CHARACTERIZATION OF CIRCULATING TUMOR DNA IN ADVANCED PROSTATE CANCER

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Sandi Alexander Kwee

Thesis Committee:
Linda Chang, Chairperson
Rosanne Harrigan
Thomas Ernst

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ABSTRACT

Background: Cell-free DNA (cfDNA) from tumors can be found in the blood of cancer patients. The clinical significance of these circulating nucleic acids is uncertain. The effects of chemotherapy on cfDNA is also not well understood, although tumor lysis may cause significant alterations in the cfDNA profile that could eventually translate into genomics-based blood tests for predicting treatment responses in cancer. Based on this premise, a pilot study was conducted testing the hypothesis that an increase in plasma cfDNA levels accompanies chemotherapy exposure. To explore the use of cfDNA profiling as a potential diagnostic complement to in-vivo molecular imaging, this pilot study was incorporated into a clinical trial of 18F-fluorocholine positron emission tomography (FCH PET) for assessing chemotherapeutic response in hormone refractory prostate cancer (HRPC).

Methods: Eight patients with HRPC receiving docetaxel chemotherapy were recruited. Blood samples were obtained on the day of whole-body FCH PET imaging performed prior to chemotherapy, and after the first and third treatment cycle. DNA was extracted from plasma and quantified by real-time quantitative PCR of genomic ALU repeats using a 115bp amplicon. Size distribution of DNA fragments was analyzed by capillary electrophoresis. The methylation status of two genes implicated in prostate cancer; GSTP1 and RARB2, was examined by PCR using primers for both methylated and unmethylated bisulfite converted sequences.

Results: Mean cfDNA levels increased significantly from a pre-chemotherapy level of 13.3 ng/ml to 46.8 ng/ml after the first chemotherapy cycle and 50.9 ng/ml after the 3rd chemotherapy cycle (ANOVA p=0.001). Plasma cfDNA fragments at baseline ranged in size from 160-180bp, consistent with DNA from apoptosis. Samples collected after chemotherapy showed additional cfDNA fragments ranging in size from 200bp-7kbs, suggesting DNA contributed by necrotic cells. All baseline samples demonstrated hypermethylation at GSTP1 or RARB2 suggesting that at least some DNA was of tumor origin. Treatment was associated with loss of GSTP1 or RARB2 methylation in 4/8 patients. Normalized plasma cfDNA level and mean tumor SUVmax were significantly correlated \((r=-0.50, p=0.01)\) and lower cfDNA levels at baseline were associated with significant tumor metabolic responses on FCH PET after the 3rd chemotherapy cycle \((8.0 \text{ ng/ml of cfDNA in PET responders vs. } 16.4 \text{ ng/ml in PET non-responders, } p=0.03)\).

Conclusions: Exposure to docetaxel appears associated with quantitative and epigenetic changes in the plasma cfDNA of patients with HRPC. Preliminary findings support a relationship between cfDNA levels and tumor activity on FCH PET encouraging further exploration of these molecular markers as potential complementary measures of chemotherapeutic response.
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ABBREVIATIONS

cfDNA  cell-free DNA
CT    Computed Tomography
DNA   Deoxyribonucleic Acids
FCH   Fluorocholine
HRPC  Hormone Refractory Prostate Cancer
GSTP1 Glutathione S-Transferase 1
LHRH  Luteinizing Hormone-Releasing Hormone
MSP   Methylation Specific Polymerase Chain Reaction
NIH   National Institutes of Health
PET   Positron Emission Tomography
PET/CT PET/ Computed Tomography
PCR   Polymerase Chain Reaction
PSA   Prostate Specific Antigen
PERCIST PET Response Criteria in Solid Tumors
RARB2 Retinoic Acid Receptor Beta-variant 2
SUVmax Standardized Uptake Value – Maximum
USP   Unmethylation Specific Polymerase Chain Reaction
Patient-individualized approaches to cancer treatment are potentially realizable with the advent of highly-sensitive and minimally-invasive techniques for molecularly characterizing malignant tumors (molecular imaging, expression analysis, gene sequencing, epigenetics). Since blood is a readily-obtained biospecimen, it would be particularly advantageous to characterize the molecular alterations in tumors affecting each individual cancer patient using blood samples. To this end, feasibility has recently been demonstrated for applying molecular-driven techniques to isolate and characterize tumor-derived cell-free DNA (cfDNA) from the circulation.

To preliminarily investigate the clinical relevance of cfDNA analysis in the management of advanced prostate cancer, I assembled a multidisciplinary team to establish methods for cfDNA isolation and analysis in our laboratories. We obtained blood samples from patients taking part in my on-going clinical trial (NCT00928252, www.clinicaltrials.gov) evaluating fluorine-18 fluorocholine positron emission tomography (FCH PET) imaging as a measure of effectiveness for chemotherapy treatment of hormone-refractory prostate cancer. The following aims were then pursued: 1) incorporate contemporary polymerase-chain reaction-based approaches to characterize serial cfDNA samples from patients with advanced prostate cancer receiving docetaxel-based chemotherapy, 2) perform epigenetic analysis to identify potential markers of response while supporting the tumor-origin of the cfDNA, and 3) correlate the results of cfDNA analysis with the results of FCH PET to explore the complementary diagnostic value that may exist between these distinct molecular approaches. The translational goal is to align multiple molecular analytic and diagnostic approaches along a common clinical strategy that will support patient-individualized treatment of cancer.
CHAPTER 1: INTRODUCTION

Extracellular deoxyribonucleic acids (DNA) of tumor origin can be found in the circulation of patients with cancer\textsuperscript{1, 2}. However, the clinical relevance of this circulating cell-free DNA (cfDNA) remains uncertain. While it has been proposed that elevated levels of cfDNA in cancer patients are the result of increased tumor cell turnover, other explanations, including the active secretion of DNA by cancer cells, have not been ruled out\textsuperscript{3, 4}. Investigations into changes taking place in the cfDNA profile of patients exposed to cytotoxic agents may enhance our understanding of the etiology of this circulating genetic material, and potentially lead to the development of novel molecular assays for guiding cancer treatment.

Prostate cancer is the second leading cause of cancer death in men behind lung cancer. We conducted a pilot study to investigate the potential clinical relevance of cfDNA in patients with advanced prostate cancer receiving chemotherapy. The use of chemotherapy to prolong survival in prostate cancer is a recent advance in clinical oncology, based on the results of two clinical trials published in 2004 demonstrating a survival benefit in hormone-refractory prostate cancer (HRPC) treated with docetaxel-based chemotherapy\textsuperscript{5, 6}. Unfortunately, the survival benefits demonstrated in these trials were small, on the order of several months. Therefore, there are continued efforts to develop better agents, including molecularly-targeted agents which could be used alone or in combination with chemotherapy to further improve outcomes in HPRC.

