Newer Forms of Diagnostic and Molecular Testing in Prostate Cancer

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Disclosures

• Relevant Financial Disclosure
  - Previous Consultant, now Collaborative Researcher: GenomeDX
    • Produces Decipher™ test for post prostatectomy risk assessment
Localized Prostate Cancer – Urologists Perspective

Decision Making Complexity
- High Prevalence
- Broad Spectrum of Localized Disease
- Relatively Long Natural History (Competing Risks)
- Morbidity of Diagnosis and Treatment

Screening and Diagnosis → Surveillance → Treatment → Prostatectomy, Radiation → Surveillance → Adjuvant/Salvage Therapy
Objectives

- Review new advances that may aid prostate cancer screening and diagnosis
- Briefly review relevant molecular biology of localized prostate cancer
- Review currently available molecular tests after diagnosis and or treatment and the evidence supporting their use
- Make recommendations regarding usage of available tests
Part 1: Screening and diagnosis
Screening and Diagnosis – Reducing Over Diagnosis

• Strategies—
  – Evidence based screening
    • Decrease intensity of screening (particularly when the screening test is suboptimal)
  – Develop superior tests
    • The ideal test would be able to identify clinically significant prostate cancer
Evidence Based Screening– AUA Statement 2013

- **No screening in men under age 40**
  - Low prevalence

- **Routine screening not recommended for men 40-54**
  - Screening can be considered on an individualized basis i.e. men at risk (AAM, +FamHx)

- **Shared decision making for screening for men age 55-69**
  - Consider routine screening intervals of 2 years or more for men who opt to be screened (PLCO)

- **No routine screening in men with <10-15 year life expectancy**

- **Consider discontinuation of screening at 70**
Emerging Approaches for Prostate Cancer Screening / Detection – FDA Approved Tests

- **Prostate Health Index (PHI)**
  - Serum test
  - Approved for use in men 50 and over with PSAs of 4-10 and negative DREs, to help determine if biopsy is indicated

- **PCA3**
  - Urine test
  - Approved for use in decision making for repeat biopsy after a prior negative biopsy (scores <25 protective)
Emerging Approaches for Prostate Cancer Screening/Detection – PHI

- **PHI – Prostate Health Index**
  - Blood test based on total PSA (tPSA), free PSA (fPSA) and the PSA isoform [-2]proPSA
  - [-2]proPSA/fPSA × √PSA = PHI
  - proPSA is expressed at greater levels in the peripheral zone (where prostate cancer develops) and also at greater levels from prostate cancer tissue
  - hK2 may be over-expressed in malignancy
  - Increases specificity over a range of sensitivities compared to PSA
  - **Cost effective = relatively cheap!**


BJU International
Above, 350 consecutive RP patients

Similar analysis of ~450 RP patients showed patients with pT3 and GS ≥ 7 have PHI of 64.9 vs 42.9 for those who don’t (p<0.0001) (Fossati et al 2014)
Emerging Approaches for Prostate Cancer Screening/Detection – PCA3

- **PCA3**
  - Urine based test for a prostate cancer specific non-coding transcript
  - FDA approved in 2012 for decision making about repeat biopsy in a man with 1 or more negative biopsies

- Bradley et al J Urol 2013:
  - A comparative effectiveness review of PCA3 vs PSA commissioned by the US Agency for Healthcare Quality and Research

- Conclusion: **PCA3 provides higher accuracy than total PSA and independent information. Evidence is insufficient that PCA3 testing improves health outcomes**
Emerging Approaches for Prostate Cancer Screening/Detection – 4K Score

- Similar to PHI
- Blood test
- Free PSA, Intact PSA, Total PSA, hK2
- AUC increased for any PCa or High Grade vs tPSA (0.79 and 0.82 vs 0.63 and 0.74)
- Prospective screening study is underway

Bryant et al JNCI 2015
Emerging Approaches for Prostate Cancer Screening/Detection – Urinary TMPRSS2:ERG

- Chromosomal rearrangement between TMPRSS2 and ETS family members (most notably ERG) seen in ~50% of prostate cancer cases
- Rearrangement produces a unique transcript (tissue based specificity 99.9%)
- Can be analyzed in urine similar to PCA3
- Increases AUC for detection of PCa and High grade disease

