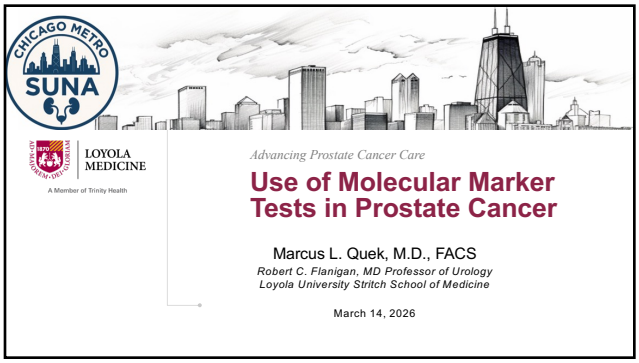
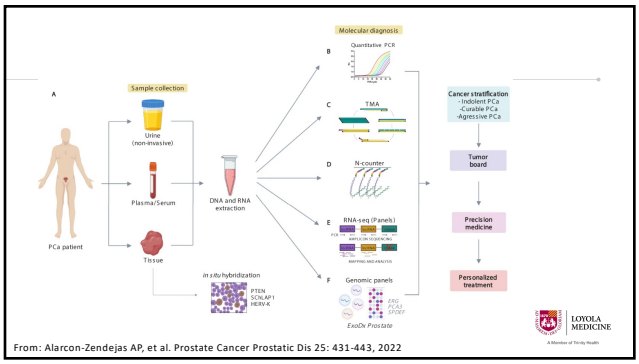


1

Learning Objectives

- Understand diagnostic, prognostic, germline, and somatic molecular tests
- Know when each test is used
- Recognize how results influence treatment decisions from biopsy to advanced disease therapy

2



From: Alarcon-Zendejas AP, et al. Prostate Cancer Prostatic Dis 25: 431-443, 2022

3

Why Molecular Testing Matters

- Prostate cancer is a heterogeneous disease (indolent → aggressive)
- Traditional tools (PSA, DRE, MRI, biopsy) may not tell the whole story
- Molecular tests help answer:
  - *Do we need a biopsy? [identify clinically-significant cancer]*
  - *Is active surveillance safe? [refine risk]*
  - *Which treatments will work best? [personalized care]*

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DIAGNOSTIC / PRE-BIOPSY TESTS
Pre-Biopsy Tests: Overview

- Traditional tests: PSA, PSA (total/%free), PSA density, PSA velocity
- Used to estimate risk of clinically-significant cancer before biopsy
- Avoid unnecessary biopsies

Common tests:
- PCA3 (urine)
- ExoDx / SelectMDx (urine mRNA)
- PHI (blood)
- 4Kscore (blood)

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Pre-Biopsy Tests [URINE]
• PCA3 (Prostate Cancer Antigen 3)

- BIOLOGY
  - PCA3 is a **long non-coding RNA (lncRNA) massively overexpressed (100x) in prostate cancer cells**
  - It is not expressed in benign prostate tissue or other organs
  - Released into urine after prostate massage because cancer cells shed RNA-containing exosomes
- ASSAY SCIENCE
  - Uses transcription-mediated amplification (TMA) to quantify PCA3 RNA
  - Reported as a PCA3 score = PCA3 RNA / PSA RNA x 1000
- CLINICAL MEANING
  - High PCA3 = higher likelihood of Gleason 7 or higher cancer on biopsy
  - Best validated for repeat biopsy decisions (prior negative biopsy)
  - **Urine-based test after DRE** (prostatic massage)

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Pre-Biopsy Tests [URINE]
- **PCA3 (Prostate Cancer Antigen 3)**

Current indication for use: prior negative prostate biopsy, persistently elevated PSA level – *should I do another prostate biopsy?*

PCA3 score from 0 – 100:
< 25 = repeat biopsy is unnecessary and prostate cancer is highly unlikely

>25 = repeat biopsy may be necessary and there's a probability of clinically significant prostate cancer

Helps to avoid unnecessary repeat prostate biopsies.

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Pre-Biopsy Tests [URINE]
ExoDx Prostate

- BIOLOGY
  - Measures **exosomal RNA shed by prostate cancer cells into urine**
  - Targets three mRNA transcripts:
    - **ERG** (ETS-related gene fusion product)
    - **PCA3**
    - **SPDEF** (prostate epithelial differentiation factor)
- ASSAY SCIENCE
  - Uses exosome isolation + RT-PCR quantification
  - Exosomes protect RNA from degradation, giving a stable signal
- CLINICAL MEANING
  - Predicts probability of Gleason GG2 or higher cancer
  - **No DRE required** – relies on spontaneous exosome shedding

