Memorial Sloan-Kettering Cancer Center
IRB Protocol

IRB#: 08-114 A(4)

A Study to Assess if a Combination of Serum Measurements of Molecular Biomarkers and Serum Protein Profiling can be used to Predict which Patients Undergoing Prostatic Biopsy will be diagnosed with Cancer

MSKCC NON-THERAPEUTIC/DIAGNOSTIC PROTOCOL

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Please Note: A Consenting Professional must have completed the mandatory Human Subjects Education and Certification Program.

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Participating Institution	PI's Name	Site's Role
New York Presbyterian Hospital, Weill Medical College of Cornell University (Manhattan) Department of Urology	Douglas Scherr, MD	Data Collection site
SUNY Downstate Medical Center (DMC) Department of Urology 450 Clarkson Avenue Brooklyn, NY 11203 Kings County Hospital Center (Affiliate) 451 Clarkson Avenue Brooklyn, NY 11203	Llewellyn Hyacinthe, MD	Data Collection
New York University School of Medicine Department of Pharmacology	Thomas Neubert, PhD	Specimen Analysis

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1.0 PROTOCOL SUMMARY AND/OR SCHEMA

Current recommendations suggest that all men over the age of 50 years should have annual prostate cancer screening with serum PSA measurement and digital rectal examination. Men with an abnormal examination and/or an elevated PSA test are generally referred for a prostate biopsy. Not all men with an elevated PSA have prostate cancer. Positive biopsy rates in a screened population will be in the order of 25-40%.[1, 2] A recent study demonstrated a positive prostate biopsy rate for African American men with an elevated PSA and/or abnormal DRE to be 33%.[3]

Incidence rates of prostate cancer for African-Americans are among the highest in the world. Men of African descent have a 60 percent higher risk of developing prostate cancer than Caucasian men and have twice the risk of dying from it.[4] Furthermore, African American men have higher levels of testosterone, which may contribute to higher serum PSA levels, worse Gleason scores, and more advanced stage of disease at diagnosis. The increased risk of advanced stage disease persists in African-American men, even after adjustment for socioeconomic, educational, and clinical variables. The patient population of SUNY Downstate Medical Center, Brooklyn offers a unique opportunity to recruit a large number of African-American men. The patient population at New York Presbyterian Hospital, Weill Medical College of Cornell University, Manhattan offers the opportunity to recruit a large Caucasian population. The patient population attending MSKCC predominately have a diagnosis of prostate cancer already on their first attendance and hence are unsuitable for this study.

In a different pilot study, of Caucasian men due to undergo radical prostatectomy at MSKCC, we are addressing the question: is there a small peptide mass proteomic profile/pattern in blood that can distinguish men with a clinically insignificant/latent prostate cancer from men with more advanced pathological features? In the current study, we propose to investigate if men of both African-American and Caucasian ethnicity have a small peptide mass proteomic profile/pattern in blood that may distinguish those with prostate cancer from those without. We will obtain a pre-biopsy blood sample from all Caucasian men and men of African-American descent, who are undergoing prostatic biopsy as part of their routine care, because of suspicion of prostate cancer. Matrix-assisted laser-desorption/ionized (MALDI) time-of-flight (TOF) mass spectrometry (MALDI-TOF MS) can determine the presence and molecular mass of polypeptides in serum.[5] We will then analyze whether those who have prostate cancer on biopsy have a different small peptide mass proteomic profile in blood to those who have a negative biopsy for prostate cancer.

We will also analyze if the proteomic profile can improve the predictive power of known serum biomarkers (PSA, hK2 and solubilized urokinase-receptor forms (su-PAR)) for prostate cancer

2.0 OBJECTIVES AND SCIENTIFIC AIMS

2.1 Primary Objectives

- To determine if men with prostate cancer have a different proteomic profile than men without cancer. Cancer-free status will be confirmed by a re-biopsy at 6 months to reduce the biopsy false negative rate to less than 5 %.
- To determine whether the peptide proteomic profile can improve the predictive ability of known serum biomarkers (PSA (free and total), hK2 and su-PAR) for prostate cancer.

2.2 Secondary Objectives

- To determine if Caucasian men and men of African-American descent with and without prostate cancer have different proteomic profiles.
- To assess reproducibility of proteomic profiles over different runs, platforms, and sites.
- To procure a DNA repository from these patients undergoing prostate biopsy for future assessment of kallikrein gene expression.
- To establish a bank of DNA, serum, and frozen lymphoblastoid cells from these patients for the purpose of enabling genetic investigations in men with a diagnosis of prostate cancer.

3.0 BACKGROUND AND RATIONALE

Prostate cancer is the most common cancer in American men. In 2003, 220,900 new cases and 28,900 deaths are expected.[4] The ratio of approximately *five newly diagnosed cases for every one death* from cancer each year-relatively constant over the past 30 years-results from the protracted natural history of the disease in most patients. Incidence rates of prostate cancer for African-Americans are among the highest in the world. Men of African descent have a 60 percent higher risk of developing prostate cancer than Caucasian men and have twice the risk of dying from it. Furthermore, African American men have higher levels of testosterone, which may contribute to higher serum PSA levels, worse Gleason scores, and more advanced stage of disease at diagnosis. The increased risk of advanced stage disease persists in African-American men, even after adjustment for socioeconomic, educational, and clinical variables.

Prostate Specific Antigen (PSA) is currently used as a marker for prostate cancer. An elevated PSA and/or an abnormal digital rectal examination, or both are indicative of increased risk of prostate cancer. Patients suspected of having prostate cancer generally undergo prostate biopsy. However, not all men with an elevated PSA have prostate cancer. Positive biopsy rates in a screened population will be in the order of 25-40%.[1, 2] A recent study demonstrated a positive prostate biopsy rate for African American men with an elevated PSA and/or abnormal DRE to be 33%.[3] Complications of prostate biopsy include pain,

hemorrhage and sepsis, and on occasion death has been reported. Aggressive, biopsy-based detection strategies create the potential for the over-diagnosis and treatment of inconsequential prostate cancer. Some prostate cancers prove to be so small, low-grade and noninvasive that they appear to pose little risk to the life or health of the host. However, the biological potential of these histologically detectable cancers is difficult to characterize with certainty. While prostate cancer is unequivocally lethal in some patients, most men do indeed die *with* rather than *of* their cancer. According to autopsy data, and further highlighting the remarkable variation in behavior of this disease, histologically apparent cancer can be found in prostates from approximately 42% of men over 50 years of age who die of other causes. This frequency of histological cancer is about 4-fold higher than the lifetime risk for American men to be diagnosed with prostate cancer which is about 11% - while the risk of dying from the disease is only 3.6%.[6-8] Consequently, most physicians recommend aggressive treatment even for small biopsy detected cancers, as biopsy may underestimate the extent of cancer present in the prostate. However, recent patient series suggest that up to 20% of men undergoing radical prostatectomy have pathologic features in the radical prostatectomy specimen consistent with an insignificant cancer that posed little threat to life or health (organ-confined cancer, tumor volume < 0.5 cc, no Gleason grade 4 or 5 component).[9-11]

