![Alberta Prostate Cancer Research Initiative logo — knowledge | action | impact]

STUDY PROTOCOL
APCaRI-05 version 5.0
CONFIDENTIAL

Clinical Validation of ClarityDX Prostate as a reflex test to PSA to refine the prediction of clinically-significant prostate cancer

Protocol Date: May 23, 2023

SIGNATURE PAGE – PROTOCOL AGREEMENT

By signing below, the investigators agree to adhere to the protocol. Any change in the study must be reviewed by a formal protocol amendment procedure and the principal investigators will submit all changes, amendments, and revisions to the Research Ethics Board (REB). Any change to the protocol that affects subject selection, safety, or changes in the conduct of the trial will require written approval from the sponsors and the REB before implementing the change.

The investigators also agree to conduct the study in accordance with the Declaration of Helsinki and the International Conference on Harmonization guidelines on Good Clinical Practice (ICH GCP).

The principal investigators also thereby agree that the study will be initiated on a subject only after the REB approves the informed consent forms. The investigators will obtain informed consent and document this process for all subjects enrolled in this study.

The investigators are Medical Experts responsible for conducting the trial and responsible for all trial-site related medical decisions

Dr. Adrian Fairey   _____   ___31May2023___

Principal Investigator   Signature   Date

Northern Alberta Urology Centre (NAUC)

11400 University Ave, Edmonton, AB T6G 1Z1

Dr. Eric Hyndman   _____   ___31May2023___

Principal Investigator   Signature   Date

Southern Alberta Institute of Urology (SAIU)

7007 14 St SW #6500, Calgary, AB T2V 1P9

Catalina Vasquez

Sponsor   _____   31May2023___

Nanostics Inc.   Signature   Date

5-142 Katz Group Centre (114 St & 87 Ave), Edmonton, AB T6G 2E1

PRINCIPAL INVESTIGATORS

Principal Investigator (KUC):   Adrian Fairey, MD

Address (Dept/Institute):   Dept. Surgical Oncology, University of Alberta

Telephone: 780-428-9405

Email: afairey@ualberta.ca

Appointment Held by PI: Assistant Professor, Division of Urology, Faculty of Medicine & Dentistry, U of Alberta

11400 University Ave, Edmonton, AB T6G 1Z1

Principal Investigator (SAIU):   Eric Hyndman, MD

Address (Dept/Institute):   Dept. Surgical Oncology, University of Calgary

Telephone: 403-943- 8910

Email: erichyndman@shaw.ca

Appointment Held by PI: Clinical Assistant Professor, University of Calgary

7007 14 St SW #6500, Calgary, AB T2V 1P9

Co-Investigators and Affiliation (all sites):

Adam Kinnaird, Assistant Professor, Division of Urology, Department of Surgery, Faculty of Medicine & Dentistry, U of Alberta, ask@ualberta.ca

Sunita Ghosh, Ph.D., P.Stat, Research Scientist / Assistant Clinical Professor, Alberta Health Services-Cancer Care/University of Alberta 780-577-8075 Sunita.Ghosh@albertahealthservices.ca

Richard Owen, Associate Professor Faculty of Medicine & Dentistry - Radiology & Diagnostic Imaging Dept., (780) 407-1210 richard.owen@ualberta.ca

Christopher Fung, Clinical Lecturer, Faculty of Medicine & Dentistry - Radiology & Diagnostic Imaging Dept., (780) 407-6907 cifung@ualberta.ca

Clinical Laboratory:

DynaLIFE Medical Labs #200, 10150 102 Street, Edmonton AB T5J 5E2

CONTENTS

1. BACKGROUND AND RATIONALE

Over-Diagnosis of Prostate Cancer

Prostate cancer is the most common non-skin cancer diagnosed in Canadian men, with an average age-standardized incidence rate of 110 per 100,000 men from 2011 to 2015 (excluding Quebec), and a 14% lifetime probability of developing the disease.[1] According to the 2017 Statistics Canada census report Alberta had 2,220 new cases of prostate cancer in 2015, which constitutes approximately one-quarter of all cancers diagnosed in men in Alberta.[1] Most prostate cancer patients are successfully treated for prostate cancer; for example, in Canada the five-year net survival rate for prostate cancer is 95%.[1] In addition, the age-standardized mortality rate has been decreasing steadily over the past two decades, in particular the rate decrease to 24 deaths per 100,000 men from 2011 to 2015.[2] This steady decline began shortly after the peak incidence of prostate cancer diagnosis occurred, around 1993, when the Prostate Specific Antigen (PSA) test became available as a common laboratory test.[3]

While the reduction of prostate cancer mortality can be attributed to the advent of therapies such as surgery, radiotherapy, and hormone treatments that are commonly employed to manage prostate cancer, there remains a fundamental problem of over-diagnosis due to PSA screening. Over-diagnosis results in patients being informed that they have prostate cancer when they do not have clinically significant prostate cancer, and this is also often associated with overtreatment. The estimated over-diagnosis rate ranges from 20-40% in the US, with an estimated mean lead time of 5 to 9 years, depending on the specific definitions used for lead time.[4]

Even though both the Canadian Task Force on Preventive Health Care and the US Preventive Services Task Force have recommended against population-based PSA screening because of evidence consistent with greater harms than benefits,[5] the uncertainties around lifetime probabilities of a common condition cannot be easily understood in order to dissuade tendencies towards screening. As Brett and Ablin (Ablin discovered PSA in 1970) aptly remarked, "the idea that physicians could initiate truly informed discussion was wishful thinking, because clinicians and patients had to consider an enormous list of probabilities estimates and uncertainties…"[6]

As health and life expectancy concerns permeate Western societies confronting an aging "baby-boomer" population, there is a pressing need to identify "a better test and better treatment options" for the management of prostate cancer.[7] The patient and the attending physician ask the following questions: Should I be worried about prostate cancer at all? Do I really need treatment for my prostate cancer? How is it that hidden prostate cancer can be dangerous to me? Not being able to definitively answer these questions contributes to significant overtreatment of prostate cancer - and a considerable negative impact on the quality of life of patients.[8] However, the concern at the other end of the spectrum is that every year many men still die of prostate cancer that was either not diagnosed early enough or treated inadequately.

