

# Prostate Cancer Biomarker Development: National Cancer Institute's Early Detection Research Network Prostate Cancer Collaborative Group Review

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## ABSTRACT

Prostate cancer remains the most common non-skin cancer and second leading cause of death among men in the United States. Although progress has been made in diagnosis and risk assessment, many clinical questions remain regarding early identification of prostate cancer and management. The early detection of aggressive disease continues to provide high curative rates if diagnosed in a localized state. Unfortunately, prostate cancer displays significant heterogeneity within the prostate organ and between individual patients making detection and treatment strategies complex. Although prostate cancer is common among men, the majority will not die from prostate cancer,

introducing the issue of overtreatment as a major concern in clinical management of the disease. The focus of the future is to identify those at highest risk for aggressive prostate cancer and to develop prevention and screening strategies, as well as discerning the difference in malignant potential of diagnosed tumors. The Prostate Cancer Research Group of the National Cancer Institute's Early Detection Research Network has contributed to the progress in addressing these concerns. This summary is an overview of the activities of the group.

See all articles in this *CEBP Focus* section, "NCI Early Detection Research Network: Making Cancer Detection Possible."

## Introduction

### Prostate cancer and early detection

Prostate cancer remains the most commonly diagnosed noncutaneous cancer in the United States and the second most common cause of cancer death in men (1). The 4-year survival rate for local regional disease is >99%, but if a man is diagnosed with distant disease, his 4-year survival rate drops to 30% (1). Two large randomized controlled trials evaluating the effect of prostate cancer screening on mortality have demonstrated a reduction in the risk of death by 20% to 40% in those undergoing screening (2, 3). Despite the potential benefits, considerable concern remains regarding overdiagnosis and ultimately overtreatment in the screened population (4). The concern for potential harm served as the impetus for the United States Preventive Services Task Force (USPSTF) to recommend against screening in 2012 (5) and later advise on informed decision-making between physician and patients to decide on screening (6). A focus of the National Cancer Institute's Early Detection Research Network (NCI-EDRN) is to allow data-driven discussions during the patient-provider interactions that guide individualized, informed decision-making. Given the grim statistics of metastatic prostate cancer, the NCI-EDRN Prostate Cancer Research Group focuses on actionable biomarkers that can be utilized in early-stage settings to prevent progression by early

intervention. Important targets of the group include the identification of aggressive, potentially lethal, cancer at an early stage and providing biomarker-driven decisional support to maximize benefit and minimize harm in prostate cancer treatment. Other targets include the identification of known prostate cancer at risk for progression and metastasis or conversion to castration-resistant prostate cancer.

### The EDRN prostate cancer research group

The EDRN network research activities are leveraged toward specific cancer types through organ-site collaborative teams. The Prostate Cancer Research Group is composed of the investigators that comprise major components and associate member programs, as well as prostate cancer expertise not directly funded through the NCI. In the current cycle, there are three Biomarker Development Laboratories (BDL); two of these programs focus on protein-based biomarkers and one focuses on nucleic acid-based biomarkers. There are also two Clinical Validation Centers (CVC), three Biomarker Reference Laboratories (BRL), statistical expertise from the Data Management Consulting Center (DMCC), and investigator teams supported through the EDRN Associate Membership program. Critical patient perspective and advocacy is provided through regular participation by the president of the National Association of State Prostate Cancer Coalitions. The collaborative group also includes representation from industry. All members of collaborative group participate in monthly video conference to build shared network expertise, discuss research progress, and evaluate programmatic activities. The group develops core projects to support critical research needs that are then reviewed by the full steering committee of the EDRN. The core projects serve to focus on the research activities across the collaborative group. For the prostate cancer research group, many of the core projects have focused on the development of unique reference sets. These reference sets are designed to assist in the validation of new biomarkers.

One of the most valuable aspects of the collaborative group is the establishment of biomarker development goals within the collective expertise of the team, which consists of patient advocate, clinicians,

