# I. Study Title: Biomarkers and clinical parameters associated with Gleason score upgrading

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Table of Contents

I. Study Title: Biomarkers and clinical parameters associated with Gleason score upgrading 1
II. Background, Significance, and Specific Aims ..... 4
III. Specific Aims, Phase I: ..... 5
A. Specific Aim 1 ..... 5
B. Specific Aim 2 ..... 5
IV. Specific Aims, Phase 2: (Not in current budget period) ..... 6
A. Specific Aim 1 ..... 6
B. Specific Aim 2 ..... 6
C. Specific Aim 3 ..... 6
V. Purpose and Objectives ..... 6
A. Primary objective: ..... 6
B. Secondary Objective: ..... 6
C. Future secondary objective: (will require additional external funding) ..... 6
VI. Research Plan ..... 6
A. Aim 1 ..... 6
1. Outcome Measures ..... 7
2. Specimens to be Collected ..... 7
B. Aim 2: ..... 9
VII. Data Analyses: ..... 9
VIII. Statistical Power: ..... 10
IX. Long-term plans for use of the Upgrading Reference Set ..... 10
X. Inclusion/Exclusion Criteria ..... 11
A. Inclusion Criteria: ..... 11
B. Exclusion Criteria: ..... 11
XI. Recruitment and consent procedures ..... 11
A. Schedule of Events ..... 12
XII. Compensation ..... 13
XIII. Risks to subjects ..... 13
XIV. Data and Safety Monitoring Oversight ..... 13
A. Monitoring Safety ..... 13
B. Reporting Requirements ..... 14
C. Assuring Compliance with Protocol and Data Accuracy ..... 14
D. Safety Definitions: ..... 14
XV. Data Management ..... 14
XVI. Appendicies ..... 16
A. Appendix 1 - Model Consent: ..... 16
B. Appendix 2 - Central Pathology Review and Consent: ..... 22
C. Appendix 3 - Data Collection Forms ..... 25

D. Appendix 4 - Specimen SOPs ..... 26
1. Blood SOP ..... 26
2. Urine SOP ..... 30
XVII. References ..... 34

## II. Background, Significance, and Specific Aims

**Background and Significance**: Since the mid-1980s, PSA has been increasingly used for the early detection of prostate cancer. While this 'national experiment' has led to a tremendous increase in detection of prostate cancer, stage migration, and may have been, in part, responsible for a 44% fall in prostate cancer mortality, there is a general consensus that screening has led to significant over-detection and over-treatment. This consequence was the primary reason why the U.S. Preventive Services Task Force in 2012 recommended against PSA testing.[1]

With the recognition that many low-grade tumors will not progress over time, many centers in the late 1990s and early 2000s began to offer active surveillance (AS) as a management approach for these tumors. With AS, patients are monitored closely with repeated PSA measures and examinations as well as periodic prostate biopsies with treatment reserved only for those who developed higher-grade disease. Follow-up approaching 10-15 years finds very high (99% at 5 years and 97% at 10 years) disease-specific survival.[2] Surprisingly, recent data suggest that fewer than 10% of patients are managed with this approach.[3] Most surprising is that as evidence supporting active surveillance increased in the past decade, an increasing proportion of men with low- risk disease has been treated with aggressive treatments including surgery and radiation.

There are a variety of reasons why patients and their physicians, when faced with a prostate cancer diagnosis, may elect surgery or radiation in lieu of a surveillance strategy. One of the most common reasons is the concern that, due to undersampling, a higher-grade tumor or a tumor that is likely to recur may be present. Simply due to the inclusion of the term 'cancer' in the diagnosis, patients and their families often opt for treatment. Rates of upgrading from biopsy to radical prostatectomy vary among series. In a Scandinavian series of patients who underwent radical prostatectomy either after initial diagnosis or after a period of surveillance, the rate of upgrading was 25% and 38%, respectively.[4] A U.S. study segregated upgrading and upstaging by different criteria: rates of upgrading were 20% versus 27% for the two criteria, and rates of upstaging, 6% and 8%, respectively.[5] Most studies have found that about a third of patients will be found to have a higher tumor grade at radical prostatectomy. As there are restrictions on upgrading (for example, a Gleason 5+5 on biopsy cannot 'upgrade' while the discovery of an element of Gleason pattern 4 on a radical prostatectomy will automatically upgrade a 3+3 on biopsy to a 3+4), it is more common that lower-grade tumors will 'upgrade' at prostatectomy, perhaps causing some of the increase in treatment.

A test or prediction tool that would allow a physician to provide an assurance to the patient that "there is a greater than 95% likelihood that you don't have a higher-grade tumor in your prostate" would be a significant contribution to clinical oncology, and could dramatically increase the utilization of active surveillance in lieu of radical prostatectomy and radiotherapy. Ultimately, if this methodology could also provide assurance that a tumor was of such a low biologic potential that the risk of biochemical recurrence would be similarly-low, active surveillance utilization would likely further increase. The ultimate benefit of such a test would be better-targeted treatment of tumors, reducing the economic burden of prostate cancer treatment and, perhaps even more importantly, reducing the morbidity due to over diagnosis of prostate cancer in the U.S.

Many of the clinical measures (volume, amount of tumor in core(s), PSA) we plan to evaluate in this study have previously been confirmed by other investigators.[6,7,8,9,10] Although many groups have noted an association between clinical risk factors and risk of upgrading, only a few have attempted to develop tools to predict an individual patient's risk. The first nomogram developed

by a group in Hamburg based on 2982 patients examined the following variables: PSA, Gleason score on biopsy, and clinical stage.[11] They found that all three variables were associated with risk of upgrading. The nomogram was further expanded thereafter examining a total of 414 patients.[12] The authors employed a small number of factors that would be anticipated to be associated with risk, including PSA, clinical stage, age, percent of positive cores, and prostate volume. All of these variables except age were associated with risk of upgrading. Unfortunately, the authors did not capture the total number of cores (expected to bias results and be related to adequacy of sampling) nor the volume of tumor in the cores themselves. Imamoto et al., in 2 Japanese institutions, evaluated 503 patients using the same Hamburg nomogram.[13] The authors found the nomogram to provide reasonably accurate predictions of upgrading but less so when applied to a Japanese patient population. Likely due to the lack of inclusion of several risk factors which are clearly associated with the volume of disease on biopsy, the lack of information related to previous biopsies, the lack of information related to total biopsies obtained, all of which are almost certainly associated with risk of undersampling and thereby risk of undergrading, none of the previously developed nomograms are in widespread clinical use because they could not provide accurate prediction of either low or high risk at individual patient level to make clinical decision with confidence.

We are proposing a multi-institutional study to identify molecular biomarkers and clinical measures that will predict presence of Gleason 7 or higher cancer (as evidence in the radical prostatectomy specimen) among patients with a biopsy diagnosis of Gleason score ≤ 6 prostate cancer. This proposal will be conducted in two phases. The first phase will assemble an "Upgrading Reference Set" that will include clinical information as well as biologics on a cohort of 600 men. The first phase will also assess the clinical parameters associated with upgrading, as well as, perform a central pathology review of both biopsies and prostatectomy specimens to confirm tumor grade. The second phase will use the biologics collected in phase 1 to evaluate a series of biomarkers to further refine the prediction of Gleason 7-10 cancer at radical prostatectomy.

## III. Specific Aims, Phase I:

### A. Specific Aim 1

We will recruit a cohort of 600 men with biopsy Gleason 3+3 prostate cancer who have elected to undergo radical prostatectomy for prostate cancer (see Appendix 1-Model Consent). Preoperative blood and urine samples, prostate biopsy slides, and blocks if available, radical prostatectomy blocks, and slides if available, as well as clinical and demographic data will be collected. Specimen collection will be coordinated by the Data Management and Coordinating Center (DMCC) of the Early Detection Research Network (EDRN). These biologics will be considered the Upgrading Reference Set (URS).

