Genetic Sequencing as a Tool for Prostate Cancer Sentinel Node Evaluation

Ramkishen Narayanan, MD
Urologic Oncology Fellow
John Wayne Cancer Institute (JWCI)

2/9/19
I do not have anything to disclose.
Outline

• **Rationale for Pelvic Lymphadenectomy (PLND) in Prostate Cancer (PCa)**

• **Review PCa Sentinel Node (SLN) Evaluation Efforts**

• **Applicability of genetic sequencing to PLND and SLN evaluation.**

• **Future Directions** for PCa SLN efforts.
1) Primary rationale for PLND in PCa is **unparalleled staging accuracy**.
   - accurate pN+ staging can mobilize adjuvant treatment shown to improve survival

2) Therapeutic efficacy, possibly from extirpation of nodal disease burden, is controversial
   - Lack of level 1 evidence
Reported incidence of occult LNI (i.e. detected at RP + PLND)

- 1970s – 1980s: 20 – 40%
- PSA screening era (FDA approval in 1986): ≤10%
  - Patient series spanning 15-20 years*:
    - 4-6% in clinically localized disease
    - <1% cT1c
- Contemporary SEER data: increasing incidence of LNM at RP from 2004 to 2014**
  - 2% → 4.7%

PLND Nomograms

• Minimal incidence of LNI among lower risk PCa patients → questioned value of routine PLND → development of LNI prediction tools → determine if PLND can be omitted

• Nomogram flaws
  – Intrinsic weaknesses of input variables (ex. prostate biopsy under-grading*)
  – Limited nodal sampling template

Bottom line: imperfect system but PLND offers most acute nodal staging

Pooled sensitivity (SN) of pre-op staging CT and MRI for PCa lymph node metastases (LNM): ~40%*

PET-CT SN on initial staging:

- $^{18}$F-choline: 10-45%
- $^{68}$Ga-PSMA PET: 66-68%**

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**Maurer et al (2016). Diagnostic efficacy of ($^{68}$)gallium-PSMA PET compared to conventional imaging for lymph node staging of 130 consecutive patients with intermediate to high risk prostate cancer. J Urol 195 (5): 1436-1443.
PLND is needed, so now what?

HOW EXTENSIVE?
Anatomic and lymphangiographic studies:

1) Laterally to **obturator** nodes

2) Laterally and medially to the **internal iliac** nodes

3) Cranially to **external iliac** nodes

4) Posterocaudally to the **lateral + subaortic nodes of sacral promontory** (i.e. presciatic and presacral nodes)
PCa LNMs, either alone or in combination, are most frequently found in the hypogastric, external iliac and obturator regions.

NO RELIABLE TIER/HIERARCHY

LEGEND:

R (right), L (left)

OB (obturator)
EI (external iliac)
II (internal iliac)
Clo (node of Cloquet)
CI (common iliac)
PC (para-caval)
IAC (inter-aortocaval)
PA (para-aortic)

AB (aortic bifurcation)
IMA (inferior mesenteric artery)
“How do you know where to cut?...”

- No regional or anatomic standardization of the PLND template in PCa.

- Dissection limited to the obturator fossa only can miss up to 50% of LNMs*

- Dawn of laparoscopic PLND in the early 1990s was associated with dissections provincialized to the OB fossa along with some extent of nodal tissue from EI region.**

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Table 1 Prostate PLND templates by nodal regions dissected

<table>
<thead>
<tr>
<th>Definition</th>
<th>Nodal regions dissected</th>
</tr>
</thead>
<tbody>
<tr>
<td>Minimala</td>
<td>Obturator (OB)</td>
</tr>
<tr>
<td>Standardb</td>
<td>Minimal template + external iliac (EI)</td>
</tr>
<tr>
<td>Extendedc</td>
<td>Standard template + internal iliac (II) aka hypogastric</td>
</tr>
<tr>
<td>Super-extended</td>
<td>Extended template + common iliac (CI) at least up to ureteroenic + presacral</td>
</tr>
</tbody>
</table>

*a Limited dissections
b E-PLND: proximal limit at least to the CI bifurcation; CI and presacral nodes optional [31, 56]
EXTENDING THE PLND TEMPLATE

- **Heidenreich et al (2002):** Extended PLND (E-PLND) comprising EI, II, OB, CI bilaterally and the presacral LNs.
  - E-PLND removed a mean of 28 LNs (range 21–42) per pt; LNI in 26.2% of pts
  - Standard (OB + EI) template: mean of 11 LNs (range 6–19) per pt and was 12% LNI detection rate.
  - Of all LNMs, 42% were outside the standard PLND template.