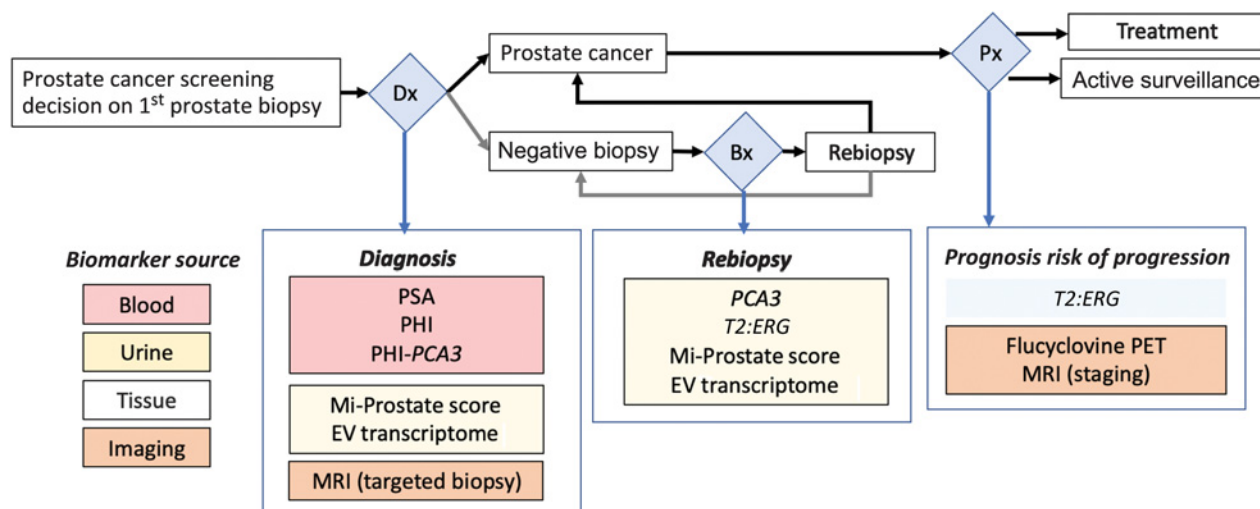
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Cancer Epidemiol Biomarkers Prev 2020;29:2454-62

doi: 10.1158/1055-9965.EPI-20-1104

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**Figure 1.**

EDRN prostate group biomarker schema. Flow chart describing decision points (blue diamonds) leading to EDRN-supported biomarkers. Shown are diagnosis (Dx), Biopsy (Bx), and Prognosis (Px). The listed biomarkers are examples of EDRN-developed tools that highlight the prostate collaborative priorities for critical clinical care decision-making. The clinical specimen type from which the biomarker is derived is color coded and indicated on the lower left.

epidemiologists, and basic scientists. In developing this focus, the team has considered the current clinical workflow within which decisions are made regarding clinical care and management of disease (Fig. 1). Diagnosis of prostate cancer is initiated through screening and confirmed through subsequent biopsy. This is a critical junction for decision-making and an area of considerable focus of the EDRN. A positive diagnosis demands accurate risk stratification between active surveillance and treatment. A negative biopsy leads to more vigilant follow-up involving repeat biopsies and risk of associated complications. Thus, early and accurate assessment of disease is critical in the clinical care continuum for prostate cancer.

#### A successful approach to biomarker development

Historically, potential biomarkers from independent laboratories were common and necessary to provide the initial leads on prostate biomarkers. However, in the absence of a supportive infrastructure, biomarker development would often stall at this point. The EDRN provides resource and expertise infrastructure to assist biomarker development from laboratory discovery through clinical validation. The EDRN Prostate collaborative group serves as the connection point between biomarker discovery (BDL), assay refinement (BRL), and performance validation (CVC) within statistically appropriate study design (DMCC). In an effort to facilitate the development process, the Prostate Cancer Research Group has assembled critical patient cohort reference sets for biomarker validation (7). Investigators are guided to the appropriate EDRN component to assist with the development phase of biomarkers. Once the biomarker performance achieves a level of accuracy and consistency, as verified through the DMCC, a larger targeted validation study involving EDRN BRL, CVC, and DMCC is developed. Sensitivity and specificity goals required for success are clearly established, and common pitfalls of biomarker development such as cost, clinical utility, and implementation are considered. EDRN specifically seeks to address these issues when a biomarker request is examined by the task force and steering committee. Cost prohibitive tests need to be considered carefully, and strategies can be discussed to investigate the same pathway by alternative means. The EDRN's network of

clinical partners, agency collaborations, and industry relationships facilitates this validation process.

## Discussion

### Refining existing blood and urine biomarkers to enhance detection of aggressive prostate cancer

Since the advent of serum PSA screening in the early 1990s, prostate cancer mortality in the United States has decreased by almost 50% (1, 8, 9). Despite conflicting evidence, the U.S. Preventive Services Task Force currently gives prostate cancer screening a "C" grade and emphasizes the discussion of risks and benefits of screening. Nonetheless, screening, diagnosis, and treatment of early-stage prostate cancer have generated intense scientific and public debate as population screening with PSA increases detection of both lethal and nonlethal cancers (10). These shortcomings promote overdiagnosis; the diagnosis of screen-detected indolent prostate cancer, left untreated, would otherwise not result in morbidity or mortality. Overdiagnosis needlessly exposes patients to the risks of prostate biopsy and the anxiety of a cancer diagnosis (10). The prostate cancer early detection paradigm using PSA screening of men at risk for prostate cancer and higher serum PSA concentrations to prompt prostate biopsy has remained unchanged for decades (11, 12). Traditionally, about 70% of men undergoing prostate biopsy are diagnosed; approximately 30% of these men are clinically significant (13–15). Overdiagnosis of low-risk prostate cancer often leads to overtreatment resulting in significant side effects of monitoring programs, surgery, and radiotherapy.