Tomlins Science 2005
Tomlins Eur Urol 2015
Emerging Approaches for Screening/Detection Multi-Parametric Prostate MRI

T2: anatomy

T2 Images

Diffusion Weighted Imaging

ADC map restricted diffusion

Dynamic Contrast Enhancement

Quantitative Parameters from ROI

10/2/15
### Multi-Parametric Prostate MRI

<table>
<thead>
<tr>
<th>PI- RADS</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Most Probable Benign</td>
</tr>
<tr>
<td>2</td>
<td>Probable Benign</td>
</tr>
<tr>
<td>3</td>
<td>Indeterminate</td>
</tr>
<tr>
<td>4</td>
<td>Probably Malignant</td>
</tr>
<tr>
<td>5</td>
<td>Highly Suspicious</td>
</tr>
</tbody>
</table>

Portalez et al, E Urol 2012
Multi-Parametric Prostate MRI

- Allowed +DRE
- Allowed PSA >10
- 57% PCA on biopsy
- 36% Reduction Unnecessary Biopsies
- 6% Un-diagnosed Gleason 7 Dz (3+4=7)

Pokomy et al, E Urol 2014
Clinical Trial of Augmented PSA Screening

PSA 4-10 ng/ml, negative DRE

200 men

A
TRUS/Bx (std 12 core)

100 men

1:1 Randomization

B
mpMRI

100 men

Serum and urine collection, PHI testing

No Cancer

Very Low / Low-Risk

Int / Hi Risk

PI-RADs ≥ 3

TRUS/MRI Fusion Bx

No Cancer

Very Low / Low-Risk

Int / Hi Risk

PI-RADs < 3

No Bx

2 years (yearly PSA/exam, PSA>10, +DRE triggers biopsy)

End of Study TRUS/Bx (std 12 core)

No Cancer

Very Low / Low-Risk

Int / Hi Risk

Serum and urine collection, PHI testing

10/2/15
Conclusions Part 1

- PHI, PCA-3, 4K Score, T2:ERG, mpMRI all present opportunities for reduction of over-diagnosis
- Use in primary unscreened populations is unclear and needs study
- Thresholds for biopsy utilizing new tests needs study
- Comparisons between biomarkers needs study

10/2/15
Part 2: Molecular Tissue testing after diagnosis
Tissue Based Advances

- Molecular understanding of localized prostate cancer has increased
- We have increased facility to obtain molecular information from routinely collected and stored formalin-fixed paraffin embedded tissue
Molecular Tissue Testing – Issues

- Confusion among patients and providers
  - Overlapping clinical spaces
  - Test assess similar molecular phenotypes
  - Aggressive marketing
Prostate cancer is characterized by extraordinary genomic complexity. Copy number alterations include deletions and amplifications. Chromosomal rearrangements and point mutations are also observed.
PTEN Loss, An Important Early Event

Baca et al. Cell 2013
PTEN Loss, An Important Early Event

- PTEN is a tumor suppressor
  - Normally halts the PI3K/AKT pathway
  - PI3K pathway members are altered in up to 40% of all primary prostate cancer and 100% of metastasis
PTEN Loss, An Important Early Event

- PTEN loss is a key component of animal models for prostate cancer
- PTEN loss can be detected in ~15-40% of primary cases and ~50% of metastasis
- Loss correlates with stage and grade

Ding, DePinhno et al Nature 2011
Lotan et al CCR 2011
Tests Based on Cell Cycle Proliferation

- **Ki-67 IHC (~$100)**
  - Nuclear protein associated with rDNA
  - Typically MIB-1 anti-body
  - Semi-quantitative, reported as %positive
  - Most studies use a dichotomous cut-off

- **Prolaris (Myriad) (~$3500)**
  - qRT-PCR
  - 31 cell cycle genes normalized to 15 house keeping genes
  - CMS approval VLR/LR men on biopsies
Ki67 Immunohistochemistry – Watchful Waiting – Transatlantic Prostate Group