Human serum contains thousands of peptides, most of which are thought to be fragments of larger proteins that have been partially degraded by endogenous, proteolytic enzymes. The complex array that is created may provide a novel and robust correlate of the biological events occurring in the entire organism. Presence and molecular mass of polypeptides in unfractionated mixtures can be directly determined by matrix-assisted laser-desorption/ionized (MALDI) time-of-flight (TOF) mass spectrometry (MALDI-TOF MS) at the sensitivities and resolution that would make it a beneficial technique for serum peptide profiling. Dr. Paul Tempst at MSKCC has recently developed a novel, automated technology platform for the simultaneous measurement of serum peptides that is simple, scalable, and generates highly reproducible patterns.[5] Peptides are captured and concentrated using reverse-phase batch processing in a magnetic particle-based format, automated on a liquid handling robot, and followed by MALDI-TOF mass spectrometric read-out. The improved sensitivity and resolution allows detection of 400 polypeptides in a single droplet of serum. A pilot study indicated that sera from brain tumor patients could be distinguished from controls based on a pattern of 274 peptides. This, in turn, served to create a learning algorithm that correctly predicted 96.4% of the samples as either normal or diseased. More recent preliminary investigations have suggested that this novel small peptide mass spectrometry can distinguish men with metastatic prostate cancer from normal subjects and from men with other advanced cancers.[12] We will utilize this technology to determine the ability of proteomics to distinguish patients (both Caucasian and African-American men) undergoing prostate biopsy with histological evidence of prostate cancer from men with no evidence of cancer.

We will perform serum assays of kallikrein family of biomarkers, namely total and free PSA, and hK2 on the same pre-biopsy serum specimens. All three of these markers have been shown to improve the accuracy of PSA in predicting prostate cancer diagnosis and outcome.

Furthermore, solubilized urokinase-receptor forms (su-PAR) have recently been demonstrated to improve the ability of the kallikrein family biomarkers to predict the probability of prostate cancer in patients undergoing prostatic biopsy.[13]

Recent studies demonstrated that both serum hK2 levels and single nucleotide polymorphisms (SNPs) of the KLK2 gene were positively associated with prostate cancer both together and individually, however they did not positively correlate with each other.[14] Thus, the relationship of the SNPs of KLK2 to serum levels of hK2 and prostate cancer remains unclear yet and requires further research. As part of the collection process of the serum samples, leucocyte DNA in the buffy coat is available for collection that would normally be discarded. This potential repository of DNA would allow future DNA genotyping to be performed to assess kallikrein gene expression. We do not plan to assess kallikrein gene expression as part of this protocol but future studies may be performed to identify changes in various genes that predict cancer risk in a representative population such as this cohort of patients. These future studies would require Institutional Review Board (IRB) approval and would be in accordance with New York State Civil Rights Law §79-1(3) (a). These DNA samples will be de-identified but there will be a coded link to the clinical information in a database.

We will examine whether the peptide proteomic profile can improve the predictive ability of known serum biomarkers (PSA – free and total, hK2 and su-PAR) for prostate cancer.

All this information should allow us to better stratify which patients at risk of having prostate cancer do require prostate biopsy and which may not. This should reduce the number of negative and unnecessary biopsies performed.

4.0 OVERVIEW OF STUDY DESIGN/INTERVENTION

4.1 Design

This study will enroll two groups of 250 patients who are to undergo prostatic biopsy as part of their routine medical care because of suspicion of prostate cancer (elevated PSA between 2 and 10 ng/ml, abnormal rectal examination, or both). The first group will contain 250 patients of African-American descent and the second will contain 250 Caucasian men. The patients (both African American and Caucasian) will be recruited at both centres - New York Presbyterian Hospital, Weill Medical College of Cornell University, Manhattan and SUNY Downstate Medical Center in Brooklyn.

4.2 Intervention

Patients will undergo standard pre-biopsy, intra-biopsy and post-biopsy treatment. This is considered standard treatment and is not part of the research study. Positive biopsy rates in a screened population will be in the order of 25-40%.1, 2 False negative biopsy rate of 20% is expected in the cohort that we plan to accrue. To account for this we will recommend that any patient who still has an elevated PSA or an abnormal DRE 6 months

after a first negative biopsy should undergo a repeat biopsy at that time. Repeating the biopsy in such a high risk cohort reduces the false negative rate from 20% to less than 5%. This false negative rate of < 5% is accounted for according to the statistical analysis.

Routine histological examination of the tissue specimens is also considered standard of care and is not performed solely for research purposes. Pre-biopsy, patients will be asked to provide five tubes (40 mL) of blood for research purposes (two "tiger" top – red-black – SST-tube, two green top tubes, one purple or lavender top/EDTA tube). The red-black "tiger" top SST-tube will provide clotted blood where serum is separated from blood cells by a gel-coat after centrifugation, while the green top tube contains heparin as anticoagulant. MALDI-TOF MS-based peptide-profiling proteomics data generated so far on serum samples in Dr. Tempst's laboratory have shown that it is critical to minimize variability in pre-analytical work-up of each patient sample as conditions during processing of the blood significantly affects the contents and composition of their proteomics profile. The samples will undergo serum peptide profiling using MALDI-TOF MS[12] (see Appendix A for details of development of a serum protein profile) and LC-MS (Liquid Chromatography-Mass Spectrometry). Serum peptide profiling will be carried out at two different sites to examine reproducibility, (The Neubert Laboratory at New York University (NYU) and the Tempst Laboratory, Sloan Kettering Institute ie the NY Consortium). This is an established consortium. We distinguish two aspects of reproducibility: *analytical* reproducibility, that is, whether a proteomics machine produces similar peaks on repeated samples, and *inferential* reproducibility, whether a proteomics experiment produces similar conclusions when repeated (see Biostats section 11).

We will analyze and compare cancer serum peptidomes using two different mass spectrometry platforms, MALDI-TOF and LC-MS (MS/MS), each at two different locations, the Tempst Lab at MSKCC and the Neubert Lab at New York University (NYU); i.e., the *NY consortium*. Four standalone instruments will therefore be used in the work described in this proposal. Systematic performance and reproducibility comparisons will be carried out between the two instruments of platform and between the two platforms.

Serum peptide samples are prepared for MALDI-TOF MS analysis using an automated liquid handling system, to form a fully integrated and previously optimized platform (Appendix A). A mirror robot/MALDI platform is established at NYU, with identical liquid handler but from a different manufacturer. Conversely, a capillary liquid chromatography (cLC) system coupled to an electrospray (ESI) hybrid quadrupole (Q)-TOF mass spectrometer will first be tested and optimized for serum peptidome analysis at NYU, and then accurately copied at MSKCC using a cLC system and Q-TOF mass spectrometer from different manufacturers.

While in general the throughput of LC-MS is lower than that of MALDI-TOF, we feel that it will be very useful to use LC-MS to evaluate the biomarker discovery platform using MALDI-TOF. We anticipate that LC-MS based peptide quantitation should provide greater depth of coverage of the peptidome than MALDI-TOF because of the extra dimension of analysis provided by separating proteins into subgroups direct coupling of HPLC to the mass spectrometer. In addition, LC-MS will enable rapid identification by MS/MS of peptides of interest- i.e., those that have diagnostic value for differentiating between disease and control groups. The Neubert lab has extensive experience in using nanoflow HPLC-Q-TOF LC-MS/MS for the identification and relative quantification of proteins and peptides.