Along with the advent of chemotherapy for HRPC, there has been increasing recognition of the difficulty associated with predicting how patients with advanced cancers will respond to treatment. Although useful as a long-term biomarker of progression, prostate specific antigen (PSA) levels measured acutely have not proven predictive of chemotherapeutic outcome, with approximately half of docetaxel
responders in HRPC clinical trials showing no significant decline in PSA initially\textsuperscript{5,6}.

Because the overall survival benefit of chemotherapy is small in HRPC, there are concerns that chemotherapy is being given to a substantial number of patients who may fail to realize any benefit. It would therefore be advantageous for there to be a “real-time” marker of chemotherapy response so that those in whom the chemotherapy is ineffective can be spared the hazards of further treatment. Early identification of treatment non-responders would also allow alternative agents to be tried. Furthermore, the ability to measure tumor responses in a timely fashion could be an accelerant for drug development.

Along these lines, we are evaluating fluorine-18 fluorocholine positron emission tomography / computed tomography (FCH PET/CT), as a means of whole-body molecular imaging for assessing metastatic tumor responses following chemotherapy treatment of HPRC. FCH is an investigational radiopharmaceutical analog of choline that can be used to measure tissue choline phospholipid synthesis in vivo. Interest in this investigational diagnostic imaging agent for cancer is based on the observation of upregulated phosphocholine metabolism in many types of cancer as another potential hallmark of malignancy\textsuperscript{7,8}. Incorporating a pilot study analysis of cfDNA into this PET imaging study of HPRC allows for a novel exploration of the potential diagnostic synergy between these distinctly different molecular markers, while also determining the feasibility of using whole-body FCH PET/CT imaging to annotate prostate cancer metastases as the source of cfDNA.
CHAPTER 2: METHODS

PATIENT SELECTION

Eight patients scheduled to receive docetaxel as chemotherapy for HRPC were enrolled. Eligibility for this pilot study required clinical confirmation of HRPC determined on the basis of two consecutive rises in PSA levels measured at greater than 1 week apart while receiving treatment with an anti-androgen (eg. bicalutamide) plus luteinizing hormone-releasing hormone (LHRH) agonist for at least 3 months duration. A testosterone level of < 50 ng/ml at the time of PSA measurements was performed to confirm biochemical castrate status. Chemotherapy treatment was initiated within 30 days of enrollment. The study was approved by Institutional Review Board and all patients gave written informed consent to participate.
**CELL-FREE DNA ANALYSIS**

**Blood Specimen Collection**

Blood samples for cfDNA analysis were collected at baseline prior to chemotherapy, and after the first and third treatment cycle. Treatment regimens were based on published clinical trial protocols\(^5,6\). However, final decisions regarding the dose and timing of all treatments rested on the discretion of the patient’s medical oncologist. All samples were collected by a trained phlebotomist using an IV Vacutainer system. To avoid the possibility of genomic DNA contamination due to a skin plug at the moment of venipuncture, 5 to 10ml of blood was first collected in a separate vial and sent to a central laboratory for PSA level determination. A specialized collection vial (Cell-Free BCT, Streck, Inc., Omaha, NE) was then used to collect 10ml of blood for the study. These vials contained preservatives to specifically inhibit degradation of cfDNA by plasma nucleases and prevent the release of cellular genomic DNA from nucleated blood cells.

**Plasma Separation and DNA Extraction**

Plasma and the buffy coat were separated by centrifugation at 1,600g for 10min. DNA was extracted from plasma using the QIAamp Circulating Nucleic Acid Kit (QIAGEN Inc., USA, Valencia, CA). Additional aliquots were frozen at -80 degrees Celsius and stored for future analysis. The protocol for biospecimen processing is illustrated in figure 1.
FIGURE 1

Blood (10ml) in cfDNA tube
Centrifuge 10min at 1,600g

Plasma       1 ml               4-5 ml
Extraction by
Qiagen Circulating
Nucleic Acid Kit
Freeze at -80 in
4-5 1ml aliquots
Thaw and Extract
Bioanalyzer analysis
Sizing range 50bp -7kbp
Quantitative range 5-500 pg/ul

Quantification by real-time quantitative
PCR and Methylation analysis by
methylation sensitive-PCR

Figure 1. Plasma Sample Preparation Protocol
Quantitative Real-time PCR

The ALU repeat is the most abundant repeated sequence in the human genome with an estimated 1.4 million copies per genome, affording a high sensitivity for DNA detection and quantification when used as the target for PCR based amplification. A 115bp amplicon was selected and used for real-time quantitative PCR (qPCR) of genomic ALU repeats. The sequence of the forward and reverse primers were 5’-CCTGAGGTCAGGAGTTCGAG-3’ AND 5’-CCCGAGTAGCTGGGATTACA-3’ respectively. Real-time PCR amplification was performed with a precycling heat activation of DNA polymerase at 95°C for 10 min, followed by 45 cycles of denaturation at 95°C for 30 sec, annealing at 64°C for 30 sec, and extension at 72°C for 30 sec using the ABI 7900HT system (Applied Biosystems, Foster City, CA). The absolute equivalent amount of DNA in each sample was determined by a standard curve with serial dilutions (1 ng to 0.01 pg) of purified DNA obtained from peripheral blood from a healthy volunteer. This relatively short amplicon was selected to allow truncated DNA (typical size range 180-200bp) from apoptotic cells to be detected. Quantitative results were expressed as nanograms of DNA per milliliter of plasma (as derived from a standard curve).

DNA Fragment Length Analysis

Capillary electrophoresis using the Agilent 2100 Bioanalyzer and High Sensitivity DNA Chips (Agilent technologies Inc., Palo Alto, CA) was performed to assess DNA fragment length. Quantification results were also obtained from the Bioanalyzer analysis. The DNA fragment length analysis was based on manufacturer recommended protocol and allows for a sizing range between 50 and 7000 base-pairs.
**Methylation Specific PCR**

Methylation specific PCR (MSP) was performed to evaluate two tumor suppressor genes reported to be frequently hypermethylated in prostate cancers: GSTP1 (glutathione S-transferase1) and RARB2 (retinoic acid receptor beta-variant 2). Sodium bisulfite treatment of genomic DNA using the EZ DNA Methylation-Direct Kit (Zymo Research, Irvine, CA) was followed by MSP and Unmethylation-Specific PCR (USP). Methylation (M) and unmethylation (U) specific primers used to amplify and detect methylated and unmethylated DNA were: GSTP1, (M) F-TTCGGGGTGTAGCGGTCGTC and R-GCCCCAATACTAAATCACGACG, (U) F-GATGTTTGGGGTGTAGTGG; RARB2, (M) F-GAACGCGAGCGATTGAGT and R-GACCAATCCAAACCAGAAAGC, (U) F-GGATTGGGATGTTGAGAATGT and R-CAACATCCAAACCAATATTGTT and R-CCACCCCAATCTAAATCACACA.

