Molecular Advances in Prostate Cancer: Beyond PSA

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Departments of Pathology, Urology and Oncology
Johns Hopkins University
Overview

• Molecular Aspects of Prostate Cancer Oncogenesis

• Molecular Biomarkers for Prognosis and Early Detection:
  • TMPRSS2-ERG
  • PTEN
  • PCA3

• Genomic Studies
## Estimated New Cases*

<table>
<thead>
<tr>
<th></th>
<th>Males</th>
<th>Females</th>
</tr>
</thead>
<tbody>
<tr>
<td>Prostate</td>
<td>238,590</td>
<td>Breast</td>
</tr>
<tr>
<td>Lung &amp; bronchus</td>
<td>118,080</td>
<td>Lung &amp; bronchus</td>
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<tr>
<td>Colorectum</td>
<td>73,680</td>
<td>Colorectum</td>
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<tr>
<td>Urinary bladder</td>
<td>54,610</td>
<td>Uterine corpus</td>
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<tr>
<td>Melanoma of the skin</td>
<td>45,060</td>
<td>Thyroid</td>
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<tr>
<td>Kidney &amp; renal pelvis</td>
<td>40,430</td>
<td>Non-Hodgkin lymphoma</td>
</tr>
<tr>
<td>Non-Hodgkin lymphoma</td>
<td>37,600</td>
<td>Melanoma of the skin</td>
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<tr>
<td>Oral cavity &amp; pharynx</td>
<td>29,620</td>
<td>Kidney &amp; renal pelvis</td>
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<tr>
<td>Leukemia</td>
<td>27,880</td>
<td>Pancreas</td>
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<tr>
<td>Pancreas</td>
<td>22,740</td>
<td>Ovary</td>
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<tr>
<td>All Sites</td>
<td>854,790</td>
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## Estimated Deaths

<table>
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<tr>
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<th>Males</th>
<th>Females</th>
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<tr>
<td>Lung &amp; bronchus</td>
<td>87,260</td>
<td>Lung &amp; bronchus</td>
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<tr>
<td>Prostate</td>
<td>29,720</td>
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<tr>
<td>Colorectum</td>
<td>26,300</td>
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<tr>
<td>Pancreas</td>
<td>19,480</td>
<td>Pancreas</td>
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<tr>
<td>Liver &amp; intrahepatic bile duct</td>
<td>14,890</td>
<td>Ovary</td>
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<tr>
<td>Leukemia</td>
<td>13,660</td>
<td>Leukemia</td>
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<tr>
<td>Esophagus</td>
<td>12,220</td>
<td>Non-Hodgkin lymphoma</td>
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<td>Urinary bladder</td>
<td>10,820</td>
<td>Uterine corpus</td>
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<tr>
<td>Non-Hodgkin lymphoma</td>
<td>10,590</td>
<td>Liver &amp; intrahepatic bile duct</td>
</tr>
<tr>
<td>Kidney &amp; renal pelvis</td>
<td>8,780</td>
<td>Brain &amp; other nervous system</td>
</tr>
<tr>
<td>All Sites</td>
<td>306,920</td>
<td>All Sites</td>
</tr>
</tbody>
</table>

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**Cancer Statistics, 2013**

Rebecca Siegel, MPH\(^1\); Deepa Naishadham, MA, MS\(^2\); Ahmedin Jemal, DVM, PhD\(^3\)
Overtreatment ????
Prostate Carcinoma
Risk Factors/Etiology

Inherited Germline Genetic Alterations

Environmental Life style Factors

Somatic Genetic & Epigenetic Alterations

PCa
Genetic Rearrangements

**TMPRSS2-ETS:**
- TMPRSS2-ERG
- TMPRSS2-ETV1
- TMPRSS2-ETV2
- TMPRSS2-ETV3
- TMPRSS2-ETV4

**Other ETS Fusion Partners**
- CADM2
- MAGI2

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**Promoter hypermethylation resulting in gene silencing**
- **GSTP1** Carcinogen detoxification

**Loss of heterozygosity and point mutation**
- **PTEN** Cell survival and proliferation
- **TP53 (also P53)** Cell survival and proliferation, genome stability

**Loss of heterozygosity and haploinsufficiency**
- **NKX3-1** Cell differentiation and proliferation
- **CDKN1B (P27KIP1)** Cell proliferation

**Point mutations**
- **COPEB (also KLR6)** Transcription regulator
- **AR** Cell proliferation, survival, and differentiation

**Amplification**
- **AR** Cell proliferation, survival, and differentiation

**Overexpressed at mRNA and protein level**
- **HTERT** Cell immortality
- **HPN** Transmembrane protease
- **FASN** Fatty-acid synthesis
- **AMACR** Fatty-acid metabolism, branched chain
- **EZH2** Transcription repressor, cell proliferation
- **MYC** Cell proliferation
- **BCL2** Cell survival

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*Modified from DeMarzo et al Nat Rev Cancer 2007*
Recurrent Fusion of TMPRSS2 and ETS Transcription Factor Genes in Prostate Cancer

Scott A. Tomlins,¹ Daniel R. Rhodes,¹,² Sven Perner,⁷,⁹
Saravana M. Dhanasekaran,¹ Rohit Mehra,¹ Xiao-Wei Sun,⁷
Sooryanarayana Varambally,¹,⁶ Xuhong Cao,⁷ Joelle Tchinda,⁷
Rainer Kuefer,¹⁰ Charles Lee,⁷ James E. Montie,³,⁵,⁶
Rajal B. Shah,¹,³,⁵,⁶ Kenneth J. Pienta,³,⁴,⁵,⁶ Mark A. Rubin,⁷,⁸
Arul M. Chinnaiyan¹,²,³,⁵,⁶*
**TMPRSS2** (Transmembrane Protease, Serine 2)

- Prostate specific **serine protease** family (PSA, hK2, prostate/PRSS18, TMPRSS2)
- **TMPRSS2** gene is located on chromosome 21q22.3, it encodes a protein of 492 amino acids
- **TMPRSS2** expression is **regulated by androgen**
- **TMPRSS2** is highly expressed in normal and neoplastic prostate epithelium.