Improving the Diagnosis and Prognostication of Prostate Cancer

While the five to ten-year cancer-specific survival for localized prostate cancer is 95% for Canada and 97% for the USA, the 5-year cancer-specific survival drops to approximately 29% for patients presenting with

distant metastatic disease.[9] Other important clinical prognostic factors include age, comorbidities, and pathologic indices of aggressiveness, in particular, Gleason grade and stage (locally advanced disease with invasion of seminal vesicles). Morbidity and mortality of prostate cancer are consequences of the biomolecular processes that lead to metastatic dissemination, which is determined by a tumour cell's ability to enter the stroma, invade surrounding vasculature, travel to a distant site, and grow in a new microenvironment.[10]

The myriad of biological processes that drive cancer progression provide the rationale for research to identify important biomarkers of metastatic potential during the latent or early phases of cancer development. Growing evidence suggests that low-risk patients can be managed with "active surveillance" which is defined as periodic assessment and continued observation of patients with low-risk, non-progressive localized prostate cancer.[11, 12] Opportunities are now available for studying the natural history of the disease, and, in turn, to search for biomarkers that may distinguish low-risk from high-risk patients. Such information would be invaluable to help clinicians and patients decide on the appropriate risk-based management strategy to follow.

Currently, the only method to diagnose prostate cancer is with a prostate biopsy, which is an invasive procedure associated with harmful side effects. In the Alberta Prostate Cancer Research Initiative (APCaRI) cohort (see section 0), of men that underwent a prostate biopsy, 60% were diagnosed with prostate cancer, but only 24% were actually found to have aggressive prostate cancer as indicated by a pathology ranking of Gleason group 3 or higher. In other words, more than 75% of men were exposed to a biopsy unnecessarily since they either did not have prostate cancer or had an indolent and clinically insignificant disease. The prostate biopsy procedure is uncomfortable and may cause fever, rectal bleeding, infection and in the worst cases, sepsis.[12] For example, in Edmonton, Alberta during 2017, the estimated post-prostate biopsy sepsis rate was close to 4%, translating into over 70 patients who developed a life-threatening infection from the prostate biopsy procedure and a good proportion of these men did not actually have prostate cancer. The above-mentioned factors have a significant weight on the quality of life of men and on the healthcare system. These numbers illustrate the need for a non-invasive test that accurately predicts which patients have clinically significant prostate cancer.

Team-Based Discovery and Validation of Non-Invasive Prostate Cancer Tests
The Alberta Prostate Cancer Research Initiative (APCaRI) is a province-wide multidisciplinary research program that was initiated in 2012 with seed funding and facilitation from the Alberta Cancer Foundation and the University of Alberta. The program's mandate is to develop, validate and translate novel tools to improve prostate cancer detection and to accurately determine the clinical significance of prostate cancer. This mandate is aimed to improve the prediction of metastatic potential that can lead to death from prostate cancer in patients who present with clinically localized disease.

Clinical validation of these tests requires large numbers of high-quality patient blood linked to accurate and complete clinical outcome data. The Alberta Prostate Registry and Biorepository was created to allow APCaRI team members to apply state-of-the-art genomic, proteomic, metabolomic and transcriptomic analyses to the development of new and better tests for prostate cancer.

The biorepository is a prospective validation study, for which blood samples that precede the diagnosis of prostate cancer, were collected. The clinical outcomes of the biospecimen donors (i.e. consenting study participants) are followed prospectively, with attention to prostate cancer related events (including diagnosis, treatment(s), relapse(s) and death). The APCaRI created the Alberta Prostate Cancer Registry (APCR) and Biorepository (APCB), which collects health information and blood samples, respectively, from a prospective cohort of men without prior diagnosis of prostate cancer who present with PSA-screened or clinical suspicion of prostate cancer requiring prostate biopsies or transurethral prostate surgery.

The ClarityDX Prostate test, developed in Dr. John Lewis' laboratory, is based on a combination of 1) micro-scale flow cytometry technology, 2) innovative and accurate nano-immunoassay and machine learning of tumour biomarker expression in extracellular vesicles (EVs) using cancer specific antibodies, called the ClarityDX Prostate Flow Score, and 3) a machine learning approach that incorporates the Flow Score with clinical features to produce the ClarityDX Prostate Risk Score. Up to six clinical features will be incorporated into the ClarityDX Prostate Risk Score, and they are listed in Table 1. This methodology was utilized to predict the results of diagnostic biopsies to distinguish those biopsies that are benign or Gleason Groups 1 and 2 (considered low grade disease that typically does not require treatment) from Gleason Group 3 and higher (an indication of aggressive or clinically significant cancer) with an AUC (Area Under the Curve) of 0.84 in pre-biopsy frozen plasma samples from 377 total patients (289 patients in training group, 88 patients in test group) from the APCaRI cohort (manuscript in preparation).

Table 1 - Clinical Features of the ClarityDX Prostate Test

|		
Age	Total & Free PSA Blood Test Result	Family History of Prostate Cancer
Ethnicity	Previous Prostate Biopsy Status	Digital Rectal Exam (DRE) Results
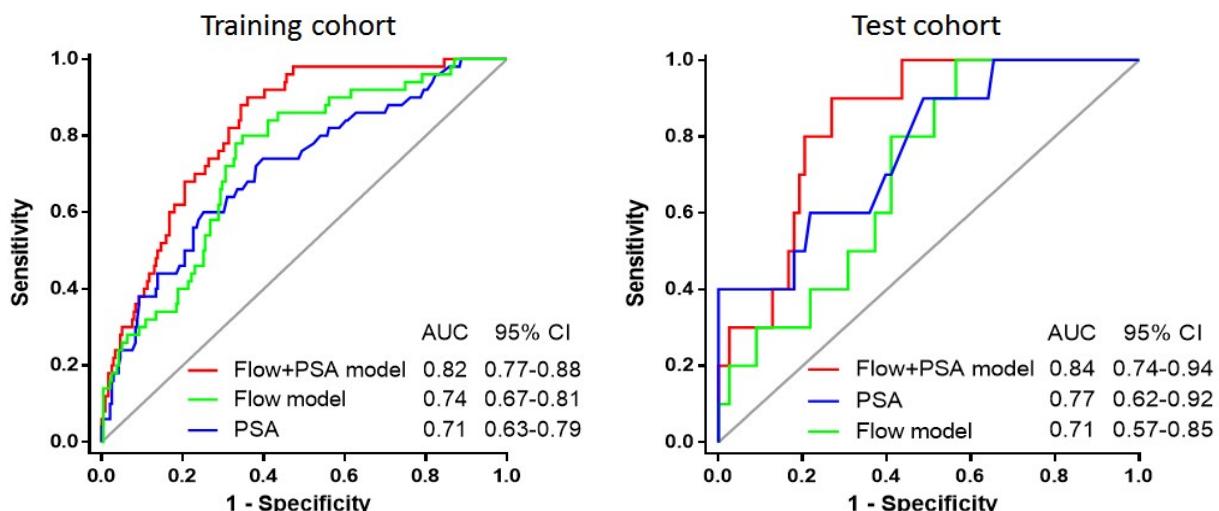
Figure 1. AUC table for validation study in cohort of 377 patients

The early test results with ClarityDX Prostate are very encouraging and now need to be validated to ensure that it can predict the prostate biopsy pathology, thereby better informing the decision process, improving

diagnosis and treatment for prostate cancer, maintaining the patient's quality of life, and saving millions of dollars to the health care system and to our patients.

