Identification of Aggressive Prostate Cancers: In-depth Proteomics of Tissues and Urines

EDRN BDL EVMS Toronto UCLA

35th EDRN Steering Committee Meeting
June 30 - July 1, 2020

Introduction

1. Current diagnostic and prognostic protocols (PSA, DRE, biopsy) are inaccurate in predicting patients' risk

2. Molecular biomarkers and/or imaging approaches could improve the decision process

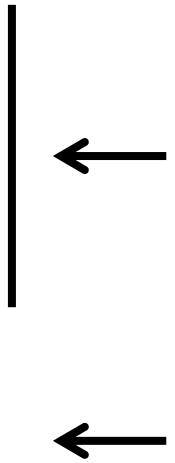
3. Molecular profiling of prostate cancer tissues and proximal fluids (post-DRE urine & dEPS) at the level of DNA, RNA and protein

4. Biomarkers for early detection of aggressive disease

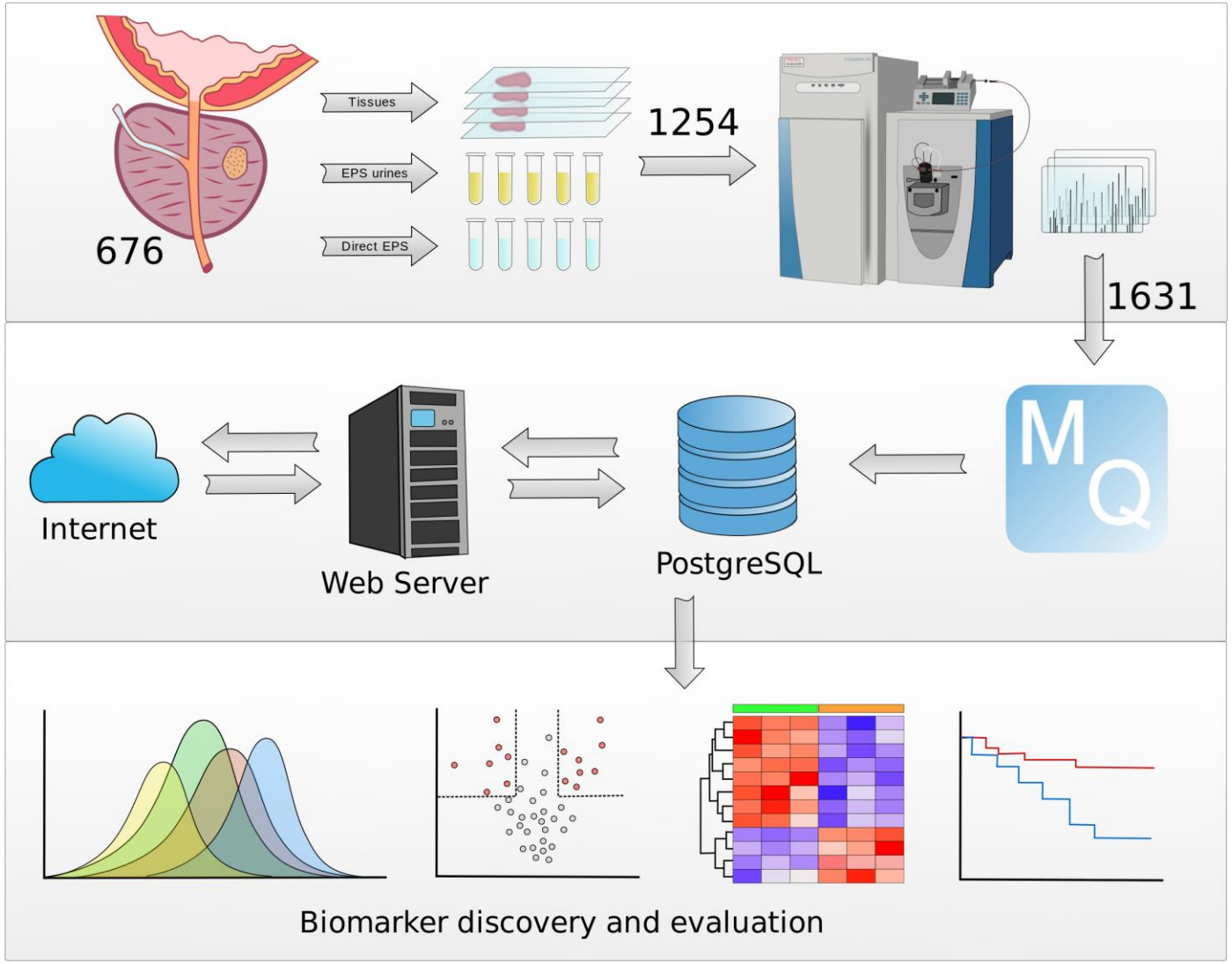
Goals & Timeline

Kim et al. *Nat Commun* 2014

Biomarker Candidate	Discovery			Pre-validation	Validation
	Discovery	Predictive Analysis	Assay Refinement	Blinded Limited Cross-Sectional	Large Cross-Sectional
PRM panel to detect Non-OC disease prior to surgery	■	■	■	■	
PRM panel to detect aggressive disease	■	■			
PRM panel to predict upgrading	■	■			
Collaborative projects					
UTHSC CVC Leach – PRM to detect PSA variant	■	■	■		
UTHSC CVC Liss – Peptide panel to assist phi and imaging	■				
UTHSC-NCI Germline Sequencing on Upgrading Study	■	■	■	■	■
UTHSC CVC Liss – MultiOmic Biomarker Ordering	■				