**ERG** (ETS Related Gene)

- ERG gene is located on Chr 21q22.2
- Member of ETS transcription factor family
  - ERG (ets-related gene) 21q22.2
  - ETV1 7p21.2
  - ETV4 17q21
- Most consistently overexpressed oncogene in malignant epithelial cells of the prostate *Petrovics et al. Oncogene. 2005*
Fusion of TMPRSS2 and ETS transcription factor genes in PCa

- Cancer outlier profile analysis (COPA) identified strong outlier profiles for ERG (21q22.2) and ETV1 (7p21.2) in prostate cancer but not in the adjacent benign epithelia.

- QPCR showed ERG overexpression in PCa cell lines (VCaP, DuCaP), patient LN metastasis (MET-28LN).

- RLM-RACE analysis and sequencing of the RT-PCR product confirmed a fusion of the complete exon 1 of TMPRSS2 with exon 4 of ERG (TMPRSS2:ERG a) or with exon 2 (TMPRSS2:ERG b).

- FISH 23/29 cases had fusions:
  - 16 ERG (55%)
  - 7 ETV1 (24%)

*Tomlins et al, SCIENCE 2005*
Split of one ERG (split red green) allele in PIN and adjacent PCAs.
ERG allele with Deletion of 5' (green)
TMRSS2-ERG Fusion & Prognosis

• FISH: fusion in 49% of 118 localized and 41% of 18 LN Mets
• Significant correlation between rearrangement through **deletion** and **stage** and presence of mets

Attard et al Oncogene 2007
• FISH, 445 cases
• Tumors without fusion had favorable PGx (90% @ 8yr)
• Tumors with fusion by **deletion** but not by **translocation** had worse OS and DSS
• Tumors with **duplication of “fusion by deletion”** had extremely poor PGx (25%@ 8 yr) independent of Gleason score
TMPRSS2-ERG Gene Fusion Is Not Associated with Outcome in Patients Treated by Prostatectomy


• 521 RRP and 40 mets
• FISH, median FU 95 months
• TMPRSS2-ERG (42%) alone associated with lower grade but not with BCR, pTNM, mets or death.
• Chrom 21 CNI (11%) associated with higher grade and stage.
• Chrom 21 CNI With E2del are more aggressive and are a reflection of generalized aneuploidy

<table>
<thead>
<tr>
<th>End points</th>
<th>Status level</th>
<th>CNID ($n=10$)</th>
<th>Other CNI + rearrangement ($n=22$)</th>
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<td>Overall survival</td>
<td>Alive</td>
<td>7</td>
<td>19</td>
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<tr>
<td></td>
<td>Dead</td>
<td>3</td>
<td>3</td>
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<tr>
<td>BCR</td>
<td>No and Alive</td>
<td>3</td>
<td>12</td>
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<tr>
<td></td>
<td>Yes</td>
<td>7</td>
<td>9</td>
</tr>
<tr>
<td></td>
<td>Dead before BCR</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Metastases</td>
<td>No and alive</td>
<td>6</td>
<td>17</td>
</tr>
<tr>
<td></td>
<td>Yes</td>
<td>4</td>
<td>4</td>
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<tr>
<td></td>
<td>Dead before metastases</td>
<td>0</td>
<td>1</td>
</tr>
</tbody>
</table>
Increased gene copy number of ERG on chromosome 21 but not TMPRSS2–ERG fusion predicts outcome in prostatic adenocarcinomas

Antoun Toubaji¹,*, Roula Albadine¹,*, Alan K Meeker¹,², William B Isaacs²,³, Tamara Lotan¹, Michael C Haffner¹,³, Alcides Chaux¹, Jonathan I Epstein¹,²,³, Misop Han², Patrick C Walsh²,³, Alan W Partin²,³, Angelo M De Marzo¹,²,³, Elizabeth A Platz²,³,⁴ and George J Netto¹,²,³

A Toubaji et al. Modern Pathology 2011
A Panel of *TMPRSS2:ERG* Fusion Transcript Markers for Urine-Based Prostate Cancer Detection with High Specificity and Sensitivity

Phuong-Nam Nguyen, Philippe Violette, Sam Chan, Simon Tanguay, Wassim Kassouf, Armen Aprikian, Junjian Z. Chen

*European Urology 59 (2011) 407-414*

Evaluation of the ETS-Related Gene mRNA in Urine for the Detection of Prostate Cancer


*Clin Cancer Res; 16(5) March 1, 2010*
Urine sediments from 55 pts with and without prior prostatic massage

Real-time RT PCR for TMPRSS2-ERG

69% post massage urine were positive for isoforms a or b, five were positive for both

24% positivity in samples without prior massage

Isoform “a” most prevalent; some pts may be positive for more than one fusion variant reflecting multifocality

TMPRSS2:ERG fusion correlated with high sPSA, pathological stage and Gleason score

Rostad K et al. APMIS 2009
mTOR Pathway

Amino acids → Energy (ATP) → Growth factors → PI3K → PTEN → T308 Akt → S473

STK11 AMPK → TSC1/2

FKBP8 GTP → Rheb GDP → Rheb

mTOR Raptor GBL → mTORC1

mTORC1 inhibitors → Sin1 mTOR Rictor GBL → mTORC2

PRAS40 4E-BP1 S6K1

mRNA translation, cellular growth, proliferation

Cell survival

Dancey J, Nat Rev Clin Ocol 2010
PTEN loss occurs in 20% of localized and over 60% of metastatic PCa and has been associated with high Gleason Sc and pT stage, BCR and castration resistance/chemoresistance.