Diagnostic Clinical Performance Study of ClarityDX Prostate to assist diagnosis of prostate cancer

The clinical performance of ClarityDX Prostate, validated using the APCaRI prospective cohort, will be assessed by carrying out a new study in partnership with DynaLIFE Medical Labs in a new cohort of up to 2,800 eligible patients that present at participating sites.

The study was designed based on guidelines from Health Canada, the US Food and Drug Administration (FDA), Standards for Reporting of Diagnostic Accuracy Studies (STARD); (13) and Transparent Reporting of a multivariable prediction model for Individual Prognosis or Diagnosis (TRIPOD) principles for diagnostic accuracy studies (14). Conduct of the study will be guided by the Tri-Council Policy Statement and ICH Good Clinical Practices E6 Guidelines.

About DynaLIFE Medical Labs

DynaLIFE is Alberta's largest privately-owned clinical laboratory. Headquartered in Edmonton (#200, 10150 102 Street, Edmonton AB T5J 5E2) they employ over 1200 medical, technical, and support staff throughout central and Northern Alberta. DynaLIFE performs over 18 million publicly funded laboratory tests annually for 1.5 million Albertans in state-of-the-art laboratories. DynaLIFE Medical Labs is accredited by the College of Physicians and Surgeons of Alberta and carries international accreditation through the College of American Pathologists. DynaLIFE is a key training facility for laboratory training in Western Canada and has demonstrated experience in partnering with research labs and small biotech enterprises to help validate new diagnostic tests. Their involvement in training programs, medical research, and the development of new and improved methodologies is extensive.

"For DynaLIFE, innovation is survival. Health care will continue to face the inevitability of aging populations and increased demands against scarcity of resources both on people and the government's ability to fund. The answer is a continuous quest for new technologies, new innovation, new processes and new products that allow us to deliver higher value health outcomes for the same or fewer resources."

Jason Pincock, CEO of DynaLIFE Medical Labs

Proposed Intended Use for the Test

The ClarityDX Prostate test is a semi-quantitative immunoassay, combining PSMA, ghrelin, polysialic acid, Cadherin-11, and prostein biomarkers via microflow cytometry performed using the Apogee GxP in SST serum or EDTA plasma to generate the ClarityDX Prostate Flow Score. The Risk Score uses a model to combine the Flow Score and clinical features, including total & free PSA (Roche Cobas), age, ethnicity, family history of prostate cancer, previous negative biopsies, and results from DRE to predict the risk of having clinically significant prostate cancer. It is a reflex test to be performed after a total PSA (Roche Cobas) result ≥ 3 ng/mL is obtained. This test is a single site assay performed at DynaLIFE Medical Labs, Edmonton Alberta Canada. The ClarityDX Prostate test is indicated in men aged 40-75 years old (inclusive) with no previous history of prostate cancer.

Men undergoing this evaluation would otherwise be well enough and have long enough expected survival to be considered for treatment of prostate cancer that unless treated would be the cause of their death. ClarityDX Prostate is used to refine the prediction of clinically significant prostate cancer, defined as Gleason Group Grade 2 or higher. A ClarityDX Prostate Risk Score of greater than a certain cut off value indicates high risk of the presence of clinically significant prostate cancer. ClarityDX Prostate Risk Scores should be used in conjunction with the information from a complete clinical evaluation including PSA, DRE or other diagnostic tests. Diagnosis of prostate cancer can only be determined by prostatic biopsy.

2. OBJECTIVES AND AIMS

Overall Aim

The aim of this investigational study is to evaluate the performance of ClarityDX Prostate as a reflex test that is intended to refine the ability to predict clinically significant prostate cancer, beyond currently available diagnostic and prognostic systems guided by biochemistry, namely the PSA test.

2.1 Objectives

The main objective of this study is to establish the performance characteristics of ClarityDX Prostate in a prospective cohort of men to be recruited at the Kipnes Urology Centre in the Kaye Edmonton Clinic, the Southern Alberta Institute of Urology in the Prostate Cancer Centre, at Whitehorse General Hospital and in selected sites in the United States of America, when they are scheduled for a biopsy as a result of an elevated PSA or other suspicion of prostate cancer and in retrospective datasets from selected sites in the United States of America and the world.

The objectives of this study are to:

- Establish a prospective study cohort of up to 2,800 men;
- Collect blood and clinical information pertaining to the diagnosis of prostate cancer;
- Assess diagnostic clinical performance of the ClarityDX Prostate test by measure(s) that quantify how closely the diagnostic output is associated with the diagnosis of a clinically significant aggressive prostate cancer at biopsy, how well it correlates with mpMRI results, and how well it predicts the results of subsequent biopsies in participants who decide to go on Active Surveillance. These measures may include sensitivity, specificity, reproducibility, likelihood ratio of positive and negative result pairs, and ROC (receiver operating characteristic) analysis along with confidence intervals and significance levels, Positive Predictive Value or PPV, Negative Predictive Value or NPV, and pre and post-test probabilities;
- Determine the cost-effectiveness of PSA plus ClarityDX Prostate testing relative to PSA testing (i.e., standard care) alone, to document the economic value of ClarityDX Prostate test technology as a supplemental test for prostate cancer diagnosis and Active Surveillance management from a health system perspective;
- Perform method assessment and analytical validation of the ClarityDX Prostate test; and
- Identify if there is interference from prostate-associated medications and co-morbidities.
-

3. SELECTION, RECRUITMENT AND WITHDRAWAL OF PARTICIPANTS

3.1 Study Population

This study will consist of up to 2,800 consenting men from Alberta in addition to up to 5,000 men from external institutions, between ages 40-75 (inclusive) years old, without prior diagnosis of prostate cancer, who have been selected to undergo a prostate biopsy to rule out prostate cancer. Only patients with total PSA ≥ 3 ng/mL will be selected to have the ClarityDX Prostate test performed as a reflex test.

Eligibility Criteria

a. Males between 40-75 (inclusive) years of age;
b. With and without family history of prostate cancer;
c. No prior prostate cancer diagnosis and who are referred to have a prostate biopsy;
d. Total PSA results ≥ 3ng/mL collected within 6m of enrollment;
e. Willing to permit local agencies (e.g. Alberta Health Services, Alberta Health, Netcare, Service Alberta or other based on recruitment jurisdiction) to disclose health-related information to study;
f. Undergoing a diagnostic prostate biopsy; and
g. Provided informed consent to participate in the study.