Three MS-based approaches will be established, optimized and applied to serum peptidome profiling and to plasma functional proteomics (i.e., protease activities) with a peptidomic read-out. The standard (S) approach we have taken until recently uses normalized MALDI ion intensities of the peptides for discriminant analysis and diagnostic peptide-signature discovery.[12] The same approach is commonly used in LC-MS-based proteomic profiling, but so far only for tryptic peptides, not for the serum peptidome. While we have compelling evidence that MALDI-based peptide ion-signatures can differentiate cancer samples from controls[12], relative quantitation of the relevant peptides by comparing the ion intensities to those of added, non-degradable, isotopically labeled reference peptides may make the data more consistent within data sets and reproducible over time. We will henceforth refer to this approach as "quantitative profiling" (Q). Relative quantitation of serum peptides of interest can be done by comparing the MS-ion intensities to those of added, isotopically labeled reference peptides, having the exact same sequence and otherwise same chemical properties as the endogenous ones (i.e. distinguishable by molecular mass only). As such, all peptide pairs will display the exact same MALD-ionization characteristics. Comparing ion intensities will therefore provide a means of normalizing the values for each peptide. While it does not provide absolute quantification in the absence of accurate calibration curves, it is an accepted way of expressing relative concentrations, or ratios, of two analytes. As reported, cancer serum peptide-signatures are surrogate markers for tumor-derived proteases.[12] Exogenous synthetic substrates, identical to the founder of each nested set of serum peptides, should degrade in an equal manner as the endogenous ones after addition to serum or plasma, with the important distinction that proteolysis can be readily controlled in terms of time, temperature and added amounts of substrates. Coupled to a MALDI read-out, such analyses amount to a blood "protease assay" (A) to monitor the tumor-dependent activities inferred from our prior studies. As explained above, addition of non-degradable reference peptides, will then also enable relative quantitation.

MALDI-TOF MS-based Standard Profiling (S): 500 samples from men undergoing prostate biopsy (estimated 150 malignant; 350 benign) will be analyzed. The number of MALDI-based analyses will be as follows: 500 samples x 3 independent analyses over

time = 1500 MALDI-analyses per site; 3000 analyses for both sites combined. Feature selection and class prediction will be performed on the data generated from these samples. Additionally, reproducibility will be checked.

MALDI-TOF MS-based Quantitative Profiling (Q): A random selection of 200 serum samples (100 malignant; 100 benign) will be analyzed at two time points at both *NY consortium* sites. These 200 samples will be the same samples examined by the remaining technologies. The number of analysis will therefore be: 200 x 2 = 400 per site; 800 for both sites combined. Statistical and reproducibility analysis will be same as described above

MALDI-TOF MS-based Protease Assays (A): A random selection of 200 serum samples (100 malignant; 100 benign) will be analyzed at two time points at both *NY consortium* sites. These will be the same as the "Q" samples above. The number of analysis will therefore be: 200 x 2 = 400 per site; 800 for both sites combined. Statistical and reproducibility analysis will be same as described above

LC-MS Ion Intensity-based Standard Profiling (S): The NYU site will use the optimized LC-MS protocol to analyze sera from 100 patients with prostate cancer on biopsy and sera from 100 patients with a benign prostate biopsy. These will be the same as the "Q" samples above. Both the NYU and MSKCC will analyze each sample, and all analyses will be repeated 3 months later for a total of (100 + 100) x 2 = 400 analyses performed at each site. All statistical analyses, including classification and comparison of repeat analyses at each site as well as results between sites, will be performed.

LC-MS Ion Intensity-based Quantitative Profiling (Q): A random selection of 100 malignant and 100 benign serum samples will be analyzed for a total of 200 LC-MS runs at both sites. These will be the same as the "Q" samples above. Evaluation of whether inclusion of the labeled standard peptides improves the sensitivity and specificity of the analyses will be performed.

The purple or lavender top/EDTA tube contains anti-coagulated venous blood. This allows anti-coagulated plasma to be separated immediately from blood cells. The cells, including buffy coat, will allow for DNA genotyping assessing kallikrein gene expression. The plasma will be analyzed for certain kallikrein-family protein forms (hK2, total PSA and free PSA) and soluble urokinase plasminogen activator receptor-forms.

As part of the collection process of the serum samples, leucocyte DNA in the buffy coat is available for collection that would normally be discarded. This potential repository of DNA would allow future DNA genotyping to be performed to assess kallikrein gene expression. We do not plan to assess kallikrein gene expression as part of this protocol but future studies may be performed to identify changes in various genes that predict cancer risk in a representative population such as this cohort of patients.

In the search and evaluation of novel diagnostics, very sensitive and specific assays were developed to detect certain kallikrein-family protein forms and soluble urokinase plasminogen activator receptor-forms. Proteases and their regulators involved in tumor invasion and metastasis comprise molecules that are candidates as prognostic or diagnostic targets. Plasminogen activation on the cell surface is catalyzed by urokinase plasminogen activator (uPA), which is secreted as an inactive proenzyme that localizes on the cell surface by binding to a specific glyco-lipid anchored receptor, uPAR. Plasmin and other proteases, e.g. trypsin, activate matrix metalloproteases (MMPs). These are capable of degrading extra-cellular matrix including collagen type IV, which is a major basal membrane component, while the destruction of the basal membrane may be crucial to allow invading cancer cells to escape from the originating organ. An assay designed to assess the heterogeneity of fPSA-fractions detects only single-chain (proPSA+mature fPSA) without cross-reaction from multi-chain fPSA cleaved at Lys_{145}-Lys_{146} (fPSA-N), and has shown that fPSA-N is significantly higher in blood from men with BPH than cancer cases. A very sensitive (detection limit $\leq$ 3 pg/mL) and specific assay for hK2 (<0.05% cross-reaction to PSA) distinguishes organ-confined cancer from cancer with extra-prostatic extension, and is a significant predictor of pathological stage by multivariate logistic regression analysis. Assays were also developed to measure intact-suPAR, suPAR (II+III), and suPAR domain I in blood, as plasmin-activation on this receptor may be critical to tumor invasion, and as kallikreins may use the urokinase plasminogen activation proteins as key targets. Levels of suPAR domain I and suPAR(II+III) have been found to be higher in men with cancer than in men without cancer, also in men with tPSA levels ≤10 ng/mL. Prediction of cancer (reported as concordance index) may be enhanced using levels suPAR-forms combined with fPSA-forms and hK2 (AUC=0.805) compared to a base model using tPSA and age (AUC=0.69).[13, 15] However, it is vital to use anti-coagulated plasma, rather than serum, for these measurements as fPSA and fPSA-fractions are prone to undergo significant pre-analytical degradation in vitro unless the sample is rapidly separated from the blood cells (i.e. within less than 6 hours) and the sample is exposed to ambient or refrigerated temperatures for less than 24 hours.

Assay for tPSA and fPSA: Free and total PSA are simultaneously measured with a DELFIA Prostatus™ Dual-label assay (Perkin-Elmer Life Sciences, Turku, Finland) in an equimolar fashion, by an assays which fully cross-reacts with hK2. Limits of detection are 0.04 ng/mL for fPSA and 0.05 ng/mL for tPSA. CV for tPSA is 13.9% at 0.34 ng/mL, and 5.5% at 20.6 ng/mL. CV for fPSA is 17.9% at 0.10 ng/mL and 4.7% at 2.9 ng/mL.

Assay for single-chain fPSA-I. The assay for single-chain fPSA uses biotinylated MoAb

5A10 immobilized to streptavidin-coated wells. Calibrators or samples (50 µl/well) are added and Eu-labeled MoAb 4D4 is added, plates are washed, and signal is measured. Recombinant proPSA is used as calibrator. Detection limit is 35 pg/mL and CV is 8.8%.