- **Schumaker et al (2008):** positive II nodes, alone or in conjunction with other LNMs:
  - 71–73% of patients; but positive EI or OB nodes w/o II involvement were seen in only 4% of patients.
  - LNM found exclusively per region: II = 21%; OB = 16%; EI = 9%.

- Tabulation across eight contemporary LNM mapping studies showed pN+ incidence in descending order from II > EI > OB.*

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• Joniau et al (2013) stratified likelihood of correctly staging pN+ patients based on PLND template extent:
  – OB only = 47%
  – EI+OB=76%
  – II + EI + OB = 94%
  – presacral + Cl + II + EI + OB = 97%

Diminishing returns for routine dissection above the aortic bifurcation and in presacral area

TAKE HOME MESSAGE ON EXTENT

- A drainage hierarchy between the three key nodal stations (EI, II, OB) cannot be ascertained from the available literature.

- “Olympic medal effect”: these regions effectively switch Olympic medals for %LNI across studies.

- The hypogastric region is a critical nodal metastatic landing site in PCa.
IS THERE A THERAPEUTIC ADVANTAGE TO EXTENDED PLND?
Battle of observational evidence

**YES**

- **Preisser et al 2018**: SEER >10 nodes → improved CSM by 1.4%

- **Schumaker et al 2008**: cN0pN+ with long-term follow-up without immediate ADT
  - ≤2 nodes positive had higher 10-yr CSS (78.6% v. 33.4%)

- **Bader et al 2003**: time to progression and CSM correlated with number of LNMs → best survival outcomes in patients with 1 or 2 LNMs

**NO**

- **Kluth et al 2014**: pN0 → # of nodes removed no prognostic significance on BCR

- **Pierorazio et al 2013**: Johns Hopkins 30-yr experience – LN counts did not predict for BCR, distant mets or death from Pca

- **Stone et al 1997**: no oncologic benefit to anatomically extended PLND – limited (OB + II) v. extended (OB + II + EI + CI)

N = 123 randomized to receive extended hemi-PLND with contralateral limited PLND

- No difference in LNI detection between templates

- Study design criticized for:
  - Underpowered
  - Majority with favorable risk disease
  - Incomplete pathologic evaluation in study protocol

Ongoing Phase 3 RCTs comparing limited versus extended PLND in PCa

- NCT01555086 (Association of Urologic Oncology, Germany)
- NCT01812902 (University of Sao Paulo, Brazil)
  - Early results: E-PLND had a six-fold increase in detecting LNI (the only template to find positive nodes in intermediate risk patients)
  - at a short follow-up of 3 years, no difference in BCR has been observed between the templates
COMPLICATIONS?
Complications of Pelvic Lymphadenectomy: Do the Risks Outweigh the Benefits?

Stacy Loeb, MD, Alan W. Partin, MD, PhD, Edward M. Schaeffer, MD, PhD
Brady Urological Institute, Johns Hopkins Medical Institutions, Baltimore, MD

**Drawbacks of PLND**

- Time, Cost
- Potential morbidity
  - Lymphocele
  - Thromboembolic events
  - Ureteral injury
  - Neurovascular injury

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**Table 1**

Lymphocele Rates in Contemporary Radical Prostatectomy Series With Limited Versus Extended Pelvic Lymph Node Dissection

<table>
<thead>
<tr>
<th>Study</th>
<th>Limited</th>
<th>Extended</th>
</tr>
</thead>
<tbody>
<tr>
<td>Allaf et al.(^1)</td>
<td>–</td>
<td>3 (0.1)</td>
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<tr>
<td>Briganti et al.(^26)</td>
<td>9 (4.6)</td>
<td>79 (10.3)</td>
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<tr>
<td>Clark et al.(^21)</td>
<td>1 (0.8)</td>
<td>3 (2.4)</td>
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<tr>
<td>Heidenreich et al.(^19)</td>
<td>9 (9)</td>
<td>9 (10.6)</td>
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<tr>
<td>Musch et al.(^2)</td>
<td>29 (3.3)</td>
<td>41 (9.4)</td>
</tr>
</tbody>
</table>