We carried out PCR after optimizing annealing temperatures for each primer set (60 °C for RARB2 and GSTP11 ul of bisulfite converted DNA was used for MSP and USP). An initial 10-minute incubation at 95 °C was followed by 45 cycles of denaturation at 95 °C for 30 s, annealing for 30 s, and extension at 72 °C for 30 s, and a final hold for 10 min at 72 °C. Genomic DNA extracted from the buffy coat DNA of each patient served as the negative control for methylation analysis.
Radiopharmaceutical Synthesis

Radiopharmaceutical-grade FCH was synthesized in accordance to an Investigational New Drug application by fluorination of ditosylmethane with fluorine-18 followed by alkylation of the fluorotosylmethane intermediate with dimethylethanolamine using a chemical process control unit (CTI/Siemens CPCU, CTI/Siemens, Knoxville, TN)\textsuperscript{15}. All synthesis products passed standard assays for radiochemical purity, radionuclidic identity, chemical purity, and pyrogenicity prior to injection. Radiochemical purity was greater than 99%.

PET/CT Imaging

PET combined with in-line computed tomography (CT) was performed using a Philips Gemini TF-64 PET/CT scanner (Philips Healthcare, Andover, MA). A CT transmission scan was first performed in the supine position from the pelvis to the skull. The 64-channel helical CT scanning parameters were: 120 kV, 50 mA/slice, rotation time 0.75 sec, slice thickness/interval 5.0mm. No intravenous contrast was used for CT. At approximately 12-15 minutes after intravenous injection of 2.6 MBq/kg (0.07 mCi/kg) of FCH, emission scans were acquired from mid-thigh to skull over 9-11 bed positions at 2-minutes per bed position. Image reconstruction employed a list-mode version of a maximum likelihood expectation maximization algorithm. CT data was used for attenuation correction.
Image Analysis

The PET/CT images were examined visually by a board-certified nuclear-medicine physician (SK) experienced in visual interpretation of FCH PET/CT images of prostate cancer patients\textsuperscript{16-18}. Focal areas of increased FCH uptake identified on the PET images were categorized based on CT localization to a visceral organ (including prostate), lymph node, or skeletal structure (Figure 1). The finding of increased uptake in inguinal or axillary nodes was an exception to this criteria given that increased uptake in benign superficial lymph nodes is known to be a possibility with FCH\textsuperscript{19}. Two-dimensional region of interest (ROI) analysis was performed on each visually identified lesion, to measure and record the maximum standardized uptake value (SUVmax) corresponding to each lesion. For each image pixel, an SUV is defined as the measured pixel activity divided by the injected radioactivity normalized to body weight. For each patient, the presence of a response on FCH PET/CT was then determined using criteria adapted from the PET Response Criteria in Solid Tumors (PERCIST)\textsuperscript{20}. A > 30\% decline in mean lesion SUVmax across all lesions between the base FCH PET/CT scan and the scan performed after the 3rd chemotherapy cycle was defined as a positive PET response.
STATISTICAL METHODS

Data analysis was performed using JMP 8.0 (SAS Institute). To achieve a normal distribution of the data, a logarithmic transformation was applied to mean tumor SUVmax, PSA values, and cfDNA quantities. However, untransformed values are given for reporting purposes. A scatterplot matrix was generated and the potential associations between cfDNA level (ng/ml), mean tumor SUVmax, and PSA level (ng/ml) were examined by correlation analysis. Mixed-model ANOVA was used to assess the significance of change across multiple timepoints. A 2-tailed p-value less than 0.05 defined statistical significance.
CHAPTER 3: RESULTS

SUBJECT CHARACTERISTICS

All patients were found to have testosterone levels of < 50 ng/ml at enrollment, consistent with chemical castration resulting from current anti-androgen therapy. All patients met criteria for the diagnosis of HRPC on the basis of two consecutive increases in PSA level while on anti-androgen therapy. The patient characteristics at enrollment and follow-up PSA values are summarized in Table 1. There were no significant differences between PSA levels measured prior to chemotherapy initiation, after the 1st cycle, and after the 3rd cycle. Patients 5 and 8 underwent radical prostatectomy as initial treatment for prostate cancer but had experienced subsequent metastatic recurrences leading to HRPC. The remaining patients had metastatic disease at clinical presentation requiring hormonal and subsequently anti-androgen therapy. These patients did not undergo primary treatment at the time of initial diagnosis, although patient 1 received radiation therapy to the prostate and pelvis approximately 1 year prior to enrollment, and patients 4 and 6 received radiation therapy for bone symptom palliation approximately 1 month before enrollment. Patient 5 received radiation therapy for bone symptom palliation immediately before receiving the 3rd chemotherapy cycle.
<table>
<thead>
<tr>
<th>Patient ID</th>
<th>Age (yrs)</th>
<th>Duration of prior hormonal treatments (months)</th>
<th>Baseline PSA at enrollment (ng/ml)</th>
<th>PSA after 1&lt;sup&gt;st&lt;/sup&gt; chemotherapy cycle</th>
<th>PSA after 3&lt;sup&gt;rd&lt;/sup&gt; chemotherapy cycle</th>
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<td>20</td>
<td>28.0</td>
<td>24.4</td>
<td>15.0</td>
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Table 1. Patient Characteristics and Serial PSA Measurements
CELL-FREE DNA QUANTIFICATION

The PCR efficiency of the 115bp ALU amplicon was confirmed at 94% by standard curve analysis using serial dilutions of genomic DNA from 1ng to 0.01 pg (Figure 2). Mean cfDNA levels based on qPCR increased from a pre-chemotherapy level of 13.3 ng/ml to 46.8 ng/ml after the first chemotherapy cycle and 50.9 ng/ml after the 3rd chemotherapy cycle (ANOVA p=0.001) (Figure 3). On pairwise testing, the changes in log cfDNA concentration from baseline measured after one and three chemotherapy cycles were significant (p=0.02 and p=0.003, respectively on paired t-tests). There was a mean 5-fold mean increase after the first chemotherapy cycle and a mean 4.7-fold increase after the third chemotherapy cycle (Figure 4). Correlation analyses between log transformed data revealed significant correlations between cfDNA concentration and mean SUVmax (r=-0.50, p=0.01) and between PSA and mean SUVmax (r=0.61, p=0.002) (Figure 5). No significant correlations were noted between cfDNA and PSA levels.
Figure 2. **Standard PCR Curve for ALU 115bp Amplicon.** Generated with serial dilutions (1ng to 0.01pg) of genomic DNA.

