Title: Clinical Validation of a Urinary Exosome Gene Signature in Men Presenting for Suspicion of Prostate Cancer

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1.0 OBJECTIVES

1.1. The purpose of this multi-center clinical study is to determine the association of an Exosome Urine Test score with the presence of high Gleason grade / score (GS>=7) prostate cancer on a prostate needle biopsy and validate the assay’s performance characteristics in men presenting to their urologist with or without a previous negative biopsy, and recommended for an initial or subsequent biopsy. The results of this study are to support the use of this assay as a laboratory developed test. A secondary objective is to develop an assay that accurately predicts the presence of any Gleason grade prostate cancer in men at risk for prostate cancer.

1.1.1. Validate an assay which can accurately predict the presence of high grade prostate cancer.

1.1.2. Development of a non-DRE urine bio-specimen platform for future investigational studies including next generation sequencing, small non-coding / Lnc RNA, miRNA analyses and possible proteomic assessment from urinary exosomes.

1.2. Study Design

1.2.1. Prospectively collect approximately 40 mL (preferred 25 mL to 40 mL) of random, non-DRE, non-catheter urine, in a standard, reduced size, clinical capture vessel. In addition, acquire clinical – pathology and patient demographic features including age, race, pathology biopsy results, and PSA values leading up to and following biopsy. Also obtain any pertinent additional clinical data including family history, evidence of a suspicious
DRE, and the use of alpha-blockers or 5-ARI’s used to either prevent cancer or treat symptoms of benign prostatic hypertrophy (BPH). It is presumed that some patients within the current biopsy protocol will undergo treatment as a result of the biopsy diagnosis. The clinical-pathology data, when accessible, will be collected for subsequent secondary analyses. A central pathology review for all diagnostic / positive biopsy results will be performed and incorporated into the final analyses.

1.3. Primary Objectives

1.3.1. Validate a non-DRE urine exosome gene expression test, with an NPV >= 90% certainty, will exclude the presence of high Gleason grade / score (>= 7) prostate cancer in a prostate needle biopsy.

1.3.2. Independent contribution of a non-DRE urine exosome gene expression test to a panel of readily available clinical variables including PSA, age, Family history and race.

1.4. Secondary Objectives

1.4.1. Validate a non-DRE urine exosome gene expression test, with an NPV >= 90% certainty, will exclude the presence of prostate cancer (any grade / score) on a prostate needle biopsy.

1.4.2. Association of a non-DRE urine exosome gene expression test with pre-biopsy therapy for BPH.

1.4.2.1. Construct a bio-specimen urine based platform for future studies including gene discovery using next-generation sequencing, miRNA array and proteomic platforms.

2.0 BACKGROUND

2.1. Study Agent(s)

2.1.1. Exosomes are small nano-vesicles (30-100nm) secreted by a wide range of mammalian cell types. The vesicles are known to be quite abundant in various extracellular fluids including blood, urine and contain proteins, and abundant mRNA and small RNA species including miRNA and snoRNA. Although described some two decades ago, recent studies have demonstrated that the exosome is involved in key physiologic processes.
including cell-cell communication, cellular adhesion, migration, invasion, angiogenesis and growth of tumor cells. The purpose of this study is to collect samples and evaluate association of a pre-determined, urine exosome gene signature with the presence of prostate cancer and secondarily with features of more high risk disease including Gleason score \( \geq 7 \) and higher volume of prostate cancer in the needle biopsy as well as outcome measures for those patients treated with radical prostatectomy. The exosome isolation and extraction of nucleic acids (RNA) will be examined for association with specific clinical-pathology features present in both the prostatectomy specimen and the diagnostic biopsy. Additionally, the urine exosome has been shown to be quite stable and contain high quality RNA enabling a completely non-invasive assessment of the transcriptome.

2.1.2. Microvesicles that exist in urine come from many different cells. Exosomes contained within multivesicular bodies (MVB) in the cytoplasm are released into the extracellular space / circulation when MVB is exocytosed. Microvesicles may also be generated by direct budding from the plasma membrane. Recent studies have demonstrated that microvesicles contain almost the entire transcriptome and that microvesicles from tumor cells have the potential to provide important diagnostic gene signatures and mutations characteristic of the tumor.