Values are number represented as whole numbers with the percentage in parenthesis.
Standardized comparison of robot-assisted limited and extended pelvic lymphadenectomy for prostate cancer

Bertram E. Yuh, Nora H. Ruel, Rosa Mejia, Giacomo Novara*, Timothy G. Wilson

Urology, City of Hope National Cancer Center, Duarte, CA, USA, and *Department of Surgical, Oncological and Gastroenterological Sciences, Urology Clinic, University of Padua, Padua, Italy
<table>
<thead>
<tr>
<th>Complications</th>
<th>LLND $n = 204$</th>
<th></th>
<th>ELND $n = 202$</th>
<th></th>
<th>All patients $N = 406$</th>
<th>Sum</th>
<th>Incidence, %</th>
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<tr>
<td></td>
<td>Minor: Clavien I–II</td>
<td>Major: Clavien III–V</td>
<td>Total</td>
<td>Minor: Clavien I–II</td>
<td>Major: Clavien III–V</td>
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<td>52</td>
<td>40</td>
<td>10</td>
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<td>102</td>
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</table>
SO WHAT DO WE ACTUALLY HAVE WITH CURRENT PLND RESEARCH?

• High yield pelvic LN stations that need to be dissected for accurate staging.

• Predominantly observational evidence about improved disease survival.

• Anatomic/templated PLND takes time, has morbidity and misses a small number of nodes outside the template borders
The Sentinel Lymph Node (SLN) Concept

The *sine qua non* of the SLN concept is avoiding unnecessary lymphadenectomy.
• 1960 Gould  
  SLN Parotidectomy  
• 1977 Cabanas  
  SLN Concept – Penile Cancer  
• 1992 Morton  
  SLN Malignant Melanoma  
• 1993 Krag  
  Gamma-Probe Localization  
• 1994 Giuliano  
  SLN Breast
Sentinel node evaluation in prostate cancer

Rammishen Narayanan and Timothy G. Wilson

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- 1960 Gould: SLN Parotidectomy
- 1977 Cabanas: SLN Concept – Penile Cancer
- 1992 Morton: SLN Malignant Melanoma
- 1993 Krag: Gamma-Probe Localization
- 1994 Giuliano: SLN Breast

Wawroschek et al 1999 (Augsburg, Germany) → feasibility of prostate SLN evaluation
**Why SLN dissection in PCa?**


2. Individualize PLND to capture nodes outside of typical dissection.

3. Targeted PLND is ideal especially since pN+ PCa patients typically have a median of few (≤ 3) nodes positive per patient.

**Key Challenges**

1. Complex pelvic lymphatic anatomy i.e. lack of orderly drainage

2. Optimal tracer / detection technique still elusive

3. Tremendous study heterogeneity among currently reported prostate SLN efforts
Challenge: ANATOMIC COMPLEXITY
- Divided LNI into pelvic and CI + RP nodes
- Observed no skip lesions to the RP without CI involvement

However, early work with patent blue V dye has shown rare direct staining of para-aortic nodes. [Smith M (1966). The lymphatics of the prostate. Invest Urol 3(5): 439-444]
Prostate Cancer Topography and Patterns of Lymph Node Metastasis

Yuji Tokuda, MD,* Lauren J. Carlino, BA,† Amuradha Gopalan, MD,* Satish K. Tickoo, MD,* Matthew G. Kaag, MD,‡ Bertrand Guillonneau, MD,‡ James A. Eastham, MD,‡ Howard I. Scher, MD,‡ Peter T. Scardino, MD,‡ Victor E. Reuter, MD,* and Samson W. Fine, MD*

FIGURE 1. A, Fifteen of 50 (30%) right lobe dominant cancers had positive lymph nodes (LN) in the left pelvis (10 exclusively left and 5 bilateral). B, Eighteen of 44 (40%) left lobe dominant cancers showed positive LN in the right pelvis (9 exclusively right and 9 bilateral). L indicates left lobe; R, right lobe.
Right sided index lesion (PIRADS 5)

LNM location:
- LEI
- LII x 2
- LOB
- RII

LNM cross-over phenomenon
Results

Median age at surgery = 68 yo. Median time from MRI to surgery = 1.9 mos.