Figure 3: **Plot of cfDNA concentrations at Different Chemotherapy Timepoints.**

Mean concentrations are indicated by green line. Timepoints correspond to pre-chemotherapy (1), after the 1st chemotherapy cycle (2), and after the 3rd chemotherapy cycle (3).
FIGURE 4

Figure 4. Patterns of change in cfDNA levels over time during the course of docetaxel-based chemotherapy. Timepoints correspond to pre-chemotherapy (1), after the 1st cycle (2), and after the 3rd cycle (3).
Figure 5. Scatterplot Examination of Correlation Between Mean Tumor SUVmax, cfDNA Concentration, and PSA.
**DNA FRAGMENT LENGTH ANALYSIS**

The majority of specimens contained DNA in a size range of 160-180bp that was consistent with digested DNA released from apoptotic cells. Samples collected after the first chemotherapy cycle from patients 1 and 2 showed additional DNA fragments ranging in size from 200bp to 7kb suggesting that this was a mixture of DNA derived via apoptotic and necrotic mechanisms (figure 6). The cfDNA concentrations determined by ALU qPCR were moderately correlated with concentrations obtained by Bioanalyzer analysis (Pearson correlation 0.58, p-value=0.004).

**FIGURE 6**

![Figure 6](image)

*Figure 6. Capillary electrophoresis analysis of cfDNA fragment length.* DNA concentrations estimated using the 2100 Bioanalyzer are shown below each lane. Samples were obtained prior to chemotherapy (timepoint 1) and after the 1st chemotherapy cycle (timepoint 2). Samples obtained at timepoint 2 from patients 1 and 2 contained substantially larger DNA fragments with a size range of 200bp-7kbp (red arrows) consistent with necrosis as a source of cfDNA in these samples.
**EPIGENETIC ANALYSIS**

Methylation assay controls were performed using methylated and unmethylated DNA samples (Figure 7). The plasma cfDNA samples from 2 patients demonstrated methylation at both *GSTP1* and *RARB2*. Methylation at only *RARB2* was demonstrated in one patient, and at only *GSTP1* in 5 patients. Baseline DNA extracted from nucleated blood cells from the same patients were unmethylated, supporting that at least some cfDNA from all samples were of malignant tumor origin. Loss of methylation at either *GSTP1* and *RARB2* after chemotherapy was observed in patients 1, 2, 4, and 7 (Figure 8). This loss of methylation was not associated with significant differences in the PSA level or change in PSA at different timepoints.

**FIGURE 7**

<table>
<thead>
<tr>
<th>Assay controls</th>
<th>1</th>
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</thead>
<tbody>
<tr>
<td>Me</td>
<td>Un</td>
<td>Me</td>
</tr>
</tbody>
</table>

**Figure 7:** Controls of methylation assay of cfDNA for *GSTP1* and *RARB2*. 1: Fully methylated DNA, 2: Fully unmethylated DNA, Me: Methylation assay, Un: Unmethylation assay.)
**Figure 8. Methylation assay results for cfDNA-associated GSTP1 and RARB2.**

Plasma cfDNA samples from 2 patients demonstrated methylation at both GSTP1 and RARB2; one at RARB2 only and 5 at GSTP1 only (at baseline and/or after chemotherapy) suggesting tumor origin of the cfDNA. The buffy coat DNAs from same patients studied at baseline were unmethylated.
**PET IMAGING**

There was a weak but statistically significant correlation between the number of abnormalities detected on FCH PET/CT and PSA level ($r=0.46$, $p=0.02$). Mean tumor SUVmax decreased from a baseline of 6.8 to 6.1 after the 1\textsuperscript{st} chemotherapy cycle and to 4.7 after the 3\textsuperscript{rd} chemotherapy cycle (Figure 9). The overall difference in mean tumor SUVmax across timepoints was not statistically significant on the basis of ANOVA ($p=0.13$). However, paired testing between individual timepoints detected a significant decrease in mean tumor SUVmax from baseline to after the 3\textsuperscript{rd} chemotherapy cycle ($p=0.0004$) and between the timepoints after the 1\textsuperscript{st} and 3\textsuperscript{rd} chemotherapy cycles ($p=0.003$). A significant PET response (>30% mean tumor SUVmax decrease across all metastases) was noted in patients 1, 2, and 8 (Figure 10). Notably, patients 1 and 2 also exhibited the largest fold-increases in cfDNA levels. Large necrotic DNA fragments were also specifically found in the post-chemotherapy samples of these two patients.

Lower baseline plasma cfDNA level was associated with significant tumor metabolic responses on FCH PET after the 3\textsuperscript{rd} chemotherapy cycle, defined as a >30% decline in mean tumor maximum standardized uptake value (SUVmax) (8.0 ng/ml of cfDNA in PET responders vs. 16.4 ng/ml in PET non-responders, $p=0.03$) (Figure 11). There were no significant differences in PSA levels and changes in PSA levels (after both the first and 3\textsuperscript{rd} cycles of chemotherapy) between PET responders and non-responders. The longitudinal changes in cfDNA and mean tumor SUVmax are summarized in Table 2.
<table>
<thead>
<tr>
<th>Patient</th>
<th>Baseline cfDNA level (ng/ml)</th>
<th>Fold Change in cfDNA After 1 Cycle</th>
<th>Fold Change in cfDNA After 3 Cycles</th>
<th>Change in Mean Tumor SUVmax After 1 Cycle</th>
<th>Change in Mean Tumor SUVmax After 3 Cycles</th>
<th>Sites of Malignancy Identified on FCH PET/CT</th>
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<td>3.0</td>
<td>3.5</td>
<td>-34%</td>
<td>-37%</td>
<td>L</td>
</tr>
</tbody>
</table>

Table 2: Chemotherapy Associated Fold Changes in cfDNA concentration and mean % Changes in Tumor SUVmax. For malignant sites identified by FCH PET/CT, S=skeletal system, L=lymph nodes, V=visceral, and P=prostate.
Figure 9. Change in Mean Tumor SUVmax with Chemotherapy. Timepoints correspond to pre-chemotherapy (1), after 1 chemotherapy cycle (2), and after 3 chemotherapy cycles (3).
Figure 10. Example of FCH PET/CT of Metastatic Prostate Cancer. Prior to chemotherapy, the FCH PET/CT image (Figure 1A) demonstrates 7 metabolically active skeletal metastases involving multiple thoracic and lumbar vertebrae (arrows). After the 3rd cycle of chemotherapy, FCH PET/CT scan performed subsequently (Figure 1B) demonstrates persistently elevated activity in only 4 of the 7 previously identified metastases (arrows). A greater than 30% mean decrease in the measured activity of all metastases was classified as a positive PET response.
Figure 11. Boxplot of cfDNA concentration by PET response.
CHAPTER 4: DISCUSSION

The presence of free nucleic acids in the peripheral blood was first reported in 1948 by Mandel and Metais, preceding by a few years the landmark discovery of the molecular structure of DNA by Watson and Crick \(^4\). It took almost three decades until this finding was also reported in cancer patients by Leon et al \(^1\). Although it was not possible to confirm the tumor origin of cfDNA in cancer using techniques available at the time, notable observations made by these investigators were higher cfDNA levels in patients at more advanced stages of cancer, and a decrease in these levels following successful anti-cancer therapy. It was only in the late 1980s and early 90s that substantial proof of the tumor origin of cfDNA in cancer patients was provided on the basis of recently developed molecular assays capable of detecting alterations such as oncogene mutations and microsatellite instability in cfDNA \(^2, 11, 21-25\).