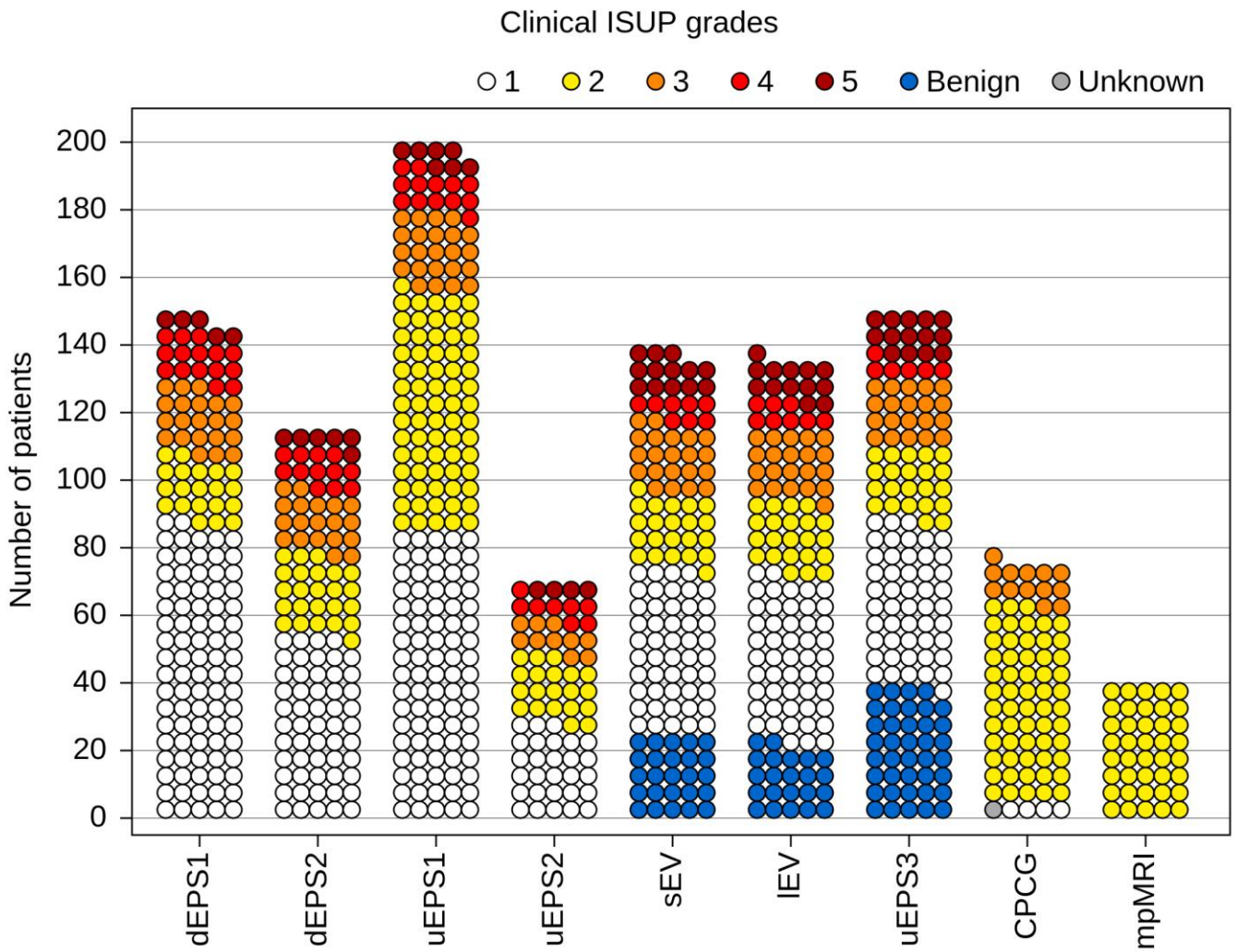
Overview

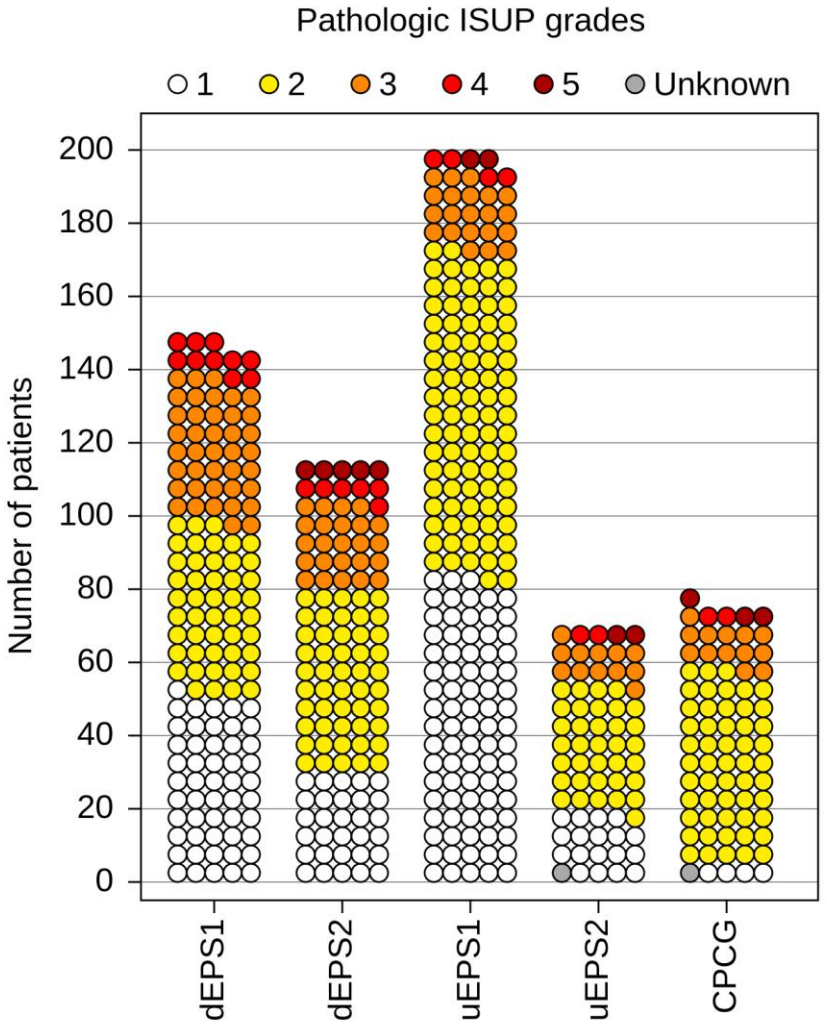

676
Tissues
EPS urines
Direct EPS
1254
1631
Internet
Web Server
PostgreSQL
MQ
Biomarker discovery and evaluation

Cohort Details

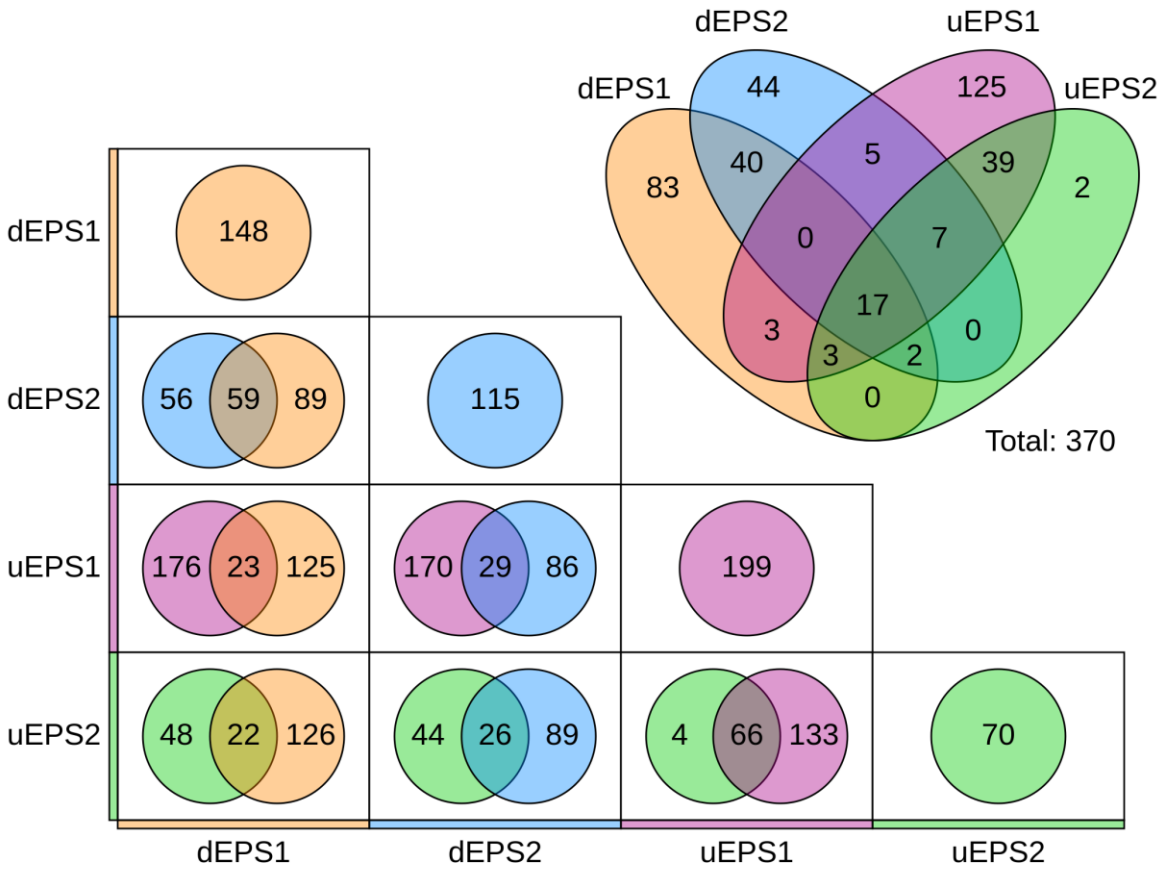
Dataset	Cohort	Site	Material	Clinical notes	RAW files	Samples	Patients	Non-redundant Patients
dEPS1	EVMS	UHN	Direct-EPS	low-high risk	148	148	148	370
dEPS2	EVMS	EVMS	Direct-EPS	low-high risk	115	115	115	
uEPS1	EVMS	UHN	EPS-urine	low-high risk	199	199	199	
uEPS2	EVMS	EVMS	EPS-urine	low-high risk	210	70	70	
sEV	SHSC	UHN	Small EV	low-high risk	128	128	113	190
sEV	EVMS	UHN	Small EV	Benign	25	25	25	
lEV	SHSC	UHN	Large EV	low-high risk	129	129	114	
lEV	EVMS	UHN	Large EV	Benign	22	22	22	
uEPS3	SHSC	UHN	EPS-urine	low-high risk	222	111	111	
uEPS3	EVMS	UHN	EPS-urine	Benign	78	39	39	
uEPS3a	SHSC	UHN	EPS-urine	pre- and post-DRE (matched) low risk	40	20	10	
uEPS3b	SHSC	UHN	EPS-urine	Active surveillanc longitudinal low risk	30	15	5	
CPCG	UHN	UHN	Tissue	localized - intermediate risk	150	76	76	76
mpMRI	UCLA	UHN	Tissue	localized - intermediate risk & adjacent normal	157	157	40	40
Total:					**1631***	**1254**		**676**