The EDRN Prostate Cancer Group has spent considerable effort over the last two decades in developing novel and clinically useful biomarkers (see Table 1). One of the earliest efforts by the collaborative was to evaluate the combination of PSA, free PSA, and the [-2] form of proenzyme PSA (pro2PSA) to develop the Prostate Health Index (PHI). In European studies, in men with a PSA between 2 and 10 ng/mL, PHI performed significantly better than PSA and the ratio of free PSA and total PSA (%free PSA) in detecting prostate cancer in general (16). However, that and other pioneering work with PHI (17)

**Table 1.** EDRN prostate collaborative group biomarker development achievements.

Biomarker	EDRN role	Clinical utility	Outcome/Status
PHI (17)	Validation	Early detection prior to initial biopsy	FDA-approved
ProPSA (20)	Validation	Early detection prior to initial biopsy	FDA-approved
<i>TMPRSS2:ERG</i> fusion ( <i>T2:ERG</i> ; ref. 28)	Discovery, validation	Early detection prior to initial or repeat biopsy	CLIA-compliant <sup>a</sup> / commercially deployed
Urine <i>PCA3</i> , <i>T2:ERG</i> (e.g., MIPs; ref. 75)	Validation	Early detection prior to initial or repeat biopsy	FDA-approved ( <i>PCA3</i> ); CLIA ( <i>T2:ERG</i> )
Tissue/urine RNA-seq (76)	Discovery to clin assay	Early detection biomarker development	CLIA-compliant
MiCheck (77)	Validation	Early detection prior to initial or repeat biopsy	CLIA-compliant
Mitochondrial deletion (78)	Discovery, validation	Early detection biomarker development (biopsy)	CLIA-compliant
Decipher ( <i>SChLAP1</i> ; ref. 79)	Discovery, validation	Salvage radiotherapy after prostatectomy	CLIA-compliant
<i>GSTP1</i> (80)	Validation	Early detection biomarker development (prostatectomy)	CLIA-compliant
Urine transcriptome (32)	Discovery to clin assay	Early detection biomarker development	CLIA-compliant
Flucyclovine PET (81)	Validation	Preoperative staging	Expanded indication

<sup>a</sup>The assay/test performance meets CLIA guidelines.

did not discern whether PHI measurement could be used to reduce the over-detection of indolent prostate cancer, which is recognized as a pivotal flaw of PSA screening for prostate cancer. The collaborative group then undertook a sequence of studies evaluating how pro2PSA could enhance effectiveness of detecting *aggressive* prostate cancer, i.e., those cancers having histopathologic Grade Group II or higher (Gleason score greater than 6), while reducing over-detection of indolent Grade Group I cancers. The EDRN collaborative group found that PHI had an AUC of 0.73 in a large cohort of community-dwelling men undergoing regular prostate cancer screening (18) and that use of PHI to select men for initial prostate biopsy significantly improved the specificity of detecting cancers having Grade Group 2 or higher (19–21).

In another series of studies that advanced the clinical deployment of a previously discovered prostate cancer biomarker, the collaborative group evaluated the long, noncoding transcript prostate cancer antigen 3 (*PCA3*), as a biomarker for prostate cancer detection. Fifteen years after its discovery as a noncoding transcript associated with prostate cancer, *PCA3* had been developed as a marker for selecting which men with prior negative biopsy should undergo repeat prostate biopsy (22, 23). However, the role of *PCA3* in selecting men for initial prostate biopsy remained elusive. The collaborative group completed a prospective trial of collecting urine and blood prior to prostate biopsy, to refine/reduce unnecessary biopsy or overdiagnosis among men with elevated PSA or abnormal digital rectal exam (DRE). These efforts established a new paradigm for using biomarkers to predict cancers having Grade Group 2 or higher, while avoiding biopsy of men predicted to have Grade Group 1 or no cancer (24). Trial outcomes showed utility of *PCA3* for this purpose, and subsequent studies showed that adding the *PCA3* urine test to the Prostate Cancer Prevention Risk Calculator (25) improved the AUCs (95% confidence intervals) for predicting high-grade cancer from 69.6% (65.6%–73.7%) to 76.3% (72.7%–79.9%).

#### Biomarker development approaches

The prostate collaborative group has leveraged state-of-the-art omics tools to analyze clinical tissues in discovery of disease-specific clinical decision-targeted biomarkers. We provide an overview of current methodologies spanning from genomic to proteomic biomarker targets. In addition to deploying high-throughput platforms for biomarker discovery, EDRN BDLs provide expertise in data analysis that is being leveraged to maximize biomarker development

through a multi-omics framework to build comprehensive data models of prostate cancer from which to refine biomarker panels for streamlined validation.