Exclusion Criteria

a. Unwilling to participate in the study;
b. Unavailable for biopsy procedure in recruitment areas;
c. Not undergoing a prostate biopsy;
d. Prior diagnosis of cancer excluding non-melanoma skin cancer; and/or
e. Under the age of 40 years of age or over the age of 75 years of age.

3.2 Participant Recruitment and Consent Process

3.3.1 Prospective Cohort

Prospective participants will be recruited at multiple sites in Canada and the United States over a 2-3-year period by one of multiple potential approaches:

i. Patients that visit specialized urology and oncology clinics in Alberta are typically given a blanket Informed Consent Form (ICF). This form was previously approved by HREBA-CC. By signing it, participants give us permission to access their health information for research and indicate if they allow us to contact them for this and other studies. If they agree to be contacted and are eligible for the study, delegated members of APCaRI will contact them to offer participation in the other portions of the study including biospecimen collection and completion of questionnaires.

ii. Patients will be referred to specialized urology clinics in Edmonton (Kipnes Urology Centre and/or Diagnostic Radiology), Calgary (Southern Alberta Institute of Urology/Prostate Cancer Centre), Whitehorse General Hospital or to selected sites in the United States of America. Patients will be pre-screened for study eligibility and if they are deemed potentially eligible, they will be approached by

their family physicians, urologists, clinical research coordinators (CRC) or clinical research nurses (CRN) for potential participation in the study.

iii. Information about the study will be put onto posters and displayed at the recruitment centre(s) and will be posted online in the APCaRI and Nanostics webpages for recruitment purposes. Interested patients can contact the study site and will get recruited by the coordinator directly.

The consenting process will be done as follows:

i. The CRC or CRN will review and explain the study, answer questions and obtain informed consent. Participants will complete the intake process; including completing a baseline survey and having biospecimen samples collected either during the visit or will be asked to arrive about 1 hour early at the imaging centre on the day of biopsy to go through the intake process.ii. In addition to the ICF an intake survey and a medication questionnaire will be given to consenting participants. The surveys will collect demographic information, use of medications for prostate-related conditions, co-morbidities, history of previous prostate biopsies, and family history of prostate cancer.

By signing the ICF, participants give APCaRI permission to access their personal information (Full name, Date of Birth, Personal Health Number, email address, phone number) and health information (co-morbidities, family history of prostate cancer, medications, and information associated with prostate cancer diagnosis) for research and agree to be contacted for present and future studies. The ICF will contain an explanation that the ClarityDX Prostate assay will be performed on their blood at DynaLIFE Medical Laboratories in Edmonton, Alberta and that the results from this test along with results from the PSA, clinical features, and biopsy will be compared to test the accuracy of the ClarityDX Prostate Risk Score in predicting prostate biopsy pathology. By signing the ICF participants agree to have results from the ClarityDX Prostate Flow Score analyzed and linked to their clinical information. The group is expecting to recruit up to 2,800 patients (please refer to section 10.2**Error! Reference source not found.** for more details).

APCaRI will provide the clinics and/or diagnostic imaging facilities with ICFs, intake surveys, and posters. It is the responsibility of the local Investigator or the research staff designated by the local Investigator (i.e., CRC or CRN) to provide each potential study participant, prior to collection of biospecimen samples and information, adequate verbal and written information regarding the objectives and procedures of the study and the possible risks involved. The patient will be informed about their right to withdraw their participation at any time and must be allowed adequate time to make an informed decision. A copy of the signed and dated consent form will be provided to the patient.

3.3.2 Retrospective Cohort

Retrospective cohorts will be obtained from sites/institutions that have existing datasets with men with elevated PSA who were referred for a prostate biopsy to rule out prostate cancer. All patients who underwent the biopsy and meet the eligibility criteria will be included. As the datasets are retrospective in nature and the data will be completely anonymized, a waiver of consent is requested.

3.3.4 Discontinuation/Withdrawal

Prospective participants may withdraw participation at any time by email, phone, in-person, or in writing. Each withdrawal will be reviewed by the person receiving it, who will communicate this to the study manager. If both biospecimen use and information are withdrawn, no further contact relevant to the study will be made with the patient. However, health information and blood samples analyzed up to that point will still be included in the study analysis. The patient's study status will be classified as "withdrawn" and no further electronic data linkage and retrieval will be performed for that case.

4.   BLOOD SAMPLE COLLECTION, PROCESSING AND STORAGE

4.1   Blood Sample Collection

Blood samples will be collected in EDTA and/or SST tubes at participating sites at baseline (i.e. when the patient is ordered a biopsy or on the day of the biopsy (pre-biopsy)) , and before confirmatory and follow-up biopsies for participants who go on Active Surveillance by trained phlebotomists following standard operating procedures (SOPs). A unique identifier number will be assigned, and after processing, the sample will be delivered to DynaLIFE Medical Labs to run the ClarityDX Prostate assay. Additionally, a total & free PSA test will also be performed on a Roche Cobas instrument.

The sample matrix (serum collected using BD SST (tube with silica clot activator, polymer gel, silicone-coated interior) and/or plasma collected in EDTA anticoagulant) will be determined by an ongoing sample stability study and SOPs and laboratory manual will be updated with this information.

4.2   Blood Sample Processing

Patient blood samples will be processed as per SOPs to obtain serum and/or plasma by the team at each site and will be shipped to DynaLIFE following specific guidelines.

4.3   ClarityDX Prostate Assay

Since PSA values will trigger the reflex test, MLTs (Medical Laboratory Technician) will know that PSA values are high, however, the exact result of the test won't be provided to them. In addition, because the decision to biopsy will be taken further along the care pathway, MLTs will not have these data at the time of running the test thus reducing the risk of bias.

4.3.1   Analytical testing will be done in accordance with applicable regulations and guidelines.
4.3.2   Proper calibration and controls will be run before the test is performed in accordance with SOPs.

4.4   Blood Sample Storage

Once PSA testing is performed and a ClarityDX Prostate Risk Score is obtained, samples may be stored at DynaLIFE at -80°C for the duration of the study in accordance with FDA and/or Health Canada regulations, and applicable local regulations. Subsequently samples will be destroyed following DynaLIFE SOPs. Samples will only be used for activities related to this study.

4.5 Data Output

ClarityDX Prostate test results will include a Risk Score. The Risk Scores represent probabilities that the patient has clinically significant prostate cancer as indicated by Gleason Group 2 or higher for the diagnostic group and how well it predicts the result of confirmatory and follow-up biopsies in the Active Surveillance group. It e uses clinical features (PSA, fPSA , age, etc.) for a more accurate prediction of clinically significant prostate cancer and will range from a low of 0% to a high of 100%.