Saal et al. PNAS 2007:
Gene expression signature of aberrant PTEN activity is associated with poor prognosis in PCa.
Aberrant ERG expression cooperates with loss of PTEN to promote cancer progression in the prostate

Brett S Carver¹,², Jennifer Tran¹, Anuradha Gopalan³, Zhenbang Chen¹,⁴, Safa Shaikh², Arkaitz Carracedo¹,⁴, Andrea Alimonti¹,⁴, Caterina Nardella¹,⁴, Shohreh Varmeh¹,⁴, Peter T Scardino², Carlos Cordon-Cardo⁵, William Gerald³ & Pier Paolo Pandolfi¹,³,⁴

Cooperativity of TMPRSS2-ERG with PI3-kinase pathway activation in prostate oncogenesis

Jennifer C King¹, Jin Xu¹, John Wongvipat¹, Haley Hieronymus¹, Brett S Carver¹, David H Leung¹, Barry S Taylor²,³, Chris Sander², Robert D Cardiff⁴, Suzana S Couto⁵, William L Gerald¹ & Charles L Sawyers¹,⁶
TMPRSS2-ERG and PTEN loss in prostate cancer

Jeremy A Squire

Diagram: Unregulated activation of AKT and downstream targets leads to overexpression of ETS targets, resulting in proliferation, anti-apoptosis, genomic instability, differentiation, angiogenesis, cell migration, and invasion, ultimately leading to normal prostate, PIN, and prostate carcinoma.
Loss of PTEN expression is associated with increased risk of recurrence after prostatectomy for clinically localized prostate cancer

Alcides Chaux¹,², Sarah B Peskoe³, Nilda Gonzalez-Roibon¹, Luciana Schultz¹, Roula Albadine¹, Jessica Hicks¹, Angelo M De Marzo¹,⁴,⁵, Elizabeth A Platz³,⁴,⁵ and George J Netto¹,²,⁴,⁵

- 541 cases / 541 controls
- IHC nested case control study
- PTEN loss predicted recurrence (OR:2.2; p0.002)
### Results

<table>
<thead>
<tr>
<th>PTEN Expression</th>
<th>Adjusted OR (95% CI)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean H-score &lt; 10</td>
<td>2.20 (1.33, 3.63)</td>
<td>.002</td>
</tr>
<tr>
<td>All TMA spots markedly decreased</td>
<td>1.67 (1.09, 2.57)</td>
<td>.02</td>
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330 RRP by High resolution 4 colors FISH

40% interstitial deletion rates

Presence of PTEN deletion by FISH is associated with earlier biochemical recurrence and increased mortality

Tumors with a homozygous deletion had strong association with hormone refractory and metastatic disease
PTEN Protein Loss by Immunostaining: Analytic Validation and Prognostic Indicator for a High Risk Surgical Cohort of Prostate Cancer Patients

Tamara L. Lotan¹, Bora Gurel¹, Siobhan Sutcliffe⁴, David Esopi², Wennuan Liu⁵, Jianfeng Xu⁵, Jessica L. Hicks¹, Ben H. Park², Elizabeth Humphreys³, Alan W. Partin³, Misop Han³, George J. Netto¹,²,³, William B. Isaacs²,³, and Angelo M. De Marzo¹,²,³
Beyond Serum PSA

Need for Better Early Detection & Management Decision Guidance Markers
Beyond Serum PSA

Need for Better Early Detection & Management Decision Guidance Markers

• Elevated serum PSA levels ~80% sensitive

• Prevalence of Cancer in the Prostate Cancer Prevention Trial (PCPT) by sPSA:
  • <0.5 ng/mL 6.6%
  • 0.6-1.0 ng/mL 10.1%
  • 1.1- 2.0 ng/mL 17.0%
  • 2.1 to 3.0 ng/mL 23.9%
  • 3.1 to 4.0 ng/mL 26.9%

• Serum PSA is of limited ~25-40% specificity for cancer detection on needle biopsy:
  NOT DIFFERENTIALLY EXPRESSED in PCA vs benign prostate tissue (1.5 fold less in PCa)
Elevated in BPH, Prostatitis etc…
  Unnecessary biopsies: morbidity not negligible

• Pressing need for markers that can differentiate aggressive from indolent disease:
  RRP morbidity
DD3: A New Prostate-specific Gene, Highly Overexpressed in Prostate Cancer

Marion J. G. Bussemakers, Adrie van Bokhoven, Gerald W. Verhaegh, Frank P. Smit, Herbert F. M. Karthaus, Jack A. Schalken, Frans M. J. Debruyne, Ning Ru, and William B. Isaacs

**DD3 (Differentially Displayed Antigen 3):**
- Discovered in 1999 using differential display analysis
PCA3

- Gene located on chromosome 9q21–22
- Originally found to contain four exons; alternative polyadenylation at 3 positions in exon 4
Schalken, J. A. et al. Urology, 62 (Suppl. 1), 34–43; 2003
### PCA3 Urine Assays & Biopsy Outcome

