Center for Prostate Disease Research

Department of Defense Prostate Cancer Center of Excellence

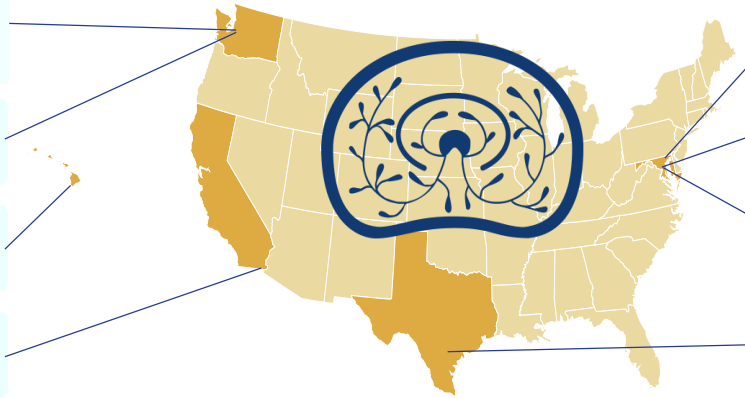
Mission: To conduct state-of-the-art clinical, epidemiologic, and basic science research with an emphasis on precision medicine to enhance the readiness of active duty personnel in conjunction with the continuum of medical care for military retirees and beneficiaries.

Virginia Mason Medical Center
Seattle, WA

Madigan Army Medical Center
Tacoma, WA

Tripler Army Medical Center
Honolulu, Hi

San Diego Naval Medical Center
San Diego, CA



Walter Reed National Military Medical Center
Bethesda, MD

Uniformed Services University
Bethesda, MD

Joint Pathology Center
Silver Spring, MD

San Antonio Military Medical Center
San Antonio, TX

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WALTER REED BETHESDA

hjf

COL (Ret) Craig Shriver, MD, MC, USA
CSO

CDR Gregory T Chesnut, MD FACS, MC, USN
Incoming Director

Contributions of CPDR to the EDRN Mission

Under our **Inter Agency Agreement** the CPDR team continues to **generate data, resources and provide services prioritized by the EDRN leadership.**

CPDR contributes to EDRN with its racially diverse patient cohort and associated biospecimens: **High representation of African American patients in the equal access Military Healthcare System with long follow up time (over 20 years).**

These tasks require high quality and well annotated biospecimens. In 2018 the CPDR Biospecimen Bank gained **accreditation from the College of American Pathologists (CAP).** Thus, the specimens CPDR provides to EDRN are collected under CAP certified protocols.

We use state of the art methods such as **next generation sequencing (NGS), fluorescent *in situ* hybridization (FISH), NanoString (including the GeoMX spatial transcriptome analysis system), Droplet Digital PCR (ddPCR), immunochemistry (IHC), multiplexed Enzyme Linked Immunosorbent Assay (ELISA), Laser Capture Microdissection (LCM).**