Assay for hK2: The current assay for total hK2 (thK2) has been modified to improve precision and linearity at low levels. The capture antibody called MoAb 7G1 has limited cross-reaction to PSA, which is eliminated by adding PSA-blocking MoAb 2C1. Detection limit is 3pg/mL, within-assay CV ranges from 4.8% to 1.5% for low to high hK2 levels, between-assay CVs ranges from 11.6% to 5.5% for low to high hK2 levels.

Immunodetection of suPAR-forms: Full-length intact uPAR assay: MoAb R2 is used as capture IgG. Calibrator (recombinant intact suPAR), sample, or controls, in duplicates are diluted and added (100 µL/well) and incubated for 1 h. Plates are washed, Eu-labeled MoAb R3 (R3-Eu) is added, incubated, washed, and fluorescence signals are measured.

Full-length intact uPAR + domain II+III assay: Protocol as above except that Eu-labeled K1 (K1-Eu) is used as tracer MoAb instead of R3-Eu. Intact uPAR is used as standard material as the assay detects uPAR domain II+III and intact uPAR with the same affinity. uPAR domain I assay: R5 is used for capture, protocol as above except: Recombinant suPAR domain I (1-92) is used as standard, sample incubation for 2 hours, and 1 µM of peptide AE120 is added together with R3-Eu to block signal from intact uPAR.

Intra-assay variation (CV) for intact, intact plus domain II+III, and domain I assays in EDTA plasma is 5.6%, 3.9% and 7.8%; and in citrate plasma 7.9%, 3.3% and 4.8%.

The samples will be drawn prior to prostate biopsy. These research blood draws will be timed such that other blood samples required for routine clinical care are drawn at the same time. In this way, the research samples should add no risk to the patient. No other research samples will be requested.

All patients will undergo prostate biopsy and have the tissue specimens histologically examined. The prostate specimen will be characterized as having either prostate cancer or not. The serum peptide profiles will be analyzed as discussed in Biostatistics section 11.

All specimens at both sites (New York Presbyterian Hospital, Weill Medical College of Cornell University, Manhattan and SUNY Downstate Medical Center in Brooklyn) will be collected, processed, stored and transported to MSKCC according to the standardized operating procedure outlined below. Therefore variability in specimen collection, processing and transportation between the two sites should be minimal due to the standardized method that both will utilize.

Lorraine Thomas, the coordinator at Manhattan and SUNY Downstate Medical Center in Brooklyn, will be responsible for the sample processing and storage on site, before transferred to MSKCC. Contact information listed below:

Lorraine Thomas
Clinical Research Coordinator
450 Clarkson Avenue, Box 52
Brooklyn, NY 11203
Phone: (718) 270-1729
Fax: (718) 270-3327
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Sagit Goldberg, the coordinator at New York Presbyterian Hospital, Weill Medical College of Cornell University, will be responsible for the sample processing and storage on site, before transferred to MSKCC. Contact information listed below:

Sagit Goldberg
Research Technician and Clinical Research Coordinator
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Standardized Serum samples preparation for serum peptide profiling using MALDI-TOF MS

1. Collect venous blood firstly into BD Vacutainer SST tube, a.k.a. tiger-top (Becton Dickinson #367988). Fill tube to top (8.5 mL).
2. Following the manufacturer's instructions:
    a. Gently invert the tube five times to mix clot activator with blood.
    b. Allow blood to clot for 1 hour at room temperature (RT) in vertical position *.
3. Place the SST tubes on wet ice in vertical position until transport.
    a. Put tubes in color-coded bag (*PROTEOMICS*).
4. Transport tubes to Clinical Chemistry laboratory in cooler within two hours after clotting.

5. Specimens at SUNY Downstate Medical Center will be processed on site (see Appendix B).
6. Specimens from New York Presbyterian Hospital, Weill Medical College of Cornell University will be processed on site (see Appendix B).
7. Specimens should be centrifuged within 2 hours after been drawn *.
8. Spin SST tubes in centrifuge at 1400-2000 RCF for 10 min, at RT.
9. Transfer the serum (upper phase) to Fisherbrand 4-mL self-standing cryovials (Fisher Scientific # 0566966), previously labeled. The volume of serum per cryovial should be approximately 1 mL.
10. Store all samples at -80°C on site, in the clinical chemistry lab until transfer.
    a. **Avoid freeze-thawing cycles**
11. When a sufficient number of samples will be available (between 1 and 6 months depending on the accrual rate), the contact person (Dr. Dipti Mehta (mehtad@mskcc.org)) from Dr Lilja's lab will collect the samples from both institutions and transfer them on dry ice to Dr. Tempst (MSKCC Protein Center, Dr. Tempst Laboratory, East 67th St., RRL-551)
    a. Samples will be stored at -80 C until time to perform mass spectrometric analysis.
    b. Contact information :

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Dr. Dipti Mehta – mehtad@mskcc.org

Standardized Plasma samples preparation for peptide profiling using MALDI-TOF MS

1. Collect venous blood into BD Vacutainer heparin tube a.k.a. green-top (Becton Dickinson #366480). Fill tube to top (8.5 mL).
2. Following the manufacturer's instructions:
    a. Gently invert the tube eight times to mix heparin with blood to prevent clotting.
3. Immediately stand sample for 1 hour on wet ice in vertical position *.
    a. Put tubes in color-coded bag (*PROTEOMICS*)

4. Transport tubes to Clinical Chemistry laboratory in cooler within two hours after clotting.
    a. Specimens at SUNY Downstate Medical Center will be processed on site (see Appendix B).
    b. Specimens from New York Presbyterian Hospital, Weill Medical College of Cornell University will be processed on site (see Appendix B).
5. Specimens should be centrifuged within 2 hours of placing the tubes on wet ice *.
6. Spin heparin tubes in centrifuge at 1400-2000 RCF for 10 min, at RT.
7. Transfer the plasma (upper phase) to Fisherbrand 4-mL self-standing cryovials (Fisher Scientific # 0566966), previously labeled. The volume of plasma per cryovial should be approximately 1 mL.
8. Store all samples at -80°C on site, in the clinical chemistry lab until transfer.
    a. Avoid freeze-thawing cycles.
9. When a sufficient number of samples will be available (between 1 and 6 months depending on the accrual rate), the contact person (**Dr**. Dipti Mehta (mehtad@mskcc.org)) from Dr Lilja's lab will collect the samples from both institutions and transfer them on dry ice to Dr Tempst (MSKCC Protein Center, Dr. Tempst Laboratory, E67th Street RRL-551).
    a. Samples will be stored at -80 C until time to perform mass spectrometric analysis.
    b. Contact information :

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* Note: If serum and plasma are collected at the same time, heparin tubes (plasma) can remain on wet ice while the serum in the SST tubes clot for 1h. Later, both serum and plasma tubes can be centrifuged together.

Standardized Plasma sample preparation for Biomarker assay, uPA receptor assay and DNA retrieval.