<table>
<thead>
<tr>
<th>Study</th>
<th>PCA3-based test methodology</th>
<th>Number of patients</th>
<th>AUC</th>
<th>Sensitivity (%)</th>
<th>Specificity (%)</th>
<th>PPV (%)</th>
<th>NPV (%)</th>
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<td>Hessels et al. (2003)</td>
<td>QRT-PCR</td>
<td>108</td>
<td>0.72</td>
<td>67</td>
<td>83</td>
<td>53</td>
<td>90</td>
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<tr>
<td>Van Gils et al. (2007)</td>
<td>QRT-PCR</td>
<td>583</td>
<td>0.66</td>
<td>65</td>
<td>66</td>
<td>48</td>
<td>80</td>
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<tr>
<td>Groskopf et al. (2006)</td>
<td>TMA</td>
<td>70</td>
<td>0.75</td>
<td>69</td>
<td>79</td>
<td>50</td>
<td>89</td>
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<td>Marks et al. (2007)</td>
<td>TMA</td>
<td>233</td>
<td>0.68</td>
<td>58</td>
<td>72</td>
<td>43</td>
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<td>Deras et al. (2008)</td>
<td>TMA</td>
<td>570</td>
<td>0.69</td>
<td>54</td>
<td>74</td>
<td>58</td>
<td>74</td>
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<td>Haese et al. (2008)</td>
<td>TMA</td>
<td>470</td>
<td>0.66</td>
<td>47</td>
<td>72</td>
<td>39</td>
<td>78</td>
</tr>
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</table>


- APTIMA® Assay; post Attentive DRE first catch urine
- Single tube whole urine sample mixed with buffer prior to overnight shipping. No centrifugation nor RNA extraction needed
- Target capture with magnetic beads \(\Rightarrow\) Transcription mediated amplification \(\Rightarrow\) Detection with Chem-illuminescent probes
- Target all PCA3 isoforms (E3/E4a junction)
PCA3
TMA based Urine Assay

- 143 pts; three Groups:
  - Pre-Bx for elevated PSA/abnormal DRE (n = 70)
  - Healthy men (n = 52)
  - Post RRP (n = 21)
- ROC curve analysis showed AUC of 0.746 with sensitivity of 69% and specificity of 79%; negative predictive value was 90%

Deras I et al J urol 2008
Haese A et al Eur Urol 2008

Gen-Probe Inc PROGENSA®
PCA3 Molecular Urine Assay Correlates With Prostate Cancer Tumor Volume: Implication in Selecting Candidates for Active Surveillance

Hiroyuki Nakanishi, Jack Groskopf,* Herbert A. Fritsche,* Viju Bhadkamkar, Amy Blase,* S. Vikas Kumar, John W. Davis,‡ Patricia Troncoso, Harry Rittenhouse* and R. Joseph Babaian‡,§

A

- $p = 0.002$: < 0.5 cc vs. 0.5-2.0 cc
- $p = 0.001$: < 0.5 cc vs. ≥ 2.0 cc
- $p = \text{N.S.}$: 0.5-2.0 cc vs. ≥ 2.0 cc

Total tumor volume in prostatectomy specimens

B

- $p = 0.007$

Low-volume/low-grade cancer

significant cancer

C

- $p = \text{N.S.}$

Biopsy Gleason score

D

- $p = 0.005$

Prostatectomy Gleason score
234 pts pre-Bx or RRP

Post Attentive DRE Urine sediment; real time Q RT-PCR

GOLPH2, SPINK1, and PCA3 and TMPRSS2:ERG fusion significant predictors of Pca

Multiplexed model outperformed sPSA and PCA3 alone
  - AUC 0.758 vs 0.662 for PCA3 alone ($P = 0.003$)
  - Sensitivity 65.9%; Specificity 76.0%
  - PPV 79.8%; NPV 60.8%
A First-Generation Multiplex Biomarker Analysis of Urine for the Early Detection of Prostate Cancer

Bharathi Laxman\textsuperscript{1,2}, David S. Morris\textsuperscript{1,3}, Jianjun Yu\textsuperscript{1,2,4}, Javed Siddiqui\textsuperscript{1,2}, Jie Cao\textsuperscript{1,2}, Rohit Mehra\textsuperscript{1,2,5}, Robert J. Lonigro\textsuperscript{1,5}, Alex Tsodikov\textsuperscript{1,6}, John T. Wei\textsuperscript{1,3,5}, Scott A. Tomlins\textsuperscript{1,2}, and Arul M. Chinnaiyan

<table>
<thead>
<tr>
<th>Variable</th>
<th>Coefficient</th>
<th>$P$</th>
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<tbody>
<tr>
<td>Univariate logistic regression analysis</td>
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<tr>
<td>$GOLPH2$</td>
<td>0.4444</td>
<td>0.0002</td>
</tr>
<tr>
<td>$SPINK1$</td>
<td>0.25</td>
<td>0.0002</td>
</tr>
<tr>
<td>$PCA3$</td>
<td>0.187</td>
<td>0.001</td>
</tr>
<tr>
<td>$TMPRSS2:ERG$</td>
<td>0.609</td>
<td>0.034</td>
</tr>
<tr>
<td>$ERG$</td>
<td>0.043</td>
<td>0.166</td>
</tr>
<tr>
<td>$TFF3$</td>
<td>0.11</td>
<td>0.189</td>
</tr>
<tr>
<td>PSA (serum)</td>
<td>0.0151</td>
<td>0.376</td>
</tr>
<tr>
<td>$AMACR$</td>
<td>0.049</td>
<td>0.45</td>
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<tr>
<td>Multivariate logistic regression analysis</td>
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<tr>
<td>$SPINK1$</td>
<td>0.308</td>
<td>7.41E-05</td>
</tr>
<tr>
<td>$PCA3$</td>
<td>0.191</td>
<td>0.003</td>
</tr>
<tr>
<td>$GOLPH2$</td>
<td>0.372</td>
<td>0.004</td>
</tr>
<tr>
<td>$TMPRSS2:ERG$</td>
<td>0.924</td>
<td>0.006</td>
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</table>