### B. Specific Aim 2

Clinical and demographic risk factors related to upgrading and/or biochemical recurrence will be collected and analyzed. Prostate biopsy slides and blocks and radical prostatectomy blocks and slides will be reviewed by the study pathologist, Mark Rubin, MD, Chair of the GU Collaborative Group. Patients will be followed to one intermediate endpoint (tumor grade and stage at radical prostatectomy). Patients will be consented for collection of follow-up data to determine use of adjuvant or salvage treatments (e.g., hormonal or radiotherapy) as well as for monitoring of disease recurrence (e.g., PSA, radiographic imaging studies). They will also be consented for potential long-term contact from either the study sites or the DMCC for collection of these data.

## IV. Specific Aims, Phase 2: (Not in current budget period)

### A. Specific Aim 1

Prioritize available progression biomarkers to identify those markers with the most promising validation data. Provide appropriate biologics to the development laboratories for assessment in a blinded manner. Data will be returned to the DMCC of EDRN for assessment.

### B. Specific Aim 2

Data from this study will be used to develop the Early Detection Research Network Upgrading Risk Assessment Tool (EURAT). The EURAT will combine clinical information and biomarker data to predict the presence of a higher-grade tumor in a patient with biopsy Gleason 3+3 prostate cancer.

### C. Specific Aim 3

Depending on additional funding available, long-term follow-up data will be collected to expand the EURAT to predict long-term risk of disease recurrence.

## V. Purpose and Objectives

### A. Primary objective:

Develop a risk prediction tool, to predict with at least 95% negative predictive value using all clinical, demographic, and biologic information, the lack of a higher tumor grade (i.e., Gleason 3+4 or greater) at radical prostatectomy. NPV depends on both intrinsic diagnostic test performance, i.e. sensitivity and specificity, and the prevalence of upgrading which differs in various populations. We also do not have a binary test ready for validation of its NPV in Phase 2. Most likely the final product of Phase 2 is EURAT, an upgrading risk calculation algorithm, and a suggested threshold corresponding to 98% sensitivity and has specificity ≥ 25%. Assuming 30% upgrading rate, this translates to NPV=96.7%. For these considerations the operational performance criterion is to have a threshold that has specificity ≥ 25% at sensitivity 98%.

### B. Secondary Objective:

Evaluate the independent predictive value of urine, blood, and tissue-based biomarkers in a risk prediction tool. Many of these biomarkers were developed with support from the EDRN.

### C. Future secondary objective: (will require additional external funding)

Develop a risk prediction tool using clinical, pathologic, and biologic data to predict risk of biochemical recurrence in patients with Gleason 3+3 cancer managed with radical prostatectomy.

## VI. Research Plan

### A. Aim 1

We will recruit a cohort of 600 men with prostate cancer who have elected radical prostatectomy for treatment of their prostate cancer within two years after prostate biopsy. This biopsy must show no greater grade than Gleason 3+3 disease as determined on central pathology review. The patient may have had previous biopsies that showed cancer but may never have had a tumor grade greater than Gleason 3+3. Urine, blood, biopsy specimens and radical prostatectomy specimens will be collected from participants. The specimens will ultimately be assessed with biomarkers associated with prognosis. Clinical and demographic information will also be collected (see Appendix 3 - Data Collection Forms). The following will be included:

1. Date of index prostate biopsy (biopsy preceding surgery)
2. Most recent PSA (within 1 year) prior to biopsy.
3. PSA values over 5 years prior to index biopsy
4. Date of birth (on Tissue Release Authorization Form)
5. Race/ethnicity
6. Family history of prostate cancer (only first-degree relatives)
7. Number of biopsy cores obtained
8. Number of cores with cancer
9. Percent of core involvement with cancer (in cancer-containing biopsy cores).
10. High Grade Prostatic Intraepithelial Neoplasia present (yes/no); # cores involved
11. Prostate volume (using formula for prolate ellipse if volume not calculated at time of biopsy)
12. Gleason score (**must be 3+3 on biopsy**)
13. Atypia present? (yes/no)
14. Perineural Invasion present? (yes/no)
15. Prior negative prostate biopsy? (yes/no) If yes, all data as above collected
16. DRE exam results/date

## 1. Outcome Measures

The primary endpoint of this study is the presence of tumor upgrading at the time of radical prostatectomy. Upgrading is defined as: Gleason 3+4 or higher grade

A secondary study endpoint is presence of prostate cancer beyond the prostate (pathologic T3 disease) at radical prostatectomy. (This finding may be considered as a rationale for treatment.) Evidence of pathologic stage T3 or higher disease will be assessed by the presence of: (1) seminal vesicle invasion *or* (2) positive surgical margins *or* (3) established extracapsular extension *or* (4) lymph node involvement by tumor. It should be noted that pathologic stage T3 disease, while generally portending a greater risk of disease recurrence, may not be an intrinsic feature of disease prognosis but can be an artifact of surgical technique. Nonetheless, a secondary risk assessment tool will include both a Gleason 7-10 endpoint plus any patients with pT3+ disease to a composite endpoint that *could* indicate disease best managed with treatment in lieu of active surveillance.

## 2. Specimens to be Collected

### *a) Biopsy and Prostatectomy specimen preparation and gene expression quantitation*

1). H&E diagnostic slides from prostate biopsies that contain tumor will be identified. Eligible FFPE blocks, if available, must contain at least one core with at least 1mm of tumor. Verification of tumor histology including Gleason pattern will be performed by central pathology review (Phase I Aim 2).
2). Blocks with highest volume of representative tumor histology from the index focus, and representative H&E slides if available, from radical prostatectomy specimens will be reviewed centrally to validate final tumor grade and pathologic stage. If FFPE blocks are available for biopsy or prostatectomy specimens, 5µm sections will be made from the block containing the greatest linear length of representative tumor.
(see Appendix 2-Central Pathology Review and Consent)