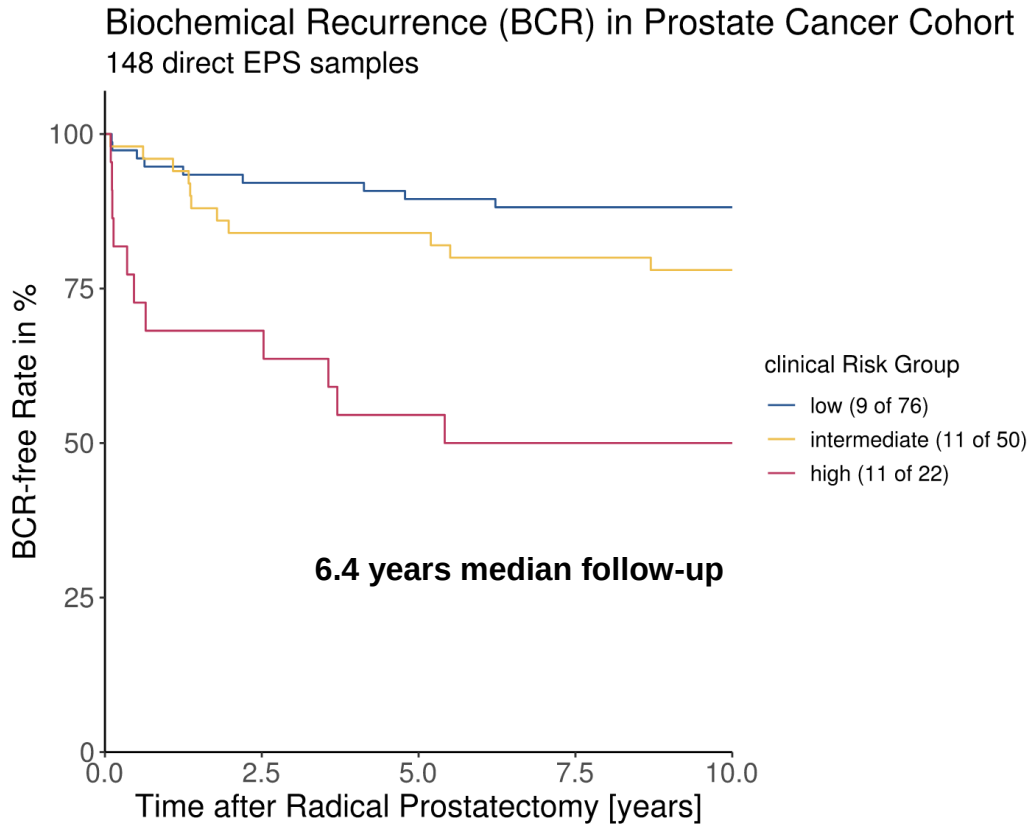
Cohort Details



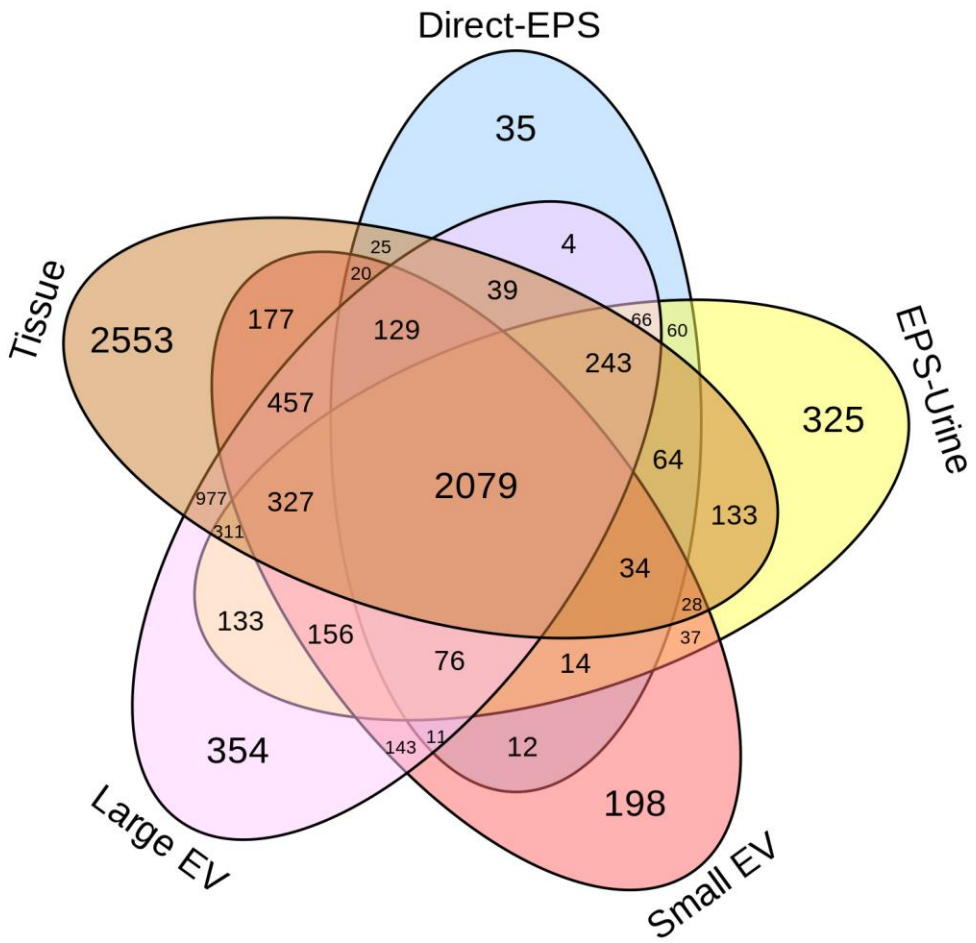


EVMS Cohort

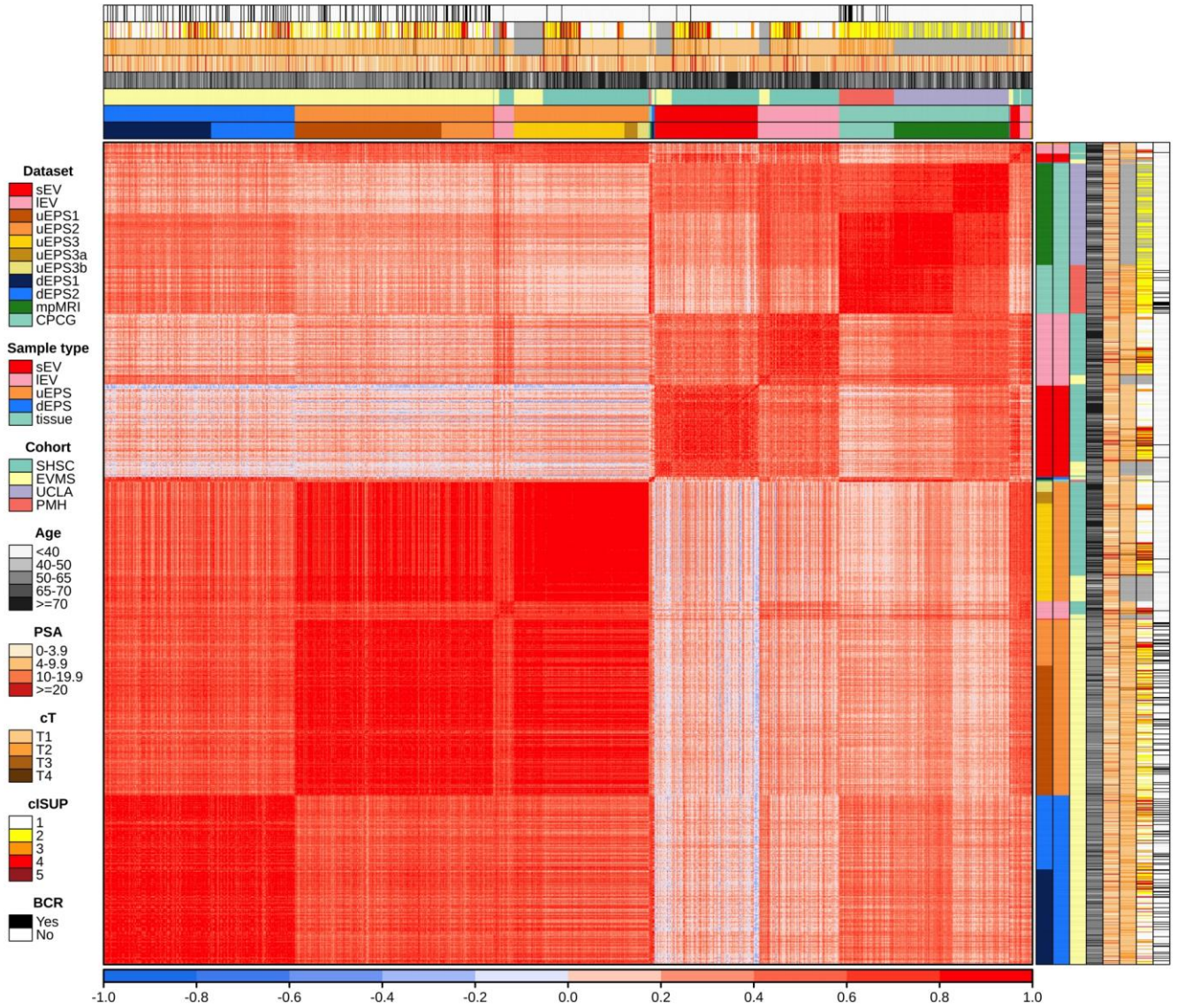

dEPS2
uEPS1
dEPS1
uEPS2
44
125
83
40
5
39
2
0
7
17
3
0
3
2
0
Total: 370
dEPS1
dEPS2
uEPS1
uEPS2
148
56
59
89
115
176
23
125
170
29
86
199
48
22
126
44
26
89
4
66
133
70
dEPS1
dEPS2
uEPS1
uEPS2


Biochemical Recurrence (BCR) in Prostate Cancer Cohort
148 direct EPS samples
BCR-free Rate in %
100
75
50
25
0
0.0
2.5
5.0
7.5
10.0
Time after Radical Prostatectomy [years]
6.4 years median follow-up
clinical Risk Group
low (9 of 76)
intermediate (11 of 50)