1. Collect venous blood firstly into BD Vacutainer K_2-EDTA tubes, a.k.a. purple or lavender-top (Becton Dickinson #367899). Fill tube to top (6.0 mL).
2. Following the manufacturer's instructions:
    a. Gently invert the tube eight times to prevent clotting.
3. Transport tubes to Clinical Chemistry laboratory in cooler within two hours after collection.
    a. Specimens at SUNY Downstate Medical Center will be processed on site (see Appendix B).
    b. Specimens from New York Presbyterian Hospital, Weill Medical College of Cornell University will processed on site (see Appendix B).
4. Specimens should be centrifuged within 2 hours of collection at 3000 RCF for 15 minutes at RT.
5. Transfer the plasma (upper phase) to Fisherbrand 4-mL self-standing cryovials (Fisher Scientific # 0566966), previously labeled. The volume of plasma per cryovial should be approximately 1 mL.
6. Harvest the buffy coat, using a non-sterile transfer pipet, by nremoving the buffy colored layer between the plasma and the cell pellet immediately, to previously labeled polypropylene cryopreservation vials (NalgeNunc #5012-0012, Rochester, NY)
7. Store all samples at -80°C on site, in the clinical chemistry lab until transfer**.**
    a. Avoid freeze-thawing cycles.
8. When a sufficient number of samples will be available (between 1 and 6 months depending on the accrual rate), the contact person (Dr**. Dipti Mehta (**mehtad@mskcc.org**)**) from Dr Lilja's lab will collect the samples from both institutions and transfer them on dry ice to Dr. Lilja (MSKCC, Dr. Lilja laboratory, East 67th St, S-910).
    a. Samples will be stored at -80 C until time to perform assay
    b. Contact information :

Dr. Dipti Mehta
Hans Lilja, MD Laboratory
Department of Urology
Memorial Sloan-Kettering Cancer Center
Zuckerman Research Building
408 E. 69th Street
Room Z-427B
10065 New York, NY
alia@mskcc.org
Tel.: 212-888-3316
Fax: 212-888-3200

Dipti Mehta – mehtad@mskcc.org

5.0 CRITERIA FOR SUBJECT ELIGIBILITY

All Caucasian and African-American men attending both the Department of Urology, New York Presbyterian Hospital, Weill Medical College of Cornell University, Manhattan, the Department of Urology, SUNY Downstate Medical Center, Brooklyn, and the Department of Urology, Kings County Hospital,who are to undergo a prostate biopsy because of suspicion of having prostate cancer are eligible for enrollment in this protocol. All patients will proceed with their planned intervention as part of their standard therapy. Serum/plasma samples will be obtained before their intervention, at the same time as samples that are part of routine care.

5.1 Subject Inclusion Criteria

- Men aged 18 years or older
- Have a PSA level between 2 and 10 ng/ml
- May or may not have an abnormal digital rectal examination
- Scheduled for trans-rectal ultrasound (TRUS) guided systematic prostate biopsy as part of routine medical care. All sites (Department of Urology at SUNY Downstate Medical Center, Brooklyn, the Department of Urology, New York Presbyterian Hospital, Weill Medical College of Cornell University, Manhattan and the Department of Urology, Kings County Hospital,) will perform a standardized 14 core biopsy protocol.
- Signed, informed consent
- Patient must be able to attend the pre-biopsy blood draw

5.2 Subject Exclusion Criteria

- Any period of prior/current treatment with hormonal therapy (LHRH agonist/antagonist, antiandrogen, 5-alpha-reductase inhibitor)
- Prior pelvic radiation
- A period of less than 6 months prior/current treatment with an alpha-blocker
- Previous diagnosis of prostate cancer

6.0 RECRUITMENT PLAN

Patients will be recruited from the Urology Clinic at Department of Urology, SUNY Downstate Medical Center in Brooklyn,the Department of Urology, New York Presbyterian Hospital, Weill Medical College of Cornell University, Manhattan and from Department of Urology, Kings County Hospital,. We will prospectively enroll 500 patients in total, 250 African-American and 250 Caucasian patients referred to both hospitals for TRUS guided

prostate biopsy. Approximately 30% will be found to have histological evidence of prostate cancer on their biopsy (150 patients) and 70% (350 patients) will not. This is based on approximately 33% expected to be positive in the African-American samples[3] and 25-30% expected to be positive in the Caucasian samples. We anticipate that accrual will be completed in two years.

Estimated breakdown of target population by race/ethnicity:

% Native American/Alaskan	0%
% Asian/Pacific Islander	0%
% Black Non-Hispanic	50%
% Hispanic	0%
% White Non-Hispanic	50%
% Other	0%

It is stated that taking part in this study is voluntary and patients have the right to withdraw at any time. Participation in the study will not have an impact on the clinical care patients receive.

7.0 ASSESSMENT/EVALUATION PLAN

Patients referred for prostate biopsy as part of their routine care are to be recruited. Patients will undergo standard medical evaluation/care by undergoing a prostate biopsy because of an elevated PSA blood test and/or an abnormal digital rectal prostate examination. The protocol involves obtaining the research blood sample at the time of routine pre-biopsy blood draw. As such, an extra five (40 mL) tubes of blood will be drawn. The serum specimens obtained will be utilized for proteomic profiling comprising MALDI-TOF MS, and serum biomarker assays.

Routine histological examination of the tissue specimens is considered standard care and not research.

8.0 TOXICITIES/SIDE EFFECTS

Patients participating in this study will be at risk for having prostate cancer. The risks and benefits of any procedures including prostate biopsy will not be altered by participation in this protocol. The plan is to obtain the research blood sample at the time of routine pre-biopsy blood draw. As such, the extra five (40 mL) tubes of blood should result in no adverse effects to the patient. If, however, the patient requires a separate blood sample to be drawn, this may result in momentary pain and possible bruising at the site where blood was drawn. There is a small risk of bruising or fainting with phlebotomy. Rarely, an infection may develop at the site from which blood is drawn.

9.0 PRIMARY OUTCOMES

See section 4.0

10.0 CRITERIA FOR REMOVAL FROM STUDY

If at anytime the patient is found to be ineligible for the protocol as designated in the section on Criteria for Patient/Subject Eligibility (i.e., a change in diagnosis), the patient will be removed from the study.

If the patient withdraws consent, refuses to provide research blood sample, or does not undergo scheduled trans-rectal ultrasound (TRUS) guided systematic prostate biopsies, the patient will be removed for the study.

11.0 BIOSTATISTICS

This is a prospective, serum proteomics study of men who are to undergo prostate biopsy. The purpose is to determine if proteomic profiles can be used to distinguish between men with prostate cancer on biopsy from men with no cancer on biopsy. Overall, 500 men will be part of the study. Based on previous experience, about 30% (150 patients) will have cancer and about 70% (350 patients) will not. Accrual is expected to take two years.

An initial biopsy will be undertaken for these patients because of a suspicion of prostate cancer. Because there is about a 20% false negative rate on a single biopsy, and a negligible false positive rate, a second biopsy will be recommended at six months only for those who are negative on the first biopsy. Patients who are positive on the first or second biopsy will be assigned to the "cancer" group, while those who are negative for all their biopsies will be assigned to the "no cancer" group. Assuming the two biopsies can be treated as independent, there would be about 4% that should be in the "cancer" group that would be assigned to the "no cancer" group if all those negative on the first biopsy got the second biopsy. We believe that over 90% of the patients that are negative on the first biopsy will get the second biopsy, leading to an expected false negative rate of 5 to 6%. Since the number incorrectly assigned should be small, we ignore it in the calculations below.