2.1.3. Urinary exosomes have also been shown to be extremely stable and contain high quality RNA enabling entirely non-invasive transcriptional analysis. The urine specimens for exosome analyses are stable at ambient temperature and do not require additives for preservation, allowing for routine shipping.

2.2. Study Disease

2.2.1. Prostate cancer is the second leading cause of cancer death among men in the United States, with an anticipated 233,000 newly diagnosed cases and nearly 29,480 deaths in 2014. Early detection of prostate cancer has relied on serum PSA testing and the digital rectal examination with neither being very useful in low risk men with non-palpable disease. PSA is acknowledged as not ideal given the overall trade-off between sensitivity and specificity based on age-specific PSA cut-offs and the inability to differentiate indolent from life-threatening disease reliably at the time of diagnosis. This limitation in diagnostic accuracy has resulted in the over-diagnosis and subsequent overtreatment of prostate cancer as represented by the approximate 1M prostate needle biopsies performed each year. A mandate for contemporary prostate cancer programs is to initially reduce the number of unnecessary biopsies (including both negative and low risk
biopsies) and secondly, improve the diagnosis (and subsequent treatment) of clinically significant disease. Such an approach would have a dramatic positive impact on health care expenses related to the prostate biopsy and treatment.

2.2.2. Of relevance, a recently published study on a cohort of men enrolled in active surveillance by Adamy et al., 2011 demonstrated that PSA levels in the absence of other indicators of tumor progression was not sufficient to initiate treatment. Therefore, a major emphasis of current prostate cancer biomarker research is to either replace and/or supplement PSA for both diagnosis and identifying aggressive disease. A variety of studies have now demonstrated that in vivo models combined with gene expression and proteomic efforts (including an assessment of complex fluids such as urine, blood and formalin-fixed paraffin-embedded (FFPE) tissue specimens) are critically important towards the identification of potentially useful prostate cancer markers although none have progressed to routine clinical practice. The exosome signature has the inherent potential to advance and ultimately improve patient stratification in the prostate cancer diagnosis and treatment paradigm.

2.2.3. Of note, current treatment options for prostate cancer include radical prostatectomy, radiotherapy, and watchful waiting with no apparent consensus on how to maximize disease control and survival, especially for men with low and intermediate-risk prostate cancer. The early published results of the only randomized clinical study which compared observation vs. surgery, demonstrated lower rates of overall death in men with T1 or T2 disease treated with radical prostatectomy; however, after 12 years of follow up this study showed no significant difference in overall survival between both groups. Additionally, in a recent review of this trial by Albertsen PC, 2009, the men who were most likely to benefit from surgery post PSA screening were those with a Gleason score of ≥7 and <60 years at diagnosis while men with a Gleason score ≤6, >65 years are probably best served by active surveillance.

2.2.4. The postoperative nomogram developed by Kattan, et al. predicts the 7 and 10 year probability of disease recurrence (PSA recurrence) for patients who have undergone radical prostatectomy as treatment for prostate cancer. These nomogram’s use a number of prognostic variables including pretreatment serum prostate-specific antigen (PSA) level, radical prostatectomy Gleason sum, prostatic capsular invasion, surgical margin status, seminal vesicle invasion, and lymph node status. Despite their widespread use and reasonable predictive accuracy, better tools are still
needed to predict an individual patient’s probability of disease recurrence, especially at the time of biopsy and prior to any definitive treatment.

2.2.5. Currently there are very limited tools which predict outcome (i.e. PSA recurrence) at diagnosis and none that are available which forecast disease progression (i.e. androgen independent state, metastasis and death due to prostate cancer). The updated Partin tables predict risk of pathologic stage (i.e. extracapsular extension, seminal vesicle invasion, and lymph node invasion); while the recent10-year preoperative nomogram predicts PSA recurrence post-surgery using preoperative variables including positive / negative biopsy core number. The nomogram in its current format; however, is limited by the use of a PSA recurrence endpoint which does not equate to systemic disease, and the absence of biologic characteristics derived from the patient’s own tumor specimen.