### *b) Blood collection*

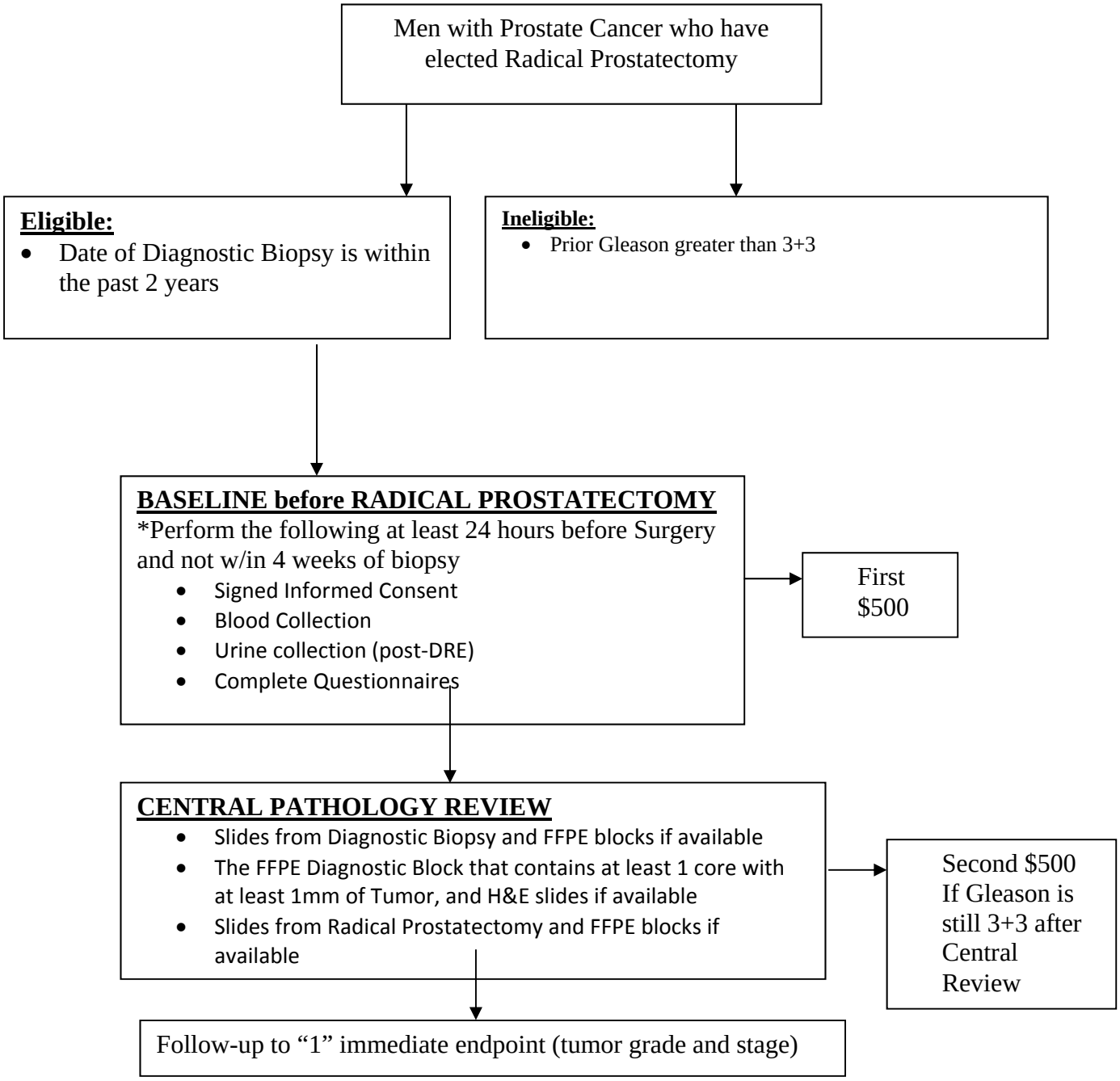
Blood will be drawn for PSA and free PSA; serum, plasma and buffy coat will be stored at -70$^{o}$C (see Appendix 4-Specimen SOPs)

### *c) Urine collection*

Post-DRE urine will be collected using standard operating procedures developed by the EDRN (see Appendix 4-Specimen SOPs).

Blood and urine collection must be at least 24 hours prior to radical prostatectomy and not within four weeks after prostate biopsy.

**SEE SCHEMA BELOW:**



### B. Aim 2:

The intended clinical application is to use clinical predictors and biomarkers (e.g., PCA3, T2-ERG, and other markers that may include tissue markers such as the Myriad Score identified by EDRN group in phase II) to accurately predict the existence of Gleason 3+4 or higher disease confirmed at radical prostatectomy. That will assist patients to making prostatectomy or active surveillance decision and would likely reduce unnecessary treatment in patients with low-grade prostate cancers. The products out of this study will be a prediction model for the risk of truly having Gleason pattern 4+ disease at prostatectomy – the 'gold standard' of tumor grading. A threshold will be chosen to "Rule out" patients from prostatectomy for active surveillance, patients whose risk score lower than this cutoff will have very low risk of having pattern 4 tumor. The risk curve and its confidence interval will also provide absolute risk estimation for patients who want to use their own estimated risk rather than suggested thresholds to make clinical decision.

The performance of upgrading risk calculator using clinical variables will unlikely have the performance adequate for making clear clinical decision because several nomograms are already available, yet most patients still do not use them for making clinical decisions. Therefore, we assume that this risk calculator based on clinical predictors will not be significantly better than the unacceptable performance criteria as defined as null hypotheses described in Section below.

## VII. Data Analyses:

Overview: The goal of this analysis will be to first determine those generally-available clinical variables that predict the risk of upgrading [Primary Objective] including PSA, age, race, ethnicity, clinical stage, and prior negative prostate biopsy. Of particular interests are the comparisons of the performance by clinical predictors alone, clinical predictors plus blood and urine based biomarkers and tumor-tissue-based markers, number of cores with cancer, % of cores involved, rectal examination findings, etc). As part of Phase 2, additional biomarkers that may be related to both objectives (e.g., Myriad Score, %free PSA, [-2]proPSA, PCA3, T2-ERG, and tissue molecular analyses) will be built into the predictive tool, EURAT, based on those biomarkers/tests that independently improve the prediction of these two outcomes. EURAT and a cutoff will be evaluated for its potential value to rule-out patients from prostatectomy.

Phase I: We will use a multivariate logistic regression model with backward model selection together with 10-fold cross-validation for Phase 1 data analyses. Goodness-of-fit will be assessed. The variables used in Phase 1 are clinical variables described above.

Phase 2: The clinical model score will be treated as a composite biomarker and used as a single predictor in the logistic regression with other new biomarkers to be added to assess their independent contribution using Likelihood Ratio test. Spline may be used for continuous predictor to accommodate possible nonlinear relationship at logit scale. Of particular interests are the comparisons of the performance by clinical predictors alone, clinical predictors plus blood and urine based biomarkers, and tumor-tissue-based markers. The final model, EURAT, will be evaluated for its goodness-of-fit in predicting upgrading risk. A cutoff will be chosen correspoinding to 98% sensitivity. The specificity at this cutoff and its one-sided 95% C.I. will be calculated. The null hypothesis of an uninformative test will be rejected if the lower bound of this C.I. is above 2%. The point estimate of the specificity indicates the portion of patients could be rule-out with high confidence that they do not have high grade tumor. NPV will be calculated at this cutoff. As a secondary analysis a high cutoff corresponding to 98% specificity will be chosen. The sensitity and its one-sided 95% C.I. will be calculated and test against null hypothesis of

25% sensitivity. This cutoff has potential to rule-in patients for prostatectomy. Patients fall in between two cutoffs are in “grey zone” that EURAT is not informative enough to make a clinical decision.

Ultimately, depending on funding for long-term follow-up of use of salvage or adjuvant therapies as well as disease recurrence, the risk assessment tool will be updated for this endpoint. Letters from Myriad and Dr. Cory Abet-Shen are included demonstrating their interested in the Upgrading Reference Set.

## VIII. Statistical Power:

The sample size depends on the primary aim of validation markers in this cohort, such as Myriad Score, and the performance of the panel, not a biomarker’s incremental value. We assume 30% upgrading at RP for patients with GS=6. The primary null hypothesis is H0: $ROC^{-1}(0.98)\geq 0.98$ for lower threshold and the secondary hypothesis is H0: $ROC(0.02)\leq 0.25$ for higher threshold. If the true false positive rate, $ROC^{-1}(0.98)\leq 0.75$ using this threshold, the study will have 90% power if we have 500 biopsy GS=6 patients. We will use the final study sample n=600 to account for various factors (lower than 30% upgrading rate, inadequate tumor tissue for assay, tissue blocks not retrievable, missing radical prostatectomy outcome). The power calculation used the formula for testing $ROC^{-1}(p)$ in Pepe et al[9] .( Pepe MS, Feng Z, Janes H, Bossuyt PM, Potter JD. Pivotal evaluation of the accuracy of a biomarker used for classification or prediction: standards for study design. J Natl Cancer Inst 100(20):1432-8, 10/2008.). Assuming 30% upgrading prevalence, 25% specificity at threshold corresponding to 98% sensitivity leads to NPV=96.7%, judged adequate for a patients with GS=3+3 to avoid radical prostatectomy and adopt active surveillance. Since this proposed study is really developing a model rather to validate an existing model, the power analysis did not consider the model building impact on performance assessment. Therefore, the model will need to be further validated in another independent cohort before it is considered as validated. However, Myriad Score alone with no other predictors added in the model can be considered as a pre-fixed model and our proposed study can be considered as a validation study for Myriad Score. Of particular interests are the comparisons of the performance by clinical predictors alone, clinical predictors plus blood and urine based biomarkers and tumor-tissue-based markers.