<table>
<thead>
<tr>
<th>Variable</th>
<th>Sens</th>
<th>Spec</th>
<th>PPV</th>
<th>NPV</th>
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<tbody>
<tr>
<td>Multiplex</td>
<td>65.9%</td>
<td>76.0%</td>
<td>79.8%</td>
<td>60.8%</td>
</tr>
<tr>
<td>PCA3</td>
<td>75.4%</td>
<td>56.3%</td>
<td>71.2%</td>
<td>61.4%</td>
</tr>
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PCa
Genomics Data
Molecular classification of prostate cancer using curated expression signatures

Elke K. Markert\textsuperscript{a}, Hideaki Mizuno\textsuperscript{b,c}, Alexei Vazquez\textsuperscript{a,d,e}, and Arnold J. Levine\textsuperscript{a,d,f,1}

Markert et al, PNAS 2011

- Swedish watchful-waiting cohort (281 pt)

- mRNA signatures:
  - ESC
  - iPSC
  - polycomb repressive complex-2 (PRC2)
  - p53 Loss; PTEN loss; MYC; ERG fusion; RAS; Inflammatory
Concordant assessment of DNA copy number, mRNA expression, and focused exon resequencing in 218 PCa.
DNA copy-number alterations robustly define clusters of low-risk and high-risk disease beyond that achieved by Gleason score.
Conclusions:

• Molecular understanding of PCa has brought us within reach of achieving the goal of stratifying management based on biomarkers that should be very soon integrated in the current clinical standards

• Refinement in early detection and screening are promising

• More work remains……..
The Johns Hopkins
Prostate
Cancer Team

The Pathology Team at Hopkins

Jonathan I. Epstein MD
Angelo Demarzo, MDPhD
Tamara Lotan, MD
Alan Meeker, PhD
Karen S. Sfanos, PhD
Michael C. Haffner MD PhD
Helen Fedor
Jessica Hicks
Marcella Southerland
Kristen Lecksell

THANK YOU !
The genomic complexity of primary human prostate cancer

- WGS of 7 PCa
- Complex chains of balanced ‘copy-neutral’ rearrangements
  - **TMPRSS2–ERG**: Rearrangement breakpoints enriched near open chromatin, AR & ERG DNA binding sites
  - link to Chromatin/ transcription regulation
- Rearrangements disrupting CADM2; PTEN or MAGI2

**Berger et al, Nature 2011**
REDUCE Trial

- 1,140 pts; largest Bx cohort evaluating PCA3

- Increased PCA3 indicated increased risk for PCa on Bx; higher Gleason Gr & predicted future Bx outcomes

- Use of PCA3 in combination with sPSA & risk factors significantly increased Dx accuracy (AUC 0.75)

Aubin et al, J Urol 2010
Lapointe et al Cancer Res 2007

- Array CGH: 22000+ genes
- 64 PCa (55 primary and 9 LN mets)
- Same cohort as previous Gene expression array study
- Three subtypes on gene expression analysis have distinct CNA (copy number alteration) changes
- Most frequent aberrations:
  - Gain: 8q (27%)
  - Loss: 13q (52%), 8p (47%), 6q (38%), 10q (28%)
**Inherited Genetic Risk Factors**

<table>
<thead>
<tr>
<th>Gene</th>
<th>Location</th>
</tr>
</thead>
<tbody>
<tr>
<td>RNASEL</td>
<td>1q24–25</td>
</tr>
<tr>
<td>ELAC2</td>
<td>17p11</td>
</tr>
<tr>
<td>MSR1</td>
<td>8p22</td>
</tr>
<tr>
<td>AR</td>
<td>Xq11–12</td>
</tr>
<tr>
<td>CYP17</td>
<td>10q24.3</td>
</tr>
<tr>
<td>SRD5A2</td>
<td>2p23</td>
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</table>

**Androgen Pathway**

- Encodes endoribonuclease that participates in an interferon-inducible 2',5'-oligoadenylate-dependent RNA-decay pathway. 
  *RNaseL−/−* mice have diminished interferon-α antiviral activity.

**Phenotypic Consequences**

- Encodes subunits of class A macrophage-scavenger receptor. 
  *Msr-A−/−* mice have an increased sensitivity to serious infection with *Listeria monocytogenes*, *Staphylococcus aureus*, *Escherichia coli*, and herpes simplex virus type 1.

- Encodes androgen receptor, an androgen-dependent transcription factor. 
  Different polymorphic alleles may be associated with different transcriptional transactivation activities.

- Encodes cytochrome P-450c17α, an enzyme that catalyzes key reactions in sex-steroid biosynthesis.

- Encodes the predominant 5-α-reductase in the prostate, converts testosterone to dihydrotestosterone.

*Nelson et al NEJM 2003*
SHORT COMMUNICATION

Diversity of TMPRSS2-ERG fusion transcripts in the human prostate

J Clark¹, S Merson¹, S Jhavar¹, P Flohr¹, S Edwards¹, CS Foster², R Eeles¹,³, FL Martin⁴, DH Phillips¹, M Crundwell⁵, T Christmas⁶, A Thompson⁶, C Fisher⁶, G Kovacs⁷ and CS Cooper¹

Clark et al. Oncogene 2007

- 14 different combinations
- Different fusions in different tumor nodules in same prostate

Soller MJ. Gen Chrom C. 2006
Distinct classes of chromosomal rearrangements create oncogenic ETS gene fusions in prostate cancer

Scott A. Tomlins, Bharathi Laxman, Saravana M. Dhanasekaran, Beth E. Helgeson, Xuhong Cao, David S. Morris, Anjana Menon, Xiaojun Jing, Qi Cao, Bo Han, Jindan Yu, Lei Wang, James E. Montie, Mark A. Rubin, Kenneth J. Pienta, Diane Roulston, Rajal B. Shah, Sooryanarayana Varambally, Rohit Mehra & Arul M. Chinnaiyan
Somatic Alterations
Genetic and Epigenetic
<table>
<thead>
<tr>
<th><strong>PTEN</strong> genomic loss by SNP array</th>
<th><strong>PTEN</strong> protein by IHC</th>
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<tbody>
<tr>
<td></td>
<td></td>
<td>Present</td>
<td>Absent</td>
</tr>
<tr>
<td>No change</td>
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<td>20</td>
<td>11</td>
</tr>
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<td>Heterozygous</td>
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<td>2</td>
<td>13</td>
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<td>Homozygous</td>
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**NOTE:** $P = 0.00026$ by Fisher's exact test.