The data from each proteomic study is a two-dimensional plot of mass over charge (m/z) and intensity. The intensity at a particular m/z is proportional to the amount of the peptide with that particular molecular weight in the sample. The m/z values are continuous, but as a first step, these values will be reduced to a finite set of "bins" based on intensity "peaks" across samples, as previously described.[12] To prevent against bias, the bins and the intensities at each bin will be computed while blinded to disease status. The value for each m/z bin for each

sample will be analyzed in two ways. One way will be to use the actual intensity measure, while the other way will be to convert it to a binary (absence or presence) value.

Primary Objectives:

Prediction models for "cancer" or "no cancer" membership will be developed. The first 250 patients accrued will be used as a training set and the second 250 will be used as a validation set. As the optimal analysis methods for these types of experiments are undefined, at least three approaches will be evaluated. One simple approach that will be used is to build a score for each patient based on forward stepwise logistic regression. The second method that will be used involves a statistical technique called "boosting" that puts extra emphasis on correct assignment of difficult cases. This approach was shown to be useful in the analysis of proteomic data in an earlier study.[16] This will be combined with a logistic regression approach. A third technique that will be used is called "logic regression".[17] Here, a search is made through a large subset of the many possible combinations of binary predictors (in this case bins) to build a good prediction rule. This will be straightforward when the data is binary, but it can be utilized for continuous data by setting optimal thresholds for the intensities. In Aim 2, proteomic data will be combined with known biomarkers (PSA and hK2) and compared to models with just the biomarkers. Therefore, for this analysis, the known biomarkers will be forced into models; for Aim 1, only proteomic data will be included. To prevent over-fitting of the training set, 10-fold cross-validation will be utilized in the modeling. The modeling results will be evaluated using the area under the ROC curve (AUC), and the best models will be validated on the validation set. Models will be constructed both with and without clinical variables such as age and family history.

Sample size considerations for the prediction rules are based on the AUC. The methods used here were introduced by Pepe.[18] Since the known biomarkers, such as PSA, give an AUC of approximately 0.8, our goal in Aim 1 is a prediction rule with an AUC of greater than 0.8. For the validation set of size 250, fixing the type I error rate at 0.05, the true AUC would have to be at least 0.86 to have at least 80% power. This is based on a one sample test. In Aim 2, we are comparing the AUC of combining the standard biomarkers with proteomic data to just using the biomarkers. In this case, the AUC of the biomarkers plus proteomic model would have to be at least 0.89 to have at least 80% power. This is a two sample test. While the data are paired, we follow Pepe's recommendation[18] to treat the samples as independent for sample size determination because the correlation is unknown without pilot data and treating the samples as independent is conservative.

In addition to prediction, the association between peak intensity and group membership ("cancer" vs. "no cancer") will be evaluated for every bin using the Wilcoxon rank sum test or Fisher's exact test, depending on whether the data is being treated as continuous or binary. Adjustment will be made for multiple comparisons using the false discovery rate methods of Benjamini and Hochberg.[19] Finally, the data will be hierarchically clustered to examine whether there are distinct peptide patterns associated with subsets of samples.

The full set of 500 samples will be examined using the standard MALDI technique at MSKCC. Thus, the main prediction rules that will be reported will be for these samples using this method at this site. As there are three time points, three separate rules will need to be reported and compared as discussed below. In addition, all data sets will be simultaneously processed in order to get the same sets of bins for reproducibility comparisons. Two other MALDI methods and two LC-MS methods will be used on a subset. For each, the same subset of 100 malignant samples and 100 benign samples will be examined. The same analyses as on the full set will be undertaken, but here 50 of each will be used as a training set and 50 of each will be used as a test set.

Secondary Objectives:

The proteomic profiles of Caucasian men and of African-American men will be compared to see if the same peaks that are predictive in one are predictive in the other. In addition, the AUCs for the two groups will be compared based on models that use all the data.[20] Finally, separate models will be built for the two groups and the AUCs will again be compared.

As mentioned in section 4, we distinguish two aspects of reproducibility: *analytical* reproducibility, that is, whether a proteomics machine produces similar peaks on repeated samples, and *inferential* reproducibility, whether a proteomics experiment produces similar conclusions when repeated.

The basic method for assessing analytic reproducibility will be through the concordance correlation coefficient (CCC)[21] of the intensity estimates. This CCC is a measure of the agreement between two readings of the same sample via the variation from the 45 degree line through the origin. It is on the same -1 to 1 scale as the standard Pearson correlation. Since there are about 600 bins per sample, there will be 600 CCCs for every comparison. Therefore, we will compute summary statistics of the CCCs. These summaries will be based on all the bins and just the discriminating bins. Since the statistic is only pair-wise, different analyses will have to be done for the three time points, the two sites, and the two platforms (MALDI and LC-MS). The only comparison between the platforms will be between standard MALDI and standard LC-MS.

Inferential reproducibility will consist of two components: determination of whether the same peaks are discriminating and comparisons of the AUCs for the prediction of cancer or no cancer. These comparisons will be made for the same varying conditions as for analytic reproducibility. For the former, discriminating peaks will be identified using a variety of thresholds and the differences will be reported as summary statistics. For the latter, formal testing of differences in AUCs will be undertaken, as well as summary statistics. A question of particular interest will be whether the difference in the estimated AUC from the two sites using the standard MALDI platform is statistically significant.

The last two secondary aims involve collection of material for future studies and no current data analyses.

12.0 RESEARCH PARTICIPANT REGISTRATION AND RANDOMIZATION PROCEDURES

12.1 Research Particpant Registration

Confirm eligibility as defined in the section entitled Criteria for Patient/Subject Eligibility.

Obtain informed consent, by following procedures defined in section entitled Informed Consent Procedures.

During the registration process registering individuals will be required to complete a protocol specific Eligibility Checklist.

All participants must be registered through the Protocol Participant Registration (PPR) Office at Memorial Sloan-Kettering Cancer Center. PPR is available Monday through Friday from 8:30am – 5:30pm at 646-735-8000. The PPR fax numbers are (646) 735-0008 and (646) 735-0003. Registrations can be phoned in or faxed. The completed signature page of the written consent/verbal script and a completed Eligibility Checklist must be faxed to PPR.

12.2 For Participating Sites:

Central registration for this study will take place at Memorial Sloan Kettering Cancer Center (MSKCC).

To complete registration and enroll a participant from another institution, the study staff at that site must contact the designated research staff at MSKCC to notify him/her of the participant registration. The site staff then needs to fax registration/eligibility document to the Department of Surgery/Urology Service at MSKCC (212) 557-1928.

The following documents must be sent for each enrollment **within 24 hours of the informed consent form being signed:**

- The completed MSKCC eligibility checklist
- The signed informed consent and HIPPA authorization form
- Supporting source documentation for eligibility questions (laboratory results,

pathology report, radiology reports, MD notes, physical exam sheets, medical history, prior treatment records and EKG report).

Upon receipt, the research staff at MSKCC will conduct an interm review of all documents. If the eligibility checklist is not complete, the patient be registered PENDING and the site is responsible for sending a completed form within 30 days of the consent.

If the eligibility checklist is complete, participant meets all criteria, all source documentation is received, the participating site IRB has granted approval for the protocol, and the site is in good standing with MSKCC, the MSKCC research staff will send the completed registration documents to the MSKCC Protocol Participant Registration (PPR) Office to be enrolled as stated in section 12.1. The participant will be registered.