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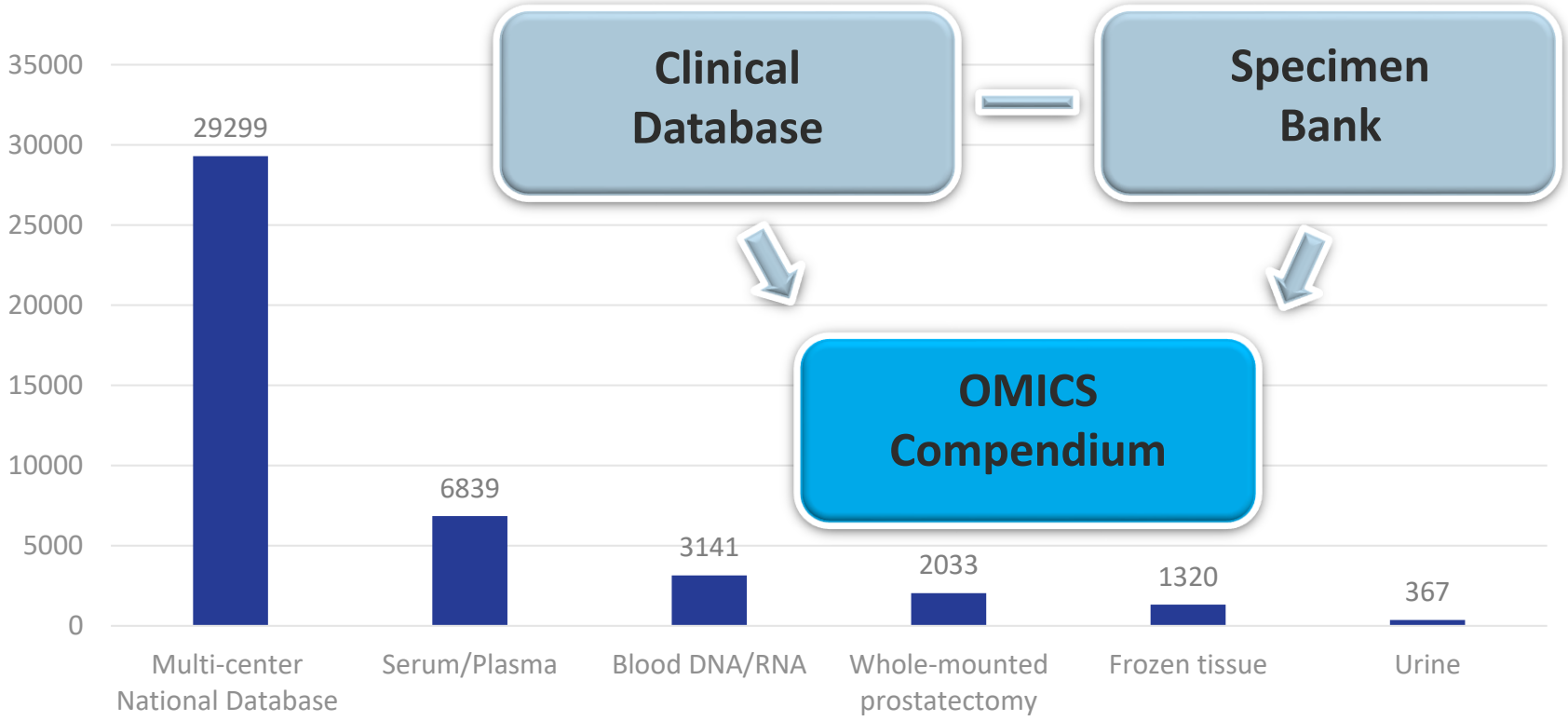
Unique CPDR Resources Supporting the EDRN Mission

Equal access Military Healthcare System; High representation of African American men;

Up to 27 years follow up time; CAP accredited biospecimen bank; Committed long term personnel



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Inter Agency Agreement between NCI-EDRN and DOD-USU-CPDR

NCI-EDRN Leadership: Dr. Sudhir Srivastava; Dr. Jacob Kagan; Dr. Wendy Wang; Dr. Richard Mazurchuk
CPDR-EDRN Leadership: COL Inger L. Rosner, MD, MC, USA; Dr. Gyorgy Petrovics; Dr. Albert Dobi

Tasks in the current cycle:

1. Provide molecular pathology resource for **tissue-based validation of EDRN discoveries** of key molecular alterations: **IHC (ETV1)** and **FISH (LSAMP, PTEN, CHD1)** validation examples.

2. Enrichment of prostate cancer epithelial cells and other cell types by Laser Capture Microdissection and Manual Microdissection to support the **development of antibody free mass spectrometry assays (PRISM)** for fast and efficient identification and verification of candidate biomarkers: **PNNL collaboration on quantitative proteomics assay for genomics markers.**

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Tasks in the current cycle:

3. **Support EDRN Genitourinary group's collaborative projects** by leveraging the unique biospecimens and database resources from the longitudinally followed and racially diverse Department of Defense patient cohort.

4. Evaluation of race and ethnicity optimized prostate cancer gene panels and assays for **early detection of aggressive prostate cancer** in biofluids, with **current focus on urine exosomal RNA based assay.**

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Key EDRN-CPDR Collaborations in the Current Cycle

Collaborating Institutions	Project
Department of Energy Pacific Northwest National Laboratory Dr. Karin Rodland, Dr. Tao Liu	Antibody free mass spectrometry assays (SRM-MS and PRM-MS) for the early identification of biomarkers for aggressive prostate cancer
Johns Hopkins University Dr. Hui Zhang	Prostate cancer protein panel for the early detection of aggressive prostate cancer at diagnostic biopsy
University of Michigan Dr. Arul Chinnaiyan, Dr. John Wei	Early prostate cancer diagnostic and prognostic marker panels using tissue and urine
Department of Defense Joint Pathology Center Dr. Isabell Sesteerhenn, Dr. Joel T. Moncur	Molecular pathology resource for tissue-based validation of EDRN discoveries of key molecular alterations
University of Texas Health Science Center San Antonio Dr. Robin Leach	Predictive prognostic markers for differentiating indolent vs. progressive disease using tissues and urine