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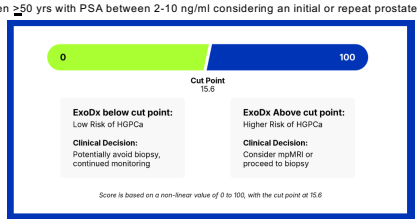
Pre-Biopsy Tests [URINE]
ExoDx Prostate

Non-invasive urine collection (no DRE), any time of day
At-Home Collection Kit (ordered by physician and sent directly to the patient's home)
For men ≥50 yrs with PSA between 2-10 ng/ml considering an initial or repeat prostate biopsy



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Pre-Biopsy Tests [URINE]
SelectMDx

- BIOLOGY
  - Measures mRNA expression of:
    - HOXC6
    - DLX1
  - These genes regulate cell differentiation and embryonic development, reactivated in aggressive prostate cancer
- ASSAY SCIENCE
  - RT-PCR quantification of mRNA from **first-catch urine (no DRE)**
  - Combined with clinical variables (age, PSA, DRE)
- CLINICAL MEANING
  - High score = higher likelihood of clinically-significant cancer
  - Helps avoid unnecessary biopsies

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Pre-Biopsy Tests [BLOOD]
PHI (Prostate Health Index)

- BIOLOGY
  - Uses PSA isoforms **in blood**:
    - Total PSA
    - Free PSA
    - [-2]proPSA (precursor form enriched in cancer tissue)
- ASSAY SCIENCE
  - Formula: PHI = ([-2]proPSA / free PSA) x √(total PSA)
  - Reflects tumor-associated PSA processing abnormalities
- CLINICAL MEANING
  - Higher PHI correlates with higher risk of Gleason 7 or higher cancer

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Pre-Biopsy Tests [BLOOD]
4Kscore

- BIOLOGY
  - Measures four kallikrein proteins **in blood**:
    - Total PSA
    - Free PSA
    - Intact PSA
    - Human kallikrein-2 (hK2), a serine protease overexpressed in prostate cancer
- ASSAY SCIENCE
  - Machine-learning model combining kallikrein levels + age + DRE + biopsy history
- CLINICAL MEANING
  - Predicts risk of high grade cancer and metastasis within 20 years

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Prognostic Genomic Tests (Genomic Classifiers)
Tissue-Based (biopsy or prostatectomy)

Why genomic classifiers?

They answer: "How aggressive is this cancer really?"

Used in low- and intermediate-risk disease to guide:
- Active surveillance
- Need for additional therapies (intensification, multi-modal treatments)
- Adjuvant therapies

These tests analyze tumor gene expression to help predict long-term outcomes.

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Prognostic Genomic Classifiers
Decipher Prostate

- BIOLOGY
  - **Analyzes 22 genes** involved in:
    - Cell cycle progression, androgen signaling, immune modulation, metastatic potential, neuroendocrine differentiation
    - Examples: MYBL2, S1PR4, UBE2C, PBX1, NFIB
- ASSAY SCIENCE
  - Whole-transcriptome microarray → machine-learning classifier → **0-1 score**
  - Validated in >50,000 patients
- CLINICAL MEANING
  - **Predicts metastasis, biochemical recurrence, and prostate cancer-specific mortality**
  - Guides: active surveillance, adjuvant vs salvage radiation, radiation intensification

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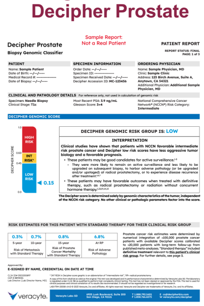
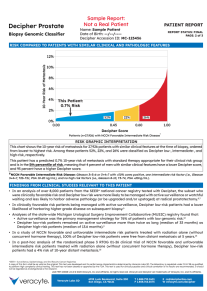
Prognostic Genomic Classifiers
Decipher Prostate


Decipher Prostate
Biopsy Genomic Classifier
Sample Report: Not a Real Patient
PATIENT REPORT


Decipher Prostate
Biopsy Genomic Classifier
Sample Report: Not a Real Patient
PATIENT REPORT

- Genomic Risk Score (0 – 1.0) – based on tumor biology alone, independent of clinical and pathological factors
- Genomic Risk Classification (low/ intermediate/ high)
- Interpretation
- Risk Estimates
- Risk Comparisons (to others with same NCCN risk category)
- Percentile rank
- Clinical studies relevant to patient

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Prognostic Genomic Classifiers
Oncotype DX GPS (Genomic Prostate Score)

- BIOLOGY
  - **17-gene RT-PCR panel** covering
    - Stromal response, cell organization, proliferation, androgen signaling
    - Key genes: ASGP1, GSTM2, TPX2, FAM13C
- ASSAY SCIENCE
  - Quantitative RT-PCR on biopsy tissue
  - Generated a **0-100 score**
- CLINICAL MEANING
  - Predicts:
    - **Adverse pathology at prostatectomy**
    - Biochemical recurrence and metastatic risk
  - Helps determine suitability for active surveillance