## IX. Long-term plans for use of the Upgrading Reference Set

After collection of the Upgrading Reference Set, the upgrading risk assessment tool will be developed, providing an immediately-valuable tool for the use of patients and physicians. Upon completion of the reference set, the set’s biologic materials will be made available for EDRN and other research institutions for proposed studies of additional biomarkers to be tested to seek markers that independently refine the prediction of high-grade disease.

Patients who are enrolled in this study will be consented for long-term follow-up of disease recurrence (i.e., PSA values) as well as use of adjuvant and salvage therapy. Upon initiation of the collection of the Upgrading Reference Set, GU Collaborative Group members will initiate submission of grant requests for the collection of this information over a 5-year period. Options for such follow-up could be at the level of the institution from whence the patient is enrolled or centrally, through the DMCC.

Potential biomarkers for prostate cancer which will be among those to be tested after collection of the reference set include:

- PCA3[14]

- [-2]proPSA[15]
- T2:ERG[16]
- Tissue-based markers (e.g., Myriad score and other markers currently under development)[17,18]
- SPOP and other mutations[19]
- Fusions of other ETS family transcription factor genes

## X. Inclusion/Exclusion Criteria

### A. Inclusion Criteria:

1. Prostate cancer, confirmed on prostate biopsy, within two years of scheduled radical prostatectomy.
2. Prostate cancer must be graded as Gleason 3+3 on the biopsy immediately prior to radical prostatectomy. (Secondary eligibility will be established on central review of pathology slides, and blocks if available, at Cornell Central Pathology Laboratory to confirm eligibility.)
3. Prostate cancer may have been detected on prior biopsy as well but must not be greater than Gleason 3+3. (Also requires Central Pathology Laboratory review.)
4. Slides must be available for Central Pathology Laboratory review on any biopsy showing prostate cancer. FFPE Blocks may also be requested if available.
5. Patient must have selected radical prostatectomy as treatment for prostate cancer.
6. Signed informed consent.
7. Blocks and/or slides from prostate biopsy and from radical prostatectomy must be available for analysis by Central Pathology laboratory.
8. Willingness to provide long-term follow-up information regarding additional treatments and cancer status.
9. Willingness to provide blood and urine specimens prior to radical prostatectomy for placement in the EDRN Upgrading Reference Set biorepository.
10. Willingness to provide demographic and clinical information related to prostate cancer and prostate cancer risk (e.g., race/ethnicity, family history of prostate cancer).

### B. Exclusion Criteria:

1. Gleason score greater than 3+3 on any prior prostate biopsy.
2. Any treatment other than radical prostatectomy planned for prostate cancer.
3. Prior treatment of the prostate with androgen deprivation, radiation, or other cytotoxic chemotherapy.

## XI. Recruitment and consent procedures

Patients will be identified by physicians and from other approved study personnel who are scheduled for radical prostatectomy at the participating clinical sites. Only those patients with a Gleason 3+3 tumor on biopsy within the prior 2 years will be approached for consent for participation.

After verifying patient eligibility, study personnel will explain the purpose of the study, the elements required for study participation, the benefits and risks.

For those patients who elect to participate, after obtaining informed consent, study related information will be obtained. At that time, blood and urine samples may be obtained or patients may opt for collection of these biospecimens at any time prior to radical prostatectomy (at least 24 hours prior to surgery) and not within four weeks after prostate biopsy.

## A. Schedule of Events

| Procedure | Baseline Visit (at least 24hr prior to surgery and not w/in 4 weeks after prostate biopsy) | Radical Prostatectomy Surgery | Post-Operative Collection |
|---|---|---|---|
| Informed Consent | ▯ | | |
| Medical authorization release | ▯ | | |
| Retroactive prostate treatment record collection (PSA values, biopsy report) | ▯ | | |
| Medical and surgical history | ▯ | | |
| Prostate Cancer Risk Factor assessment (family history of PCA) | ▯ | | |
| Blood collection<br>*Note: obtained by trained research personnel only* | ▯ | | |
| Digital Rectal Exam | ▯ | | |
| Urine collection (post-DRE) | ▯ | | |
| Collection of initial positive biopsy tissue (retroactive) | ▯ | | |
| Radical Prostatectomy | | ▯ | |
| Pathology Review of biopsy and prostatectomy materials | | | ▯ |
| *Long-Term Follow up chart review | | | ▯ |

*Will be completed if funding for phase 2 is received
** Red check mark indicates research procedure; black check mark indicates standard of care

## XII. Compensation

No compensation will be provided for study participation.

## XIII. Risks to subjects

This study is a non-interventional trial, examining the relationship of studies of biologic materials combined with clinical and demographic information and the risk of higher grade prostate cancer than that available from prostate biopsy.

As such, the primary risks to the subjects include:

a) There is a risk of bruising, bleeding, and pain from obtain blood specimens. This risk will be mitigated by ensuring that qualified personnel are conducted phlebotomy.
b) There is a risk that clinical or other personal information related to the study could be inadvertently released, compromising the patient's privacy. This risk will be mitigated by ensuring that only qualified and trained personnel participate in this study and that all of these personnel have been trained in privacy and other regulatory areas. Data from the study will be maintained in such a fashion to minimize the release of this information.

## XIV. Data and Safety Monitoring Oversight

A Data and Safety Monitoring Plan (DSMP) is required for all an individual protocols conducted under the leadership of University of Texas Health Science Center at San Antonio. All protocols conducted at UTHSCSA are covered under the auspices of the UTHSCSA Institutional Data Safety Monitoring Plan.

The UTHSCSA Institutional DSMP global policies provide individual trials with:

- institutional policies and procedures for institutional data safety and monitoring,
- an institutional guide to follow,
- monitoring of protocol accrual by the UTHSCSA Protocol Review Committee,
- review of study forms and orders by the Forms Committee,
- tools for monitoring safety events,
- monitoring of events by the Director of Quality Assurance and DSMC,
- determining level of risk (Priority of Audit Level Score – PALS) ,
- oversight by the Data Safety Monitoring Committee (DSMC), and
- verification of protocol adherence via annual audit for all Investigator Initiated Studies by the UTHSCSA Quality Assurance Division.

## A. Monitoring Safety

Due to the low risk associated with participation in this protocol, The Principal Investigator will conduct independent biannual review and report any findings to the UTHSCSA Data Safety Monitoring Committee (DSMC) and the UTHSCSA IRB. It is not anticipated that any safety issues will arise from this study because it is an observational study.

## B. Reporting Requirements

As per the UTHSCSA DSMP, any protocol modifications, problematic safety reports, unanticipated problems, and suspension or early termination of a trial must be reported to all members of the research team, participating clinical sites, and DMCC at Fred Hutchinson Cancer Research Center. Suspension and early termination of a trial must also be reported immediately to the Director of Quality Assurance (DQA) who will promptly notify the sponsor and the UTHSCSA IRB.

## C. Assuring Compliance with Protocol and Data Accuracy

As with all studies conducted at UTHSCSA, the PI has ultimate responsibility for ensuring protocol compliance, data accuracy/integrity and responding to recommendations that emanate from monitoring activities. PI/study coordinator will complete source verification of data, to be performed every 16 weeks. Protocol compliance, data accuracy and reporting of events is further ensured by an annual audit conducted by the Data Safety Officer, whose audit report is shared with the PI, the research team, and will be reviewed by the CTRC DSMC.