high (11 of 22)

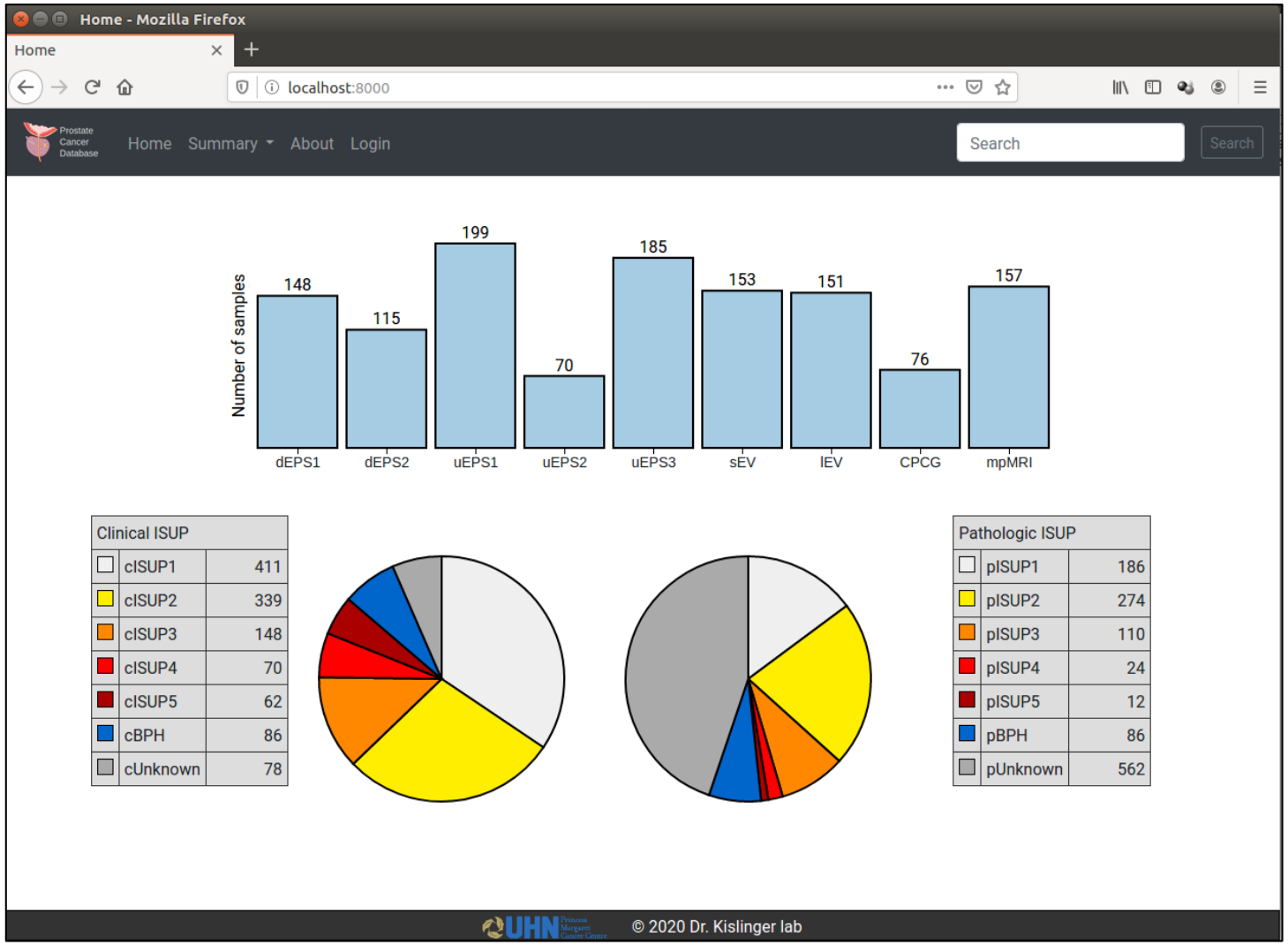
Prostate Cancer Proteome



Detected proteins



Prostate Cancer Proteome



- **Web-portal**
- **Clinical data for all patients**
- **Protein, peptide and spectral data**
- **Will enable independent PRM assay development**

Identification and Pre-validation of a Clinically Relevant PSA Variant in Post-DRE Urines by a Targeted Mass Spectrometry Assay

Set-Aside Collaborative Project

EVMS/Toronto/UCLA/UTHSA (Robin Leach)

- Despite specificity and sensitivity issues, PSA serum level quantitation is still the most commonly used biomarker for prostate cancer.