**Overall LNM cross-over rate:** 39% (16/41) of men had LNM contralateral to any index MRI lesion.

LNM cross-over rate increased to 55% (16/29) when only men with completely lateralized MRI lesions were included.
<table>
<thead>
<tr>
<th>Patient ID</th>
<th>Location of LNM</th>
<th>Site of LNM</th>
<th># of x-over nodes</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>LEI, RCI</td>
<td>LEI</td>
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<td>2</td>
<td>LCI, LEI x3, LII x5, RII</td>
<td>RII</td>
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</tr>
<tr>
<td>3</td>
<td>LOB x2, RCI x 1; REI x 1; ROB x2</td>
<td>LOB x2</td>
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<tr>
<td>4</td>
<td>LEI, LII x2, LOB, RII</td>
<td>LEI, LII x2, LOB</td>
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<td>5</td>
<td>LEI</td>
<td>LEI</td>
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<td>LCI, LEI x2, LII x1, RCI</td>
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</tr>
<tr>
<td>16</td>
<td>ROB</td>
<td>ROB</td>
<td>1</td>
</tr>
</tbody>
</table>

= exclusively contralateral to the index prostate lesion
Challenge: TRACER
Variety of Tracers:
- Radiotracer
  - $^{99m}$Tc-nanocolloid
  - $^{99m}$Tc-tilmanocept
- Blue dye
- Fluorophores (ex. ICG)
- Hybrid tracers / conjugates
  - ICG-$^{99m}$Tc-nanocolloid
  - $(111)$In-PSMA
- USPIO

Tracers are subject to operator technique and lymphatic congestion (nodal tumor plug)
Digitization of an Analog Biologic Signal (pH) allows amplification of fluorescence without introducing noise or distortion, which in turn allows detection of low volume disease as in clinically N0 nodes. By tuning the activation pH the nanoprobe can be made to selectively target Sentinel Nodes only, cancer involved nodes only, or both.
The Value of $^{99m}$Tc-PSMA SPECT/CT-Guided Surgery for Identifying and Locating Lymph Node Metastasis in Prostate Cancer Patients