PCR-based approaches for characterizing the genome, along with recently developed molecular imaging techniques for visualizing tumor biochemical and molecular processes in-vivo, are recent examples of the unprecedented capabilities at hand for molecularly characterizing tumors in a minimally invasive manner. With large investments being made in pharmaceutical development pipelines for target-specific anti-cancer agents, it has become desirable to have available minimally-invasive assays that could potentially serve as the basis of drug selection by identifying the specific molecular targets in tumors afflicting each individual cancer patient. The analysis of cfDNA as a source of genetic information for serial tumor assessment is particularly attractive for this role given that tumor biopsy is often impractical to perform on a longitudinal basis in the clinical setting, especially in patients with metastatic disease. With the advent of chemotherapy that can prolong the survival of patients with prostate cancer, we sought to preliminarily examine changes in the cfDNA profile (in terms of
quantity as well as fragment length distribution) that could potentially be the result of chemotherapy-induced tumor cell lysis. The integration of this pilot study with an on-going clinical trial of an investigational form of PET imaging (being tested as an imaging marker of prostate cancer treatment response) allowed changes in whole-body tumor metabolic activity to be correlated with the results of cfDNA analysis.

Using qPCR of repeated ALU sequences as a highly sensitive means of DNA detection and quantification, we noted a significant increase in the levels of plasma cfDNA after both the first and third cycles of chemotherapy. Not surprisingly, these changes in cfDNA level did not correlate with changes in the PSA level, since PSA is produced but not necessarily stored in large amounts by prostate cancer cells. In contrast, the release of nuclear DNA into the circulation may conceivably follow any event leading to cell lysis, including the exposure of sensitive tumor cells to cytotoxic chemotherapeutic agents. The finding of large DNA fragments (in the range of 200bp – 7kbp) in several of the post-treatment samples in this study supports cell lysis as the mechanism underlying the increase in cfDNA levels observed after chemotherapy initiation. In contrast, cfDNA arising from apoptotic cells have been shown to be smaller than 200bp in length due to their digestion by apoptosis-associated endonucleases. A similar increase in the amount of large non-apoptotic cfDNA fragments has also been reported following neo-adjuvant chemotherapy treatment of breast cancer. The mechanism of action of docetaxel as a microtubule inhibitor is consistent as a cause of cell lysis that may lead to an increase in both cfDNA amount and size. However, these findings amount to a proof of concept, and additional studies will be needed to determine the potential clinical prognostic relevance of these changes in chemotherapy-treated prostate cancer.

In order to explore potential clinical applications of cfDNA profiling, further work is needed to identify the optimal techniques for reliable quantification and characterization
of cfDNA. Currently, there is little consensus on approaches for characterizing tumor-derived cfDNA\textsuperscript{31-36}. Issues that must be dealt with before standardization can occur include the lysis of nucleated blood cells leading to contamination by cellular genomic DNA, and nucleic acid degradation over time related to serum nuclease activity\textsuperscript{19-21}. The use of collection vials designed specifically to address these issues, as used in this study, should hopefully help improve standardization efforts. While PCR based on ALU repeats affords a high level of sensitivity for DNA detection and quantification, alternative targets for PCR quantitation based on a single-copy gene could conceivably be more directly related to the magnitude of tumor burden, or cell-kill in the case of post-treatment cfDNA measurement. For example, PCR-based cfDNA quantitation using single copy genes such as hTERT have already been used in studies of lung cancer\textsuperscript{37}. It is worth pointing out that a limitation of any PCR-based quantitation method is that it can only quantify amplifiable DNA, while older methods (eg. spectrophotometry) may detect all DNA fragments, but with less sensitivity. These points underscore the need for further research to identify an optimal cfDNA quantitation scheme for clinical applications.

The methylation of cytosines at CpG islands contained in the 5' region of genes is known to be an important controller of gene expression. These so-called epigenetic alterations are observed with great frequency in cancer, and are recognized as a cause of tumor suppressor gene silencing that may lead to classic tumorigenic phenomenon such as altered signal transduction and loss of apoptotic signaling\textsuperscript{38,39}. Hypermethylated promoter regions at GSTP1 and RARB2 are among the most frequently found epigenetic alterations in prostate cancer\textsuperscript{13,14,40}. In this study, increased methylation at GSTP1 and/or RARB2 was detected in the baseline plasma cfDNA samples of each patient, providing evidence that at least some of the cfDNA obtained in this study was tumor-derived. In 4/8 patients, there was a change in the promoter methylation status after the first cycle of docetaxel chemotherapy. Several
hypotheses may explain this observation. First, docetaxel exposure may have caused
the demethylation of these gene promoter regions directly. The demethylation of gene
promoters has been proposed as a potential strategy against cancer. However, DNA
demethylation is not known to be a primary mechanism of action for docetaxel. Thus,
this appears to be an unlikely explanation for the methylation changes observed in this
study. Another potential hypothesis is that cells exhibiting GSTP1 or RARB2
hypermethylation are more sensitive to the chemotherapy, leading to the preferential
destruction of a hypermethylated tumor cell sub-population. In support of this
hypothesis, other gene methylation patterns have been associated with increased
sensitivity to taxanes in both endometrial and cervical cancer\(^{41,42}\), and various
methylation changes have also been found to be associated with response to docetaxel
in breast cancer\(^{43,44}\). Although notably, one study of breast cancer patients reported no
association between GSTP1 methylation status and primary tumor response to
docetaxel\(^{45}\). A third hypothesis to explain the DNA methylation changes in this study is
that the majority of tumor cells were eliminated after the 1\(^{st}\) chemotherapy cycle and that
the cfDNA found in subsequent samples were derived from non-tumor cells. This
hypothesis engages the possibility that increases in cfDNA could also be a reflection of
normal tissue toxicity, given that docetaxel may cause side effects to normal tissues (eg.
hemotoxicity) that may result in the lysis of rapidly dividing normal cells. However, it
appears somewhat doubtful that all cfDNA producing tumor cells would be eliminated
after just one dose of docetaxel (which is given once every 3-4 weeks in a typical
treatment cycle for HRPC). Additional studies in chemotherapy treated cancer patients
will be needed to properly test these hypotheses, with such studies potentially benefiting
from recently introduced genome-wide methylation assays that may allow for more
global assessments of epigenetic targets in cancer.
In addition to significant changes in the cfDNA profile after chemotherapy, a significant change in overall tumor metabolic activity measured by FCH PET/CT was detected after the 3rd chemotherapy cycle. In exploratory analyses, mean tumor SUVmax correlated significantly with both cfDNA concentration and PSA level, supporting a possible interrelationship between these potential measures of chemotherapeutic response. A lower baseline cfDNA concentration was also associated with a PET response measured after the 3rd chemotherapy cycle, lending further support to the possibility that information contained within the cfDNA profile may aid in predicting metabolic outcomes related to chemotherapy. In addition, the feasibility of identifying potential tumor sources of cfDNA was shown on the basis of FCH PET/CT imaging, with significant changes in cfDNA levels observed in both the patients with PET-confirmed bone-dominant metastases as well as those with lymphotrophic-predominant metastases. Thus, FCH PET/CT has the potential to serve as a guide to select biopsy targets in correlative studies examining the relationship between tumor and cfDNA.