4.6 Data Analysis

4.6.1   **Training Phase**: During the Training Phase of our clinical trial, processed common clinical features such as total and free PSA test result, age, ethnicity, previous negative biopsy status, family history of prostate cancer, and/or DRE findings will be analyzed using machine learning to predict clinically significant prostate cancer, with the output being the ClarityDX Prostate Risk Score.

Machine learning will identify the minimum number of clinical features needed for this prediction, as well as the optimal probability threshold to provide optimal model performance based on a model ROC area under the curve (AUC) and maximum specificity with at least 90% sensitivity. The probability output from this logistic regression model is the ClarityDX Prostate Risk Score and is presented as both a quantitative and qualitative (High and Low) probability Risk Score. The Risk Score probability thresholds that separate these two qualitative risk groups will be determined at the end of the training phase of the clinical trial. For each patient, total PSA values will be automatically verified to be performed in a Roche Cobas instruments and to be ≥ 3 ng/mL. Any patient with a total PSA value outside of the cut-off will be removed from analysis. The training machine learning phase will be closed and locked in prior to the clinical validation phase.

4.6.2   **Validation Phase:** During the Validation Phase of the study, patient data and clinical features will be used as an input for the previously made models in the Training Phase. Patient status for clinically significant prostate cancer will be determined using the optimal probability threshold determined in the Training Phase. Results will be presented in a manner similar to the Training Phase, and these results will be considered investigational and not communicated or used in patient management. Performance of the ClarityDX Prostate test will be determined for patients of most representative ethnicities as well as for individual ethnic groups including Caucasian, Asian, African American, Latin American, and Native American.

The final model requires up to six common clinical features relevant to prostate cancer. If any clinical feature other than total and free PSA is missing, median imputation may be performed using the median values in the training phase dataset.

4.6.3   Interim analysis will be done to assess recruitment rate and benign vs. cancerous biopsy ratios and sample size will be updated as/if applicable.

5. PATIENT MANAGEMENT

This study will <u>not</u> provide results of the investigational diagnostic test to the medical team or to patients for the management of the prostate condition. Study results will not impact decisions about prostate biopsy. Standard of care will not be altered. Instead, a thorough analysis of the care pathway followed for each patient will be performed to assist in the performance analyses.

6. DATA COLLECTION

6.1 Range and Scope of Patient-level Health Information

6.1.1 Personal Information

Patients will be asked to provide personal information including full name, Date of Birth, Personal Health Number, email address, and phone number. Information will be used as unique identifiers to i. match data during linking process with different data repositories listed in section 6.2 and ii. communicate with participants with information about the study, or other related studies.

6.1.2 Clinical Information

The information below will be collected to: i. validate the performance of ClarityDX Prostate test, ii. perform health economics and cost-benefit analysis of the test, and iii. identify if medications have an effect in the results of the ClarityDX Prostate test.

- Prostate pathology: cancer or non-cancerous prostate pathology;
- Adverse events that resulted from performing the prostate biopsy;
- Cancer diagnosis: cancer staging, standard prognosticators, MRI (e.g. PSAs, Gleason groups);
- Use of medications for: i. urologic conditions and ii. other conditions.

6.1.3 Patient-reported Information

- Basic demographics, race-ethnicity, date of birth to calculate age, PHN, full name, email address, phone number, comorbidities, medications, previous history of prostate biopsies, and family history of prostate cancer.

6.2 Data Sources

Participants will consent to having their health information disclosed by their family physicians, urology clinics, Alberta Health Services, Alberta Health, Service Alberta, health facilities in global participating sites.

- From the Kipnes Urology Centre: Connect care or other similar EMR, will be accessed for data regarding consultation, digital rectal examination, laboratory and pathology, medications, and diagnostic imaging.
- From Alberta Health Services: cancer diagnosis information may be retrieved via the Cancer Measurement Outcomes Research and Evaluation (CMORE) and the Alberta Cancer Registry (ACR). Hospitalization, emergency room visits, and other health care resource utilization associated with adverse events from biopsy may be retrieved from Data Integration, Management and Reporting (DIMR). ARIA MO, ARIA RO, and Connect care or other similar EMR, may be used to collect information

associated with the disease for analysis and data related to diagnosis (PSA, DRE, pathology), treatment (Active Surveillance, Radical Prostatectomy & pathology report from prostatectomy, EBRT, Brachytherapy, Chemotherapy, ADT, antiandrogens, gland ablation and watchful waiting) for the Health Economics analysis.

- From Alberta Health: NetCare may be accessed for data regarding medications, laboratory and pathology, diagnostic imaging, and treatments, Alberta Blue Cross, Pharmaceutical Information Network, Population Registry, and Practitioner Claims. These databases will be used should elements not be available through Connect care or other similar EMR.
- From Service Alberta: Vital Statistics, date of death, causes of death, location of death may be accessed for the health economics study.
- Laboratory services: Total and free PSA, testosterone, CBCD, ALP, Potassium, Bilirubin, Creatinine, GFR, ALT/AST, LDH levels, and biopsy results may be accessed for health economics analyses if not available from HealthQuest/NetCare.

6.3 Data Storage

Patient-reported data and clinical parameters will be captured using REDCap (Research Electronic Data Capture). REDCap is a secure web application for building and managing clinical databases via the internet. It is designed to meet the security and privacy needs of clinical research. Access restrictions are assigned individually, user activities are logged for auditing, and a de-identifying feature is available for data extraction. These tools are built into the REDCap platform and greatly simplify the management challenges associated with these kinds of databases. REDCap is hosted and maintained by The University of Alberta Faculty of Medicine and Dentistry. For easy data querying and visualization, an online clinical information system called PROSPeCT that was developed by APCaRI will be implemented. Working closely with the REDCap system, anonymized data is hosted securely in an encryption protected, java-based web application. The system allows its applicable users to extract information from and answer questions about the data using a variety of intuitive and helpful interfaces. Data will be retained for 15 years as per Health Canada and FDA regulations.

Hard copies are stored within 3 secured areas. The file cabinet that stores the source documents are located:
a) at participating sites within facilities with secured main door entry access
b) housed in the secure Research Unit department(s) which has restricted access
c) within rooms that have key access
d) filed within the delegated filing cabinets

Electronic information will be kept in secured, encrypted, firewall protected servers that have obtained PIA approval.

