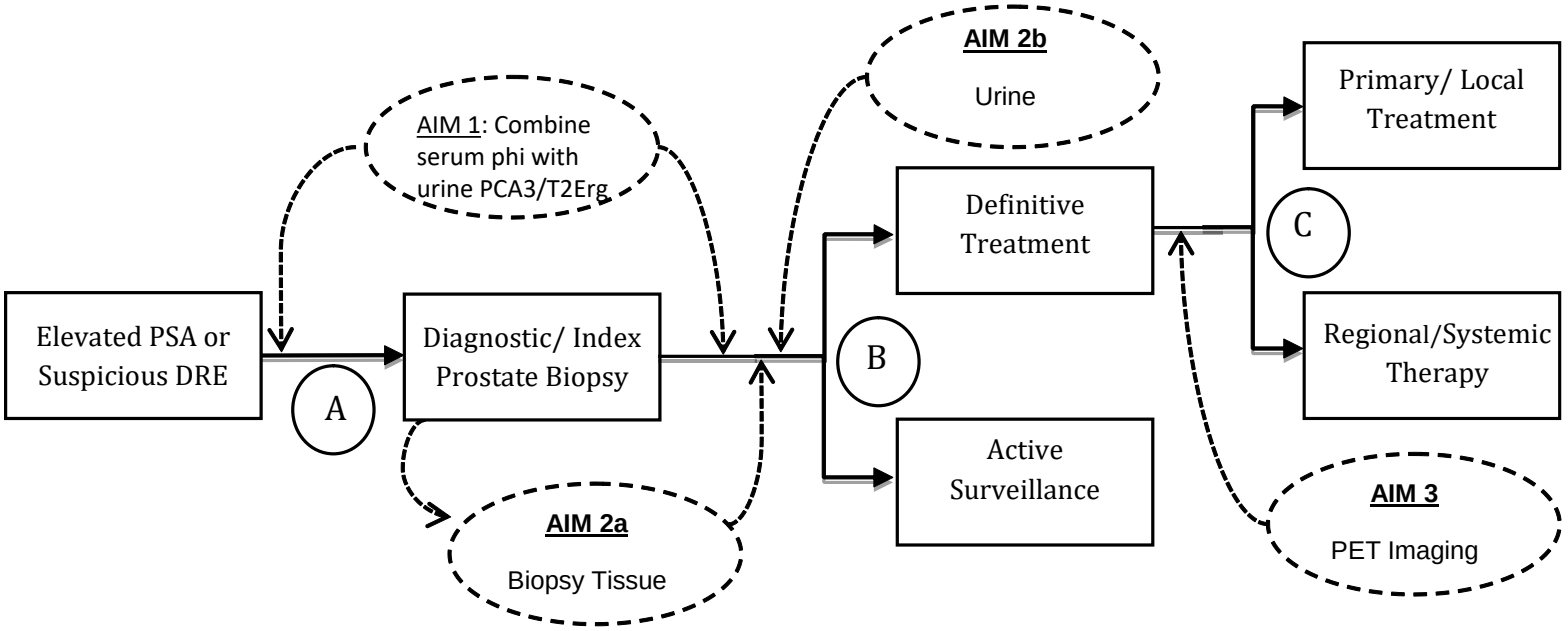
Emory-Harvard-University of Washington Prostate Cancer Biomarker Center (an EDRN CVC)



HYPOTHESIS: early detection targeting aggressive prostate cancer, will enhance survival benefit of treating lethal disease yet reduce harms of over-treating indolent Pca

AIMS:

- Validate combining phi, PCA3, T2Erg to predict biopsy (PCA3 Trial) and AS progression-PASS
- Validate multiplex RNA signatures (tissue, urine) to discern aggressive PCa (PASS, URS)
- FACBC-PET to detect occult metastatic in high risk primary prostate cancer

INVESTIGATORS: Emory: Sanda (PI), Huang (co-PI), Moreno, Schuster, Goodman, Alemozaffar, Petros, Pellegrini; Harvard SPH: Mucci; Univ Washington: Lin (PASS)