The rationale for a network structure was to integrate discovery, assay refinement, and validation. One example of streamlining the pathway from discovery to clinical translation via EDRN partnership was the targeting of the transmembrane protease serine 2:*v-ets* erythroblastosis virus E26 oncogene homolog (*TMPRSS2:ERG* or *T2:ERG*) fusion as a biomarker for prostate cancer detection. Leveraging the discovery of *T2:ERG* (26), the BDL partnered with CVC investigators to evaluate whether this gene rearrangement could be detected in urine to refine prostate cancer detection. Initial prevalidation studies showed that combining *T2:ERG* with *PCA3* performed better than urinary *PCA3* alone or serum PSA without urinary testing in predicting aggressive prostate cancer (27, 28). Subsequent multicenter validation analysis following Prospective Randomized Open Blinded End-Point design (of locking predictive rule parameters before validation analysis) affirmed that combining *T2:ERG* measurement with *PCA3* in post-DRE urine, using a Clinical Laboratory Improvements Amendments (CLIA) certified, commercially scalable platform enhanced specificity of detecting aggressive prostate cancer by one third. This strategy provided a means of reducing unnecessary prostate biopsy and mitigating over-detection of indolent prostate cancer, while enhancing identification of men with aggressive disease for which treatment is advisable (29). This paradigm led to the development of two commercially available assays that are now in widespread clinical use (28, 30). Building upon this success, EDRN investigators and international collaborators identified the extracellular vesicle fraction of urine collected after DRE was suitable for detection strategies targeting the entirety of the prostate cancer-associated transcriptome. The subsequently developed clinical assay targets the next generation of multiplex urinary transcript to further refine prostate cancer detection (31, 32).

The collaborative team has also developed powerful data visualization methodology to help link independent molecular analysis as recently highlighted in a study focused on curable intermediate risk disease in which a cohort of tumors was analyzed by genomic, epigenomic, and proteomic approaches (33). The study revealed a previously unrealized pivotal role of *ETS*-fusion events in driving numerous proteogenomic pathways. Performance analysis of study biomarker events clearly revealed the value of combined multi-omics approaches and demonstrated that multimodal assays

consistently outperform single modality measures. This same group also sought to define the relationship between genomic risk loci and epigenomic changes in prostate cancer, revealing AKT1 expression as predictive of relapse (34). Understanding the complex network of proteogenomic factors that drive disease will help focus biomarker discovery toward development of effective tools to improve clinical decision-making.

#### Proteomics-based biomarker development

The Prostate Collaborative Group is well represented by expertise in the application of proteomics to biomarker development. There are two BDLs employing innovative strategies in proteomics supported by two CVCs and one BRL. Each BDL has developed approaches for discovery, verification, and validation with a clear path toward clinical assay development that maximizes the network resources. Significant advances in mass spectrometry over the past 20 years have accelerated the field of clinical proteomics toward greater capacity and rigor in discovery, as well as achieving unprecedented accuracy/precision targeted quantitation (35). There has also been greater attention paid to developing analytical strategies tailored to the analysis of tumor and tumor-proximal fluids that have accelerated discovery through reduction in processing associated with protein analysis. Many of these advances in the application of proteomics technologies have been leveraged by the group with the goal of developing biomarkers for the early identification, stratification, and management of disease.

#### Discovery approaches

Directly assessing human prostate tissues encompassing tumor and nontumor partitions is a clearly logical strategy for biomarker discovery. This is true whether the eventual application of the disease-specific biomarker relies on pathologic assessment of tissue or measurement in body fluids as reasoned that tumor biology is a leading source of disease-specific changes. Earlier efforts at tissue-based proteomic analysis have been limited to fresh or fresh-frozen samples and were plagued with variable tissue pathology and low protein identification content (36). The bulk of these obstacles have been overcome with improved tissue lysis and proteolytic digestion methods that allow for comprehensive identification of proteins from formalin-fixed paraffin-embedded (FFPE) tissues and retain annotation by a clinical pathologist (37–40). Significant expansion in the proteome space available for interrogation has been achieved through improvements in mass spectrometry instrumentation in signal resolution, analytical speed, fragmentation, and downstream data analysis. Speed in this case refers to the ability to select ions for subsequent analysis in LC-MS/MS workflows and has a direct impact on the assessable volume of a targeted proteome. Strategies to capture more physical data from a single analysis include data-independent acquisition that bypasses the data-dependent selection criteria used in typical LC-MS/MS (41, 42). One such strategy termed Sequential Window Acquisition of all Theoretical Mass Spectra (SWATH-MS) defines mass windows within which all data are acquired and subsequently analyzed resulting in unprecedented numbers of proteins identified in a defined proteome (43, 44). A recent application of SWATH-MS to prostate cancer involved a novel modification that allowed for targeting glycoproteins derived from tissues that offered insight into potential markers of aggressive disease (45). The developers of this innovative approach colead one of the prostate collaborative BDLs. The biomarker development strategy from this team focuses on employing comprehensive SWATH-MS analysis of tumor tissues to discovery of protein biomarkers that stratify with disease. The most promising candidates are

targets for antibody-based assays for the appropriate tissue or fluids assessment.