## D. Safety Definitions:

For this study, the following safety definitions will be applicable:

Unanticipated Problems Involving Risks to Subjects or Others Definition: Unanticipated problem involving risk to subjects or others includes any incident, experience or outcome that meets all of the following criteria:

| **Reporting Requirements** | | |
|---|---|---|
| Type of Event | Report to | Timeframe |
| Life threatening | IRB and DMCC (DMCC will inform UTHSCSA) | w/in 48 hours of PI determining “life threatening” |
| Non-life threatening | IRB and DMCC (DMCC will inform UTHSCSA) | w/in 7 days of PI determining “non-life threatening” |

A. unexpected (in terms of nature, severity, or frequency) given (a) the research procedures that are described in the protocol-related documents, such as the IRB-approved research protocol and informed consent document; and (b) the characteristics of the subject population being studied (note: the unfounded classification of a serious adverse event as “anticipated” constitutes serious non-compliance);
B. definitely related or probably related to participation in the research; and
C. suggests that the research places subjects or others at a greater risk of harm (including physical, psychological, economic, or social harm) than was previously known or recognized

All events will be reported following UTHSCSA institutional guidelines.

## XV. Data Management

Data will be entered into centralized database Validation Study Information Management System (VSIMS), a secure database maintained by the Early Detection Research Network

(EDRN) Data Management Coordinating Center (DMCC) at Fred Hutchinson Cancer Research Center. Data will be entered by participant ID only and will not be linked to personal information maintained securely at each study site. Individuals are granted access once they have completed VSIMS and protocol training and submitted a signed pledge of confidentiality. Sites and users are assigned unique ID's for data entry tracking.

## XVI. Appendicies

### A. Appendix 1 - Model Consent:

IR Number:_____ IRB Consent Approval Date:_____ IRB Project Approval Expiration Date: _____

**Institution Name**

**Consent to Be Part of a prostate Upgrading Reference Set for the Early Detection Research Network**

**Researchers' statement**

We are asking you to be in a research study. The purpose of this consent form is to give you the information you will need to help you decide whether to be in the study or not. Please read the form carefully. You may ask questions about the purpose of the research, what we would ask you to do, the possible risks and benefits, your rights as a volunteer, and anything else about the research or this form that is not clear. When we have answered all your questions, you can decide if you want to be in the study or not. This process is called "informed consent." We will give you a copy of this form for your records.

**PURPOSE OF THE STUDY**

The purpose of this study is to understand how prostate cancer behaves. One of the major goals of this research is to find unique markers and clinical parameters associated with aggressive prostate cancer. We are hopeful that these new markers can be used for developing better diagnostic tests and predicting the outcomes of prostate cancer.

You have been diagnosed with prostate cancer and have decided to have a radical prostatectomy. In this study, we will be gathering information about your medical history, obtaining various samples (blood, urine, tissue), and storing them for research related to prostate upgrading.

**STUDY PROCEDURES**

At least 600 men will take part in this study at multiple different study locations in the United States.

In this study your care will not differ from the care that you would receive if you do not participate in the study. However, in this study we will obtain blood, urine, and tissue that will be collected for research on prostate cancer. We will also ask you several questions about your health and medical history. You may skip any questions that you do not wish to answer.

If you agree to take part in this study, you will be asked to visit your urologist once before your prostatectomy to complete the study questionnaire and have blood drawn and urine collected. We will draw about two tablespoons of blood from a vein in your arm and provide you with a urine collection cup. These procedures need to be done at least 24 hours before your prostatectomy surgery.

We will ask you to allow us to retain your excess tissue collected during your surgery for research purposes. If you agree or not agree to participate in this study your surgery will proceed as usual. The same procedures (e.g. amount of tissue removed) will be followed regardless of whether you decide to participate in this study. Once the tissue is removed, we will work closely with the pathologist in obtaining excess tissue. This study will have no effect on the length of

your surgery or your diagnosis. Tissue is only obtained if it is not needed by the pathologist for a diagnosis.

We would like to collect data about your health status after your Study visit has ended. Study staff will, with your permission, obtain updated information about your health status from your medical records. You will not be asked to come into the clinic, and no specimens will be collected. You may request that this review stop at any time.

## RISKS, STRESS, OR DISCOMFORT

### ***Blood Draw Risks***

You may experience a little discomfort or have a temporary bruise from having blood drawn. All effort will be made to minimize this risk. Occasionally you may feel lightheaded or feel faint when having blood drawn. If you feel faint, tell the person drawing your blood and she or he will have you lie down until the feeling goes away.

### ***Invasion of privacy/confidentiality***

You may feel anxious or worried about research tests being done on your blood, urine and prostate tissue without the results being given to you.

You may feel uncomfortable about answering personal questions on the study questionnaire.

## ALTERNATIVES TO TAKING PART IN THIS STUDY

Participating in this study is strictly voluntary. If you choose not to participate it will in no way affect your medical care.

## BENEFITS OF THE STUDY

You will not benefit directly from participating in this study. The information gained from this research may help us further understand the dynamics of prostate cancer, thereby improving treatment and methods of early detection in the future.

## OTHER INFORMATION

We will ask you for permission to examine your medical records indefinitely following your entry into this study. We will use this information to link research findings from your samples to any prostate problems that are diagnosed. Your study data will be kept confidential. All study data and specimens will be labeled with a study number only. A personal information database, available only to study researchers, and kept in a locked area, will link your name to a study number. This link may be kept indefinitely.

It will be necessary for us to share information about your disease and study results with the following research team members and collaborators/sponsors, but all patient identifiers will be removed and confidentiality will be maintained:

- [Study Site Institution]
- the Early Detection Research Network's Data Management Coordinating Center at the Fred Hutchinson Cancer Research Center in Seattle, WA.
- government agencies that regulate research such as the Food and Drug Administration (FDA) or the Department of Health and Human Services (DHHS)

- the [Study Site Institution] committees that oversee research, including the Institutional Review Board that oversees the safety and ethics of [Study Site Institution] studies

Government or Sponsor staff some times review studies such as this one to make sure they are being done safely and legally. If a review of this study takes place, your records may be examined. The reviewers will protect your privacy. The study records will not be used to put you at legal risk of harm.

The tests we do on the blood and urine samples and the extra prostate tissue will be used for research on prostate disease. We plan to examine many characteristics, including levels of hormones and proteins. We will also examine many hundreds of genes, to see which ones are "turned on" and "turned off." We may also examine the DNA in your blood and in your prostate tissue. We cannot say exactly which genes or proteins we will examine, because research in this area is advancing very fast. To best perform research on prostate disease we will share the specimens we collect with other investigators in academia and industry under guidelines established by an Institutional Review Board (IRB, a committee responsible for protecting research subjects). Scientists using samples will not have access to your name or any other identifying information. The study Executive Committee will be responsible for the use of all samples.

Studies, such as this one, make it possible to test many different research questions, including ones for which we do not currently have funding or the technology. Thus, the blood and urine samples and prostate tissue specimens collected in this study may be stored indefinitely in a repository. You will be asked to sign separate consent forms for your samples to be stored in this repository.

***Ending the Study***

You can withdraw from this study at any time without loss of any non-study related benefits to which you would have been entitled before participating in the study. There are no dangers to leaving this study early. If you want to withdraw, you may do so by notifying the study staff. If you withdraw from the study review of your medical records will cease, however information and specimens obtained prior to your withdrawal will continue to be used for the purposes described in this document.

***Financial Information***

It is conceivable that during the course of these studies, results or products will be generated from the study and use of specimens provided by you that are of potential commercial value. There are no plans to provide financial compensation to you should this occur, nor will you be paid for participation in this study.

*Insert your site's compensation language and your site's language pertaining to care in the case of injury related to research.*

| Printed name of study staff obtaining consent | Signature | Date |
|---|---|---|

**Subject's statement**

I give permission for the prostate tissue and blood and urine specimens that I donate to be analyzed for research purposes. I understand that I will not receive the results of these tests. I understand that research using my blood may lead to the development of commercial products, and that I will not share in any profits that this work may produce.

I am aware that I and/or my insurance carrier are responsible for the costs of the prostatectomy that would happen as part of routine clinical care.