Clinical Deployment of New Biomarkers (Highlights): *Emory-Harvard-University of Washington CVC*

(Aim) Biomarker	1. Readiness	2. Intended Use	3. Type of biomarker	4. Specimen	5. Parameters
1) Phi-PCA3 combination	in clinical use (expanded indication)	Screening, surveillance (reduce un-necessary bx)	Protein (phi), RNA (PCA3)	Serum *(phi)*, post-DRE urine (PCA3)	Increase specificity at high sensitivity
2) Urine EV Transcriptome	CLIA, clinical grade platform	Screening, surveillance (reduce un-necessary bx)	RNA	post-DRE urine	Increase sensitivity, specificity
3) Flucyclovine (Axumin) PET	in clinical use (expanded indication)	Detect occult metastases	Metabolic Radiotracer	PET imaging	Increase sensitivity over conventional imaging

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Aim 4 Biospecimen Distribution: 7955 samples from 1923 subjects (2015-2020)

Impact: 32 publications (original research articles, PubMed, 2015-20)

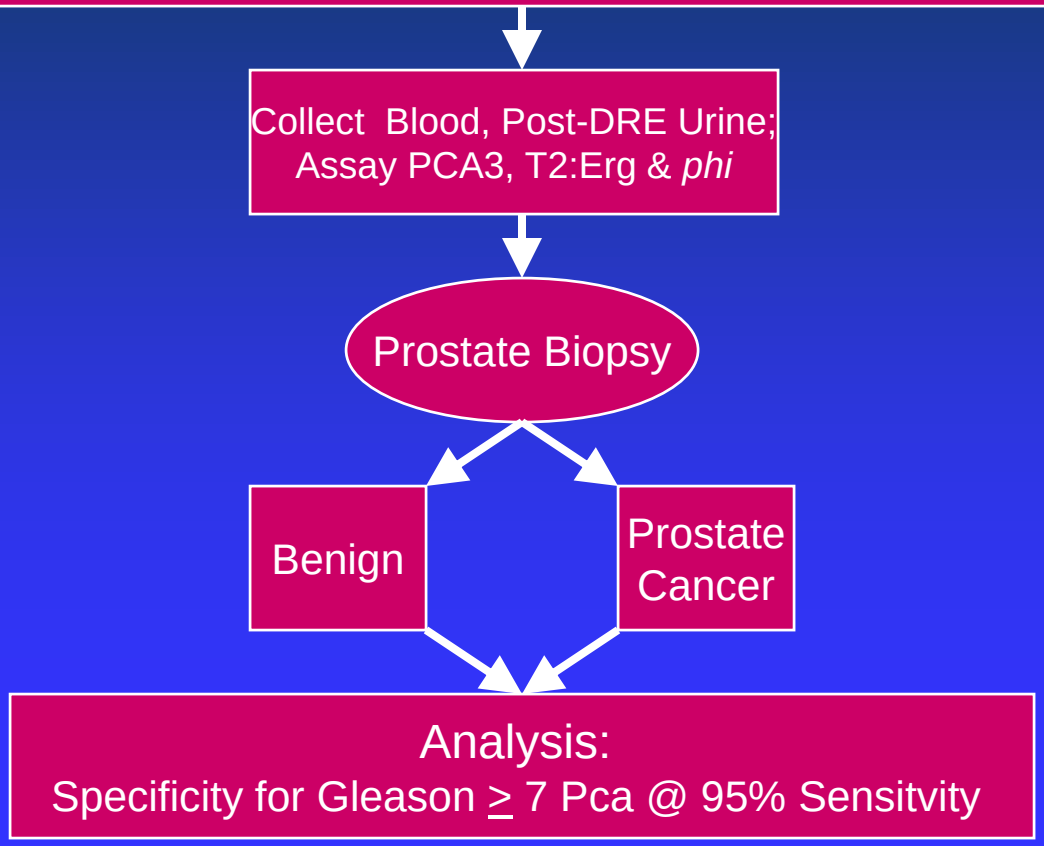
Year	Recipient PI / Institution	Sample	Biomarker / Project	# Subjects	# sample
2015	A. Perry / Dublin, Ireland	Urine Pellets	epiCaPture	91	91
2015	H Whitaker/Cambridge, UK [1]	Whole Urine	Movember GAP Study	181	181
2015	H Pandha/Surrey, UK [2]	Whole Urine			181
2015	E. Diamandis/Toronto, Canada[3]	Whole Urine			179
2015	E. Davicioni/ San Diego [4]	Urine EV cDNA & FFPE	Compare urine & prostate transcriptomes	15	30
2015	J. Clark / Newcastle, UK [5]	Urine EV cDNA	Prostate Urine Risk Signature	100	100