2.2.6. The scope of this study is to confirm an exosome signature that is able to reliably identify the presence of high grade / score (>=Gleason score 7) prostate cancer. For those men treated with surgery, an additional association with the test and characteristics of the prostatectomy specimen will be evaluated. For the study cohort we anticipate working with 15 or more institutions to obtain urine samples from approximately 2000 patients from different geographic regions in the US. We will also require a central pathology review of all diagnostic / positive biopsies to occur at Memorial Sloan-Kettering Cancer Center. H&E stained sections from individual sites representing all patients enrolled (with positive biopsy results) will be sent to Dr. Victor Reuter, MD at Memorial Sloan-Kettering Cancer Center, NYC, NY for an official independent review and generation of a summary and individual biopsy Gleason grade and score. At termination of the study, the database containing the specific diagnostic information will be sent to Exosome Dx, in Minneapolis, MN, (BLINDED) such that personnel at Exosome will not be able to link this information with patient specific results of the Exosome test.

2.3. Rationale

2.3.1. Exosomes with intact mRNA for several prostate specific genes (e.g. SPDEF, SPARCL1, ERG, PCA3, TMPRSS2: ERG, and KLK3) have been previously isolated from the urine in patients diagnosed with prostate cancer. We have demonstrated that this method and analytical approach will be successful across multiple stages of prostate cancer and provide an abundant source of high-quality RNA for identifying and monitoring disease course.
2.4. Pilot Data in Prostate Cancer

2.4.1. Several multi-center pilot studies have been completed on the urine exosome profile of 176 and 251 consecutive patients from two different cohorts (under appropriate institutional IRB) including those with and without definitive prostate cancer as determined by prostate needle biopsy, patients post-surgery, patients prior to a diagnostic biopsy and a control population of men under 35 years old. Utilizing PCA3 and ERG expression levels normalized with SPDEF in urine volumes of 20-40ml, for first biopsy patients in the PSA gray zone (2-10 ng/mL), the AUC for predicting presence of cancer achieved a consistent ROC >0.7, and when predicting >=GS7 in this same group the NPV was 94% with a sensitivity of 84%. A defined threshold for this three gene signature has been used for the current AUC and NPV values. 90% of the total patient cohorts provided high quality exosome material for gene transcription studies confirming the ability to isolate and extract mRNA from comparable patients in the current study protocol. In addition, the results also demonstrated the ability to identify and use selected gene expression profiles to segregate biopsy positive from negative patients and the down regulation / absence of specific genes post-surgery.

2.5. Correlative Studies Background

2.5.1. The purpose of this study is to extract prostate specific RNA from urine exosomes for molecular analysis. Therefore, the entire study is correlative in relation to patient outcomes in the absence or presence of other therapies.

3.0 PARTICIPANT SELECTION

3.1. Eligibility Criteria

3.1.1. Participants must meet the following criteria on screening examination to be eligible to participate in the study:

3.1.1.1. Male, ≥50 years of age with a clinical suspicion for prostate cancer based in part on an elevated PSA (limit range: 2.0 - 20 ng/ mL), and or suspicious DRE, with or without the clinical history of a single prior negative biopsy, and who have been recommended for a repeat and or first time biopsy.
3.1.1.2. The subject must be able to comprehend and sign an approved informed consent form and other applicable study documents.

3.2. Exclusion Criteria

3.2.1. Participants who exhibit any of the following conditions at screening will not be eligible for admission into the study.

3.2.1.1. Use of medications or hormones that are known to affect serum PSA levels within 3-6 months of study enrollment.

3.2.1.2. Clinical symptoms of urinary tract infection (including prostatitis) at the time of enrollment.

3.2.1.3. History of prostate cancer.

3.2.1.4. History of invasive treatments for benign prostatic hypertrophy (BPH) or lower urinary tract symptoms within 6 months of study enrollment.

3.2.1.5. Medical history or concurrent illness that the investigator considers sufficiently serious to interfere with the conduct, completion or results of this trial, or constitutes an unacceptable risk to the subject.

3.2.1.6. Participation in pharmaceutical or treatment related clinical study within 6 months of study enrollment. Exception: Trials for non-prostate conditions may be acceptable, with approval by the investigator and Sponsor.

3.2.1.7. No known hepatitis (all types) and/or HIV documented in patient’s medical record.

3.2.1.8. Patients with history of concurrent renal/bladder tumors within 6 months of study enrollment.

3.3. Inclusion of Women, Minorities and Other Underrepresented Populations

3.3.1.1. Patients are eligible regardless of race, and ethnicity.
4.0 REGISTRATION PROCEDURES

4.1. General Guidelines for Other Participating Institutions

4.1.1. Not applicable.

4.2. Registration Process for Other Participating Institutions

4.2.1. Not applicable.

5.0 TREATMENT PLAN

5.1. Patients who have agreed to participate in the study will donate 25-40 mL of urine as described in Section 8. The urine will be processed as per standard operating procedure outline in Section 8.1. It is anticipated that up to 150 patients will be enrolled per participating institution/site.