- Needle Biopsies (1990-96)
- 243 analyzable men
- 5% have Ki-67 >10%, 1 with GS 6 Disease
Ki67 Immunohistochemistry – Post-treatment

- **RTOG 92-02** – Phase III RCT
  RT+STAD vs RT+LTAD (~600 men)
  - ~25% cohort had Ki-67 >11.3
  - HR 2.95 (1.89-4.6) for metastasis on MVA
- **Mayo Clinic RP Series** (~450 men)
  - ~10% Ki-67 hi
  - HR mets 2.6 (1.7-4) HR death (9.0 (3.5-23) on MVA

Khor et al JCO 2009
Prolaris- Watchful Waiting
Transatlantic Prostate Group

- 349 men on WW, Needle Bx
- CCP score 1 (IQR 1.4-1.7)
- HR death 1.7 per unit increase

Cuzick et al Br J Cancer 2012
582 men undergoing RP, Needle Bx
- ~60% GS 6, 33% GS 7, 7% GS 8; 61% T1c
- HR per unit increase in score, 4.2 (2.1-8.5) MVA

141 men undergoing XRT, Needle Bx
- 73% int or hi risk
- HR BCR (Phoenix) per unit increase in score, 2.1 (1.05-4.25) MVA
PTEN Testing

- IHC (PREZEON, JHH)
- FISH (Prostavysion)
- Phospho-Protein Proxy? ProMark
  - phospho-S6 and phospho-PRAS40

Shipitsin et al Proteome Science 2014
PTEN Testing – Watchful Waiting, Transatlantic Prostate Group

- 675 men on WW
- PTEN IHC & FISH (IHC better) [cheaper too, $95 at JHH]
- TURP specimens; 18% PTEN loss; 3% among low risk

<table>
<thead>
<tr>
<th>Gleason</th>
<th>HR (95% CI)</th>
<th>N (D) L</th>
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<tbody>
<tr>
<td>&lt; 7</td>
<td>8.13 (2.84–23.24)</td>
<td>327 (40) 10</td>
</tr>
<tr>
<td>= 7</td>
<td>2.04 (1.16–3.58)</td>
<td>181 (59) 37</td>
</tr>
<tr>
<td>&gt; 7</td>
<td>1.10 (0.74–1.64)</td>
<td>167 (98) 72</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>PSA (ng ml⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>≤ 10</td>
</tr>
<tr>
<td>&lt; 10–25</td>
</tr>
<tr>
<td>&gt; 25</td>
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</table>

<table>
<thead>
<tr>
<th>Extent of disease</th>
</tr>
</thead>
<tbody>
<tr>
<td>≤ 21% (Median)</td>
</tr>
<tr>
<td>&gt; 21% (Median)</td>
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</table>

<table>
<thead>
<tr>
<th>Clinical score</th>
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<tr>
<td>≤ Median</td>
</tr>
<tr>
<td>&gt; Median – q₇₅%</td>
</tr>
<tr>
<td>&gt; q₇₅%</td>
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</table>

<table>
<thead>
<tr>
<th>Overall (unadjusted)</th>
</tr>
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<tbody>
<tr>
<td>3.51 (2.60–4.73)</td>
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</tbody>
</table>
PTEN Testing – Upgrading

- 174 men with GS 6 disease undergoing RP
- PTEN IHC on biopsy tissue
- PTEN lost in 18% of upgraded cases with 7% of cases that did not upgrade
- OR 3 (1.1-8.6) for upgrading
### HPFS Cohort Multivariate Modeling

<table>
<thead>
<tr>
<th></th>
<th>N</th>
<th>Lethal</th>
<th>HR for lethal prostate cancer</th>
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<tbody>
<tr>
<td><strong>PTEN positive</strong></td>
<td>735</td>
<td>46</td>
<td>1</td>
</tr>
<tr>
<td><strong>PTEN mixed</strong></td>
<td>90</td>
<td>7</td>
<td>1.2 (0.6-3.0)</td>
</tr>
<tr>
<td><strong>PTEN negative</strong></td>
<td>139</td>
<td>28</td>
<td>1.9 (1.2-3.0)</td>
</tr>
</tbody>
</table>