A main limitation of this study is that it was designed as a pilot study involving few patients. These preliminary findings will require confirmation in the context of a larger study. Still, it is intriguing in that the two patients that demonstrated large amounts of necrotic-size cfDNA fragments after chemotherapy experienced a significant PET response. In addition, the 3 patients experiencing a significant PET response were among those with the largest fold-changes in cfDNA level over time. While longitudinal assessment of these observations in relation to survival are beyond the scope of this pilot study, these preliminary associations encourage further investigation in larger studies. The techniques developed in this project may also prove applicable to studies of other tumors such as breast cancer, where minimally-invasive molecular phenotyping can also be of great potential clinical benefit.
CONCLUSION

These preliminary results show a substantial increase in cfDNA levels after docetaxel treatment of advanced prostate cancer. The finding of promoter region methylation involving the GSTP1 or RARB2 genes in cfDNA samples supports at least partial tumor origin of the DNA extracted from the plasma. Changes in the methylation status of these genes may also be observed following chemotherapy. Changes in tumor metabolic activity measured in advanced prostate cancer using FCH PET/CT appear to correlate with changes in cfDNA and PSA levels. The clinical significance of these interrelationships, and particularly the potential prognostic value of these molecular marker in the management of advanced prostate cancer, is to be determined in larger studies.
November 24, 2010

TO: Sandi Kwae, M.D.
   Principal Investigator
   Department of Medicine

FROM: Nancy R. King
   Director

SUBJECT: CHS # 17604, "Chemotherapy Response Monitoring with 18F-Choline PET/CT in Hormone Refractory Prostate Cancer"

This is to acknowledge receipt of your email response received November 17, 2010, to the stipulations issued by the Committee on Human Studies (CHS) during its review of the project identified above at its meeting on November 15, 2010. The information you provided satisfactorily addressed CHS stipulations, and the project, including the revised protocol and consent form, is approved for one year effective November 23, 2010.

This project qualified for review by the Cooperative IRB, and unless notified by the participating institutions, this approval is accepted by the University of Hawaii, Hawaii Pacific Health Adult Research, the Queen’s Medical Center, Castle Medical Center and Hawaii Medical Center.

This memorandum is your record of CHS approval of this study. Please maintain it with your study records.

CHS approval for this project will expire on November 22, 2011. If you expect your project to continue beyond this date, you must submit an application for renewal of this CHS approval. CHS approval must be maintained for the entire term of your project.

If, during the course of your project, you intend to make changes, you must obtain CHS approval prior to implementing them. Unanticipated problems that are likely to affect study participants must be promptly reported to the CHS.

You are required to maintain complete records pertaining to the use of humans as participants in your research. This includes all information or materials conveyed to and received from participants as well as signed consent forms, data, analyses, and results. These records must be maintained for at least three years following project completion or termination, and they are subject to inspection and review by CHS and other authorized agencies.

Please notify this office when your project is completed. Upon notification, we will close our files pertaining to your project. Reactivation of CHS approval will require a new CHS application.

Please contact this office if you have any questions or require assistance. We appreciate your cooperation, and wish you success with your research.

C: The Queen’s Medical Center IRB
   Hawaii Pacific Health Adult Research IRB
   Hawaii Medical Center IRB
   Castle Medical Center IRB

1960 East-West Road, Biomedical B104, Honolulu, Hawaii 96822-2303
Telephone: (808) 956-5007, Facsimile: (808) 956-8683, Website: www.hawaii.edu/irb
CHEMOTHERAPY RESPONSE MONITORING WITH 18F-CHOLINE PET/CT IN HORMONE REFRAC TORY PROSTATE CANCER

Principal Investigator: Sandi Kwee, MD
Co-investigator: Marc Coel, MD
Address: The Queen’s Medical Center, PET Imaging Department
1301 Punchbowl Street
Honolulu, HI 96813
Phone: Investigator: (808) 585-5466, Scheduling: (808) 537-7077
Sponsor: National Institutes of Health / National Cancer Institute
Bethesda, MD

Why am I being asked to take part in this research study?
You are invited to take part in this research study because you have prostate cancer and are showing signs that your disease is resisting treatment. Because of this, your doctor may prescribe to you chemotherapy treatment with a drug called docetaxel. The purpose of this research study is to see if the effects of the chemotherapy on prostate cancer can be detected by a medical imaging test called PET/CT. PET/CT stands for Positron Emission Tomography / Computed Tomography. PET/CT produces images by measuring the body's metabolism (use) of nutrients given in the form of an radioactive drug called a tracer. The tracer that will be used in this study is called 18F-choline. In other research studies, 18F-choline has been used to successfully detect areas of the body involved with prostate cancer. The United States Food and Drug Administration (FDA) allows for 18F-choline to be used only for research. This research study will be conducted only at The Queen’s Medical Center.

What is informed consent?
Before you decide whether or not to take part in this study, you must understand its purpose, how it may help, any risks to you, and what you have to do. Your doctor or a researcher will talk to you about the study and go over information contained in this form. This process is called informed consent. The consent form also gives you information about what health information will be collected as part of the research study and how that information will be used or disclosed. If you sign this form you are agreeing to take part in this study and allow the use and disclosure of your medical records, diagnostic tests, and health information collected in connection with your participation in this study. You will be given a copy of this consent form to keep. If you do not wish to take part in this study, you will continue to receive medical care, but not as part of this study.

Before you learn about the study, it is important that you know the following:
• Taking part in this study is of your own free will. You have rights that cannot be taken away by signing a consent form.
• You may decide not to take part in the study or stop being in the study at any time without it making any difference to your care now or in the future, or to any benefits that you are allowed.
• If the study changes in any way which could make a difference to your taking part, you will be told about the changes and may be asked to sign a new consent form.

**Why is this study being done?**
The goal of this study is to determine whether 18F-choline PET/CT can measure the effects of chemotherapy treatment for prostate cancer.

**How many people will take part in the study?**
Twenty-one (21) men with prostate cancer will be recruited for this particular study.