5.2. Medical records of the participating patients will be reviewed as needed to collect correlative information about patient demographics, disease status, and relevant clinical-pathology variables. See Appendix A regarding clinical variables.

5.3. Pre-treatment Criteria

5.3.1. Not applicable.

5.4. Agent Administration

5.4.1. Not applicable.

5.5. Definition of Dose-Limiting Toxicity

5.5.1. Not applicable.

5.6. General Concomitant Medication and Supportive Care Guidelines

5.6.1. Not applicable.

5.7. Duration of Therapy

5.7.1. Not applicable.
5.8. **Duration of Follow Up**

5.8.1. Patients will be followed until pathology of clinically directed biopsy pathology report.

5.9. **Criteria for Removal from Study**

5.9.1. Participants will be removed from study if they request to do so. The reason for study removal and the date the participant was removed must be documented in the study-specific case report form (CRF) or received clinical database. Alternative care options will be discussed with the participant.

5.9.2. In the event of unusual or life-threatening complications, participating investigators must immediately notify the Principal Investigator at the participating institution.

6.0 **EXPECTED TOXICITIES AND DOSING DELAYS/DOSE MODIFICATIONS**

6.1. **Anticipated Toxicities**

6.1.1. Not applicable.

6.2. **Toxicity Management**

6.2.1. Not applicable.

6.3. **Dose Modifications/Delays**

6.3.1. Not applicable

7.0 **DRUG FORMULATION AND ADMINISTRATION**

7.1. Not applicable
8.0 CORRELATIVE/SPECIAL STUDIES

8.1. Specimen acquisition, storage and shipping (see Appendix A for a complete Standard Operating Procedure for specimen collection)

8.2. Urine sample processing:

8.2.1. First voided random urine taken in 60 mL urine collection container; target 40mL.

8.2.2. Sample is stored at 4°C.

8.2.3. Ship the collected samples (BLINDED to outcome data) under ambient or ice pack conditions to Exosome Diagnostics, Minneapolis, MN. within 24 hours of collection following the directions and requirements of the courier or shipping service

8.3. Shipment of Urine Samples for Analysis:

8.3.1. Samples will be transported (or couriered) to Exosome Diagnostics, Inc., 1000 Westgate Drive, Suite 117, St Paul, MN 55114 (BLINDED to clinical outcome data) from all sites; accompanying clinical data will be stored on Exosome secure servers and available only to individual laboratory directors and scientific personnel with permissions to view and append data but not involved in the direct processing of samples.

8.3.2. Information on individual subjects will be limited to subject study ID, enrollment site, disease state, age, and sample acquisition site. All clinical outcome data will be BLINDED.

9.0 MEASUREMENT OF EFFECT

9.1. Not applicable

10.0 ADVERSE EVENT REPORTING REQUIREMENTS

10.1. Reporting to the Food and Drug Administration (FDA)

10.1.1. Not applicable
10.2. **Reporting to the NIH Office of Biotechnology Activities (OBA)**

10.2.1. Not applicable

10.3. **Reporting to the Institutional Biosafety Committee (IBC)**

10.3.1. Not applicable

10.4. **Reporting to Hospital Risk Management**

10.4.1. Participating investigators will report to their local Risk Management office any subject safety reports or sentinel events that require reporting according to institutional policy.

10.5. **Monitoring of Adverse Events and Period of Observation**

10.5.1. Not applicable.

11.0 **DATA AND SAFETY MONITORING**

11.1. **Data Reporting**

11.1.1. **Method**

11.1.1.1. Exosome has developed highly customized software that has the ability to store and maintain different types of data including clinical, molecular biomarkers, and images files in an Oracle database. The software tracks each sample and associated information during its lifecycle. Samples are de-identified at the institution and assigned unique identifiers upon acquisition. For this particular study the unique identifier will have been pre-assigned and all samples and accompanying clinical data will arrive at Exosome (samples are handled and processed **BLINDED** to outcome data) with the unique identifier. Details, such as their stored location number, current status (in storage, released to analysis, etc.) and image details, are tracked electronically. The quality of the data entered is ensured by data review through a pathologist and a data manager who both have the privilege to overwrite the data. Access to this software is restricted to select users and is controlled by an administrator. Every change performed to tissue records is logged along with
the user-ID and tissue-ID, and is available for audit. The entire database is backed up on a daily basis.