2015	A. McDonald/ Penn State [6]	Plasma	microRNA project	34	102
2016	W. Catalona / Northwestern	Buffy Coat	Genomics of men on AS	30	51
2016		Saliva		71	71
2016	E. Riley / Freenome	Plasma	Genomic Analysis	20	176
2017	J. Codington / Emory	Serum	Diagnostic Sr. Antibody	31	31
2017	T. Liu / Pacific Northwest National Lab[7]	Whole Urine	Proteomics of Whole Urine	30	30
2017	C. Moreno / Emory [8]	Serum	Acuray platform test	10	10
2017	R. Arnold / Emory	Urine Pellets	mtDNA Mutations	45	45
2017-18	NCI Central Repository	Serum	URS Study	92	810
2017-18		Plasma			563
2017-18		Whole Urine			244
2017-18		Supernatant			48
2017-18		PBS Pellet			17
2017-18		RNALater Pellet			26
2017-18		Buffy coat			67
2017-19	FHCRC Central Repository	Frozen Tissue	PASS Study	105	112
2017-19		Serum			1143
2017-19		Plasma			1620
2017-19		Buffy Coat			125
2017-19		Whole Urine			324
2017-19		Supernatant			320
2017-19		PBS Pellet			22
2017-19		RNALater Pellet			58
2018	R. Cummings / BIDMC [9]	Serum	Glycomics analysis	50	60
2018	R. Arnold / Emory	Urine EV RNA	mtDNA Mutations	13	13
2018	Lori Sokoll/ JHU[10]	Serum	PASS patients sr. PHI	557	657
2019	H. Kissick / Emory	Fresh tissue	T-cell response	18	18
2019	B. Olson / Emory	Fresh tissue	T-cell proliferation project	30	30
2018-19	E. Davicioni/ San Diego [11]	Urine EV cDNA	Urine Classifier Development	220	220
2019	Lori Sokoll/ JHU [12]	Serum	EDRN-BRL dev. Collab	90	90
2019	E. Davicioni/ San Diego [11]	Urine EV cDNA	Urine Classifier Development	90	90
Total				1923	7955