**What happens during my involvement on this research study?**
If you decide to take part in this study, you will be asked to sign this consent form. Your oncologist (cancer doctor) will then provide us with a schedule of your chemotherapy treatment. Chemotherapy is usually given in cycles. Each cycle typically lasts 3 weeks. Your oncologist will determine the total number of cycles you will be receiving. Based on your treatment schedule, you will have to make three (3) appointments at the PET/CT Imaging Center at The Queen’s Medical Center. At each appointment, you will receive a PET/CT scan of your body. You will also be given a questionnaire about your cancer symptoms (pain and quality of life). It will take about 10 minutes to fill out this questionnaire. If you cannot complete the questionnaire, you can still take part in the rest of this study. The first appointment takes place before you start chemotherapy. The next two appointments will take place after you finish the first and third cycle of chemotherapy. You will also have blood samples taken to check your PSA (prostate specific antigen) level. The PSA is a blood test that measures the amount of prostate cancer in your body. The schedule of PET/CT scans and blood tests is shown below:

<table>
<thead>
<tr>
<th>When?</th>
<th>What will take place?</th>
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<tbody>
<tr>
<td>Within two weeks before starting chemotherapy</td>
<td>First PET/CT scan, symptom questionnaire, and PSA blood test.</td>
</tr>
<tr>
<td>Within a week after your first chemotherapy cycle</td>
<td>Second PET/CT scan, symptom questionnaire, and PSA blood test.</td>
</tr>
<tr>
<td>Within a week after your third chemotherapy cycle</td>
<td>Third PET/CT scan, symptom questionnaire, and PSA blood test.</td>
</tr>
<tr>
<td>After chemotherapy is finished</td>
<td>The final symptom questionnaire and PSA Blood Test will be done 1 month after your last chemotherapy.</td>
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If for some reason you do not finish 3 chemotherapy cycles, your 3rd and 4th appointments will be scheduled approximately 30 and 90 days after your last treatment. Each appointment for the PET/CT scan will last between one and one and a half hours. You will be asked to not eat or drink for at least two hours before your appointment. After you arrive and before you leave the PET center, your vital signs (temperature, pulse, and blood pressure) will be checked. A small plastic tube (intravenous catheter) will be placed into an arm vein. You will receive an injection of 18F-choline through this tube. The amount of 18F-choline you receive will depend on your body weight. This drug will give off a small amount of radioactive energy that can be picked up by the PET/CT scanner. The PET/CT scanner is a large donut shaped machine. You will lie on a bed as it moves slowly through the PET/CT scanner to produce an image of your body from your head to your thighs. The actual scan will take approximately 45 minutes to 1 hour. You will be asked how you are feeling during and after the scan. After the scan, the intravenous catheter will be removed and you will be asked to void (urinate).
What possible side effects or risks do I need to be aware of?

This research study will expose you to a small amount of radiation. The amount of radiation is about as much as that from a standard (diagnostic) CT scan of the body. Diagnostic CT scans are common and widely used X-ray tests. The total radiation exposure from having three 18F-choline PET/CT scans falls below limits set by the FDA for radiation exposure to medical workers and research volunteers. It is not known for certain whether this low level of radiation exposure can increase the risk of developing cancer by a small amount. If you are receiving radiation therapy at this time, the amount of radiation from this imaging test will not increase your risk of side effects from radiation. Voiding after the scan will safely minimize the radiation exposure to your bladder.

The safety of 18F-choline as a drug has been studied in animals and human beings. As of this time, there are no known side effects related to 18F-choline. Like any drug, there may be unknown side effects.

The scanner never comes into contact with you directly. However, it is possible to feel claustrophobic (afraid of being in a small space) or uncomfortable during the scan. This does not happen very often. If it does happen, you can ask at any time to stop the scan.

Blood draws or putting in the intravenous line may cause some discomfort and possibly some bruising, swelling, or infection. Also, some people get dizzy and on rare occasions, may faint. Only 1 to 2 teaspoons of blood will be taken for each PSA blood test.

The images produced by the PET/CT scan are experimental. We do not know if these scans can detect all of the disease in your body. If something found on your PET scan is felt to be important for your medical care, the research doctors will tell your own doctor about what was found. This is done out of concern for your well being, but it may make you worried. It may also cause your doctor to recommend other tests to you. You or your insurance company will have to pay for tests not performed as part of this study.

There is a risk to your privacy since you will be asked to share your personal health information with the researchers in this study. This personal information includes medical test results. Your doctor will be asked to provide some of this information.

Are there benefits to taking part in the research study?

This study is not part of your doctor’s plan for treating you. You should not expect to directly benefit from taking part in this study. However, if something found on the PET/CT is felt to have a good chance of helping you, it will be shared with your doctor. Knowledge gained in this study may help other people with your disease in the future.

What other choices do I have if I do not take part in the research study?

You can receive treatment without taking part in a research study. Your decision to take part or not take part in this study will not make any difference in your treatment. You may choose to not take part in this study without it making a difference in the care you get now or in the future.

Will my medical information be kept private?

Federal Privacy Regulations provide safeguards for privacy, security, and authorized access to health information. Information gained during this study and information known about you will be confidential (private) to the extent permitted by state and federal law. The results of this research may be presented at meetings or in publications; however, your identity will not be disclosed.
Who can receive or use the protected health information collected by this research study?

By signing this form you are authorizing the collection, use and release of your personal health information in medical records and diagnostic imaging and any health information gathered about you as part of this study. Your information will only be used/disclosed as described in this consent form and as permitted by state and federal laws. Your personal health information is health information about you that could be used to identify you. This information may include information about AIDS or HIV infection, treatment for alcohol and/or drug abuse, or mental health or psychiatric services.

Your protected health information will be used to collect the data needed to complete the research, to properly monitor (watch) how the study is done, and to answer research questions related to this study. There is no expiration date to this authorization.

Who may receive, use or release information:
Your medical records and any health information related to this study may be used or released in connection with this research study to the following:

- Dr. Marc Coel, Dr. Sandi Kwee, and their research associates (Douglas Prager, MD; James Davis, PhD; Manoj Narayanan, PhD; Maarit Tiirikainen, PhD; Liam C’Sullivan, BS; Finbar O’Sullivan, PhD; Eric Wolzinski, PhD; Damita Thomas, MD) and The Queen’s Medical Center research personnel for the purposes of conducting this research study.
- The Research and Institutional Review Committee of The Queen’s Medical Center and staff members of the Research Regulatory Office, and the University of Hawaii Committee on Human Studies, for purposes of overseeing the research study and making sure that your ethical rights are being protected.
- Providers and other healthcare staff involved in your care.
- The Cancer Research Center of Hawaii Clinical trials Unit for the purposes of conducting this research study.

Who may receive the information from the above groups:
The individuals or groups named above may release your medical records, this consent form and the information about you created by this study to:

- The sponsor of this study and their designees
- Federal, state and local agencies having oversight over this research, such as The Office for Human Research Protections in the U.S. Department of Health and Human Services, Food and Drug Administration, the National Institutes of Health,
- Representatives of outside groups hired by the hospital research department for audits to make sure studies are done as required.
- Providers and other healthcare staff involved in your care.

There is a possibility that your information may be released again by the sponsor of the study or governmental agencies described above and no longer covered by federal privacy rules.