Once eligibility has been established and the participant is registered, the participant will be assigned an MSKCC Clinical Research Database (CRDB) number (protocol participant number). This number is unique to the participant and must be written on all data and correspondence for the participant. This protocol participant number will be relayed back to study staff at the registering site via e-mail and will serve as the enrollment confirmation.

13.0 DATA MANAGEMENT ISSUES

A Research Study Assistant (RSA) will be assigned to the study. The responsibilities of the RSA include project compliance, data collection, abstraction and entry, data reporting, regulatory monitoring, problem resolution and prioritization, and coordinate the activities of the protocol study team.

The data collected for this study will be entered into a secure database. Source documentation will be available to support the computerized patient record. Minimal data set will be entered into CRDB.

Participating sites will store specimens until needed for analysis.

13.0.1 Data Management Requirements for Participating Sites

Data and Source Documentation

Data:
Blank data collection tools will be sent to the study staff at each participating site for use. The participating Site PI is responsible for ensuring these forms are completed accurately, legibly and in a timely manner. MSKCC will maintain the central database for data collection. Participating sites are responsible for maintaining screening and study match lists.

Source Documentation:

Source documentation refers to original records of observations, clinical findings and evaluations that are subsequently recorded as data collection tools. Relevant source documentation to be submitted throughout the study, if applicable, includes:

- Diagnosis
- Patient history/MD notes
- Transcripts

13.0.2 Data and Source Documentation Submission

Participating sites should e-mail completed data collection tools files and source documentation to the MSKCC contact provided below. Submissions should include a cover page listing all documents enclosed per participant.

EMAIL: Tanya Milan at **MilanRoT@mskcc.org** and Mike Nieves at nievesm@mskcc.org

13.0.3 Data and Source Documentation Submission Timelines:

	Baseline	Follow up Visit(s)
Source Documentation	Within 24 hours	14 days from visit date

Data collection tools and source documentation (if applicable) to support data should be submitted to MSKCC on a monthly basis according to chart 1:

Chart I: Sata and Source Documentation Submission Requirements and Timelines

	(see section 13.1.1)	
CRFs	Within 7 days of visit	
Eligibility Form	X	
Follow-up Evaluation		X*
Biopsy Evaluation	X	X*
Treatment Evaluation		X*
Prostatectomy Deatils		X*
Prostatecomy Pathology		X*
New Cancer Evaluation		X*
Survival Status		X *
*if form is applicable according to Follow-up CRF		

13.1 Quality Assurance

Weekly registration reports will be generated to monitor patient accruals and completeness of registration data. Routine data quality reports will be generated to assess missing data and inconsistencies. Accrual rates and extent and accuracy of evaluations and follow-up will be monitored periodically throughout the study period and potential problems will be brought to the attention of the study team for discussion and action

Random-sample data quality and protocol compliance audits will be conducted by the study team, at a minimum of two times per year, more frequently if indicated.

13.2 Quality Assurance for Participating Sites

Each site participating in the accrual of participants to this protocol will be audited by the staff of the MSKCC study team for protocol and regulatory compliance, data verification and source documentation. Audits may be accomplished in one of two ways: (1) selected participant records can be audited on-site at participating sites or (2) source documents for selected participants will be sent to MSKCC for audit. Audits will usually be determined by participant accrual numbers and rate of accrual, but can also be prompted by reported SAEs or request of MSKCC PI.

Audits will be conducted at least once shortly after initiation of participant recruitment at a site, annually during the study (or more frequently if indicated), and at the end or closeout of the trial. The number of participants audited will be determined by available time and the complexity of the protocol.

The audit will include a review of source documentation to evaluate compliance for:

- Informed consent documents and procedures

- Adherence to eligibility criteria
- Protocol defined treatment
- Required baseline, on study and follow-up protocol testing
- IRB documents (submitted amendments, annual continuing review reports, SAEs)
- Required specimen submission
- Pharmacy review, if applicable

A wrap-up session will be conducted at the participating site and preliminary findings will be discussed with the participating site PI and research team. The preliminary results will be sent to the MSKCC PI.

Each audit will be summarized and a final report will be sent to the PI at the audited participating site within 30 days of the audit. The report will include a summary of findings, participant by participant case review, specific recommendations on any performance and/or shortcomings and request for corrective action, when necessary. When corrective action is required, the participating site must reply within 45 days of receipt of audit report with their corrective action plan.

A copy of the audit report and corrective action plan (if applicable) submitted by the participating site must be sent to the MSKCC IRB/PB, CRQA and maintained in the department's protocol **regulatory binder.**

13.3 Data and Safety Monitoring

The Data and Safety Monitoring (DSM) Plans at Memorial Sloan-Kettering Cancer Center were approved by the National Cancer Institute in September 2001. The plans address the new policies set forth by the NCI in the document entitled "Policy of the National Cancer Institute for Data and Safety Monitoring of Clinical Trials" which can be found at:

http://cancertrials.nci.nih.gov/researchers/dsm/index.html.

The DSM Plans at MSKCC were established and are monitored by the Office of Clinical Research. The MSKCC Data and Safety Monitoring Plans can be found on the MSKCC Intranet at:

http://mskweb5.mskcc.org/intranet/_assets/_tables/content/359709/DSMPlans07.pdf

There are several different mechanisms by which clinical trials are monitored for data, safety and quality. There are institutional processes in place for quality assurance (e.g., protocol monitoring, compliance and data verification audits, therapeutic response, and staff education on clinical research QA) and departmental procedures for quality control, plus there are two institutional committees that are responsible for monitoring the activities of our clinical trials programs. The committees: *Data and Safety Monitoring*

Committee (DSMC) for Phase I and II clinical trials, and the *Data and Safety Monitoring Board (DSMB)* for Phase III clinical trials, report to the Center's Research Council and Institutional Review Board.

During the protocol development and review process, each protocol will be assessed for it's level of risk and degree of monitoring required. Every type of protocol (e.g., NIH sponsored, in-house sponsored, industrial sponsored, NCI cooperative group, etc.) will be addressed and the monitoring procedures will be established at the time of protocol activation.

13.3.1 Regulatory Documentation

Prior to implementing this protocol at MSKCC, the protocol, informed consent form, HIPAA authorization and any other information pertaining to participants must be approved by the MSKCC Institutional Review Board/Privacy Board (IRB/PB). Prior to implementing this protocol at the participating sites, approval for the MSKCC IRB/PB approved protocol must be obtained from the participating site's IRB of Record.
The following documents must be provided to MSKCC before the participating site can be initiated and begin enrolling participants:

- Participating Site IRB approval(s) for the protocol, appendices, informed consent form and HIPAA authorization
- Participating Site IRB approved consent form
- Participating Site IRB membership
- Participating Site IRB's Federal Wide Assurance number and OHRP Registration number
- Curriculum vitae/biosketch and medical license (if applicable) for each investigator and consenting professional
- Documentation of Human Subject Research Certification for investigators and key staff members
- Laboratory certifications and normals (if applicable)

Upon receipt of the required documents, MSKCC will formally contact the site and grant permission to proceed with enrollment.

13.3.2 Amendments

Each change to the protocol document must be organized and documented by MSKCC and first approved by the MSKCC IRB/PB. Upon receipt of MSKCC IRB/PB approval, MSKCC will immediately distribute all non expedited amendments to the participating sites, for submission to their local IRBs.

Participating sites must obtain approval for all non expedited amendments from their IRB of record <u>within 90 calendar days</u> of MSKCC IRB/PB approval. If the amendment is the result of a

safety issue or restricts the eligibility criteria, sites will not be permitted to continue enrolling new participants until IRB approval has been granted.