A separate but complimentary approach to prostate cancer biomarker discovery targets tumor-proximal fluids (46, 47). In this strategy, direct expressed prostatic secretions (EPS) are tumor-proximal fluid from which enrichment of prostate tumor-specific proteins has been demonstrated. An additional attractive feature to utilization of EPS in biomarker discovery is the ability to conveniently collect EPS in urine following DRE and thus a readily available tumor-proximal clinical assay fluid. The use of post-DRE urine as a biomarker source was pioneered by researchers in the EDRN prostate collaborative and has been an area of successful transition to clinical utility (48–50). A comprehensive effort to mine EPS from disease-stratified cohorts is being conducted by this same BDL following an approach this team demonstrated to be successful in identification of potential biomarkers of aggressive disease (46). This approach involves large-scale discovery with Orbitrap class LC-MS/MS instrumentation coupled with a rapid 96-well-based processing method (51). The combined approach allows for reproducible identification of over 3,000 high-confidence proteins from 200  $\mu$ L of unfractionated EPS/post-DRE urine. The second phase leverages the discovery data to build targeted Parallel Reaction Monitoring (PRM-MS) assays for hundreds of candidate proteins so as to maximize the quantitation through subsequent validation study (52, 53). The result has been the realization of unprecedented surveys of large statistically powered cohorts.

#### Targeted verification/validation approaches

The traditional path toward measurement of protein expression as a clinical biomarker is the subsequent development of targeted immunoassays. Examples of the successful implementation of clinical immunoassays include current tests for serum PSA and Promark tissue-based assay for aggressive disease in low-risk groups. This tried and true approach has been adopted by the Prostate Collaborative Group and is pursued either directly or in parallel utilizing the strengths of network laboratories with experience in building such assays. A particularly innovative strategy for incorporation of immunoassay into biomarker development workflow was recently described by an EDRN BDL/BRL collaborative team (54). This team incorporated a previous discovery of increased serum levels of fucosylated PSA in patients with aggressive disease (55). Using this *a priori* finding, they developed a tandem immune-assay, lectin-based targeting of fucosyl residues and subsequent antibody-based targeting of PSA, that resulted in better discrimination between disease aggressiveness. The strength of this discrimination was observed to be in intermediate Gleason score 7 disease.

In many instances, immunoassay reagents that specifically target protein-based biomarkers are either nonexistent or ineffective. This is especially true as the field explores more nuanced proteome variability that extends beyond simple protein expression levels toward post-translational modifications, isoform selection, protein cleavage/processing/degradation, and functionally associated protein interactions. A promising technical approach for quantitative analysis of these events employs a targeted mass spectrometry methodology referred to as selected reaction monitoring (SRM; for review, see ref. 56). A variant of SRM, PRM-MS, leverages the recent advances in high-resolution mass spectrometry to allow for SRM with parallel detection of all ion transitions in a single run. A major focus of the prostate collaborative BDLs is the incorporation of PRM-MS to improve assay stability. The approach involves a two-phase biomarker development strategy in which discovery is conducted on appropriately powered retrospective

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cohorts, multianalyte PRM-MS assays are built using the collected empirical data, and the resulting PRM-MS tools are used in all subsequent validation efforts (46).