What if I decide to stop taking part in the research study?

If you decide to end your part in the study or you are removed from the study, you may revoke (take away) your authorization. In order to take away this authorization, you must send a letter/notice to the researcher and address listed on the first page of this consent form.

If you take away your authorization, your part in the study will end and the study staff will stop collecting medical information from you and about you. The researchers and sponsor will continue to use information that has already been collected, but no new information about you will be collected unless the information is about an adverse event (a bad side effect) related to
the study or to keep the scientific integrity of the study. If an adverse event happens, the investigators, federal agencies, and hospital Research and Institutional Review Committee may need to review your entire medical record. If you choose not to be in the study, or choose to withdraw from the study, or if you refuse to sign the authorization, it will not make a difference in your usual treatment, or your payment, and it will not change your eligibility for any health plan or health plan benefits that you are allowed.

Do I have access to the information collected about me?
You may see the information in your medical record; however, the information related only to the study is kept separately and will not be available to you until the study is finished. This is designed to maintain the validity (integrity) of the study. If you wish to review your study records after the completion of the study, you should request this from the research doctor.

What happens if I am injured because I took part in this study?
If you are injured as a result of being in this study, you will get immediate medical care and treatment including hospitalization, if needed. The sponsor of the study and the study doctor do not have any funding (money) to pay for treating any injury or illness. If your insurance company does not pay for some (or all) of the treatment of your injury, you may be responsible for payment. You have legal rights if you feel you have been wrongfully injured.

Can I be removed from the research study?
You take part in this study of your own free will. You may be taken off the study without your consent if you do not keep your study visit or follow study directions.

What are the costs of taking part in this study?
The Queen’s Medical Center will pay for the PET/CT scans and PSA blood tests performed as part of this research study. You will not be charged for taking part in this study.

Will I be paid for taking part in this study?
Taking part in the study requires you to make four study visits. For your time and effort, you will receive $25 for each visit. The total amount of $100 will be sent to you in about 4 weeks after you complete the study. The study will pay for parking at The Queen’s Medical Center. If you live on another island and need to travel to Oahu to make your study visit, you will also receive $300 to help pay for airfare, incidental expenses, and taxi for each appointment.

Will I be notified of any new findings?
Any important new information learned during this study will be given to you if that information will make a difference to your willingness to continue in this study.

Who can I contact for questions or concerns?
If you have any questions about your treatment, your rights as a volunteer or any other matter relating to this study, you may call Dr. Sandi Kwee at (808)-585-5466 and talk about any questions that you might have. If you cannot get satisfactory answers to your questions or you have comments or complaints about your treatment in this study, you may contact:

Committee on Human Studies
The University of Hawaii at Manoa
1960 East-West Road, B-104
Honolulu, HI 96822
Phone: (808) 956-5007  Email: uhrb@hawaii.edu

Research & Institutional Review Committee
The Queen's Medical Center
1301 Punchbowl Street
Honolulu, HI 96813
Phone: (808) 547-4512
AGREEMENT TO TAKE PART AND CERTIFICATION AND AUTHORIZATION OF PROTECTED HEALTH INFORMATION

I, or my legally authorize representative (the legal person who cares for me) have read and understand the description of this study such as the purpose and nature of this study, its expected length, the procedures to be done, reasonably known risks and discomforts, benefits to expect, other treatments I may have, release of my medical records, payment and medical treatment for injury, and removal without my consent for this research study.

I am taking part in this study of my own free will. I may withdraw (stop taking part) and/or withdraw my authorization for use and release of protected health information at any time after signing this consent form without it making a difference to my care now or in the future or any loss of benefits that I am allowed. My consent does not take away my legal rights in case of carelessness or negligence of anyone connected with this study. My signature means that I have read the information above or that it has been read to me, my questions have been satisfactorily answered, and at any time I have other questions, I can contact the researcher listed on the first page.

Specially Protected Health Information
I agree to the release of the following information should it be contained in my medical records: Acquired Immune Deficiency Syndrome (AIDS or HIV), alcohol and/or drug abuse treatment, or behavioral or mental health services.

cc: Signed copy of consent to patient

Subject's Name (Print)          Subject's Signature          Date/ Time

Witness' Name (Print)          Witness' Signature          Date/ Time

In my judgment the subject is voluntarily and knowingly giving informed consent and has the legal capacity to give informed consent to take part in this research study.

Individual Obtaining Consent (Print)          Consenter's Signature          Date/ Time

(Investigator: fax a copy of this signed page to Research Regulatory Office at 537-7897 within 24 hours of signing.)
STORAGE AND USE OF BLOOD SAMPLES FOR USE IN FUTURE RESEARCH

This is an optional part of the research study. If you agree, a small amount of extra blood (1 to 2 teaspoons) will be collected when your PSA (prostate specific antigen) blood tests are performed. This extra sample will be used only for cancer research. You can still take part in the main study (described on pages 1 to 6) even if you do not allow a sample of your blood to be used for research. The goal of studying blood samples is to develop better tests for cancer.

How will your blood be used for other research studies in the future?

Your blood samples will be frozen and stored safely in a laboratory freezer at The Queen’s Medical Center or Diagnostic Laboratory Services (where your blood was drawn). Tests on the blood sample will be run by the Cancer Research Center of Hawaii to look for cells or other material (for example, DNA) that may allow us to learn more about your cancer.

Are there any risks or benefits with storing blood samples for research?

There is always a risk of losing privacy when medical information is shared for research. To protect your privacy, the vial containing your blood sample will be labeled with only a code instead of your name. Your blood may be used for genetic research (research about diseases that can be passed on in families). If your samples are used for genetic research, the findings will not be shared with anyone not involved in research. In the future, there may be other research studies that could benefit from using your blood sample. These studies will be required to contact you to get your permission (consent) before they can use your blood sample. To protect your privacy and welfare, all research using your blood samples will need to have the approval from an institutional review board.

You should not expect to benefit from this study. This research may help other patients with cancer in the future. There is no financial benefit from taking part in this research study. Your blood will not be sold. There is no plan to develop new products from your blood. In any case, you should not expect payment if the research leads to new products in the future.

Your blood sample may be stored indefinitely (forever). If you decide later that you want your samples destroyed, you may contact the The Queen's Medical Center Clinical Trials Office at (808) 537-7614 or write to: Queen’s Cancer Clinical Trials Office, The Queen’s Cancer Center, 1301 Punchbowl St., Honolulu, HI 96813. The Queen’s Medical Center and its researchers may throw away or end the storage of your blood without telling you.

Making your choices about future research

The choice to give a sample of your blood for future research is up to you. No matter what you decide to do, it will not affect your care.

I allow a sample of my blood and the health information collected from this study to be used for cancer research.

_________ YES  _________ NO

Subject’s Name (Print)  Subject’s Signature  Date/Time

Researcher’s Name (Print)  Researcher’s Signature  Date/Time

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REFERENCES