I acknowledge that I will receive a signed copy of this consent form. My signature below indicates that I have voluntarily decided to participate in this study.

This study has been explained to me. I volunteer to take part in this research. I have had a chance to ask questions. If I have questions later about the research, I can ask one of the researchers listed above. If I have questions about my rights as a research subject, I can call the Human Subjects Division at [insert phone number]. I give permission to the researchers to use my medical records as described in this consent form. I will receive a copy of this consent form.

| Printed name of subject | Signature of subject | Date |
|---|---|---|

**PLEASE PROCEED TO NEXT PAGE**

**********

## WE WOULD LIKE YOU TO DONATE SOME OF YOUR SPECIMENS FOR OTHER RESEARCH

After we do tests on your specimens in this study, some specimens may be left over. We would like you to donate these leftover specimens for future research. This may include genetic research.

You do not have to donate your specimens for research. You are free to say yes or no. Your regular medical care will not change. If we want to use your specimens for other research or share them with other scientists for research, we will ask the IRB. The IRB will decide if we need to ask for your consent to do the research.

Your donated specimens will be stored at NCI-Frederick National Laboratory for Cancer Research. They will be used for research only. Researchers will not report their results to you or your doctor. The research results will not appear in your health record. They will not affect your care.

Your specimens will not be sold. Research on your specimens may help develop new products. If these products make money, there is no plan to share the money with you.

If you donate your specimens for research, you can change your mind anytime. Just call Dr. [Site PI] at [phone] and tell us you do not want us to use your specimens. There is no penalty for changing your mind. Your regular medical care will not change. However, if you do change your mind, we cannot return donated specimens. We may be able to destroy specimens we know are yours. But if they are stored or shared anonymously (without any label saying who they belong to), we cannot destroy them. In this case they would still be used for research, but no one would know they were yours.

Read each question and think about your choice. When you decide on each question, please circle **YES** or **NO**.

| | | | |
|---|---|---|---|
| Do you agree to donate your specimens to study cancer? | | | |
| (circle one) | | | |
| **YES** | **NO** | Initials: | Date: |

| | | | |
|---|---|---|---|
| Do you agree to donate your specimens to study other health problems, such as diabetes, Alzheimer's disease, or heart disease? | | | |
| (circle one) | | | |
| **YES** | **NO** | Initials: | Date: |

| | | | |
|---|---|---|---|
| Is it OK if someone contacts you in the future for research purposes? | | | |
| (circle one) | | | |
| **YES** | **NO** | Initials: | Date: |

**FUTURE GENETIC RESEARCH:**

Several genetic databases are available to help researchers understand different diseases. These databases contain DNA samples and other information helpful to study diseases. DNA comes from cells in your body and contains all y our genetic information. As part of this study we would like to put your genetic information into these databases. Your information may benefit future research.

All of your personal information would be removed. Your name, address, etc will not be in the database. Only genetic information and information about your condition will be sent to the database.

There is a small risk that your genetic information could be matched against other genetic databases to get your name. Once we release your data to the central database we are no longer in control of the information.

| Is it OK if we send your genetic information to one or more databases for future research? | | | |
|---|---|---|---|
| (circle one) | | | |
| **YES** | **NO** | Initials: | Date: |

Printed name of subject Signature of subject Date

Printed name of study staff obtaining consent Signature Date

Copies to: Researcher
Subject

## B. Appendix 2 - Central Pathology Review and Consent:

### EDRN CENTRAL PATHOLOGY REVIEW:

Early Detection Research Network

ARCHIVAL MATERIAL PREPARATION INSTRUCTIONS

☐ H&E slides. Diagnostic H&E slides **of initial and subsequent biopsies** containing tumor are requested. When applicable, representative H&E slides of **prostatectomy** are also requested.

AND

☐ FFPE block(s). For **biopsies**: All blocks with tumor should be sent.

If primary **prostatectomy** is available: The blocks with the highest volume of representative tumor histology from the index focus should be sent.

*FFPE blocks should be placed within a ziplock bag. To ensure that the FFPE blocks do not break, the ziplock bag should be wrapped in bubble wrap. If bubble wrap is not available, the ziplock bag can be placed within a cryobox.*

OR

☐ Unstained slides. If blocks cannot be sent, a minimum of 15 x 5um unstained sections on unbaked, charged slides from the aforementioned blocks (biopsy/prostatectomy) should be sent.

**Tissue should be fixed in neutral buffered formalin and embedded into a paraffin block. If sending slides, these should be 5-micron thick, unstained, charged and unbaked, with accompanying H&E.*

Please send material to:

***Mark A. Rubin, M.D.***
*Weill Cornell Medical College*
*Department of Pathology and Laboratory Medicine*
*413 East 69th Street*
*Office 1402, 14th Floor*
*New York, NY 10021*
*Tel: 646-962-6166*

Once the material is shipped, please notify Ms. Diana Hernandez at dih2012@med.cornell.edu

Tracking number(s) and any related sample information should also be communicated.

Please feel free to contact us at any time should you have any questions.

**EDRN Pathology Tissue Request Template of Archival Material**

Date

Name: [Physician]
Address: [Institution]

Re: [Patient name, date of birth]

Dear Dr. [Last Name],

The patient above, who is currently enrolled in the Early Detection Research Network Study, Biomarkers and Clinical Parameters Associated with Gleason Score Upgrading, at [Site], had a previous surgical procedure at your institution on [Date].

Because analysis of archival tissue from the prior operation is essential for the study, we would greatly appreciate your assistance in providing the corresponding pathology material. **The patient's consent form is attached.**

We would be very grateful if the material could be prepared and sent to us at the earliest. Please use the FedEx pack provided, following the enclosed preparation instructions.

Thank you in advance for your time and assistance.

Sincerely,

Mark A. Rubin, MD
Professor, Dept. of Pathology
Weill Cornell Medical College
Study Pathologist
EDRN Study

**EDRN Release Authorization Template**

# AUTHORIZATION FOR RELEASE OF INFORMATION FORM

**Patient Name:**_____ **DOB:** _____

**Address:** _____

**City:** _____ **State:** _____ **Zip Code:** _____ **Phone #:** _____

I authorize _____ to release pathology material to
(Name of Institution)

EARLY DETECTION RESEARCH NETWORK STUDY: Biomarkers and Clinical Parameters Associated with Gleason Score Upgrading.

Type of material to be released:

☐ Pathology Slides/Reports: _____
☐ Archival tissue/FFPE blocks (See Instructions): _____
☐ Other (Please Specify): _____

Please send all the above material to:

***Mark A. Rubin, M.D.***
*Weill Cornell Medical College*
*413 East 69th Street*
*Office 1402, 14th Floor*
*New York, NY 10021*
*Tel: 646-962-6166*
*Fax: 646-962-0576*

_____ _____
Signature of Patient/Guardian/Legal Representative Date

_____ _____
If not patient, state relationship Witness

## C. Appendix 3 - Data Collection Forms

Separate document

## D. Appendix 4 - Specimen SOPs

Blood and urine collection must be at least 24 hours prior to radical prostatectomy and not within four weeks after prostate biopsy

## 1. Blood SOP

If the two (2) specimen types (blood and urine) are collected on the same day, then they must be processed in the following order: Pre-DRE blood, DRE, Post-DRE urine.

If procured on different days, the order for blood and urine collection does not matter.

Do not collect blood (or urine) from a patient who has evidence of urinary tract infection (treated or untreated) within the past 2 days.

Do not collect blood (or urine) from a patient who has had a cystoscopy or catheter within the past 2 days.

**Phlebotomy Materials and Procedure (recommendation from DMCC at FHCRC)**

**Materials:**
Gloves, sharps Container, 21- gauge Butterfly needle, attached tubing and Luer adapter, Tourniquet, Antiseptic wipes, bandages, centrifuge as per routine phlebotomy procedures at clinical sites

**Collection Procedure:**

Do not collect blood (or urine) from a patient who has evidence of urinary tract infection (treated or untreated) within the past 2 days.