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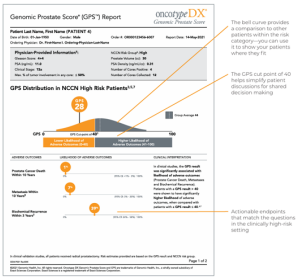
Prognostic Genomic Classifiers
Oncotype DX GPS (Genomic Prostate Score)


Genomic Prostate Score® (GPS™) Report

oncotype DX Genomic Prostate Score

GPS Distribution in NCCN High Risk Patients[1,2,3]

The best curve provides a comparison to other patients within the risk category—you can use it to show your patients where they fit

The CPS cut point of 40 helps simplify patient discussions for shared decision making

Actionable endpoints that match the questions in the clinically high-risk setting

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Prognostic Genomic Classifiers
Prolaris (Cell Cycle Progression Score)

- BIOLOGY
  - Measures expression of 31 CCP genes controlling:
    - G1/S transition, DNA replication, mitotic entry
  - Examples: CDC20, CDKN3, BUB1B
- ASSAY SCIENCE
  - RT-PCR quantification → CCP score → integrated with CAPRA clinical score
- CLINICAL MEANING
  - Predicts disease-specific mortality
  - Helps decide between active surveillance vs definitive therapy

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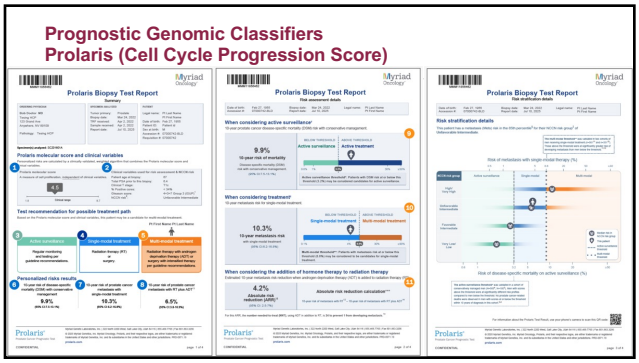
Prognostic Genomic Classifiers
Prolaris (Cell Cycle Progression Score)



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Prognostic Marker – Pathology Images
ArteraAI – AI-based Multimodal Biomarker

- BIOLOGY
  - Instead of measuring genes, ArteraAI analyzes digital **histopathology images (H&E slides)**
    - Tumor architecture
    - Nuclear morphology
    - Glandular patterns
    - Stromal interactions
    - Spatial relationships between cancer cells
  - These features correlate with:
    - Radiosensitivity
    - Hormone therapy responsiveness
    - Metastatic potential

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Prognostic Marker – Pathology Images
ArteraAI – AI-based Multimodal Biomarker

- ASSAY SCIENCE
  - Deep convolutional neural networks (CNNs) trained on >5,000 patients
  - Integrates:
    - Pathology images
    - Clinical variables (PSA, Gleason, stage)
  - Produces:
    - **ADT Benefit Score**
    - **Radiation Intensification Score**
- CLINICAL MEANING
  - Identifies patients who benefit from ADT + radiation
  - Identifies patients who do NOT need ADT, sparing toxicity
  - Predicts metastasis risk
  - FDA-cleared for **radiation decision support**

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GERMLINE GENETIC TESTING (Inherited Mutations)
Germline DNA Repair Genes

HRR Pathway Genes:
- BRCA1/2
- ATM
- CHEK2
- PALB2

BIOLOGY
- Maintain high-fidelity DNA repair
- Loss → genomic instability → aggressive cancer

CLINICAL USE
- PARP inhibitor
- Family testing

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GERMLINE GENETIC TESTING (Inherited Mutations)
Mismatch Repair Deficiency (MSI-High)

GENES:
- MLH1
- MSH2
- MSH6
- PMS2

BIOLOGY:
- Loss → microsatellite instability → hypermutated tumors
- High neoantigen load → immunotherapy sensitivity

CLINICAL USE:
- *Predicts response to checkpoint inhibitors (pembrolizumab)*
- *Suggests Lynch Syndrome*

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SOMATIC TUMOR TESTING (NGS)
Somatic Mutations with Therapeutic Impact

Important when managing advanced stage metastatic hormone-refractory disease

HRR defects (BRCA1/2, ATM): PARP inhibitor sensitivity
CDK12 loss: Immune-responsive phenotype
PTEN loss: PI3K/AKT pathway activation
TP53/RB1 loss: neuroendocrine differentiation
AR-V7: resistance to AR-targeted therapy

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SOMATIC TUMOR TESTING (NGS)
AR-V7: A Splice Variant Driving Resistance

BIOLOGY:

- *Androgen receptor lacks ligand-binding domain*
- *Constitutively active*
- *Drives growth without androgens*