6.4 Data Linkage and Management

We may link the study database with other databases at regular intervals (usually every 6 months to a year). These other databases include:
- DynaLIFE Medical Labs and/or Alberta Public Labs

- Alberta Health, Alberta Cancer Registry and Cancer Measurements Outcomes Research and Evaluation
- Discharge Abstract Database
- National Ambulatory Care Reporting System

While the precise process for matching data with the databases listed above will vary, we generally see this process involving:
- Extracting the data elements required for uniquely identifying participants in the other database from the database. These elements may include personal health number, date of birth, first name, and last name.
- Transmitting these sensitive data through a secure website or physical media transported by an approved courier service. All files containing identifiable data elements will be password-protected, with the password being sent separately.
- Matching participants from the registry with their respective records in the other database. The matched data set will be extracted and transmitted back to APCaRI as in step 2.

6.5 Data Access & Retrieval
Identifiable information will be accessible only to authorized study personnel, including: study principal investigators, APCaRI program manager, clinical research coordinator/nurse, data entry/analysis staff, database administrator, biosample collection personnel, and monitor/auditor(s) as needed. Applicable users of the PROSPeCT platform will have secured access to de-identified data.

For some quality improvement, health economics, and translational research questions, date of birth and postal code may be necessary variables for age calculation and socioeconomic status correlation. In which case, these non-unique identifiers will be released to investigators for explicit scientific purposes. Because this cohort study is targeted at large samples, it is unlikely that disclosure of these non-unique identifiers will incur a significant risk to participants' privacy and confidentiality. Furthermore, investigators will sign confidentiality agreement with APCaRI, with explicit understanding that individual patient identity will not be re-established.

6.6 Data Reporting
Aggregate reporting: APCaRI may use the linked data sets as the basis for reporting aggregated, de-identified data to its stakeholders, including clinicians, Health Canada, The US Food and Drug Administration, European health regulatory agencies (CE Mark), and funding agencies nationally and internationally. These reports will present de-identified data for those patients recruited for participation.
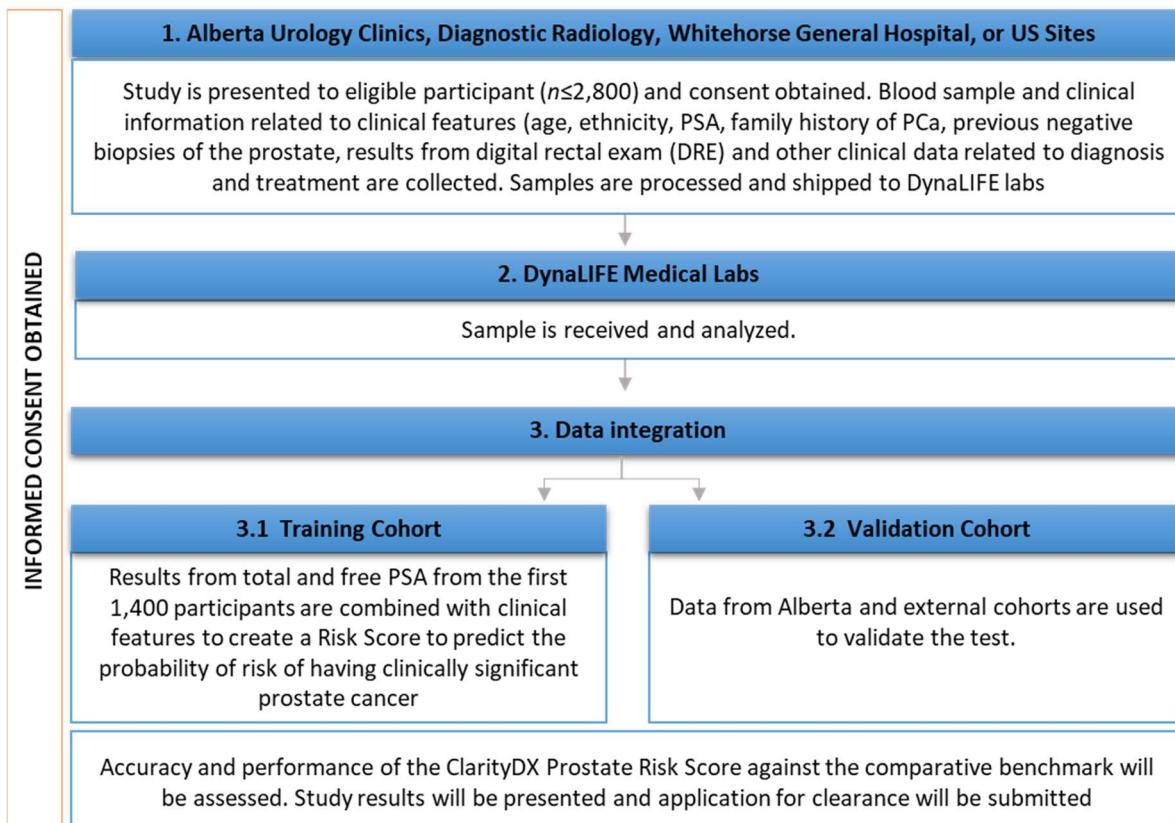
Figure 2. Study Outline

7. END-POINTS

7.1 Primary End-Points

Diagnostic Clinical Performance: prediction of clinically significant prostate cancer at biopsy

Active Surveillance: prediction of Gleason Grade Group on confirmatory/follow-up biopsies for participants on Active Surveillance

MRI: prediction of PI-RADS pre-diagnostic biopsy

7.2 Secondary End-Points

- Health economics / Cost-benefit of the test;
- Effect of selected medications and co-morbidities on the predictive value of the test; and
- Method assessment and analytical validity of the ClarityDX Prostate test.

8. STUDY OBSERVATION PERIOD

Longitudinal clinical data collection will continue until the biopsy and/or prostatectomy results are obtained.

	Intake	3-6m post enrollment	6-12m post enrollment	Annually pre-F/U biopsy for 3 years
Informed Consent	X			
Signed Sample collected	X			
Intake survey and medication questionnaire	X			
Results from PSA & biopsy	When available			
MRI	X	X		
Prostatectomy Pathology			X	
Active Surveillance data follow-up				
				X

9. ADVERSE EVENTS

There are no anticipated significant adverse events associated with participation in this study. Due to the nature of the blood sample collection process, potential adverse events caused by or related to the ClarityDX Prostate test are identical to those associated with the current SOC PSA test, which is also a blood test.