<table>
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<tr>
<th><strong>PTEN</strong> genomic loss by FISH</th>
<th><strong>PTEN</strong> protein by IHC</th>
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<tr>
<td></td>
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<td>Present</td>
<td>Absent</td>
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<td>Normal</td>
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<tr>
<td>Deleted</td>
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<td>6</td>
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</table>

**NOTE:** $P = 0.0044$ by Fisher's exact test.
Berger et al, Nature 2011
Berger et al, Nature 2011
Gene expression profiling identifies clinically relevant subtypes of prostate cancer

Jacques Lapointe\textsuperscript{a,b,c}, Chunde Li\textsuperscript{d}, John P. Higgins\textsuperscript{a}, Matt van de Rijn\textsuperscript{a}, Eric Bair\textsuperscript{e}, Kelli Montgomery\textsuperscript{a}, Michelle Ferrari\textsuperscript{f}, Lars Egevad\textsuperscript{d}, Walter Rayford\textsuperscript{f}, Ulf Bergerheim\textsuperscript{g}, Peter Ekman\textsuperscript{d}, Angelo M. DeMarzo\textsuperscript{h}, Robert Tibshirani\textsuperscript{i,j}, David Botstein\textsuperscript{l}, Patrick O. Brown\textsuperscript{b,k}, James D. Brooks\textsuperscript{c,l}, and Jonathan R. Pollack\textsuperscript{a,m}

Lapointe et al PNAS 2004

- 26,000 genes cDNA microarrays
- 62 PCa and 41 Normal, 9 LN mets
- Unsupervised hierarchical clustering separated benign from PCa and Identified three PCa subgroups (2 aggressive subgroups)
• Surrogate markers MUC1 and AZGP1 applied to TMA from 225 additional cases

• Strong predictors of recurrence independent of grade, stage and PSA
Prostate Carcinoma
Environmental Risk Factors/Etiology

Environmental/Lifestyle Factors:

- Geographic variation in PCa Incidence
- Immigrant changing risk (Asian → USA)
- Red meat consumption? PhIP
- Obesity risk?
- Selenium, Soy, Vitamin D, E
- STD’s

DeMarzo et al Nature Reviews 2007
Exome sequencing identifies a spectrum of mutation frequencies in advanced and lethal prostate cancers

Akash Kumar\textsuperscript{a}, Thomas A. White\textsuperscript{b}, Alexandra P. MacKenzie\textsuperscript{a}, Nigel Clegg\textsuperscript{b}, Choli Lee\textsuperscript{a}, Ruth F. Dumpit\textsuperscript{b}, Ilsa Coleman\textsuperscript{b}, Sarah B. Ng\textsuperscript{a}, Stephen J. Salipante\textsuperscript{a}, Mark J. Rieder\textsuperscript{a}, Deborah A. Nickerson\textsuperscript{a}, Eva Corey\textsuperscript{c}, Paul H. Lange\textsuperscript{c}, Colm Morrissey\textsuperscript{c}, Robert L. Vessella\textsuperscript{c}, Peter S. Nelson\textsuperscript{a,b,c,1}, and Jay Shendure\textsuperscript{a,1}

Kumar et al, PNAS 2011

- WES of 23 prostate cancers (lethal metastatic)
- Mice xenografts passage
- \sim200 novel nonsynonymous variants
- Few recurrent alterations: TP53, DLK2, GPC6, and SDF4
- CR model: wnt alterations
Acknowledgement
Study Funding Sources

“Patrick C. Walch”
Prostate Cancer Foundation

“David H. Koch”
Prostate Cancer Foundation

NCI/NIH – JHU “SPORE”

Thank You…..
Gene expression profiling identifies clinically relevant subtypes of prostate cancer

Lapointe et al PNAS 2004
• 26,000 genes cDNA microarrays
• 62 PCa and 41 Normal, 9 LN mets
• Unsupervised hierarchical clustering separated benign from PCa and
  Identified three PCa subgroups (2 aggressive subgroups)

Genomic Profiling Reveals Alternative Genetic Pathways of Prostate Tumorigenesis

Lapointe et al Cancer Res 2007
• Array CGH: 22000+ genes
• Three subtypes on gene expression analysis have distinct CNA (copy number alteration) changes
• Most frequent aberrations:
  - Gain: 8q (27%)
  - Loss: 13q (52%), 8p (47%), 6q (38%), 10q (28%)
PCA3 Molecular Urine Assay Correlates With Prostate Cancer Tumor Volume: Implication in Selecting Candidates for Active Surveillance

Hiroyuki Nakanishi, Jack Groskopf,* Herbert A. Fritsche,* Viju Bhadkamkar, Amy Blase,* S. Vikas Kumar, John W. Davis,† Patricia Troncoso, Harry Rittenhouse* and R. Joseph Babaian‡,§

![Graphs showing sensitivity and 1 - specificity for different variables.](image)