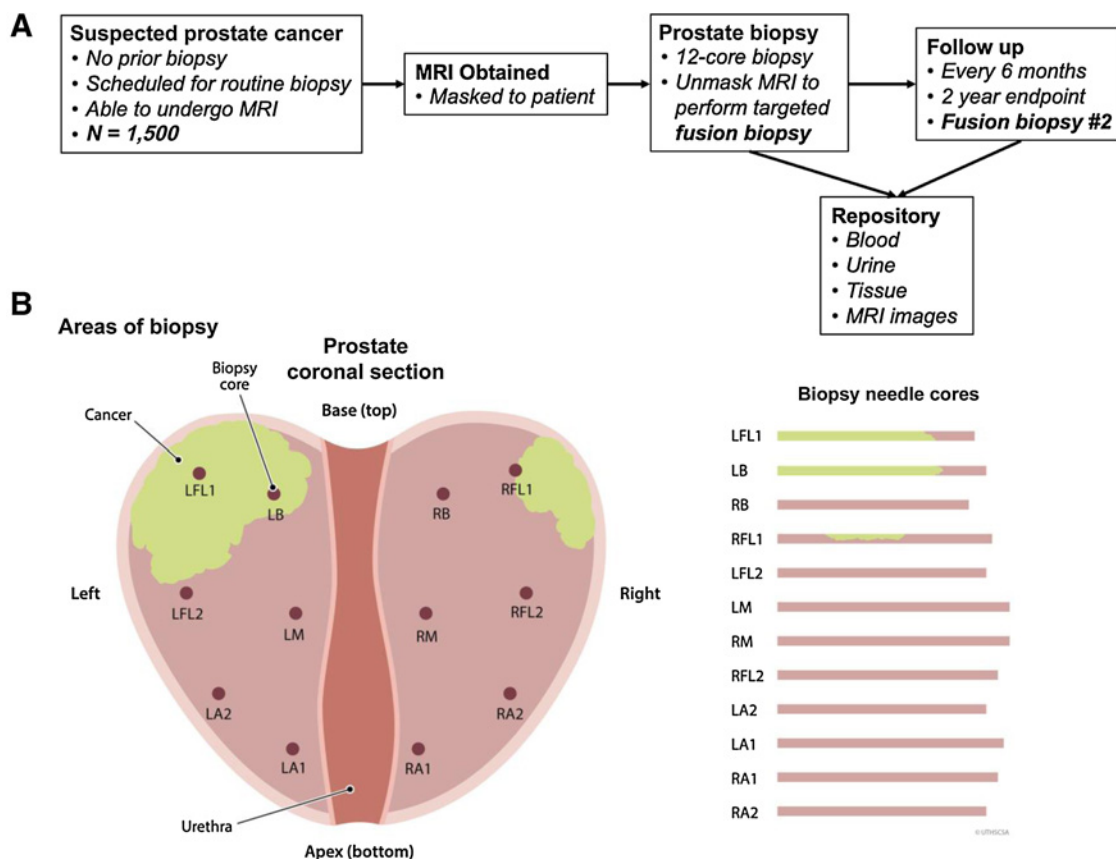
One advantage of the SRM/PRM-MS pipeline is the ability to readily incorporate biomarker discovery from outside the EDRN network to include in collaborative group validation efforts. For example, prostate collaborative group researchers employed high-Pressure high-Resolution separation with intelligent Selection and Multiplexing (PRISM)-SRM, that allows for unprecedented sensitivity without affinity enrichment (57), to evaluate over 50 candidate tissue-based gene expression markers for correlation with prostate cancer outcome. They employed PRISM-SRM to target the corresponding proteins and discovered a 5-protein panel that effectively predicted biochemical recurrence and metastasis in tumor tissue (58). This team is currently working to move this assay into body fluids and evaluate its potential utility in early detection of aggressive disease. Similarly, group members employed a PRM-MS strategy to develop a protein-based assay for the direct quantitation of genomic SNP. One such SNP encodes a PSA variant (rs17632542) that results in a single amino acid change in the PSA protein associated with reduced relative secretion into blood and association with lower serum PSA levels (59, 60). In a recent collaborative group study, investigators developed a PRM-MS assay that

could accurately detect and quantify PSA wild-type and variant proteins in patient urine (61). Although genotyping is readily available, it is not routinely ordered and does not address the relationship between heterozygosity and protein expression. In addition, such protein-direct assays of the expression of genomic variants allow for research into the actual biological roles of the gene products.

## Current prostate collaborative group core studies

### Upgrading of men diagnosed with low-risk disease

The majority of men with low-risk prostate cancer are currently being managed on active surveillance. Because prostate cancer is multifocal, most prostate biopsies are conducted without knowledge of the location of the tumor. Although MRI technology can increase the accuracy of biopsies, patients and their families are often apprehensive that the biopsy may have missed most aggressive disease. This concern results in many men electing to undergo additional therapy, despite their low-grade cancer diagnosis. Their anxiety is clearly warranted as numerous studies find evidence of more aggressive disease in a subset of their patients (62–66). The prostate cancer can be either upgraded (i.e., the Gleason score is higher in the prostatectomy than in the biopsy) or upstaged (i.e., the tumor–node–metastasis stage is higher at time of surgery than was originally recorded). These



**Figure 2.**

EDRN MRI biomarker study. **A**, The most recent prospective clinical cohort developed by EDRN is the MRI Biomarker study, which is inserted at the diagnostic decision point in **Fig. 1**. Men scheduled for prostate biopsy will undergo a blinded MRI, systematic biopsy, then unblinded to MRI for a targeted biopsy. Full biomarker assessment will include blood, urine, tissue (via tissue prints), and imaging acquisition. **B**, A standard template systematic biopsy. The figure demonstrates the locations of a standard systematic biopsy usually directed toward the peripheral zone of the prostate. A major issue with standard systematic prostate biopsy is sampling error and allocating a cancer diagnosis in a subject that may have a false negative. Cancer (green) or more importantly high grade can be missed by standard biopsy if located outside of the standard core template.

studies have focused on retrospectively evaluating patients with prostate cancer diagnosed with Gleason score 6 disease who proceed to have a prostatectomy; the majority of the studies gathered clinical cases over a long period of time from a single institution. More than one of the studies has concluded that upgrading is associated with older age individuals (62, 63, 66); however, because they are retrospectively evaluated, the studies do not have matching biologics that could be used to identify biomarkers that predict upgrading/upstaging.