10. STATISTICAL CONSIDERATIONS AND DATA ANALYSIS PLAN

10.1 Analysis Plan

As described in section 4.6, an initial group of patients will be recruited into a training cohort. Patient data and clinical features from the training cohort will be used to create machine learning models that create the ClarityDX Prostate Score. These models, will be used on patient data from the validation cohort to determine the performance of the ClarityDX Prostate test for predicting Gleason Grade Group 2 and greater prostate cancer, how well the risk score correlates with mpMRI results, and how well it predicts the results of subsequent biopsies in participants who decide to go on Active Surveillance.Performance analysis may include sensitivity and specificity pairs, likelihood ratio of positive and negative result pairs, and ROC analysis along with confidence intervals, significance levels and pre and post-test probabilities. Analysis will also include determination of the predictive value of a positive result (sometimes called positive predictive value or PPV) and predictive value of a negative result (sometimes called negative predictive value or NPV) pair.

This is a validation study with the primary objective of evaluating the performance of the non-invasive ClarityDX Prostate test as a reflex test to PSA (Roche Cobas) that would help refine prediction of clinically significant prostate cancer beyond currently available diagnostic and prognostic systems guided by biochemistry (PSA) , imaging (MRI), and histopathology (Gleason grouping at diagnosis and in the Active Surveillance setting).

The final model requires up to the six common clinical features relevant to prostate cancer. If any clinical feature other than PSA and ClarityDX Prostate Flow Score is missing, median imputation may be performed using the median values in the training phase dataset.

10.2 Sample Size Calculation and Test Interpretation

10.2.1 Sample Size of Training Set

Based on a population-based study of patients with prostate cancer diagnosis in Alberta in 2017, 3328 men underwent a prostate biopsy and 1156 were diagnosed with prostate cancer indicating that the prevalence of prostate cancer based on biopsy is about 35% in Alberta. The calculations for the sample size were based on a prevalence of 35%.

A total sample size of 1400 (which includes 490 subjects with the disease) achieves 83% power to detect a change in sensitivity from 95% to 97.5% using a two-sided binomial test and 86% power to detect a change in specificity from 41% to 46% using a two-sided binomial test.[15,16] The target significance level is 0.05. The actual significance achieved by sensitivity is 0.0484 and achieved by specificity is 0.0467. The following formula was used to calculate the sample size based on sensitivity.

$$TP + FN = \frac{z^2 * SN(1 - SN)}{w^2}$$

Where

TP=True Positive
FN=False Negative
SN=Sensitivity
w=accuracy
z=confidence interval normal distribution value, for 95% z=1.96

$$N(S_n) = \frac{TP + FN}{p}$$

Where p=prevalence of disease in the test population.

SN=95%
w=0.025
p=35%

TP+FN=490
$N(S_n) = 1400$

10.2.2 Recruitment distribution per site for training set

Participants will come frommultiple sites as follows:

a. Prostate Cancer Centre: ~500 participants

b. United States Site: ~1500 participants

10.2.3 Participant's Ethnic Representation

Based on data from the Alberta Prostate Registry and Biorepository, HREBA.CC-18-0513 (Total subjects: 1882), our team is expecting the following ethnic distribution in Alberta:

Caucasian, (%)	1599 (85.0%)
Black, (%)	53 (2.82%)
Asian, (%)	122 (6.48%)
Native American, (%)	11 (0.584%)
Other, (%)	47 (2.50%)
Missing/Refused, (%)	50 (2.66%)

If ClarityDX Prostate validation results demonstrate superior performance, these will be submitted to the FDA for review and clearance in order to be commercialized in the US. The Albertan cohort will likely have under representation of African American and Latin American groups when compared with the US population. In order to address this, participants from additional US sites will be added to the study. These participants will be recruited at sites with high ethnic representation, however, to avoid risk of bias, all eligible participants will be recruited in the selected site without regard to their ethnicity and recruitment will continue until the desired ethnic percentage is reached.

Enrichment plan:
African American: 8% ~100 participants recruited during training
Latin American: 8% ~100 participants recruited during training

10.2.4   Participant Replacement
Enrolled participant maybe replaced with newly enrolled participants in case of relevant missing data, unfit flow cytometry sample runs, protocol deviations, and/or participant withdrawals. Estimating an error of ~20%, about 280 extra participants may be recruited in the training cohort to account for any participants whose data is incomplete. These replacements will be discussed and decided by the Data Monitoring Committee at the end of the training set recruitment.

10.2.5   Sample Size of Validation Cohort
For the validation cohort, it is estimated that a similar sample size, recruitment distribution, ethnic representation/enrichment plan would be needed. The sample size may be modified based on the results obtained from the training set.

a.   Kipnes Urology Centre/Diagnostic Radiology: ~1,000 participants
b.   Other sites globally: ~2,000 participants

11. INTERIN ANALYSIS
Members of the APCaRI-05 Data Monitoring Committee, as well as sponsor data analysis team will review accumulating data and data integrity, at least every six months after data from first patient has been obtained.

The committee will recommend re-evaluation of the study if during test validation results among patients are substantially different than estimated in the sample size calculation, the committee can recommend increasing or decreasing the target accrual to maintain statistical power. Additionally, the committee will evaluate the need to replace withdrawing participants or patients with relevant missing data. The committee will also define end of training cohort and beginning of testing cohort. This analysis will be used to determine the sample size for the validation cohort, which will remain unchanged throughout the validation study.

12. QUALITY CONTROL AND QUALITY ASSURANCE PROCEDURES

The APCaRI-05 Data Monitoring Committee (composition and terms of reference available upon request) will monitor the study activities, to ensure scientific integrity, quality of data, safety of the participants and compliance with ethical principles. Monitoring assures that study staff adhere to the protocol, study data are entered completely and accurately, records are accurate, regulatory and ethical requirements are followed, the investigator' facility supports the study procedures and it progresses according to the timeline.

Data quality will be checked at least annually by a random selection of cases (participants) and validated through a review of the source documents following APCaRI SOPs. Missing follow-up data will be monitored as part of a semi-annual data review and assessment. Missing data, especially related to clinical outcomes of interest, will be queried through available data repositories, or urology and cancer clinics. Where data is completely missing or non-retrievable, the reason(s) will be logged.

This committee will also ensure research ethics approval status through Health Research Ethics Board of Alberta Cancer Committee, , and applicable ethics boards in the United States are maintained by annual renewals. This includes amendments to the study protocol or brochure that may arise over the lifetime of this study.

The designated study monitor will visit the study site(s) regularly. A final monitoring visit (study close-up visit) will be made after locking the database. The investigator will inform the monitor about participant recruitment. A standardized quality check will be performed for all study procedures.

13. KNOWLEDGE DISSEMINATION & TRANSLATION

For the validation phase,participants maybe asked if they would like the results of the tests to be shared with their Doctor/urologist Research progress will be published in scientific journals and through the APCaRI and/or Nanostics web portals, press releases, and newsletters. Participants will have the option to choose if they want to hear more from this and other studies by receiving our newsletters and/or blogs. Only those patients who respond positively to the email will be sent updates through email. No identifiable information will be released.