<table>
<thead>
<tr>
<th>Variables</th>
<th>AUC</th>
<th>Asymptotic significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total Tumor Volume &lt; 0.5 cc</td>
<td></td>
<td></td>
</tr>
<tr>
<td>PCA3 score</td>
<td>0.757</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>PSA</td>
<td>0.632</td>
<td>0.051</td>
</tr>
<tr>
<td>% positive cores</td>
<td>0.733</td>
<td>0.001</td>
</tr>
<tr>
<td>Maximum tumor length</td>
<td>0.700</td>
<td>0.003</td>
</tr>
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</table>

<table>
<thead>
<tr>
<th>Variables</th>
<th>AUC</th>
<th>Asymptotic Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dominant Tumor Volume &lt; 0.5 cc</td>
<td></td>
<td></td>
</tr>
<tr>
<td>PCA3 score</td>
<td>0.673</td>
<td>0.006</td>
</tr>
<tr>
<td>PSA</td>
<td>0.812</td>
<td>0.076</td>
</tr>
<tr>
<td>% positive cores</td>
<td>0.682</td>
<td>0.004</td>
</tr>
<tr>
<td>Maximum tumor length</td>
<td>0.723</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

Abbreviations: AUC, area under the curve; PSA, prostate specific antigen.
PCa cells are shed into urethra via prostatic ducts
Yield of routine microscopic urine cytology is very low (not currently pursued)
Fujita K et al; Hum Pathol 2009: Immunofluorescence labeling with prostate specific markers (NKX3.1; AMACR) and nucleolin could enhance specificity of microscopic urine cytology
HGPIN and Intraductal carcinoma arguably can gain access to the urethra
PCA3 assays on urine sample
PCA3: A Molecular Urine Assay for Predicting Prostate Biopsy Outcome

Ina L. Deras,*,† Sheila M. J. Aubin,*,,† Amy Blase,† John R. Day,† Seongjoon Koo,† Alan W. Partin,† William J. Ellis,‡ Leonard S. Marks,§ Yves Fradet,‖ Harry Rittenhouse† and Jack Groskopf†,‖

Logistic Regression Model:
- Log PSA
- Log PCA3
- TRUS Volume
- DRE Results

Deras I et al, J urol 2008
Prostate Cancer Antigen 3 (PCA3)

- Gene structure/biology
- PCA3 molecular assays development and performance characteristics
- PCA3 as a predictor of needle biopsy outcome
- PCA3 as Prognosticator/Guidance of Active Surveillance (AS)
- Synergistic interaction with other molecular markers assays
PCA3

- PCA3 cDNA sequence contains large number of stop codons; noncoding RNA (ncRNA)
- 4 New transcription start sites, 2 differentially spliced exons and 4 additional poly-adenylation sites
- New transcription start sites allow for 4 new putative ORFs (70 to 82 AA)??
PCA3
TMA based Urine Assay
**PCA3 Function?**

- ncRNAs include several types of infrastructural RNA (e.g., tRNAs, rRNAs, spliceosomal uRNAs) and small ncRNA such as miRNAs and siRNAs.

- ncRNA implicated in gene regulation:
  - chromatin structure modification
  - transcriptional/post-transcriptional silencing
  - RNA splicing regulation

*Day J et al Cancer Letters 2011;*
Detection of TMPRSS2-ERG Fusion Transcripts and Prostate Cancer Antigen 3 in Urinary Sediments May Improve Diagnosis of Prostate Cancer

Daphne Hessels,¹ Frank P. Smit,¹ Gerald W. Verhaegh,¹ J. Alfred Witjes,¹ Erik B. Cornel,² and Jack A. Schalken¹

- 78 pts with posBx and 30 negBx
- Post DRE voided urine sediment
- PCA3 by TRF RT-PCR
- TMPRSS2-ERG by semi-quantitative RT-PCR + SB
- TMPRSS2-ERG: sensitivity 37% / Specificity 93%
- Combination improved PCA3 sensitivity (from 62% to 73%)
- TMPRSS2-ERG positivity had a 94% PPV in a subset of persistent elevated sPSA/Bx neg pts suggesting a role in determining need for re-biopsy
PCA3
RT-PCR TRF Urine Assay

Hessels et al. Eur Urol 2003
- Time Resolved Fluorescence (TRF) RT-PCR
- Post Massage urine Sediment in 108 men with elevated serum PSA (>3)
- PCA3 normalized using urine PSA mRNA
- Prediction of outcome on Bx: Sensitivity: 67%; Specificity 83%; Negative predictive value: 90%.

- Findings confirmed in larger multicenter (583 pts) European study by Van Gils Cancer Res 2007
PCA3
NASBA Real Time PCR Urine Assay

Fradet Y et al. Urology 2004
- UPMA® Assay; multicenter Canadian study; 517 pts
  - Post DRE urine sediment in men with elevated serum PSA undergoing needle Bx
  - Isothermal NASBA and qRT-PCR
  - PCA3 normalized to urine PSA mRNA
  - Performance consistent across serum PSA categories; overall accuracy 81% vs 47% for PSA

Tinzl et al Eur Urol 2004:
- 201 pts; Sensitivity: 83%; Specificity 76%
PCA3: A Molecular Urine Assay for Predicting Prostate Biopsy Outcome

Ina L. Deras,*,† Sheil M. J. Aubin,*,† Amy Blase,† John R. Day,† Seongjoon Koo,† Alan W. Partin,† William J. Ellis,‡ Leonard S. Marks,§ Yves Fradet,¶ Harry Rittenhouse† and Jack Groskopf†,||

---

**A**

<table>
<thead>
<tr>
<th>Prostate Volume</th>
<th>Serum PSA (ng/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;30 cc (153)</td>
<td></td>
</tr>
<tr>
<td>30 - 50 cc (248)</td>
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<tr>
<td>&gt;50 cc (151)</td>
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</table>