The following documents must be provided to MSKCC for each amendment within the stated timelines:

- Participating Site IRB approval
- Participating Site IRB approved informed consent form and HIPAA authorization

13.3.3 Additional IRB Correspondence

Continuing Review Approval
The Continuing Review Approval letter from the participating site's IRB must be submitted to MSKCC at the time re-approval is granted. The most current approved version of the consent form should also be submitted to MSKCC within 7 days of expiration. Failure to submit the re-approval in the stated timeline will result in suspension of study activities.

Deviations and Violations

A protocol deviation on this study is defined as a request to treat a research participant who does not meet all the eligibility criteria, pretreatment evaluation, or who requires alteration in their study plan. If a deviation from this protocol is proposed for a potential or existing participant at MSKCC or a participating site, approval from the MSKCC IRB/PB is required prior to the action. Participating sites should contact the MSKCC PI who will in turn seek approval from the MSKCC IRB/PB.

A protocol violation is anything that occurs with a participant, which deviated from the protocol without prior approval from the MSKCC IRB/PB. For protocol violations that are identified after they occur, the participating site should report to MSKCC as soon as possible. The MSKCC PI will in turn report the violation to the MSKCC IRB/PB.

Participating sites should report deviations and violations to their institution's IRBs as soon as possible per that site's institutional guidelines. Approvals/acknowledgments from the participating site IRB for protocol deviations and violations should be submitted to MSKCC as received.

Other correspondence
Participating sites should submit other correspondence to their institution's IRB according to local guidelines, and submit copies of that correspondence to MSKCC.

13.3.4 Document maintenance

The MSKCC PI and the Participating Site PI will maintain adequate and accurate records to enable the implementation of the protocol to be fully documented and the data to be subsequently verified.

The participating sites will ensure that all participating site IRB correspondence (IRB approval letters referencing protocol version date and amendment number, IRB approved protocol, appendices, consent forms, deviations, violations, and approval of continuing reviews) is maintained in the regulatory binder on site and sent to MSKCC.

A regulatory binder for each site will also be maintained at MSKCC; this binder may be paper or electronic.

After study closure, the investigator will maintain all source documents, study related documents and CRFs for 3 years.

13.4 Noncompliance
If a participating site is found to be noncompliant with the study protocol, accrual privileges may be suspended and/or contract payments may be withheld (if applicable), until the outstanding issues have been resolved

14.0 PROTECTION OF HUMAN SUBJECTS

As described previously there are no risks, benefits or toxicities attributable to the study protocol for the patients participating in the study. As described, the only possible side effects would be venipuncture site bruising. This study will not affect patient treatment options. There will be no financial burden or benefit for patients in this study.

All patient identifiers will be removed from serum/tissue samples and replaced with a coded identification number. The investigators receiving serum or nucleic acid samples will be unable to identify the patient. A database containing clinical, pathological and molecular data will be Investigator Anonymized.

It is stated that taking part in this study is voluntary and patients have the right to withdraw at any time. Participation in the study will not impact on the clinical care the patient will receive. If participants decide to withdraw from the study, specimens that have not yet been analyzed will not be used in this study and any remaining portions of samples that have not been used for research will be, if requested by the patient, destroyed.

14.1 Privacy

MSKCC's Privacy Office may allow the use and disclosure of protected health information pursuant to a completed and signed Research Authorization form. The use

and disclosure of protected health information will be limited to the individuals described in the Research Authorization form. A Research Authorization form must be completed by the Principal Investigator and approved by the IRB and Privacy Board.

14.2 Serious Adverse Event (SAE) Reporting

Only SAEs related to the protocol intervention will be reported to the IRB. Any SAE must be reported to the IRB/PB as soon as possible but no later than 5 calendar days. The IRB/PB requires a Clinical Research Database (CRDB) SAE report be submitted electronically to the SAE Office at sae@mskcc.org containing the following information:

Fields populated from the CRDB:

- Subject's name (generate the report with only initials if it will be sent outside of MSKCC)
- Medical record number
- Disease/histology (if applicable)
- Protocol number and title

Data needing to be entered:

- The date the adverse event occurred
- The adverse event
- Relationship of the adverse event to the treatment (drug, device, or intervention)
- If the AE was expected
- The severity of the AE
- The intervention
- Detailed text that includes the following information:
  - A explanation of how the AE was handled
  - A description of the subject's condition
  - Indication if the subject remains on the study
  - If an amendment will need to be made to the protocol and/or consent form

The PI's signature and the date it was signed are required on the completed report.

14.3 Serious Adverse Event (SAE) Reporting for Participating Sites

Responsibility of Participating Sites

- **Participating sites are responsible for reporting all SAEs** MSKCC PI via fax or e-mail within 3 calendar days of learning of the event.
- Participating sites should report any grade 5 event to the MSKCC PI immediately.

- **Participating sites should** use the SAE Report Template to report SAEs to MSKCC.

Responsibility of MSKCC

- The MSKCC Research Staff is responsible for submitting all SAEs to the MSKCC IRB/PB as specified in 14.2
- The MSKCC PI is responsible for informing all participating sites about unexpected SAEs within 30 days of receiving the stamped SAE from the MSKCC IRB/PB
- Any report pertaining to a grade 5 event will be distributed to the participating site as soon as possible.

15.0 INFORMED CONSENT PROCEDURES

Before protocol-specified procedures are carried out, consenting professionals will explain full details of the protocol and study procedures as well as the risks involved to participants prior to their inclusion in the study. Participants will also be informed that they are free to withdraw from the study at any time. All participants must sign an IRB/PB-approved consent form indicating their consent to participate. This consent form meets the requirements of the Code of Federal Regulations and the Institutional Review Board/Privacy Board of this Center. The consent form will include the following:

1. The nature and objectives, potential risks and benefits of the intended study.
2. The length of study and the likely follow-up required.
3. Alternatives to the proposed study. (This will include available standard and investigational therapies. In addition, patients will be offered an option of supportive care for therapeutic studies.)
4. The name of the investigator(s) responsible for the protocol.
5. The right of the participant to accept or refuse study interventions/interactions and to withdraw from participation at any time.

Before any protocol-specific procedures can be carried out, the consenting professional will fully explain the aspects of patient privacy concerning research specific information. In addition to signing the IRB Informed Consent, all patients must agree to the Research Authorization component of the informed consent form.

Each participant and consenting professional will sign the consent form. The participant must receive a copy of the signed informed consent form.

15.1 For Participating Sites

The investigators listed on the cover page and their qualified designees at each participating institution may obtain consent and care for the participants according to good clinical practice and protocol guidelines.

Signed copies of the informed consent should be distributed as follows: One copy will be given to the participant to be retained for their personal records. One copy will be maintained on file at the MSKCC. The third copy will be confidentially maintained by the participating institution.

A note will be placed in the medical record documenting that informed consent was obtained for this study, and that the participant acknowledges th
e risk of participation.

16.0 REFERENCE(S)

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17.0 APPENDICES

Appendix A: Villanueva J, Philip J, Entenberg D et al. Serum peptide profiling by magnetic particle-assisted, automated sample processing and MALDI-TOF Mass Spectrometry. 2004 Anal Chem 76(6): 1560-70.

Appendix B: Sample processing document

Appendix C: Case report forms