Aim 1a): Combining Urinary PCA3, T2:Erg & Serum *phi* to Refine PCa Detection: Cohort & Biospecimen Collection

Development Cohort: 3 Sites, N = 516

Validation Cohort: 10 Sites (EDRN PCA3 Trial Group) N = 561

(Urine PCA3/T2:Erg in validation cohort previously reported, Wei et al JCO Sanda et al JAMA Oncology



Prostate Health Index (*phi*)

EDRN Team Participants: BRL, CVC, DMCC

Parameter	Sensitivity	Specificity
Development Cohort		
Phi >24	96%	35%
PSA > 2.5	96%	17%
Validation Cohort		
Phi >24	92%	30%
PSA > 2.5	98%	18%

- *PHI* improved detecting aggressive PCa with higher specificity
- Using *PHI* ≥ 24 to select men for biopsy can:
  - Avoid unnecessary biopsies in 36% of biopsy candidates

J Urology 2015

Combining Urinary T2:Erg and PCA3 RNA

EDRN Team Participants: BDL, CVC, BRL,DMCC

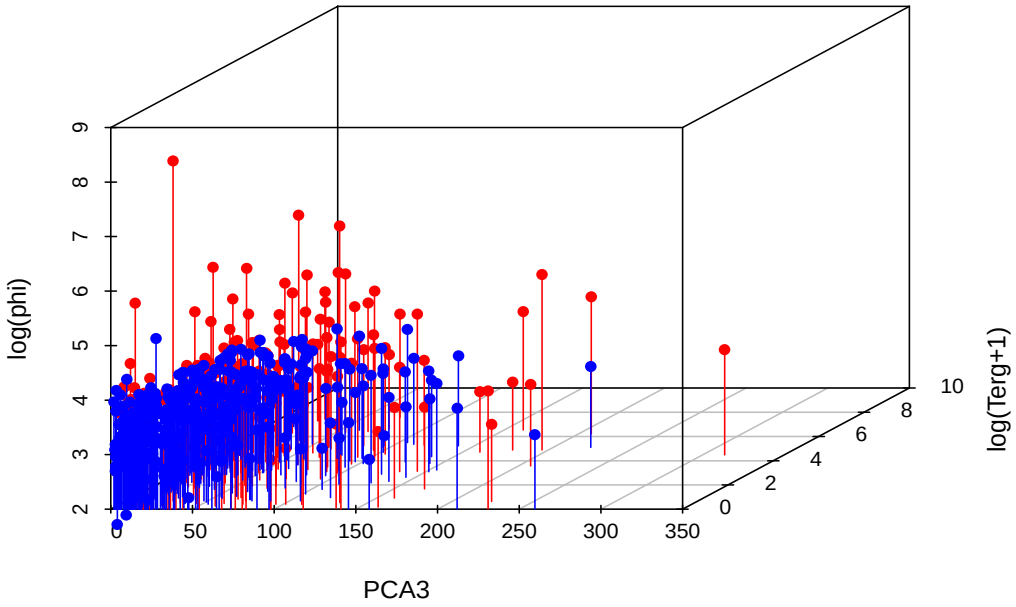
Biomarker	Assay Threshold for > 95% Sensitivity To Predict Gleason ≥ 7	Specificity
PSA	3.0	**18%**
PCA3	6.3	17%
T2-erg*	---	0%
PCA3-T2erg	19.1, 7.6	**39%**

**T2:erg maximum sensitivity is <95% because it exists in ~60% of PCa*

JAMA Oncology 2017

Aim 1: Combining Serum Prostate Health Index with post-DRE Urine PCA3 and T2Erg Clinical Assays to Predict Aggressive Prostate Ca (E Huang et al)

Serum Prostate Health Index (y axis), post-DRE Urine PCA3 (x axis), and T2Erg (z axis) clinical assay results stratified by aggressive Gleason 7+ prostate cancer (red) vs benign or indolent (blue) N=512



Biospecimen Substrate	Biomarker Combination	Sensitivity	Specificity
Blood (serum)	Prostate Health Index (*phi)*	95	27
Urine (post-DRE)	PCA3 + T2Erg	92	36
Blood *and* Urine	*phi* + PCA3 + T2erg	97	41
1st Blood, then Urine	*phi* > PCA3 + T2erg	97	45
1st Urine, then Blood	PCA3 + T2erg > *phi*	96	46

Aim 1 (Set-aside Funds): Combine serum *phi* with Urinary RNA Testing to Refine Prostate Cancer Detection

a) To determine if combining *phi* with urine PCA3, T2:Erg improves specificity for detecting 'aggressive' PCA (biopsy Gleason score $\geq$ 7) on *initial* biopsy

- *EDRN CVC Training Set (completed, E Huang)*
- *EDRN PCA3 Trial Validation (Y Zheng and E Huang, ongoing)*

b) To predict progression during active surveillance: PASS Cohort

- *PASS Activated at Emory Site*
- *Samples for phi to Hopkins BRL (N=657)*
- *Model training: Phi effective to reduce unnecessary biopsy*
- *Next step: Set aside proposal with BRL for validation (N=920)*