**B**

<table>
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<tr>
<th>Prostate Volume</th>
<th>PCA3 Score</th>
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<tbody>
<tr>
<td>&lt;30 cc (154)</td>
<td></td>
</tr>
<tr>
<td>30 - 50 cc (254)</td>
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</tr>
<tr>
<td>&gt;50 cc (151)</td>
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</tr>
</tbody>
</table>

---

*Deras I et al, J urol 2008*
**PCA3: A Molecular Urine Assay for Predicting Prostate Biopsy Outcome**

Ina L. Deras,* ‡ Sheila M. J. Aubin,* ‡ Amy Blase,† John R. Day,† Seongjoon Koo,† Alan W. Partin,† William J. Ellis,‡ Leonard S. Marks,§ Yves Fradet,¶ Harry Rittenhouse† and Jack Groskopf†,||

<table>
<thead>
<tr>
<th>Serum PSA (ng/ml)</th>
<th>No. Pts (% biopsy pos)</th>
<th>PCA3 Assay (95% CI)*</th>
</tr>
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<tbody>
<tr>
<td></td>
<td></td>
<td>% Sensitivity</td>
</tr>
<tr>
<td>Less than 4</td>
<td>131 (26)</td>
<td>50 (36–63)</td>
</tr>
<tr>
<td>Greater than 4–10</td>
<td>346 (38)</td>
<td>53 (46–59)</td>
</tr>
<tr>
<td>Greater than 10</td>
<td>86 (48)</td>
<td>61 (50–69)</td>
</tr>
<tr>
<td>Overall</td>
<td>563 (37)</td>
<td>54 (49–59)</td>
</tr>
</tbody>
</table>

* Sensitivity and specificity at a PCA3 score cutoff of 35.
PCA3: A Molecular Urine Assay for Predicting Prostate Biopsy Outcome

Ina L. Deras,*,† Sheila M. J. Aubin,*,† Amy Blase,† John R. Day,† Seongjoon Koo,† Alan W. Partin,† William J. Ellis,‡ Leonard S. Marks,§ Yves Fradet,‖ Harry Rittenhouse† and Jack Groskopf†,||

Deras I et al, J urol 2008
**DD3^PCA3**, a Very Sensitive and Specific Marker to Detect Prostate Tumors

Jacques B. de Kok, Gerald W. Verhaegh, Rian W. Roelofs, Daphne Hessels, Lambertus A. Klemency, Tilly W. Aalders, Dorine W. Swinkels, and Jack A. Schalken

- Expressed almost exclusively in prostate epithelial cells
- On average, 60 fold higher expression levels in PCa vs benign prostatic gland tissue

<table>
<thead>
<tr>
<th>Tumor pool</th>
<th>n</th>
<th>rRNA (×10^8)</th>
<th>DD3</th>
<th>Normalized DD3</th>
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<tbody>
<tr>
<td>Lung</td>
<td>5</td>
<td>13.0</td>
<td>0</td>
<td>0</td>
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<tr>
<td>Esophagus</td>
<td>6</td>
<td>7.6</td>
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<td>0</td>
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<td>Ileum</td>
<td>4</td>
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<td>92.0</td>
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<td>Testis</td>
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<td>15.0</td>
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<td>Breast</td>
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<td>8.0</td>
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<td>Melanoma</td>
<td>3</td>
<td>8.8</td>
<td>0</td>
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ERG “split-apart” probes

Fusion due to Erg Deletion (E del)

Fusion due to Erg Split (E split)

Normal
<table>
<thead>
<tr>
<th>Case/Control</th>
<th>Cases</th>
<th>Controls</th>
<th>P value</th>
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</thead>
<tbody>
<tr>
<td>Age at surgery, yrs</td>
<td>Mean (SD)</td>
<td>58.7 (6.1)</td>
<td>58.9 (5.8)</td>
</tr>
<tr>
<td>Race, %</td>
<td>Caucasian</td>
<td>85</td>
<td>88</td>
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<tr>
<td>Pre-operative serum PSA, ng/mL</td>
<td>Mean (SD)</td>
<td>12.3 (10.4)</td>
<td>11.2 (8.5)</td>
</tr>
<tr>
<td></td>
<td>Median (IQR)</td>
<td>9.1 (8.6)</td>
<td>8.7 (7.2)</td>
</tr>
<tr>
<td>Follow-up time, yrs</td>
<td>Mean (SD)</td>
<td>2.5 (1.9)</td>
<td>5.9 (2.4)</td>
</tr>
<tr>
<td></td>
<td>Median (IQR)</td>
<td>2 (2)</td>
<td>6 (4)</td>
</tr>
<tr>
<td>Gleason score, %</td>
<td>≤ 6</td>
<td>15</td>
<td>15</td>
</tr>
<tr>
<td></td>
<td>7</td>
<td>61</td>
<td>63</td>
</tr>
<tr>
<td></td>
<td>≥ 8</td>
<td>24</td>
<td>22</td>
</tr>
<tr>
<td>Pathologic stage, %</td>
<td>T2</td>
<td>13</td>
<td>13</td>
</tr>
<tr>
<td></td>
<td>T3a</td>
<td>51</td>
<td>51</td>
</tr>
<tr>
<td></td>
<td>T3b or N1</td>
<td>36</td>
<td>36</td>
</tr>
<tr>
<td>PTEN mean H-Score</td>
<td>Mean (SD)</td>
<td>105.5 (93.6)</td>
<td>112.4 (85.3)</td>
</tr>
<tr>
<td></td>
<td>Median (IQR)</td>
<td>100 (175.8)</td>
<td>102.5 (166)</td>
</tr>
<tr>
<td>PTEN expression, %</td>
<td>Mean H-score = 0</td>
<td>15.7</td>
<td>11.3</td>
</tr>
<tr>
<td></td>
<td>All TMA spots markedly decreased a</td>
<td>39.8</td>
<td>31.4</td>
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