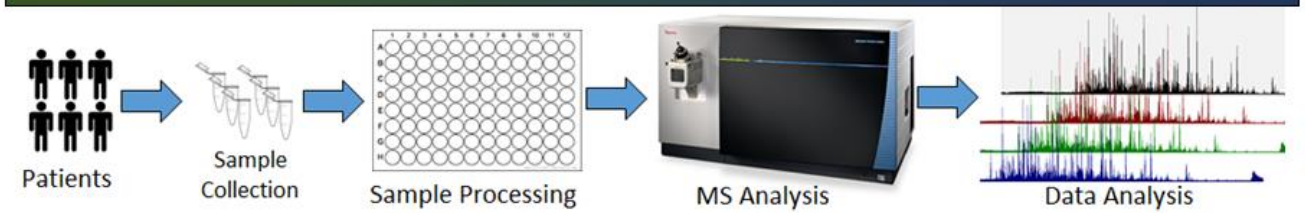
- The PSA rs17632542 SNP with a **I179T** substitution results in lower serum PSA levels which may further mitigate against its clinical utility as a prostate cancer biomarker.

- Post-DRE urine is a minimally invasive fluid that is currently utilized in prostate cancer diagnosis. We have developed a targeted MS method to detect and quantitate the variant protein in urine using small samples volumes and a high-throughput assay.

- The assay provides a tool to evaluate the utility of PSA variant (rs17632542) assessment in parallel with current and forthcoming urine biomarker panels.

Discovery Experiments

A. MSTERN



Post-DRE urines from the EVMS Biorepository processed by an optimized MStern approach (Berger et al. 2015)

EXPERIMENTAL WORKFLOW

B

Step 1: Discovery

Patient Samples N=75
DDA in Triplicate

Custom Database Searching in Mascot

PSA Observed in 75/75
I179T PSA Variant Identified in 3/75 Patients

I179T
K.LQCVDLHVTSNDVCAQVHPQK.V
WT
K.LQCVDLHVISNDVCAQVHPQK.V

16226 Prostate-specific antigen
8476 Prostate-specific antigen variant

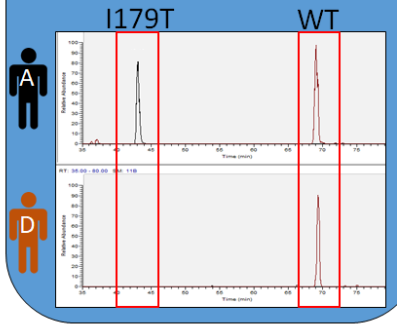
C

Step 2: Predictive Analysis

New Preparation of Six Samples
I179T Previously Observed in 3/6 DDA

Mascot and Xcalibur for Data Analysis

XIC Example



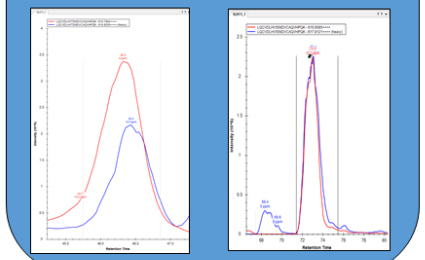
D

Step 3: Assay Refinement

New Samples (N=14)
Spiked with Stable-Isotope Labeled Peptides

$^{13}C_6$,$^{15}N_2$ C-Terminal Lysine
I179T
K.LQCVDLHVTSNDVCAQVHPQK.V
WT
K.LQCVDLHVISNDVCAQVHPQK.V

Targeted-MS: PRM
Parallel Reaction Monitoring
Skyline for Data Analysis



Pre-validation Experiments

EXPERIMENTAL WORKFLOW

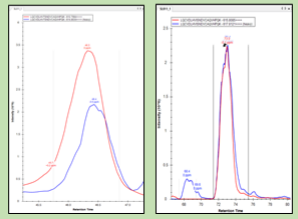
- Post-DRE urine samples from **rs17632542** genotyped patients were obtained from the UTHSA SABOR cohort and processed in a double blinded manner using Mstern (2015).
- The SNP alters a codon **ATT** to **ACT** leading to an amino acid substitution of an **isoleucine** to a **threonine** at position **179 (I179T).** Genotypes of **TT** are homozygous wild-type, **CT** are heterozygous, and **CC** are homozygous variant.

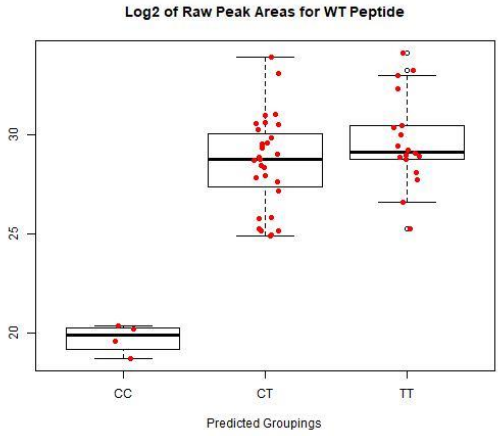
Pre-validation Assays

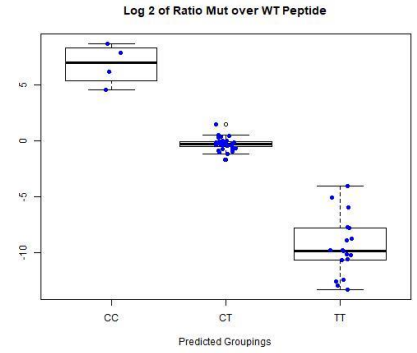
53 genotyped samples (N=53)
Spiked with Stable-Isotope Labeled Peptides