CLINICAL USE:

- *Predicts resistance to enzalutamide/ abiraterone*
- *May be better treated with chemotherapy*

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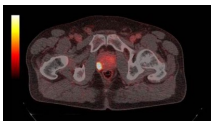
DIAGNOSTIC MOLECULAR IMAGING – PSMA PET-CT

- PSMA = transmembrane glycoprotein overexpressed in prostate cancer
- Radiotracers bind extracellular domain
- PET detects gamma radiation emissions from tracer decay
- High tumor-to-background contrast



- Clinical use:
  - Staging for high risk and unfavorable intermediate risk disease
  - Detects recurrences at PSA <0.5
  - Guides metastasis-directed therapy
  - Identifies candidate for PSMA radioligand therapy (Pluvicto)

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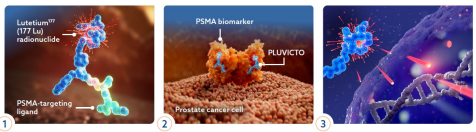
MOLECULAR THERAPEUTICS – Radioligand Therapy

- Targeted radiation delivered directly to cancer cells (hopefully sparing normal tissue)
- Uses a ligand that binds a tumor-specific surface protein
- In prostate cancer, the target is PSMA
- "Smart Bomb": Radioactive payload → internalized → DNA damage → cell death



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MOLECULAR THERAPEUTICS – Why PSMA is the Ideal Target

- PSMA is highly overexpressed in:
  - Metastatic disease
  - Castrate-resistant disease
  - High grade tumors
- Low expression in normal tissues
- Internalizes rapidly after ligand binding

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MOLECULAR THERAPEUTICS
PSMA-Directed Radioligand Therapy

Mechanism of Action:

1. Ligand binds PSMA on cancer cell surface
2. Ligand-PSMA complex internalizes
3. Radioisotope emits radiation inside the cell
4. Causes double-strand DNA breaks
5. Triggers apoptosis and cell death
6. "Bystander effect" damages nearby cancer cells

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MOLECULAR THERAPEUTICS
PSMA-Directed Radioligand Therapy

Radioisotopes Used:

ISOTOPE	PARTICLE	RANGE	IDEAL USE	NOTES
Lutetium-177 (Lu-177)	Beta-emitter	1-2 mm	Bulky disease	FDA-approved
Actinium-225 (Ac-225)	Alpha-emitter	<100 microns	Micrometastatic disease	Investigational

Beta particles travel farther and are "gentler"

Alpha particles are extremely potent, but have a short range

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MOLECULAR THERAPEUTICS
PSMA-Directed Radioligand Therapy

- FDA-approved therapy: Lu-177 PSMA-617 (Pluvicto)
  - *Approved for mCRPC after ARPI and taxane-based chemotherapy*
  - *Requires PSMA-positive disease on PSMA PET-CT*
    - *PSMA-negative lesions won't respond*
  - *Given every 6 weeks x 6 cycles*
  - *Outpatient infusion (Nuclear Medicine Dept)*

- Predictors of Response:
  - *High PSMA uptake*
  - *Homogeneous PSMA expression*
  - *HRR mutations (BRCA2) – may increase radiosensitivity*
  - *Neuroendocrine differentiation – low PSMA, poor candidates*

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MOLECULAR THERAPEUTICS
PSMA-Directed Radioligand Therapy

- Expected clinical benefits
  - *PSA decline in ~60-70% of patients*
  - *Radiographic response*
  - *Delay in disease progression*
  - *Improved overall survival*
  - *Symptom relief, especially bone pain*

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MOLECULAR THERAPEUTICS
PSMA-Directed Radioligand Therapy

Common Side Effects:
- Fatigue
- Nausea
- Xerostomia (dry mouth)
- Mild bone marrow suppression
- Transient pain flare
- Rare renal toxicity

Precautions:
- Encourage hydration for 48 hrs
- Avoid close contact with children or pregnant individuals
- Flush toilet twice
- Good hand hygeine

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Explaining Tests to Patients

- Diagnostic tests → "Do we need a biopsy?"
- Genomic classifiers → "How aggressive is this tumor?"
  - ArteraAI --? "Do you need hormone therapy with radiation?"
- Germline tests → "Was this inherited? " "Should I get my family tested?"
- Somatic tests → "What treatments might work now?"

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KEY TAKEAWAY POINTS

- Molecular biomarker tests reveal tumor biology beyond PSA and Gleason grading
- Pre-biopsy diagnostic tests may help reduce unnecessary biopsies
- Genomic classifiers guide surveillance and treatment intensity
- Germline and somatic mutation testing is important in advanced stage disease to help guide targeted therapy
- PSMA is not just used for diagnostic PET imaging, but can also be utilized in the treatment of advanced stage disease

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Thank you!

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