Supplementary figure 2

Indocyanine Green (ICG) fluorophore-based sentinel pelvic lymphadenectomy (PLND) at time of robotic radical prostatectomy (RARP)
Challenge: STUDY HETEROGENEITY
<table>
<thead>
<tr>
<th>Study</th>
<th>Pt N; (% LNI)</th>
<th>Tracer Injection Method; prostate location</th>
<th>Tracer</th>
<th>No. traced LNs per pt (median)</th>
<th>Total LNs per pt (median)</th>
<th>FN rate</th>
<th>LN level</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Miki et al (2017)</td>
<td>50; (12% LNI)</td>
<td>TRUS; bilateral mid-prostate PZ</td>
<td>Free ICG</td>
<td>4 (range 0-16)</td>
<td>16 (range 6-33)</td>
<td>0%</td>
<td>LN level = 17%</td>
<td>Miki et al (2017)</td>
</tr>
<tr>
<td>*van den Berg et al (2017)</td>
<td>10; (30% LNI)</td>
<td>TRUS*; either unilateral or b/l lobes</td>
<td>ICG-99mTc-nanocolloid hybrid + Fluorescein</td>
<td>3.6 (mean; range 1-5)</td>
<td>13.2 mean (range 6-22)</td>
<td>10%</td>
<td>LN level = NS</td>
<td>*van den Berg et al (2017)</td>
</tr>
<tr>
<td>*van der Poel et al (2011)</td>
<td>10; (20% LNI)</td>
<td>TRUS; PZ</td>
<td>ICG-99mTc-nanocolloid hybrid</td>
<td>3 (range 2-4)</td>
<td>11 (range 4-16)</td>
<td>0%</td>
<td>LN level = 0%</td>
<td>*van der Poel et al (2011)</td>
</tr>
<tr>
<td>Chennamsetty et al (2016)</td>
<td>20; (35% LNI)</td>
<td>TRUS guided TP**; base, mid, apex</td>
<td>Free ICG</td>
<td>4 to 6 (varied with ICG dose)</td>
<td>20.5 (range 11-46)</td>
<td>NS</td>
<td>LN level = 38%</td>
<td>Chennamsetty et al (2016)</td>
</tr>
<tr>
<td>Ramirez-Backhaus et al (2016)</td>
<td>84; (29% LNI)</td>
<td>TRUS guided TP; ¹⁵³Tb/l lobes</td>
<td>Free ICG</td>
<td>7 (range NS)</td>
<td>22 (range 8-47)</td>
<td>8%</td>
<td>LN level = 40%</td>
<td>Ramirez-Backhaus et al (2016)</td>
</tr>
<tr>
<td>*Hruby et al (2015)</td>
<td>38; (40% LNI)</td>
<td>TRUS guided TP; PZ b/l lobes</td>
<td>Free ICG</td>
<td>12 (range 0-37)</td>
<td>18 (range 7-38)</td>
<td>7%</td>
<td>LN level = 2%</td>
<td>*Hruby et al (2015)</td>
</tr>
<tr>
<td>Jeschke et al (2012)</td>
<td>26; (8% LNI)</td>
<td>TRUS; PZ b/l lobes</td>
<td>Combo (¹⁹⁵mTc + free ICG)</td>
<td>10 (range 0-36)</td>
<td>22 (range 11-36)</td>
<td>50%</td>
<td>LN level = NS</td>
<td>Jeschke et al (2012)</td>
</tr>
<tr>
<td>Manny et al (2013)</td>
<td>50; (8% LNI)</td>
<td>Robot-guided percutaneous into b/l anterior base</td>
<td>Free ICG</td>
<td>Specified in pN+ pts: 6 (range 5-16)</td>
<td>14.2 mean (range 10-20)</td>
<td>0%</td>
<td>LN level = 0%</td>
<td>Manny et al (2013)</td>
</tr>
</tbody>
</table>

**False negative (FN):**

“Patient level”: accurately staging a patient as pN+ alone (even 1 metastatic SLN) = TP
- Untraced nodes do not affect FN rate

“Nodal level”: patient is considered a FN if any untraced metastatic nodes
Is PCa SLN worth the effort?

Utility of SLND in PCa is best appreciated in the context of **appropriate patient selection**.

1) pN+ PCa patients, in our review of the contemporary literature, typically have a median of a few (≤ 3) positive nodes per patient
   - an important counterpoint is that the upper limit in the range of positive nodes can be dramatically increased with higher risk disease and extent of dissection.

2) Second, fewer positive nodes are associated with improved survival and reduced disease progression.

3) The median number and range of nodes traced with some available agents appears adequate to encompass the median number of positive nodes in PCa patients.

**Take home**: patients with a risk ceiling of no more than only a few positive nodes can undergo a completely targeted PLND that would extract only, or little more than, those malignant nodes.
JWCI Urology PCa SLN endeavor

1. Novel tracer administration

2. Molecular Translation medicine collaboration for prostate lymph node sequencing.
PCa SLN efforts currently use heterogenous tracer injection techniques but do not reconcile disease multifocality in the gland.
Identifying aggressive prostate cancer foci using a DNA methylation classifier

Kamilla Mundbjerg¹, Sameer Chopra¹, Mehrdad Alemozaffar¹, Christopher Duymich¹, Ranjani Lakshminarasimhan¹, Peter W. Nichols², Manju Aron², Kimberly D. Siegmund³, Osamu Ukimura¹, Monish Aron¹, Mariana Stern³, Parkash Gill⁴, John D. Carpten⁵, Torben F. Ørntoft⁷, Karina D. Sørensen⁷, Daniel J. Weisenberger⁶, Peter A. Jones¹,⁸, Vinay Duddalwar⁹, Inderbir Gill¹* and Gangning Liang¹*
Fig. 1 Strategy and sample selection. a A prostate gland with four cancer foci (green and orange areas) and a pelvic lymph node with metastasis marked by a purple star. Our hypothesis is that we can determine the primary focus of metastasis origin based on matching DNA methylation in the lymph node metastasis, and this in turn will represent the most aggressive cancer subclone. By determining the aggressive subclone in multifocal PCs, we will obtain groups of aggressive and non-aggressive samples, which will form the basis for developing a classifier to determine the aggressiveness of primary PC foci. b An overview of the samples from patient 41 is shown in the upper left corner. P patient, T primary tumor focus, NL tumor-negative lymph node, PL tumor-positive lymph node. The physical location of the five prostate samples and the two lymph node samples collected are shown on schematics of the dissected prostate gland (middle) and the lymphatic system (lower left corner), respectively.
Bottom line: potential for DNA methylation biomarker panel with potential clinical applicability
AIM:

1. Utilize next-general sequencing (NGS) to correlate gene expression profiles of primary tumor lesion(s) and positive pelvic LNs

2. If the dominant/index histopathologic lesion matched the malignant LN more than secondary lesions, efforts to identify the dominant lesion preoperatively will be an important component of prostate SLND technique
NGS of the dominant/primary lesion & sites of LNMs may help guide SLN efforts for PLND in PCa

Ramkishen Narayanan, MD, Matthew Salomon, PhD, Darryl Nousome, PhD, Michael Tyler, MD, David Krasne, MD, Joshua Hanelin, MD, Kevin Tran, Jennifer A. Linehan, MD, Dave Hoon, PhD, Timothy G. Wilson, MD

Methods

• Paired primary tumor and malignant LN archival tissue (FFPE) collected from N = 9 men after RP + PLND

• Dominant/index lesion defined based on tumor volume in the RP specimen

• N = 6 men with multifocal intra-prostatic primary lesions defined as the dominant tumor and a discrete second lower volume lesion.

• 33 total samples were sharply micro-dissected and assayed via NGS

• Expression profiles were then used to make phylogenetic relationships and network analyses among intra-prostatic tumor foci and lymph node metastases
Results
Translating NGS findings to anatomic dissection

Key: L=left; R=right

Nodal regions (relative to arterial anatomy):
CI = common iliac
EI = external iliac
Clo = node of Cloquet
OB = obturator
II = internal iliac
Transcriptome deconvolution analysis:
Significantly lower number of CD8+ T-cells in the dominant foci

Lower number of CD8 positive T-cells in the primary foci as compared to the secondary foci and positive lymph nodes (ANOVA, p=0.002).

Potentially greater immune system evasion in primary foci.
Conclusions

• The dominant/index histopathologic lesion has a higher genetic expression profile match with lymph node metastases.

• The lymph node metastases have greater similarity among themselves.

• Risk for lymph node metastases may be related to poor immune response to tumor infiltration in the primary site.
NGS of the dominant/primary lesion & sites of LNMs may help guide SLN efforts for PLND in PCa

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Limitations / Future Direction

• Small sample size in our pilot analysis*
• Only internal pathology review
• Need to add negative lymph nodes to the analysis
Frequent clonal relations between metastases and non-index prostate cancer lesions

Jeroen Kneppers,1,2 Oscar Krijgsman,2,3 Monique Melis,4 Jeroen de Jong,2,3 Daniel S. Peep,2,3 Elise Bekers,1 Henk G. van der Poel,4 Wilbert Zwart,1,3 and Andries M. Bergman1,4

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4Division of Molecular Genetics, 5Division of Molecular Pathology, and 6Division of Urology, Netherlands Cancer Institute, Amsterdam, Netherlands. 1Laboratory of Chemical Biology and Institute for Complex Molecular Systems, Department of Biomedical Engineering, Eindhoven University of Technology, Eindhoven, Netherlands.

23.3% of cases the index lesion was not the clonal origin of the LN metastasis in a high-risk cohort of patients who underwent a prostatectomy and a pelvic lymphadenectomy.
miR-133a3p, miR-133b, miR-196a-5p, miR-143-5p and miR-143-3p represent the top 5 differentially expressed miRNAs from our analysis.

Of these, the top miRNA (miR-133a-3p) is involved in human transcriptional misregulation in cancer.

Figure 1 delineates the gene targets of miR-133a-3p: **CDKN1A(p75)** which is involved in prostate cancer cell growth and IGF1R which is involved in PC cell colony migration.
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• Pathology – Dr. David Krasne, Dr. John Jalas
• Radiology – Dr. Joshua Hanelin
THANK YOU!