Do not collect blood (or urine) from a patient who has had a cystoscopy or catheter within the past 2 days.

See http://www.bd.com/vacutainer/pdfs/blood_collection_tubes_product_insert_VDP40035.pdf
NOTE: Tubes with additives must be thoroughly mixed. Erroneous test results may be obtained when the blood is not thoroughly mixed with the additive.

Step 1. Assemble the supplies to be used in obtaining the specimen. Do not label the vacutainer tubes until specimen is obtained.

Step 2. Put on disposable gloves.

Step 3. The patient should be comfortably seated in a venipuncture chair. The arm should be positioned on a slanting armrest in a straight line from the shoulder to the wrist. The arm should not be bent at the elbow.

Step 4. Apply a tourniquet 2 inches above the antecubital fossa or above area to be drawn with enough pressure to provide adequate vein visibility. Have the patient form a fist. Select the site for venipuncture.

Step 5. Clean the forearm of the patient with antiseptic wipe in a circular motion beginning at the insertion site. Allow the antiseptic to dry.

Step 6. Anchor the vein by placing the thumb 2 inches below the site and pulling the skin taut to prevent the vein from moving. The holding finger is placed below the site, not above, to prevent accidentally sticking the finger with the needle.

Step 7. Using the dominant hand, insert either the butterfly needle Push the evacuated tube onto Luer adapter

Step 8. Release the tourniquet once blood flow is established.

Step 9. Carefully remove the tubes when full without dislodging the needle. The tube will automatically stop filling when the vacuum is gone leaving the tube approximately three-fourths full.

Step 10. Lightly place a sterile gauze pad over the venipuncture site. Gently remove the needle.

Step 11. Apply pressure to the site with sterile gauze. Apply bandage. Instruct the patient to leave the bandage on for at least 15 minutes.

Step 12. Dispose of the needle in a sharps container.

Step 13. Remove gloves and wash hands.

**Serum, Plasma and Buffy Coat Collection, Processing and Storage**

**Objective:**

To collect biologic samples on men prior to radical prostatectomy, in association with clinical information and common data elements appropriate for evaluation of prostate upgrading. All blood collection supplies are being provided by the University of Texas Health Science Center at San Antonio, or will be purchased directly by the sites themselves. Labels and worksheets will be provided by the DMCC.

**Materials:**

- Serum: One 10ml red top glass tube, no additive, no clot activator with silicone coated interior, (BD366430). http://catalog.bd.com/bdCat/viewProduct.doCustomer?productNumber=366430
- Plasma-EDTA: Two 6 ml EDTA plastic tube (367899) http://catalog.bd.com/bdCat/viewProduct.doCustomer?productNumber=367899
- Aliquot containers for serum, buffy coat & plasma specimens: 0.5ml Polypropylene Micro Tubes, screw top, conical skirted (Sarstedt 72.730) http://www.sarstedt.com/php/main.php. For whole blood, 2mL polypropylene tubes (DS C3172). http://www.denvillescientific.com/?q=searchs&title_op=allwords&k=C3172&submit_search=.
- 2-D barcode labels and worksheets for each subject contributing specimens (provided by the DMCC)

1. **Serum Specimens (processing consistent with** http://www.bd.com/vacutainer/pdfs/blood_collection_tubes_product_insert_VDP40035.pdf)

   Serum is obtained from whole blood collected in red top vacutainer tube with no additives or clot activators. The blood specimen should be allowed to clot for minimum of 30 minutes and maximum of 60 minutes at room temperature, stored at $4^0$C for up to 4 hours and then centrifuged 20 minutes at 1300g-force, 4 degrees Celsius. Serum (supernatant post-centrifugation) will then be placed in Eight 250µl and Ten 100µl aliquots and stored in 0.5 ml tubes.

   The goal is for the serum to be centrifuged and transferred to the Micro Tubes <u>within 4 hours of collection</u>, (however up to 18 hours is acceptable; time at $4^0$C will be recorded as a specimen-specific CDE). Samples are then frozen at –$70^0$C or colder until shipping to NCI-Frederick. Hemolyzed serum samples are to be excluded.

2. **Plasma and cellular fraction (Buffy Coat) specimens (processing consistent with** http://www.bd.com/vacutainer/pdfs/blood_collection_tubes_product_insert_VDP40035.pdf)

   Plasma and cellular fractions are being collected using a 6 mL EDTA containing tube. Plasma is obtained from whole blood collected in plastic vacutainer tubes containing EDTA from Becton-Dickinson. Tubes are inverted for mixing as per phlebotomy routine and BD instructions. The blood specimen should be placed immediately on ice or $4^0$C.. Centrifuge EDTA tube 10 minutes at 1500g-force at $4^0$C. Aliquot 0.2ml of plasma and store in 0.5ml tubes to get ten 200µl aliquots.

The goal is for the plasma to be centrifuged and transferred to the Micro Tubes within 4 hours of collection, however up to 18 hours is acceptable. Plasma samples are then frozen at –70$^0$C or colder until shipping to NCI-Frederick.

After removal of EDTA-plasma do not vortex or mix. Carefully take out buffy coat (between plasma and RBC), about 1mL. Divide 1ml buffy coat evenly between two 0.5ml tubes and then freeze at –70$^0$C or colder for future RNA extraction.. One 0.5ml tube can be kept locally.

3. **Whole Blood for future DNA isolation**
http://www.bd.com/vacutainer/pdfs/blood_collection_tubes_product_insert_VDP40035.pdf). A second EDTA tube will be used for banking whole blood that can be used for total DNA isolation analysis. Tubes are inverted for mixing as per phlebotomy routine and BD instructions but not centrifuged. A total of 4 mL of whole blood will be distributed between two 2mL tubes. Any remaining blood can be processed for local storage with proper IRB approval. Whole blood samples are then frozen at -70$^0$C or colder until shipped to NCI-Frederick.

4. **Sample Aliquots**
   a) Sites are being asked to collect a minimum of Eight 250µl and Ten 100µl of serum aliquots to NCI-Frederick. The remaining serum can be kept locally. If a site is unable to provide eighteen aliquots, please send what is obtained. Serum is stored in 0.5 mL microfuge tubes.
   b) Sites are being asked to collect a minimum of ten 200µl EDTA plasma aliquots to NCI-Frederick. Any remaining plasma from the EDTA tube can be kept locally. If a site is unable to provide fifteen aliquots, please send what is obtained. Plasma is stored in 0.5 mL microfuge tubes.
   c) Sites are being asked to collect two EDTA Buffy Coat, one will be provided to NCI-Frederick. The remaining EDTA Buffy Cells can be kept locally. Buffy coat is stored in 0.5mL tubes.
   d) Sites are being asked to collect two whole blood specimens for DNA isolation. One will be provided to NCI-Frederick the remaining tube can be kept locally. Whole blood is stored in 2.0 mL tubes.

## 2. Urine SOP

Do not collect urine (or blood) from a patient who has evidence of urinary tract infection (treated or untreated) within the past 2 days.

Do not collect urine (or blood) from a patient who has had a cystoscopy or catheter within the past 2 days.

Materials for collecting urine sample and labeling the urine specimens: Some of the supplies for processing urine are supplied by Gen-Probe and University of Texas Health Science Center at San Antonio.