11.1.2. **Data Submission**

11.1.2.1. The schedule for completion and submission of case report forms (paper or electronic) will be determined on an institutional basis and may include receipt of clinical database. Clinical outcome data for individual urine specimens will be kept in a separate database accessible only to approved personnel.

11.2. **Safety Meetings**

11.2.1. Not Applicable

11.3. **Monitoring**

11.3.1. Involvement in this study as a participating investigator implies acceptance of potential audits or inspections, including source data verification, by representatives designated by the Institution. The purpose of these audits or inspections is to examine study-related activities and documents to determine whether these activities were conducted and data were recorded, analyzed, and accurately reported in accordance with the protocol, institutional policy, Good Clinical Practice (GCP), and any applicable regulatory requirements.

11.3.2. All data will be monitored for timeliness of submission, completeness, and adherence to protocol requirements. Monitoring will begin at the time of participant registration and will continue during protocol performance and completion.

12.0 **REGULATORY CONSIDERATIONS**

12.1. **Protocol Review and Amendments**

12.1.1. This protocol, the proposed informed consent and all forms of participant information related to the study (e.g., advertisements used to recruit participants) and any other necessary documents must be submitted, reviewed and approved by a properly constituted IRB governing each study location.
12.1.2. Any changes made to the protocol must be submitted as amendments and must be approved by the IRB prior to implementation. Any changes in study conduct must be reported to the IRB.

12.1.3. All decisions of the IRB concerning the conduct of the study must be made in writing.

12.2. Informed Consent

12.2.1. All participants must be provided a consent form describing this study and providing sufficient information for participants to make an informed decision about their participation in this study. The formal consent of a participant, using the IRB approved consent form, must be obtained before the participant is involved in any study-related procedure. The consent form must be signed and dated by the participant or the participant’s legally authorized representative, and by the person obtaining the consent. The participant must be given a copy of the signed and dated consent document. The original signed copy of the consent document must be retained in the medical record or research file.

12.3. Ethics and Good Clinical Practice (GCP)

12.3.1. This study is to be conducted according to the following considerations, which represent good and sound research practice:

12.3.1.1. Good Clinical Practice: Consolidated Guidance


12.3.1.2. US Code of Federal Regulations (CFR) governing clinical study conduct and ethical principles that have their origin in the Declaration of Helsinki

12.3.1.2.1. Title 21 Part 11 – Electronic Records; Electronic Signatures

   www.access.gpo.gov/nara/cfr/waisidx_02/21cfr11_02.html

12.3.1.2.2. Title 21 Part 50 – Protection of Human Subjects

   www.access.gpo.gov/nara/cfr/waisidx_02/21cfr50_02.html
12.3.1.2.3. Title 21 Part 54 – Financial Disclosure by Clinical Investigators
www.access.gpo.gov/nara/cfr/waisidx_02/21cfr54_02.html

12.3.1.2.4. Title 21 Part 56 – Institutional Review Boards
www.access.gpo.gov/nara/cfr/waisidx_02/21cfr56_02.html

12.3.1.2.5. Title 21 Part 312 – Investigational New Drug Application
www.access.gpo.gov/nara/cfr/waisidx_02/21cfr312_02.html

12.4. State Laws

12.4.1. It is understood that deviations from the protocol should be avoided, except when necessary to eliminate an immediate hazard to a research participant. In such case, the deviation must be reported to the IRB according to the local reporting policy.

12.5. Study Documentation

12.5.1. The investigator must prepare and maintain adequate and accurate case histories designed to record all observations and other data pertinent to the study for each research participant. This information enables the study to be fully documented and the study data to be subsequently verified. Such clinical data variables can be part of a larger clinical data base provided to Exosome.

12.5.2. Original source documents supporting entries in the case report forms include but are not limited to hospital records, clinical charts, laboratory and pharmacy records, recorded data from automated instruments, microfiches, photographic negatives, microfilm or magnetic media, and/or x-rays.