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Milestones of Collaborative Team Projects (2018-20)

1. Early prognostic markers of metastatic progression (**PNNL, Dr. Karin Rodland and Dr. Tao Liu**, *Gao et al., Cancers, 2020*)
2. Independent validation of CPDR findings on early predictive mRNA markers for BCR using NanoString platform (**UTHSCSA, Dr. Robin Leach**, in last step of analysis)
3. Independent validation of candidate glycoproteins for early detection of high-grade CaP (**JHU, Dr. Hui Zhang**, in progress)
4. International collaboration with EDRN-China through EDRN leadership: Deep genomic analysis of prostate cancers from diverse populations (**SIBS, Guo-Ping Zhao, Yixue Li, Hong Li,** *Xiao et al., Scientific Reports, 2018*)
5. With **University of Michigan, Dr. John Wei**, independent pre-validation of urinary mRNA marker panel for detection of aggressive CaP (in IRB process)
6. With **University of Michigan, Dr. Arul Chinnaiyan**, lncRNA markers of early prognostic value focusing on African American prostate cancer. (in IRB process)
7. Long-term collaboration with **Joint Pathology Center, Dr. Isabell Sesterhenn and Dr. Joel Moncur**, to support EDRN tissue-based prognostic marker validation studies.

Milestones of CPDR Projects (2018-20)

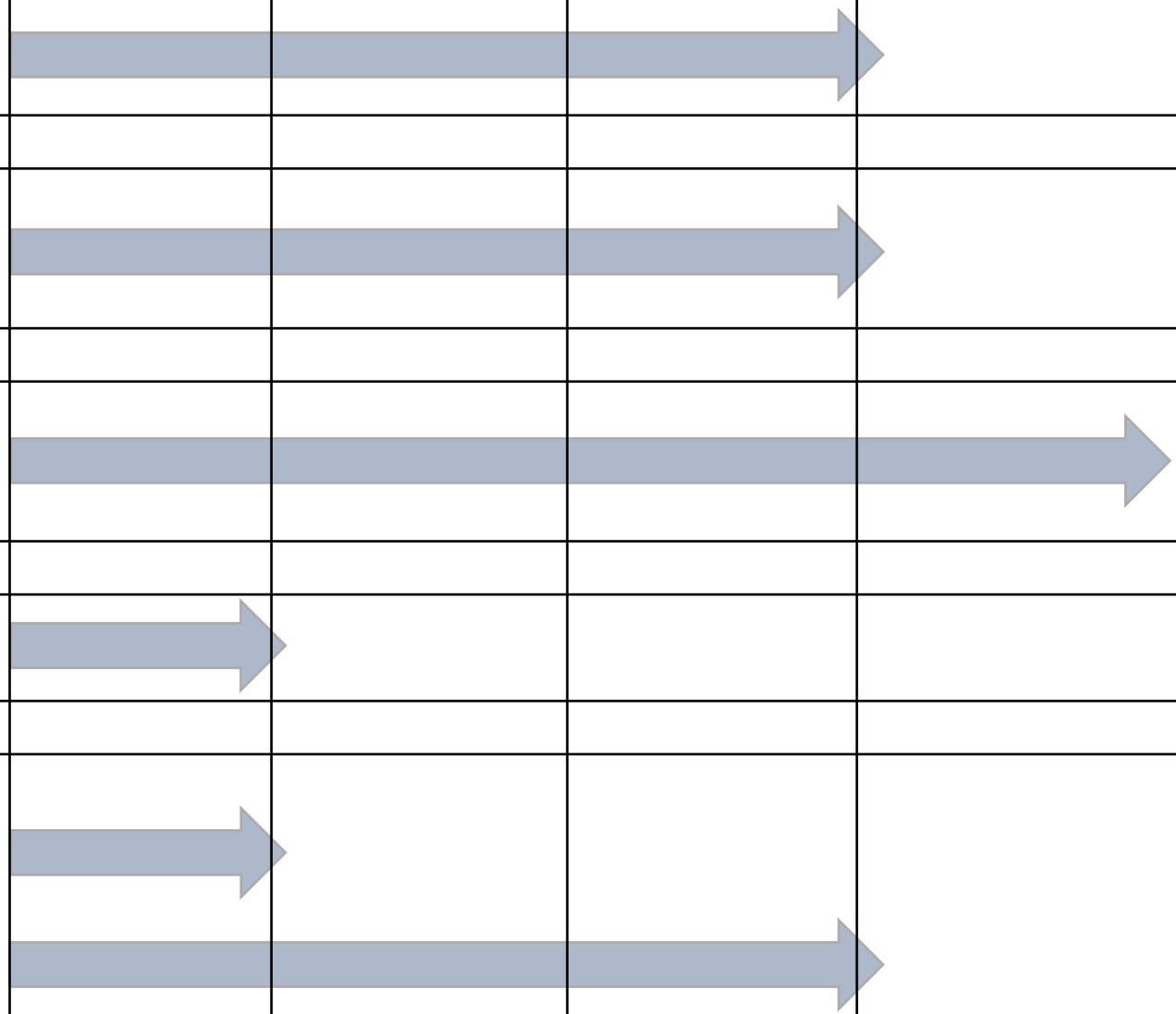
1. Development of race informed marker panel and **urine exosomal RNA-based** assay for the **early detection of aggressive prostate cancer**: Developed a sensitive and reproducible regular urine (not post-DRE) exosome-based gene expression assay platform; the objective was to differentiate high from low-grade disease in a racially diverse patient cohort (*under review PNAS*).