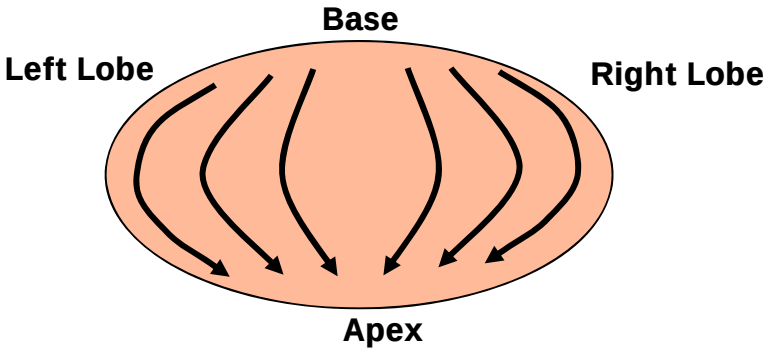
- 100 mL uncoated plastic urine collection container
- Transfer pipette for processing whole urine
- Five Gen-Probe/PROGENSA Urine Specimen Transport Tubes for processing whole urine
- 1.5 mL eppendorf tubes for processing and storing urine sediment
- Two 5ml tubes for storing supernatant
- ID Labels: One set of 2-D barcode labels for labeling all original and child urine specimens provided by the DMCC.
- Urine Specimen Collection Worksheet: contains copies of 2-D barcode labels and information that is necessary to record about urine specimen collection and processing. This worksheet will stay with the specimens until they are put in the freezer, at which point it will be put into the participants study chart. Worksheets provided by the DMCC.

Step 1. At least 24 hours before prostatectomy but not within 4 weeks after a prior prostate biopsy collect a post-DRE urine specimen will be collected. The following materials for urine sample collection are needed:

- 1- 100 mL uncoated plastic urine collection container (supplied by Michigan)
- Transfer pipettes
- Gen-Probe Urine Specimen Transport Tubes
- 1 Set of Urine Specimen ID labels (provided by DMCC)

Step 2. **Attentive DRE Procedure:**
The urine sample will be collected following an attentive DRE. The clinician will perform a standard of care attentive DRE as indicated below and document any suspicious nodules or other abnormalities (Figure 1). Apply firm pressure on the prostate from the base to the apex and from the lateral to the median line of each lobe as shown below. Apply enough pressure to slightly depress the prostate surface. Perform exactly three strokes per lobe.

Figure 1: Attentive Digital Rectal Exam of the Prostate




Step 3. **Whole Urine Procurement Procedure:**
Following the DRE, the subject will collect up to 100 mL of urine in a 100 mL urine collection cup labeled with provided Kit# or specimen ID. Men should be instructed to fill the urine cup starting with the first drop of urine until the cup is full or their bladder is empty. If the subject is unable to provide this quantity, collect at least 12.5 mL. Record the time and volume of urine collection. A sterile technique is not required for the procurement of the urine specimens.

- The first 12.5 mL of urine will be prepped in Gen-Probe Urine Specimen Transport Tubes (see step 4 below). If less than 12.5 mL is procured, then the entire specimen will be prepped in Gen-Probe Specimen Transport Tubes.
- The remainder of the voided urine will be prepped for urine sediment isolation (see sediment processing steps)

Step 4. In order to test the urine sample, the Initial Voided sample must be processed with the Gen-Probe Urine Specimen Transport Tubes. If not processed immediately, the urine sample should be placed promptly on ice or at 4°C and refrigerated for no longer than four hours.

Step 5. Instructions for processing urine:

1. Invert Initial Voided urine sample (in urine collection cup) 5 times to resuspend cells. Do not shake as it will cause foam and bubbles.
2. Using the transfer pipette, transfer 2.5 mL of urine to an appropriately labeled Gen-Probe Urine Specimen Transport Tube. The correct volume of urine has been added to the transport tube when the fluid level is between the black fill lines.
3. Screw on the transfer tube cap tightly then invert the transport tube 5 times to mix.
4. Four (4) additional aliquots of processed urine specimens will be made by following the same procedures 1 through 3 above, volume permitting. There should be a total of 5 processed urine specimens. Label the specimens with the provided Specimen Labels. All processed urine specimens should be frozen at -70°C or colder following processing/labeling.
5. Enter information into VSIMS Specimen Tracking System

**Urine Sediment**

After the whole urine has been processed as described above, the remainder will be used for urine sediment processing (if there is < 30 ml of urine after whole urine procurement (Step 5), then urinary sediment processing will not be possible).

Urine samples should be placed on ice or at 4°C immediately and refrigerated for no longer than 4 hours if not processed sooner.

Step 1. Invert the urine collection cup 5 times to resuspend the cells.

Step 2. Depending on the remaining volume of urine, follow the following plan for creating urinary sediment:

Step 3. If there is 60-90 ml – divide sample into two equivalent samples, one for RNA later prep and the second one for a PBS pellet

Step 4. If there is 30< 60 ml then create just one sample 30-40 ml for RNA later sample (PBS pellet will not be created).

Step 5. If there is <30 ml, then no urine sediment will be processed

**RNAlater sediment**

Step 1. Transfer 30-40 mL urine to a 50 mL conical and process as described below.

Step 2. Centrifuge: 10 min, 1000 x g, at 4°C. Do not use brakes.

Step 3. Remove supernatant, taking care not to disturb the pellet

Step 4. Decant the supernatant into separate tubes as individual 5 ml aliquots. Label with provided Specimen IDs.

Step 5. Add 5 mL ice cold PBS (PBS must be free of calcium and Magnesium Chloride) to the remaining pellet

Step 6. Re-suspend pellet thoroughly by pipetting up and down with a sterile pipet.

Step 7. Transfer suspension to a 15 mL conical tube.

Step 8. Centrifuge: 10 min, 1000 x g at 4°C. Do not use brakes.

Step 9. Remove and discard this supernatant, taking care not to disturb the pellet

Step 10. Add 1 mL ice cold PBS.

Step 11. Re-suspend pellet thoroughly by pipetting up and down with a sterile pipet.

Step 12. Transfer suspension to 1.7 mL labeled eppendorf tube.

Step 13. Microfuge: 10 min, 700 x g at 4°C.

Step 14. Decant.

Step 15. Resuspend (vortex) pellet in 250 microliters of RNAlater. Store at 4°C for 24 hours. After 24 hours, transfer the sediment tube to -70°C or colder in appropriate freezer box until shipping. NOTE: If specimen is collected on a Friday, the sample may be stored for up to 72 hours.

Step 16. Label specimen with provided Specimen IDs

Step 17. Enter information into VSIMS Specimen Tracking System

**PBS sediment –**

Step 1. Transfer at least 30 mL urine to a 50 mL conical and process as described below.

Step 2. Centrifuge: 10 min, 1000 x g, at 4°C. Do not use brakes.

Step 3. Decant supernatant, taking care not to disturb the pellet

Step 4. Add 5 mL ice cold PBS (PBS must be free of calcium and Magnesium Chloride) to the remaining pellet

Step 5. Re-suspend pellet thoroughly by pipetting up and down with a sterile pipet.

Step 6. Transfer suspension to a 15 mL conical tube.

Step 7. Centrifuge: 10 min, 1000 x g at 4°C. Do not use brakes.

Step 8. Remove and discard this supernatant, taking care not to disturb the pellet

Step 9. Add 1 mL ice cold PBS.

Step 10. Transfer suspension to 1.7 mL labeled eppendorf tube.

Step 11. Microfuge: 10 min, 700 x g at 4°C.

Step 12. Decant, Label specimen with provided Specimen IDs, and store at -70°C in appropriate freezer box until shipping.

Step 13. Enter information into VSIMS Specimen Tracking System

**Urine Specimen Transport and Storage Conditions**

Urine samples (in the urine collection cup) must be processed immediately or stored on ice or at 4°C and processed within 4 hours of collection. The processed urine specimens should be frozen at -70°C or colder until shipment.

Frozen processed urine specimens (in the transport tube) may be shipped overnight, on dry ice, to NCI-Frederick. Once received, these specimens must be stored at -70°C or colder. The processed urine specimens may be stored -70°C or colder. Processed urine specimens must be thawed to room temperature before testing and may be subjected to no more than one freeze/thaw cycles.

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