Adjusted for age at diagnosis, BMI at diagnosis, Gleason grade, and cTNM.
PTEN Testing – Outcomes s/p RRP (Health Professionals F/U Study and Physicians Health Study)

<table>
<thead>
<tr>
<th>PTEN / ERG status</th>
<th>N</th>
<th>Lethal</th>
<th>MVA HR</th>
<th></th>
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<tbody>
<tr>
<td>PTEN+ and ERG-</td>
<td>306</td>
<td>20</td>
<td>REF</td>
<td></td>
</tr>
<tr>
<td>PTEN+ and ERG+</td>
<td>260</td>
<td>12</td>
<td>0.9 (0.4-1.8)</td>
<td></td>
</tr>
<tr>
<td>PTEN- and ERG-</td>
<td>48</td>
<td>13</td>
<td>3.1 (1.5-6.3)</td>
<td></td>
</tr>
<tr>
<td>PTEN- and ERG+</td>
<td>132</td>
<td>12</td>
<td>0.7 (0.4-1.5)</td>
<td></td>
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</table>

Product-Limit Survival Estimates
With Number of Subjects at Risk

<table>
<thead>
<tr>
<th>pten_erg</th>
<th>PTEN+ / ERG-</th>
<th>PTEN+ / ERG+</th>
<th>PTEN- / ERG-</th>
<th>PTEN- / ERG+</th>
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</thead>
<tbody>
<tr>
<td>PTEN+ / ERG-</td>
<td>371</td>
<td>306</td>
<td>60</td>
<td>1</td>
</tr>
<tr>
<td>PTEN+ / ERG+</td>
<td>291</td>
<td>241</td>
<td>69</td>
<td>0</td>
</tr>
<tr>
<td>PTEN- / ERG-</td>
<td>63</td>
<td>48</td>
<td>7</td>
<td>0</td>
</tr>
<tr>
<td>PTEN- / ERG+</td>
<td>163</td>
<td>120</td>
<td>40</td>
<td>0</td>
</tr>
</tbody>
</table>
Tests on Multiple Features of Prostate Cancer
OncotypeDX Prostate – Genomic Health

- qRT-PCR of 12 genes (derived from 732 genes which correlated with poor oncologic outcome) and 5 housekeeping genes
  - Run on biopsies
  - Each 20-point increase (~IQR) in Genomic Prostate Score (GPS) equals ~2 fold risk of ≥ 4+3 or pT3 disease at RP
  - Marketed for men with low to low-int risk considering AS
  - Not tested in AS or WW populations
  - ~$3800.00
Tests on Multiple Features of Prostate Cancer
ProMark– MetaMark

- Multiplexed proteomics assay
- Run on biopsies
- Score ranges form 0-1, each 0.25-point increase equals ~3 fold risk of unfavorable pathology
- Thresholds of <0.33 and >0.8 appear useful and include ~40% of patients
- Marketed for men with low to low-int risk considering AS
- Not yet tested in AS or WW populations
Tests on Multiple Features of Prostate Cancer
Decipher – GenomeDX Biosciences

- High density (Affy) array performed on FFPE prostate tissue
  - 1.4 million probes (coding and non-coding)
  - CLIA certified

- 22 genomic markers selected for ability to predict rapid mets s/p RP
  - Outputs a genomic classifier (GC) score (0-1)
  - CMS approved for post RP testing

Abdueva J Mol Diag 2010
Erho J Onc 2012
Tests on Multiple Features of Prostate Cancer
Decipher – GenomeDX Biosciences – Post RP

• Metastasis signature validation in 219 high risk men s/p RRP at Mayo Clinic
• Categorical cut-offs at <0.4 (60%), 0.4-0.6 (20%) and >0.6 (20%) ass with HR for mets on MVA of 1, 2.4 (1.1-5.2) and 7.3 (3.5-15.1)
• Mayo cohort with high amount of adjuvant and salvage tx post-RP

Karnes et al J Urol 2013

10/2/15
Tests on Multiple Features of Prostate Cancer
Decipher – GenomeDX Biosciences – Post RP