2. Stratification by **ERG in a large racially diverse (AA, CA) DOD prostate cancer cohort**: Comprehensive analyses of the ERG oncoprotein expression in 930 whole mounted prostate specimens in the context of multi-focal prostate cancers (*Cullen et al, Eur Urol Focus, 2018*).

3. Molecular profiling of radical prostatectomy tissue from patients with no sign of progression identifies ERG as the strongest independent **predictor of recurrence** using **NanoString technology** (*Yan et al, OncoTarget, 2019*)

4. Development and validation of a **diagnostic grade antibody to detect the ETV1** oncoprotein: developed and assessed ETV1 rabbit monoclonal antibody for detection and stratification of prostate cancer (*Manuscript in preparation*).

Candidate Biomarker	Discovery			Pre-validation	Validation
	Discovery	Predictive Analysis	Assay Refinement	Blinded limited Cross-Sectional	Large Cross-Sectional
CPDR Urine RNA Marker Panel (*PCA3, PCGEM1*), in non-DRE urine					
CPDR-PNNL Protein Marker Panel (TGF-b and SPARC) by SRM to predict metastasis					
CPDR-UTHSCSA RNA Marker Panel (ERG + 7 genes) by NanoString assay of low grade CaP					
CPDR ETV1 rabbit monoclonal antibody for IHC					
CPDR-JHU Urine Glycoprotein Markers by glycoproteomic assay (Mass spec/ELISA) in non-DRE urine (Two projects)					



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Significant CLIA/LDT Assay

CPDR intellectual property (prostate cancer mRNA panels selected from various gene expression analysis platforms) was **licensed to Exosome Diagnostics** (BioTechne Inc) for the clinical use of a **urine based prostate cancer detection assay** (EPI, ExoDX Prostate IntelliScore Assay), which has been **commercialized and is covered by BlueCross BlueShield and Medicare**.

CPDR has been further developing this urine exosome panel focusing on mRNA markers with **similar performance in African American and Caucasian American** patients.

The state of your marker/s. In which phase is your marker? Did you move your marker/s from development to verification, etc.?

- **Tissue RNA** NanoString panel: **finishing independent verification** (with UTHSCSA).
- **Urine RNA, tissue protein** (SRM with PNNL), and **urine glycoprotein** (MS with JHU) panels: **starting verification** in independent cohorts.

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EDRN Questions

What was accomplished in this cycle of EDRN?

- Early detection of aggressive CaP urine exosomal RNA assay (*Under review in PNAS*)
- Protein marker panel of progression to metastasis with PNNL (*Gao et al, Cancers, 2020*)
- Early predictive RNA markers of biochemical recurrence (*Yan et al, OncoTarget, 2019*)
- Completed a deep genomic analysis of prostate cancers from diverse populations (*Xiao et al., Scientific Reports, 2018*)

What is the progress and the current state of your trans-network collaborative projects (collaborative projects funded through set-aside funds)?

