder cancer"
 - 2007 International Congress on Kidney and Bladder Cancer, Orlando, FL. Invited Lecture, "Current neoadjuvant and adjuvant therapies for bladder cancer"
 - 2008 Eighth International Congress on Genitourinary Malignancies, Washington, DC. Invited Lecture, "Current neoadjuvant and adjuvant therapies for bladder cancer"

E. Report of Clinical Activities

1. Description of clinical practice

From 2003 to 2008, I was a medical oncologist at the UCSF Comprehensive Cancer Center caring for patients with adult urologic cancers (kidney, bladder, prostate, and testis). At UCSF, I saw patients as a consultant, as well as for ongoing care. I spent two full days per week in clinic, and saw 6-7 new patients and approximately 30-35 follow-up patients per week. I also attended on the inpatient consultation service and followed patients admitted to the hospital. I currently see patients at the Lank Center for Genitourinary Oncology at the Dana Farber Cancer Institute, where I see 8 new patients and up to 30 follow-up patients each week. I consult on inpatients with genitourinary cancers, as well as spend 6 weeks annually leading an inpatient medical oncology service.

2. Patient load

I saw approximately 175 new patients or consultations and approximately 1100 follow-up visits in FY2006. Each year, I attended on the UCSF inpatient oncology consultation service for 4-6 weeks per year. In addition to consulting responsibilities, during that time I managed the inpatient chemotherapy administration for solid tumor oncology patients. At the Dana Farber, I attend as the inpatient oncology attending 6 weeks per year managing a team of 6-16 patients.

3. Clinical contributions

At UCSF, I supervised a nurse practitioner, a fellow, and a registered nurse as part of my daily practice duties. I have participated in initiatives to begin the transfer of the UCSF

outpatient oncology practice to an online medical record, and have worked with the administrative staff to improve clinic patient flow and access. I coordinated the care for patients with muscle invasive bladder cancer who require multimodality therapy, and actively participated in the UCSF Urologic Oncology Tumor Board. At the Dana Farber Cancer Institute, I will be supervising a nurse practitioner as well as rotating fellows in the Lank Center for Genitourinary Oncology.

4. Other relevant information about clinical role

I have participated as a member of the UCSF-Stanford Northern California Tumor Board, a regional event that educates community oncologists in the multidisciplinary care of oncology patients, and devoted time to patient outreach events educating patients regarding kidney cancer.

PART III: BIBLIOGRAPHY

Original Articles

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2. Li S, **Rosenberg J**, Donjacour AA, Botchkina IL, Hom D, Cunha GR, and Blackburn EH. Rapid inhibition of cancer cell growth induced by lentiviral delivery and expression of mutant-template telomerase RNA and anti-telomerase short-interfering RNA. *Cancer Res* 2004; 64:4833-40.
3. **Rosenberg J**, von der Maase H, Seigne JD, Mardiak J, Vaughn, DJ, Moore M, Sahasrabudhe D, Palmer PA, Perez-Ruixo JJ, Small, EJ. A phase II trial of R115777, an oral farnesyltransferase inhibitor (FTI), in patients with advanced urothelial tract transitional cell carcinoma. *Cancer* 2005; 103:2035-41.
4. **Rosenberg J**, Galsky M, Rohs N, Oh W, Kelly WK, Small EJ. A retrospective evaluation of second-line chemotherapy response in hormone-refractory prostate carcinoma: second line taxane-based therapy after first-line epothilone-B analog ixabepilone (BMS-247550) therapy. *Cancer* 2006; 106:58-62.
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7. Garcia J, **Rosenberg JE**, Weinberg V, Scott J, Frohlich M, Park JW, Small EJ. Evaluation and significance of circulating epithelial cells in hormone refractory prostate cancer patients. *BJU Int* 2007; 99:519-24.
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9. Lin, AM, Rini BI, Kakefuda M, Weinberg V, Randall M, Ryan CJ, **Rosenberg JE**, Bubley G, Small EJ. A phase I trial of estramustine, docetaxel, and imatinib mesylate in hormone refractory prostate cancer patients. *Clin Genitourinary Cancer* 2007; 5: 323-8.
10. Motzer RJ, Michaelson MD, **Rosenberg JE**, Bukowski RM, Curti BD, George DJ, Hudes GR, et al. Sunitinib efficacy against advanced renal cell carcinoma. *J Urol* 2007; 178:1883-87.
11. Ryan CJ, Weinberg V, **Rosenberg JE**, Lin AM, Fong L, Kim J, Small EJ. Phase II Study of Ketoconazole Plus Granulocyte Macrophage Colony Stimulating Factor in Prostate Cancer: Effect of Extent of Disease on Outcome. *J Urol* 2007; 178: 2372-77.
12. Bradley DA, Dunn R, Nanus D, Stadler W, Dreicer R, **Rosenberg J**, Smith DC, Hussain M. Randomized blinded phase II trial of maintenance SU011248 versus placebo postchemotherapy for patients with advanced urothelial carcinoma: Scientific rationale and study design. *Clin Genitourinary Cancer* 2007; 5:460-3.
13. Ryan CJ, Harzstark A, **Rosenberg JE**, Lin AM, Claros C, Goldfine ID, Kerner JF, Small EJ. A pilot dose escalation study of the effects of nordihydroguareacetic acid (NDGA) on hormone levels and prostate specific antigen in patients with relapsed prostate cancer. *BJU Int* 2008; 101:436-9.
14. **Rosenberg JE**, Halabi S, Sanford BL, Himelstein AL, Atkins JN, Small EJ, et al. CALGB 90207: Phase II trial of bortezomib in patients with previously treated advanced urothelial tract transitional cell carcinoma. *Annals of Oncology* 2008; 19(5): 946-50.
15. Rini BI, Michaelson DM, **Rosenberg JE**, Bukowski MR, Sosman JA, Stadler WM, Hutson TE, Margolin KA, Harmon CS, DePrimo SE, Kim ST, Chen I, George DJ. Antitumor Activity and Biomarker Analysis of Sunitinib in Patients with Bevacizumab-Refractory Metastatic Renal Cell Carcinoma. *J Clin Oncol* 2008; 26(22):3743-8.
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6. **Rosenberg JE**. Current neoadjuvant and adjuvant therapies for bladder cancer. *Expert Review of Anticancer Therapy* 2007;7:1729-36.
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