The team will meet yearly to discuss news and scientific progress in prostate cancer research. As clinical observations within the prospective cohort study accumulate over time, it is expected that results will be

reported periodically to the AHS Cancer Control provincial Genito-Urinary Tumor Group of clinicians that provide clinical care to patients. Where research results have larger implications to more general, non-specialist medical audience, the team will disseminate new knowledge through other faculty educational updates or outreach programs. Reportable scientific progress will be made through scientific meetings in basic science and clinical oncology, and urology. Publications through peer-reviewed journals are expected for this study.

Where future funding and opportunities allow, research results may generate information of interest to the public, including many donors who support the Alberta Cancer Foundation and the University Hospital Foundation. Formal or informal educational sessions will be conducted through appropriate public venues.

Throughout the lifespan of this validation study, it is expected that APCaRI investigators will meet with each other and with researchers from other groups. Comments and feedback to this cohort study from the public, including study participants, and from the scientific community will be reported to the Steering Committee ad hoc.

Should results from the study prove the superior performance of ClarityDX Prostate, Health Canada, The Food and Drug Administration and European pre-market approvals will be pursued to make the test available in North America and Europe.

14. PUBLICATION POLICY

The multidisciplinary team coordinated by the sponsor and principal investigators will undertake primary responsibility for the preparation of the clinical study report. The clinical report will include a discussion of study objectives, methodology, clinical observations, and conclusions in relation to the study objectives. APCaRI recognizes the right of the investigator to publish, however, all publications, presentations, and communications concerning findings of this clinical study will be based on data validated and released within reasonable timelines. The Principal Investigator will have the leading role in any publication related to this study, and the authors of this protocol will be included in the list of authors.

APCaRI has established a well-thought publication policy (available upon request).

15. BUDGET AND FUNDING

This study is funded by multiple partnerships including: i. The Bird Dogs in partnership with the Alberta Cancer Foundation, ii. The University Hospital Foundation through The Kaye Fund, iii. The Yukon Motorcycle Ride for Dad and iv. Alberta Innovates, Nanostics Inc., The Alberta Cancer Foundation and DynaLIFE Medical Labs through the Alberta Small Business Innovation and Research Initiative (ASBIRI) funding program.

- APCaRI will partially cover costs associated with study oversight, patient recruitment, data collection and data QC at the Kaye Edmonton Clinic through funding from the Alberta Cancer Foundation, the Kaye Fund, and the Yukon Motorcycle Ride for Dad and will participate in the interpretation and publication of research results.

- DynaLIFE will provide in-kind services including facilitating translational research space at its headquarters, providing trained Medical Laboratory Technologists to perform the assay at DynaLIFE, partially covering the costs of sample collection, processing and shipping, sharing logistical infrastructure to run the ClarityDX Prostate test in their facilities, and offering expertise for i. Study design, conduct and interpretation of research results, and ii. Creation and implementation of quality management system and SOPs.
- Nanostics & ASBIRI will cover the remaining costs associated with study coordination, monitoring, data management, sample analysis, including a portion of salaries for sample collection, processing, and analysis, equipment and reagents to perform the test, creation and implementation of quality management system and test-specific SOPs, Health Economics studies and reporting to Health Canada and the US FDA, and will participate in the interpretation and publication of research results.

16. REFERENCES

1. Xie, L., R. Semenciw, and L. Mery, Cancer incidence in Canada: trends and projections (1983–2032). Health Promotion and Chronic Disease Prevention in Canada: Research, Policy and Practice, 2015. 35(Suppl 1): p. 1-187.
2. Committee, C.C.S.A., Canadian Cancer Statistics 2018. 2018, Canadian Cancer Society: Toronto, ON.
3. Fradet, Y., et al., The burden of prostate cancer in Canada. Canadian Urological Association Journal, 2009. 3(3 Suppl 2): p. S92-S100.
4. Neutel, C.I., et al., The changing age distribution of prostate cancer in Canada. Can J Public Health, 2007. 98(1): p. 60-4.
5. Fenton, J.J., et al., U.S. Preventive Services Task Force Evidence Syntheses, formerly Systematic Evidence Reviews, in Prostate-Specific Antigen-Based Screening for Prostate Cancer: A Systematic Evidence Review for the U.S. Preventive Services Task Force. 2018, Agency for Healthcare Research and Quality (US): Rockville (MD).
6. Brett, A.S. and R.J. Ablin, Prostate-Cancer Screening — What the U.S. Preventive Services Task Force Left Out. New England Journal of Medicine, 2011. 365(21): p. 1949-1951.
7. Heidenreich, A., et al., EAU guidelines on prostate cancer. Part 1: screening, diagnosis, and treatment of clinically localised disease. Eur Urol, 2011. 59(1): p. 61-71.
8. Drost, F.-J.H., et al., Can active surveillance really reduce the harms of overdiagnosing prostate cancer? A reflection of real-life clinical practice in the PRIAS study. Translational Andrology and Urology, 2018. 7(1): p. 98-105.
9. Board, P.A.T.E., Prostate Cancer Treatment (PDQ(R)): Health Professional Version, in PDQ Cancer Information Summaries. 2002, National Cancer Institute (US): Bethesda (MD).
10. Willetts, L., et al., Quantitative Analysis of Human Cancer Cell Extravasation Using Intravital Imaging, in The Tumor Microenvironment: Methods and Protocols, J. Ursini-Siegel and N. Beauchemin, Editors. 2016, Springer New York: New York, NY. p. 27-37.
11. Klotz, Clinical results of long-term follow-up of a large, active surveillance cohort with localized prostate cancer. 2010.
12. Pepe, P. and F. Aragona, Morbidity After Transperineal Prostate Biopsy in 3000 Patients Undergoing 12 vs 18 vs More Than 24 Needle Cores. Urology, 2013. 81(6): p. 1142-1146.

13. Murthy, V., et al., Clinical impact of prostate specific antigen (PSA) inter-assay variability on management of prostate cancer. Clin Biochem, 2016. 49(1-2): p. 79-84.

14. Logozzi, M., et al., Increased PSA expression on prostate cancer exosomes in in vitro condition and in cancer patients. Cancer Lett, 2017. 403: p. 318-329.

15. Brant, R. Inference for Proportions: Comparing Two Independent Samples. [cited 2018 September 20]; Available from: https://www.stat.ubc.ca/~rollin/stats/ssize/b2.html.
    Pink, D.B., et al., A novel extracellular vesicle blood test to distinguish indolent and aggressive prostate cancer at diagnosis. 2018. Manuscript in preparation, available upon request.