- JHH cohort, 260 NCCN Int or Hi risk men, 99 with metastatic progression
  - Natural history (no tx p RP until met)
- Median GC score 0.34 (IQR 0.22-0.52)
- HR met MVA 1.5 (1.3-1.7) per 0.1 increase in score

Ross et al Eur Urol 2015
Tests on Multiple Features of Prostate Cancer Decipher – GenomeDX Biosciences – Post RP

Decipher c-index 0.76 (0.65-0.86)

md-CCP c-index 0.63

md-GPS c-index 0.62

34 Literature Based Prognostic Prostate Cancer Signatures

Ross, Schaeffer in preparation
Decipher – GenomeDX Biosciences – Post RP Radiation therapy Decision

Karnes et al. (2013), N=219
Design: Case-cohort

Den et al. (2014), N=139
Design: Cohort

Ross et al. (2015), N=260
Design: Case-cohort

Freedland et al. (2015), N=117
Design: Cohort

Exclusion:
1) N+ (n=61)
2) Received any neo-adjuvant prostate cancer treatment prior to surgery (n=3)
3) Received hormone-only treatment prior to clinical evidence of metastasis (n=67)
4) Received salvage radiation for PSA above 10ng/mL (n=8)

Inclusion:
1) Patients in the randomly selected sub-cohort of case-cohort studies
2) Achieved PSA nadir after surgery
3) Complete clinical data
4) Received either radiation or no radiation treatment prior to clinical evidence of metastasis
5) pT3 or SM+ patients

---

applying inclusion and exclusion criteria

Karnes et al. (2013), N=86

Den et al. (2014), N=118

Ross et al. (2015), N=114

Freedland et al. (2015), N=104

Final Cohort (N=422)

No evidence of clinical metastasis during study follow-up (n=385)

Experienced metastasis (n=37)
Decipher – GenomeDX Biosciences – Post RP Radiation therapy decision

![Graph showing the 10-Year Risk of Metastasis for CAPRA-S 0-5 and 6-12](image)

- CAPRA-S 0-5:
  - n=269
  - Metastasized=9

- CAPRA-S 6-12:
  - n=153
  - Metastasized=28

Graph legend:
- ART
- Early SRT
- Delayed SRT
- No RT
Tissue Based Molecular Tests

USE BY CLINICAL CONTEXT
Surveillance VLR Men-Nothing Preferred

- NO molecular testing recommended in VLR >65
Surveillance of Low Risk Men—PTEN IHC Preferred (GPS, ProMark option)

- Most testing will be non-informative
- OR 3 for upgrading if PTEN lost
- HR death if WW performed, 8
- Ki-67/Prolaris not informative in this population
- OncotypeDx (GPS) and ProMark have limited data in this population
- IHC is >30X cheaper

Alam et al J Urol 2015
Watchful Waiting-Int or Hi Risk
Ki-67 or Prolaris Preferred

- NO molecular testing recommended in low risk men with <10 year life expectancy
- Ki-67 or Prolaris predictive in PSA >10 and GS ≥ 7
- Ki-67 much cheaper, Prolaris probably more reproducible

Intensity/Use of Primary Radiation Therapy: ?

- Unlike RP, RT is biologic and modified based on predicted cancer aggression
- ADT adds morbidity
- RTOG 0815: RT +/-ADT for Int Risk Men
- SADT vs LADT for Hi Risk men
- Needs research: Prolaris or Decpiher the best candidate?
Most men with APF after RP do not benefit from adjuvant XRT
Men with APF after RP may be harmed by adjuvant XRT (long term results EORTC 22911, Bolla et al. Lancet 2012)
Adjuvant Radiation after RP – 2 Step Process – Nomograms First