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Aim 2: Interrogating Multiplex RNA in Extracellular Vesicles from Post-DRE Urine for Pca Detection (K Pellegrini, C Moreno et al)

- EV's from post-DRE Urine as a source of prostate-derived RNA
- Targeted RNA-Seq (Precise Assay) for CLIA intermediate density RNA panels (several hundred)
- Whole-transcriptome analysis (clinical grade assay)

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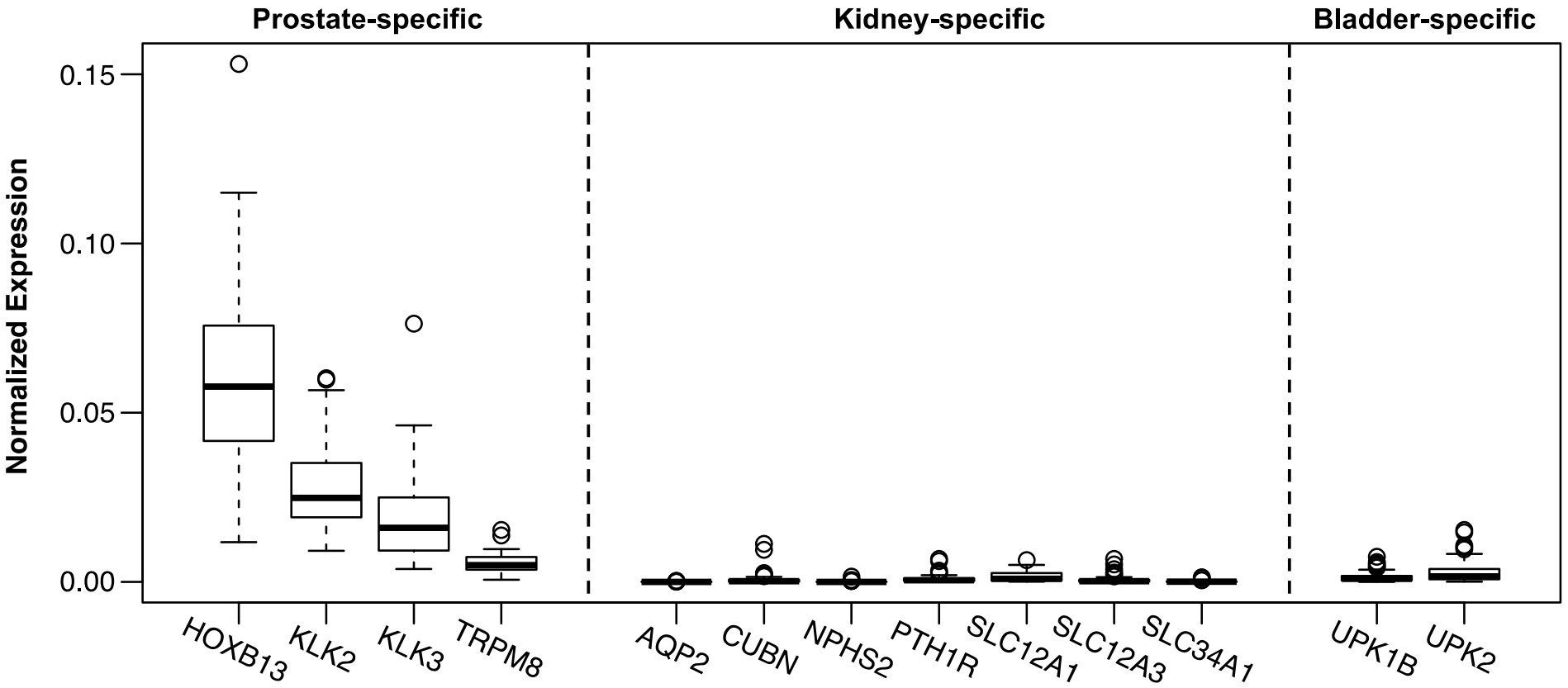
Urine Vesicles Enriched for Prostate RNAs