To assist in counseling men with low-risk prostate cancer, the EDRN prostate research group began gathering a cohort of men with low-grade disease (defined as Gleason score 6) who ultimately chose to have a prostatectomy. The goal of this cohort was to identify pretherapeutic biomarkers (urine, serum, and tissue) that could predict upgrading. These biological samples comprise the Upgrading Reference Set (URS) and have been recruited using EDRN core funds. The cohort enlists ten clinical recruiting sites. In addition, we introduced a specific protocol for gathering patient urines, requiring that all of the preprostatectomy urines be gathered post-DRE. Another important aspect of the study is that all of the biopsies and prostatectomy specimens are centrally reviewed by a single pathology laboratory. We currently have over 80% of the URS reference set gathered with additional subjects consented but awaiting central pathology review.

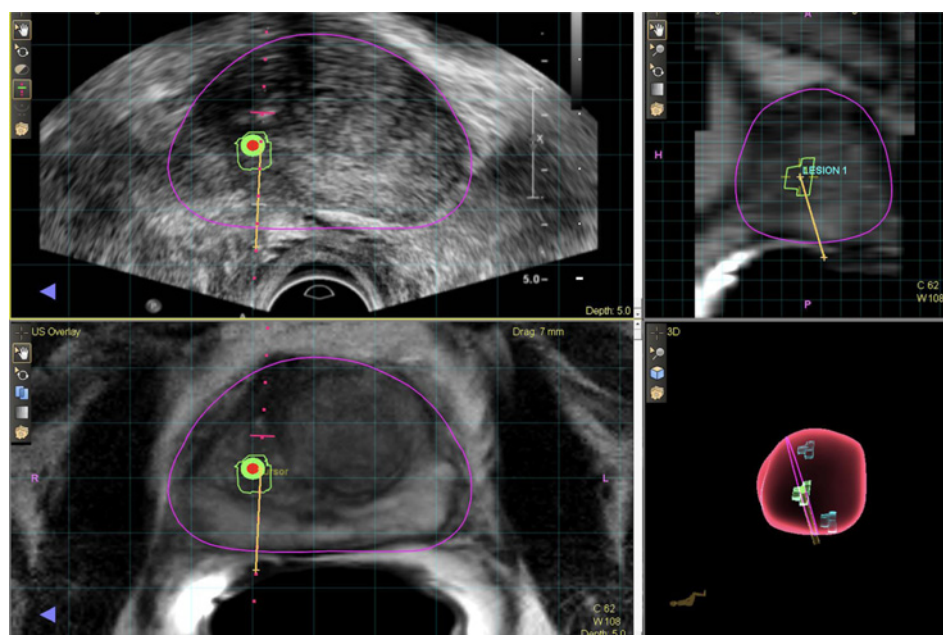
In addition to having plasma, serum, and urine on all of the subjects enrolled in URS, we have banked PBMCs that can be used for genetic studies. EDRN resources have been provided to isolate DNA from each subject and perform whole-genome sequencing. This information will be useful for evaluating newly described polygenetic risk score, such as those developed by the PRACTICAL consortium (67, 68), and can also

be used for evaluating men with DNA damage repair gene mutations such as *BRCA2* and *ATM*, which have been shown to be associated with grade reclassification in men on active surveillance (69). We plan to bank both biopsy and prostatectomy specimens that can be evaluated with various omics technologies.

#### Evaluation of MRI combined with biomarkers improves detection of aggressive disease

Unlike diagnostic biopsy for most other solid organ tumors, standard-of-care prostate biopsy has traditionally been performed without image guidance or selective targeting of suspected lesions. The systematically directed biopsies suffer from diagnostic inaccuracy, poor positive predictive value, and high false-negative rates. In addition, approximately 30% of men initially diagnosed with low-risk cancer on biopsy who undergo surgery are subsequently found to have aggressive tumors, indicating that standard prostate biopsy often fails to detect potentially lethal cancer (70). In the last several years, a number of commercial products have become available allowing the "fusion" of MRI images to prostate ultrasound, making it simple to biopsy MRI-detected lesions. A growing body of literature examining these "targeted" fusion biopsies supports incremental value of fusion biopsy over standard template biopsy in the detection of clinically significant prostate cancer (71, 72).