- Independent verification of prognostic markers in low-grade disease (*Yan et al, OncoTarget, 2020)* with Dr. Robin Leach, UTHSCSA)
- Independent validation of glycoproteins for early detection of high-grade CaP in progress (Dr. Hui Zhang, JHU)

How does the Network structure benefit your biomarkers development and validation study (how is it better than R01 mechanism)?

- The EDRN network operates through collaboration and provides framework for biomarker development and validation.
- EDRN enables us to collaborate with EDRN members, which made the discovery and verification of biomarkers much more straightforward and robust

Each investigator should submit 1-2 bullet points as potential areas that should be included in the EDRN GU renewal discussions.

- Explore the potential of germline mutation testing in early detection of more aggressive and earlier onset malignancies
- Increase focus on integrating biomarker discovery and validation with imaging technologies

Future Directions

Goals for the next year:

- CPDR **urine exosome-based** early markers of aggressive prostate cancer in racially diverse population
- Validate **biofluid and tissue based** markers developed by EDRN collaborators (PNNL, Johns Hopkins University, University of Michigan, UTHSCSA)

Goals for 3 years:

- Focus on **validating** and progressing biomarker candidates **towards clinical application**
- **New seminal discoveries/developments related to biomarkers:** Under a DOD grant CPDR **discovered novel germline mutations in DNA Damage Repair Genes (DDRG) in African American prostate cancer patients.** We have successfully completed the discovery phase evaluating the **whole genome sequencing of germline DNA of 300 African American and 300 Caucasian American patients.** We propose to EDRN the **independent validation** and **genetic test development** to complement early detection biomarkers developed by EDRN investigators.

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Publication Highlights (2018-20)

1) Proteomic Tissue-Based Classifier for Early Prediction of Prostate Cancer Progression. Gao Y, Wang YT, Chen Y, Wang H, Young D, Shi T, Song Y, Schepmoes AA, Kuo C, Fillmore TL, Qian WJ, Smith RD, Srivastava S, Kagan J, Dobi A, Sesterhenn IA, Rosner IL, Petrovics G, Rodland KD, Srivastava S, Cullen J, Liu T. ***Cancers,*** **2020 May, 12(5):1268.**

2) Molecular profiling of radical prostatectomy tissue from patients with no sign of progression identifies ERG as the strongest independent predictor of recurrence. Yan W, Jamal M, Tan SH, Song Y, Young D, Chen Y, Katta S, Ying K, Ravindranath L, Woodle T, Kohaar I, Cullen J, Kagan J, Srivastava S, Dobi A, McLeod DG, Rosner IL, Sesterhenn IA, Srinivasan A, Srivastava S, Petrovics G. ***Oncotarget,*** **2019 Nov, 10(60):6466-6483.**

3) Predicting Prostate Cancer Progression as a Function of ETS-related Gene Status, Race, and Obesity in a Longitudinal Patient Cohort. Cullen J, Young D, Chen Y, Degon M, Farrell J, Sedarsky J, Baptiste W, Rosen P, Tolstikov V, Kiebish M, Kagan J, Srivastava S, Kuo HC, Moncur JT, Rosner IL, Narain N, Akmaev V, Petrovics G, Dobi A, McLeod DG, Srivastava S, Sesterhenn IA. ***Eur Urol Focus,*** **2018 Dec, 4(6):818-824.**

4) Systematic analysis reveals molecular characteristics of ERG-negative prostate cancer. Xiao Q, Sun Y, Dobi A, Srivastava S, Wang W, Srivastava S, Ji Y, Hou J, Zhao G-P, Li Y, Li H. ***Scientific Reports,*** **2018 Aug, 8:12868.**

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PNNL: Tao Liu, Karin Rodland and their group

UTHSCSA: Robin Leach and her group

JHU: Hui Zhang and her group

SIBS: Guoping Zhao and his group

UMICH: Arul Chinnaiyan, John Wei

CPDR: Albert Dobi, Indu Kohaar, Shyh-Han Tan, Jennifer Cullen, Yongmei Chen, Claire Kuo, Denise Young, Yingjie Song, Isabell Sesterhenn, Shiv Srivastava, Inger Rosner

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