$^{13}C_6$,$^{15}N_2$ C-Terminal Lysine
I179T
K.LQCVDLHVTSNDVCAQVHPQK.V
WT
K.LQCVDLHVISNDVCAQVHPQK.V

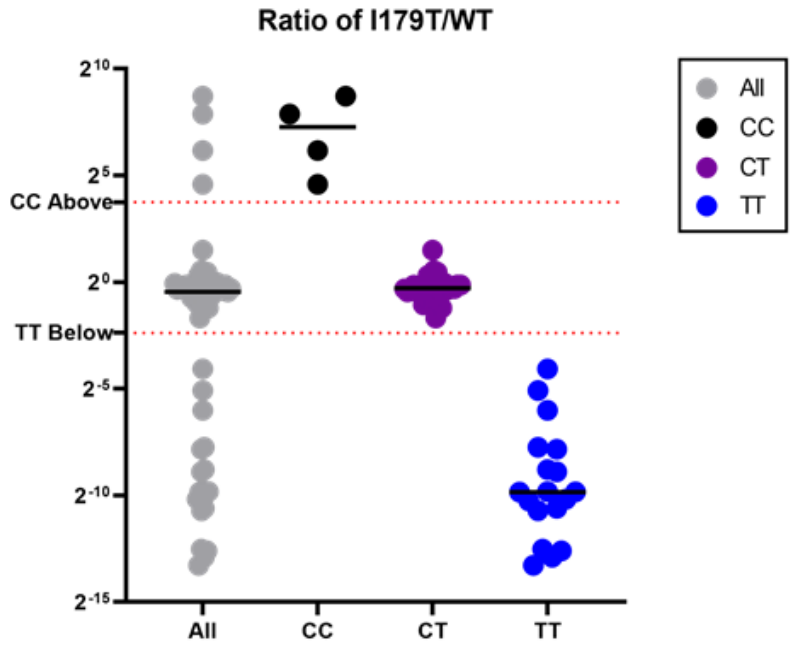
Targeted-MS: PRM
Parallel Reaction Monitoring
Skyline for Data Analysis and Quantitation



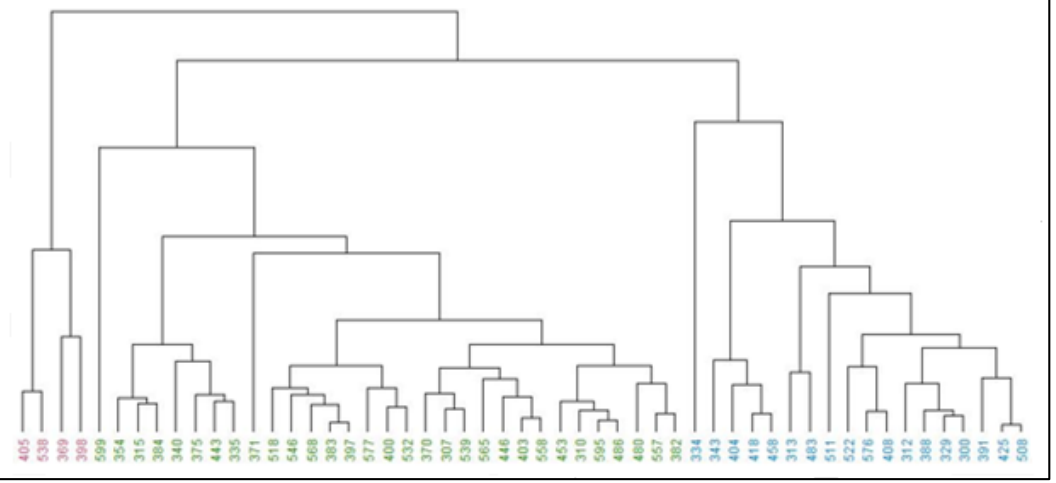


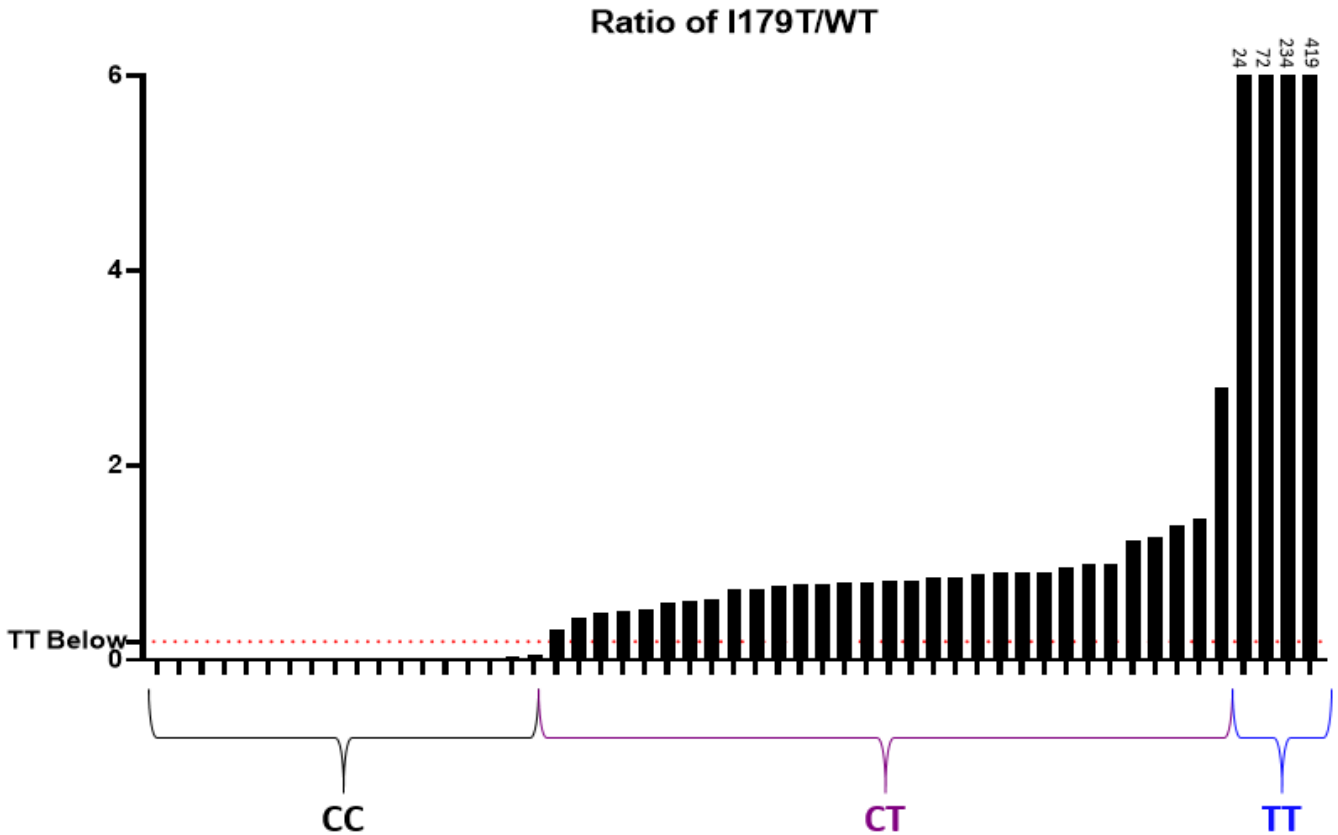


Pre-validation of Clinically Relevant PSA Variant in the SABOR Cohort Post-DRE Urines by a Targeted Mass Spectrometry Assay



Hierarchical clustering identifies the 3 genotypes.