Modern multiparametric (mp) MRI of the prostate conventionally uses three primary imaging sequences (T2 weighted, diffusion weighted, and dynamic contrast-enhanced imaging). These three sequences are combined in a scoring system referred to as the Prostate Imaging Reporting And Data System (PIRADS; ref. 73). For MRI-Ultrasound



**Figure 3.**

MRI-Ultrasound Fusion-targeted prostate biopsy. The images are snapshot pictures of the UroNav urologist user workstation during an MRI-Ultrasound Fusion prostate biopsy. MRI ROI is outlined in a radiology workstation (DynaCAD) and imported into an MRI-Ultrasound Fusion machine. The targeting system connects the biopsy needle location in space relative to ROI, and a needle core is then obtained. The top left plot is the real-time ultrasound image. The green outline is the ROI, and the bullseye is the center of that lesion. The yellow line represents the biopsy needle path. The bottom left plot is the corresponding MRI image obtained prior to the biopsy. The ultrasound and MRI image are aligned through a series of segmentation, alignment, and rotational adjustments. The top right plot is the MRI in sagittal view with corresponding ROI (blue circle) and biopsy core (yellow line). The bottom right plot is a 3D representation of the prostate. The pink line represents the plane of view, and in this case, there were a total of three 3D target lesion (green) structures within the 3D prostate (pink) image.

Fusion-targeted biopsies, the radiologist, using proprietary software, contours the suspicious lesions and records the PIRADS for each region of interest (ROI). The software links the MRI images to a live ultrasound at the time of a transrectal prostate biopsy. Once the images have been “fused” digitally, the clinician can easily direct the needle into the MRI ROIs. Studies have identified significant diagnostic yield, upward of 30% more high-grade prostate cancer, using this technique compared with standard biopsy (71). The higher the PIRADS score, the greater is the risk of identifying clinically significant cancer.

The commercialization of MRI fusion biopsies has resulted in a dramatic increase in the use of MRI imaging for prostate cancer. Given the ability of mpMRI to target a lesion, the role of laboratory biomarkers has been increasingly questioned. Conversely, how much MRI adds to the value of laboratory biomarkers has not been thoroughly investigated. The few studies published to date have suggested a role for a combination of blood, urine, and imaging biomarkers (74).

We hypothesize that addition of prostate MRI fusion biopsy will significantly improve specificity for high-grade prostate cancer over PSA, PCA3, and T2:ERG. The prostate collaborative has undertaken a systematic study of a range of biomarkers and their role given the expanding use of prostate MRI. The primary aim of this study is to see if the addition of prostate MRI to a panel including PSA, PCA3, and T2:ERG will significantly improve specificity for high-grade prostate cancer. The other objective of this cohort is to create an optimal panel of urine and blood biomarkers that will select those cases most likely to benefit from an MRI-targeted biopsy. In addition, the study seeks to optimize MRI imaging to improve test performance, observe longitudinal changes, and create a prospective reference sample set for future imaging and biomarker studies.

In order to accomplish these goals, we have initiated a multisite, prospective, cross-discipline cohort study to investigate prostate MRI in the context of developed prostate cancer biomarkers. Subjects will have no previous biopsies and consent to prostate biopsy prior to MRI imaging (Fig. 2A). The group consensus is that MRI may guide decisions regarding whether or not to obtain a prostate biopsy despite limited evidence to support this decision-making. At the time of the biopsy, the 12-core systematic standard biopsy will be performed (Fig. 2B), then the MRI lesion will be unmasked to the provider and patient. The provider will then obtain two to three targeted cores of the

lesion specified by the radiologist using an MRI-Ultrasound Fusion-guided technique (Fig. 3). The unmasking process is important because the groups' consensus is that if the target is performed first, the target area could be avoided on the systematic biopsy. Avoiding the target area may provide a different area of tissue to examine but will not allow for robust comparisons between systematic and targeted biopsy because the systematic biopsy has been altered. The systematic biopsy may have detected the lesion without targeting if placed in the normal position. This study has begun recruitment and specimen accrual.

## Conclusions

The research activities of the Prostate Collaborative Group are focused toward providing tools to improve the clinical management of men with prostate cancer. The EDRN-supported infrastructure of both resources and expertise is leveraged by the group to facilitate discovery, guide progress through biomarker development, provide unbiased evaluation of progress and design, and implement appropriate validation studies. State-of-the-art omics technologies provide comprehensive data-driven discovery with an eye toward combined multiomics assays that can be integrated into current clinical decision-making. The strong focus on biomarker application optimizes the development of biomarkers with clinical utility as well as the early adoption of disruptive technologies, such as MRI imaging, into biomarker development workflows. Likewise, efforts to validate findings from laboratories outside of the EDRN, such as polygenic risk scores and capture of in-depth data from clinical cohorts, provide unique resources to the biomarker community.

## Authors' Disclosures

No disclosures were reported.

## Acknowledgments

This work was supported by funds from the NCI Early Detection Research Network (U01 CA 113913, to M.G. Sanda), NIH U01 CA214194 (to O.J. Semmes), and NIH U01 CA086402 (to M.A. Liss and R.J. Leach).

Received July 22, 2020; revised September 29, 2020; accepted October 15, 2020; published first October 22, 2020.

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# Cancer Epidemiology, Biomarkers & Prevention

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*Cancer Epidemiol Biomarkers Prev* 2020;29:2454-2462. Published OnlineFirst October 22, 2020.

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