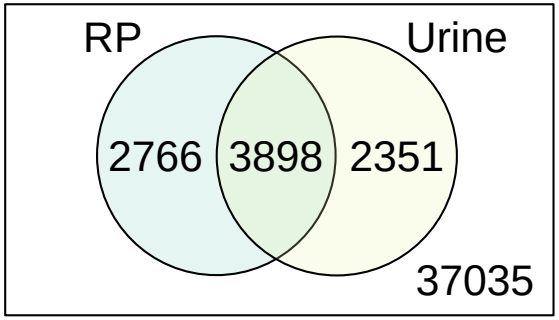
Prostate-specific transcripts were detectable in post-DRE urine at higher levels than kidney/bladder-specific genes



Transcripts Detected in Both RP and Urine

- Pilot evaluation of matched tumor (RP) and post-DRE urine EV specimens were collected from 15 patients
  - Gene expression assessed by whole transcriptome microarray (Affymmetrix)
- Initial (Pilot) Analysis:
  - Comparison of transcript detection in tumor and urine specimens
  - Correlation of RP and urine transcript levels

Genes detected for all 15 patients



- Over 9000 genes detected in RP tissue or post-DRE urine EV
  - 3898 transcripts were in BOTH prostate tissue AND Evs (i.e. target biomarkers)
- Ongoing:
  - Case-control study to train model (N=303); prevalidation in CVC Biopsy cohort

Aim 3: Detection of Prostate Cancer Micrometastatic Disease by FACBC-PET

D Schuster, M Alemozaffar, et al in press

- Patients with high grade prostate cancer, no metastases on standard imaging (CT or MRI, bone scan) enrolled
- On-study Flucyclovine (Axumin) PET (has current FDA approval for recurrence, NOT initial diagnosis) then prostatectomy with extended lymphadenectomy (pelvic+lower retroperitoneal)
- Endpoint: Flucyclovine PET Accuracy in detecting occult metastases
- Specificity of regional mestastases noted on Axumin-PET = 99%

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Prostate Ca Detection:
Biomarkers From Discovery to Clinical Assay by the EDRN

(32 publications from Emory CVC from 2015-2020)

Biomarker	EDRN Role	Type of EDRN Site (Location)	Outcome/ Status
Prostate Health Index (*phi)*	Validation	BRL (Hopkins) CVC (Cornell, Harvard, Hopkins, UTHSC-SA)	FDA-approved
TMPRSS2:Erg Fusion (T2:Erg)	Discovery, Validation	BDL (Michigan) CVC (Harvard-Cornell)	CLIA/ Commercially Deployed
Urine PCA3, T2:Erg (eg MIPs)	Validation	DMCC (FHCC), BRL (Hopkins) CVC's (Harvard, UTHSCSA) Trial Consortium (10 sites)	FDA-approved (PCA3); CLIA (T2:Erg)
Tissue/Urine RNA-Seq	Discovery to Clin Assay	BDL (Michigan)	CLIA
Urine Transcriptome	Discovery to Clin Assay	CVC (Emory-Harvard-Univ Washington)	CLIA

Ongoing Team Science Biomarker Development:

EDRN Team Participants: BDL, CVC, BRL, DMCC

Multi-site Trial	EDRN Sites	Endpoint	Biomarkers
Upgrading Study *Thompson, Leach, Sanda, et al*	DMCC; CVC (Emory, UTHSC-SA); Multisite Trial Group; BDL's (CPDR, EVMS, Hopkins Michigan); BRL's (Hopkins, PNNL)	Outperform biopsy in discerning aggressive vs indolent cancer (Upgrading at Prostatectomy)	Serum: *phi,* Tissue: RNA, RNA-Seq Urine: PCA3, T2:Erg, Targeted RNA-Seq, Transcriptome, Proteoglycomics
Combining MRI+ Serum and Urine Biomarkers *Wei et al*	DMCC; New Multi-center Imaging Group, BDL's, BRL, CVC's	Aggressive Prostate cancer at initial biopsy	Serum: *phi,* Urine: PCA3, T2:Erg, Transcriptome, Targeted RNA-Seq, Proteoglycomics

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