<table>
<thead>
<tr>
<th>Nomogram Cut-point</th>
<th>No. of APF Patients (%)</th>
<th>APF Patients (at 10 yrs)</th>
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<tbody>
<tr>
<td>CAPRA-S &lt;3</td>
<td>200 (26%)</td>
<td>3%</td>
</tr>
<tr>
<td>CAPRA-S 3-5</td>
<td>435 (56%)</td>
<td>4%</td>
</tr>
<tr>
<td>CAPRA-S &gt;5</td>
<td>139 (18%)</td>
<td>30%</td>
</tr>
<tr>
<td>Eggener &lt;2.5%</td>
<td>302 (39%)</td>
<td>1%</td>
</tr>
<tr>
<td>Eggener 2.5-5.0%</td>
<td>264 (34%)</td>
<td>5%</td>
</tr>
<tr>
<td>Eggener 5-15%</td>
<td>133 (17%)</td>
<td>15%</td>
</tr>
<tr>
<td>Eggener 15-25%</td>
<td>48 (6%)</td>
<td>31%</td>
</tr>
<tr>
<td>Eggener &gt;25%</td>
<td>27 (4%)</td>
<td>59%</td>
</tr>
</tbody>
</table>

- Natural history cohort of men undergoing RP at JHH

(B) pT3 or SM+ Patients

Eggener 15 year probability Log Scaled

Ross et al. Eur Urol 2015
Adjuvant Radiation after RP – 2 Step Process
Decipher if Eggener >2.5% <25%

- Strong consideration to treat with adjuvant XRT vs early salvage if Decipher >0.45, particularly if <70 years old
Conclusions Part 2: Tissue Based Molecular Testing

- Our molecular understanding of prostate cancer is increasing exponentially
  - PTEN loss appears to be an early and important event for the development of aggressive prostate cancer
- Tests have been developed that may help treatment decisions
- Current evidence suggests selective use of these tests may be beneficial
Part 3: Molecular Imaging
Prostate Membrane Specific Antigen (PSMA)

- Dimerized type II transmembrane glycoprotein
- Detectable in 95% of prostate cancer specimens
- Expression increases with tumor grade and stage
Molecular Imaging—PET PSMA

A Serpin
c-PSA

\(^{89}\text{Zr-5A10}\)

B AR

\(^{18}\text{F-FDHT}\)

DHT

Test.

Extracellular

Intracellular

PSMA

\(^{64}\text{Cu-J591}\)

\(^{89}\text{Zr-J591}\)

\(^{18}\text{F-DCFBC}\)

C f-PSA

10/2/15

Holland et al. JNM 2013; 53: 1333.
18F-DCFPyL – Small Molecule for PET/PSMA Imaging

- High binding affinity for PSMA with rapid clearance from the bloodpool
- Straightforward radiochemical synthesis
Intra- and Peri-Prostatic Findings

- Seminal Vesicle Invasion
- Intraprostatic Lesions
- Prostate Bed Recurrence
Clinical Trial in Men with BCR

PSA ≥0.2 ng/mL s/p RRP

- Continued Surveillance
- Salvage External Beam Radiation
- Androgen Deprivation Therapy
- Salvage PLND
- SABR to Oligometastatic Sites
Clinical Trial in Men with BCR

Men with an Elevated Serum PSA (≥0.2 ng/dL) Following Radical Prostatectomy

- Screening Assessment
- Informed Consent
- $^{18}$F-DCFPyL PET/CT
  - Management Per M.D. x 6 Months
  - $^{18}$F-DCFPyL PET/CT
- Study Completion
Clinical Trial in Men with BCR

18F-DCFPyL PET/CT

Standard CT

10/2/15
Clinical Trial in Men with BCR – Median Enrollment PSA 0.3ng/ml

Summary of Findings

<table>
<thead>
<tr>
<th>Finding</th>
<th>Conventional n (%)</th>
<th>18F-DCFPyL n (%)</th>
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</thead>
<tbody>
<tr>
<td>Local / Prostate Bed</td>
<td>1 (8.3)</td>
<td>3 (25)</td>
</tr>
<tr>
<td>Pelvic Lymph Nodes</td>
<td>0</td>
<td>3 (25)</td>
</tr>
<tr>
<td>Bone</td>
<td>3 (25)</td>
<td>2 (16.7)</td>
</tr>
<tr>
<td>Pelvic Lymph Nodes + Bone</td>
<td>0</td>
<td>1 (8.3)</td>
</tr>
<tr>
<td>Any Positive</td>
<td>4 (25%)</td>
<td>9 (75%)</td>
</tr>
</tbody>
</table>
Thanks and Questions