12.6. Records Retention

12.6.1. All study-related documents must be retained for the maximum period required by applicable federal regulations and guidelines or institutional policies.
13.0 STATISTICAL CONSIDERATIONS

13.1. Study Design/Endpoints

13.1.1. The primary endpoint of this study is to correlate an exosome gene expression signature with the presence or absence of high grade prostate cancer in the prostate needle biopsy. For this purpose, samples will be collected from patients to determine the sensitivity of a urine exosome RNA signature and relate it to clinical-pathology evidence of disease.

13.1.2. Sample Size

13.1.2.1. We hypothesize that prostate specific RNA biomarkers from urine-associated exosomes will be readily detected in the majority of patients with localized and locally advanced prostate cancer. Given the current specimen utilization success rate of 95% from the pilot study, and the anticipated positive diagnostic biopsy rate of ~40%, we anticipate collecting up to ~2000 patients which will provide n=760 ‘event’ patients to validate the exosome test. Additional parameters including historical positive biopsy rate will be calculated collectively from the participating institutions files and then used to adjust patient numbers as necessary.

13.2. Stratification Factors

13.2.1. None

13.3. Analysis of Primary Endpoint

13.3.1. All statistical analyses will be handled through an external statistical analysis group under the direction of Dr. Mike Kattan of the Cleveland Clinic with initial oversight by Dr. Mark Thornquist of the Fred Hutchinson Cancer Research Center. It is anticipated that a variety of strategies will be employed when evaluating correlation of a gene expression profile with a specific clinical end-point including linear regression, t-test and ANOVA. Optimized thresholds for specific genes will be performed with sensitivity analysis to optimize stratification of patients in the training set. Multivariate models (logistic regression models) will also be employed to evaluate the combination of exosome
gene expression profiles and pre-treatment clinical variables such as PSA, biopsy Gleason grade/score and clinical stage.

13.3.2. Our goal is to validate various thresholds utilizing the exosome RNA gene signature to stratify patients based on the likelihood of having any Gleason pattern 4 disease present within the biopsy (or when available the prostatectomy specimen surgical pathology report) for first time biopsy patients in the PSA gray zone of 2.0-10 ng/mL. In addition, we will associate this gene signature with a dominant Gleason 4 pattern present in the biopsy (and available prostatectomy specimen if appropriate). In our earlier cohort analyses and in the literature there is an expected 20-25% of patients who will present with Gleason score \( \geq 7 \) and the prevalence of a dominant Gleason 4 varies considerably dependent upon studies from 17-30%. We anticipate based on the number of patients (~n=190, 25% of 760) that will be enrolled in the study (and that approximately 75-80% will be in the intended use population of 2-10 ng/mL PSA) we will have sufficient numbers of dominant pattern 4 and or Gleason score \( \geq 7 \) to accurately predict. An initial evaluation will be performed on the first collected 1000 patients, which will provide a sufficient number of patients in our intended use population to determine preliminary performance of the assay.

13.4. Analysis of Secondary Endpoint

13.4.1. All statistical analyses will be handled through an external statistical analysis group under the direction of Dr. Mike Kattan of the Cleveland Clinic and Dr. Mark Thornquist of the Fred Hutchinson Cancer Center. It is anticipated that a variety of strategies will be employed when evaluating correlation of a gene expression profile with a specific clinical end-point including linear regression, t-test and ANOVA. Optimized thresholds for specific genes will be performed with sensitivity analysis to optimize stratification of patients in the training set. Multivariate models (logistic regression models) will also be employed to evaluate the combination of exosome gene expression profiles and pre-treatment clinical variables such as PSA, biopsy Gleason grade/score and clinical stage.

13.4.2. Our goal with this endpoint is to validate the performance of the test to identify any Gleason grade prostate cancer both in the intended use population and all patients presenting to their urologist with a suspicion of prostate cancer. For this endpoint we will focus on both the NPV and area under the curve to assess performance of the test. Given the current
prevalence of prostate cancer in this group from prior studies (47%) we anticipate having sufficient numbers of patients from the both initial and prior negative groups to determine validation performance characteristics.

13.5. **Exploratory Analyses**

13.5.1. Exploratory models will be used to estimate the relationship between exosome RNA expression, tumor burden and outcome in both needle biopsies and when available outcome data from the surgical pathology report. Further statistical approaches will be included in a provided statistical analysis plan.

13.6. **Reporting and Exclusions**

13.6.1. All patients from whom samples are obtained (and with complete clinical data) will be included in the analyses if they pass quality control based on pre-defined attributes of RNA content and quality.