- Ratio of integrated peak areas for the I179T variant peptide and WT peptide.
- The genotype specification of TT (homozygous variant) can be seen below the dotted line.

Correlative Proteomic Analysis of Men on Active Surveillance Receiving Magnetic Resonance Imaging (MRI)

Set-Aside Collaborative Project

EVMS/Toronto/UCLA/UTHSA (Michael Liss)

Objective: Discovery and refinement of protein-based "liquid biopsy" assays for use in radiomic approaches for the early identification of aggressive prostate cancer

Step 1: Discovery

- **AS** Patient Post-DRE Urine Samples (N=90)
- MStern Processing
- DDA LC/MS/MS

↓

Database Searching and LFQ Analysis in MaxQuant

↓

Statistical Analysis to Identify Differentially Expressed Proteins

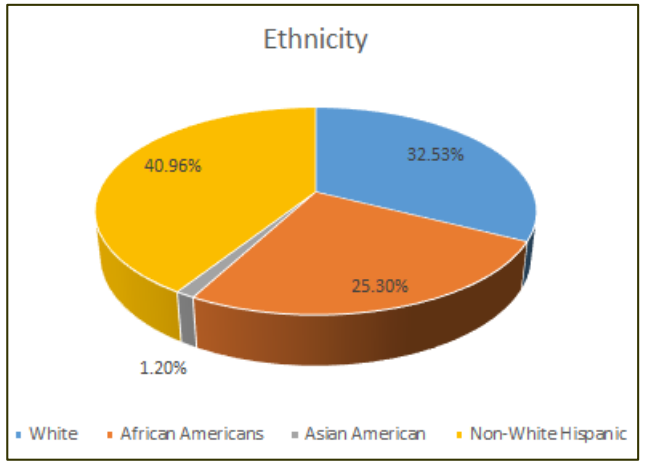
↓

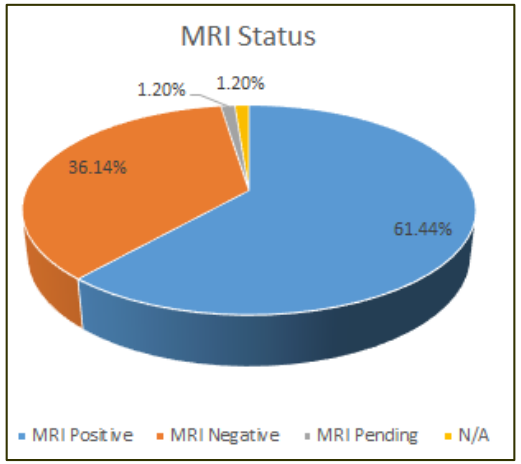
Develop PRM-MS Assays for Absolute Quantification of Targets in Post-DRE Urines

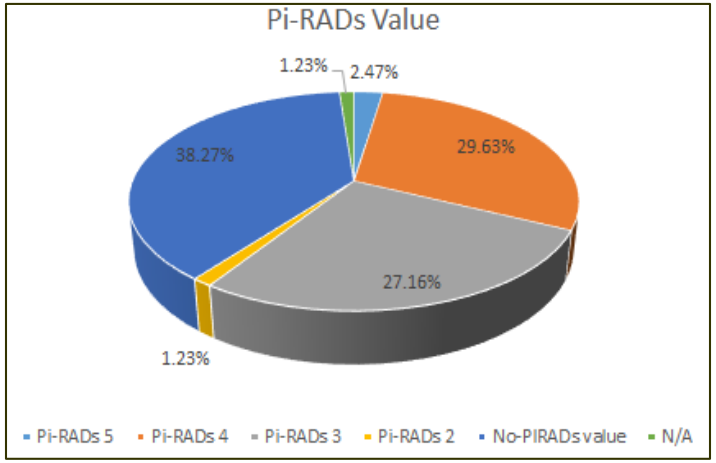
↓

Identification of Prognostic MRI Correlated PDU **AS** Biomarkers for Aggressive Disease

Demographic and Clinical Information of Active Surveillance Patient Cohort







Status:

- Currently running Discovery DDA LC/MS/MS analysis to identify differentially expressed proteins.

Pending:

- Identification of men upgraded and determine performance in predicting such outcomes in active surveillance populations in combination with MRI and MIPS.
- Machine-learning approaches to integrate a unified predictor to discriminate patients.

UCLA - Toronto

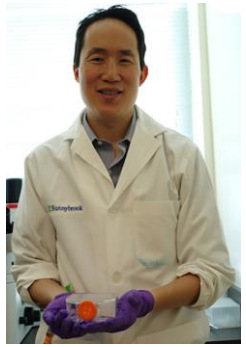
Zhuyu Qui
Vincent Huang
Julie Livingstone
Michael Fraser
Jouhyun Jeon

Toronto

Yunee Kim
Ankit Sinha
Lydia Liu
Amanda Khoo
Kathi Fritsch
Andrew Macklin
Salvador Mejia
Natalie Kurganovs

SLRI

Michelle Downes
Stan Liu



EVMS

John Semmes
Julius Nyalwidhe
Brian Main
Joseph Otto
Lifang Yang
Vanessa Correll

CIHR IRSC
Canadian Institutes of Health Research
Instituts de recherche en santé du Canada

Canadian Cancer Society
Société canadienne du cancer

UHN Princess Margaret Cancer Centre

Prostate Cancer Canada

NIH NATIONAL CANCER INSTITUTE
Early Detection Research Network