14.0 **PUBLICATION PLAN**

14.1. The results will be made public within 24 months of the end of data collection. The initial release may be an abstract that meets the requirements of the International Committee of Medical Journal Editors. A full report of the outcomes will be made public no later than three (3) years after the end of data collection. Exosome Diagnostics will be given 60 days to provide comments on all publications.

15.0 **REFERENCES**


15.24.1.1.


16.0 APPENDICES

16.1. Appendix A – Clinical and Pathological Features/Available Outcome Data

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Appendix A: Clinical and Pathological Features/Available Outcome Data

Note: Prostatectomy clinical features are for those patients who have surgery for definitive treatment.

1. Age at diagnosis (years)
2. Contemporary Biopsy Gleason Score (dominant plus secondary for all positive cores)
3. Contemporary Dominant Biopsy Gleason Grade
4. Contemporary Secondary Biopsy Gleason Grade
5. PSA at time of diagnostic biopsy (complete PSA record file prior to and including time of diagnostic biopsy)
6. PSA density at the time of diagnosis
7. Tumor Volume-clinical (including how determined – TRUS, MRI etc)
8. TNM (clinical)
9. Race (White / Black / Hispanic)
10. Weight / BMI
11. Number of positive cores
12. Total number of cores
13. Percentage of tumor in each needle biopsy core (contemporary value)
14. Percent involvement of all individual positive cores
15. Anatomic Location(s) of positive needle biopsy core(s) (Right/Left/Unknown) (optional)
16. Anatomic location of all positive cores
17. Palpable on DRE
18. Disease progression (yes / no)
19. Time in months from diagnostic biopsy to radical prostatectomy
20. PSA undetectable post radical prostatectomy (or other primary therapy) (yes/no)
21. Serial PSA Measurements before and after primary therapy with dates*
22. PSA Recurrence (definition: one rise equaling 0.4 ng/ml) (non-censored/censored) (yes or no)
23. Time to PSA Recurrence from prostatectomy (in months)

24. PSA Velocity/Doubling Time (to be discussed – i.e., post-prostatectomy and prior to treatment)

25. Hormonal therapy post-prostatectomy (yes / no and give months after prostatectomy). Define type of therapy, if combination used, duration, type, etc

26. Salvage RT post prostatectomy (yes / no and months after prostatectomy). Include type, dose / gray level, duration if available.

27. Secondary therapy (i.e., second line of ADT/additional salvage RT) post-PSA recurrence/relapse (yes/no); time in months from prostatectomy.

28. Time to PSA rise post-Adjuvant Therapy (in months from definitive treatment –combined as hormonal therapy +/- salvage RT and as individual treatments)

29. Positive MRI/CT scan (non-censored/censored) (yes/no)

30. Time to positive MRI / CT scan (in months) – time to metastasis from prostatectomy

31. Death Status (non-censored/censored)

32. Death due to prostate cancer (on death certificate) (yes or no)

33. Time to Death (in months) (from prostatectomy)

34. Radiation prior to Prostatectomy or other definitive therapy (yes/no)

35. Hormones prior to Prostatectomy or other primary therapy (yes/no)

36. Lymph Node Status (if prostatectomy; # nodes positive / total)

37. Number of dissected lymph nodes (if prostatectomy)

38. Surgical Margin Status (if prostatectomy)

39. Extra Capsular Extension (if prostatectomy)

40. Seminal Vesicle Invasion (if prostatectomy)

41. Contemporary Prostatectomy Gleason Score (see above)

42. Contemporary Dominant Prostatectomy Gleason Grade (see above)

43. Contemporary Secondary Prostatectomy Gleason Grade

44. Pathologic stage (pTNM)

45. Initiation of systemic chemotherapy (yes/no, date if yes)
46. Time in months to initiation of systemic chemotherapy, post radical prostatectomy.

47. Date of last follow-up

48. Historical Biopsy Gleason Score (dominant plus secondary)

49. Historical Dominant Biopsy Gleason Grade

50. Historical Secondary Biopsy Gleason Grade

51. Historical Prostatectomy Gleason Score (see above)

52. Historical Dominant Prostatectomy Gleason Grade (see above)

53. Historical Secondary Prostatectomy Gleason Grade

*All PSA measures collected from baseline to PSA recurrence and post-treatment.