Molecular analysis of circulating tumor cells in prostate cancer: moving toward a liquid biopsy and personalized medicine

Edwin M. Posadas, MD FACP
Medical Director, Urologic Oncology Program
Samuel Oschin Comprehensive Cancer Institute
Cedars-Sinai Medical Center

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Prostate cancer is a significant health problem

<table>
<thead>
<tr>
<th>Estimated New Cases*</th>
<th>Estimated Deaths</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Prostate</strong></td>
<td>233,000</td>
</tr>
<tr>
<td>Lung &amp; bronchus</td>
<td>116,000</td>
</tr>
<tr>
<td>Colorectum</td>
<td>71,830</td>
</tr>
<tr>
<td>Urinary bladder</td>
<td>56,390</td>
</tr>
<tr>
<td>Melanoma of the skin</td>
<td>43,890</td>
</tr>
<tr>
<td>Kidney &amp; renal pelvis</td>
<td>39,140</td>
</tr>
<tr>
<td>Non-Hodgkin lymphoma</td>
<td>38,270</td>
</tr>
<tr>
<td>Oral cavity &amp; pharynx</td>
<td>30,220</td>
</tr>
<tr>
<td>Leukemia</td>
<td>30,100</td>
</tr>
<tr>
<td>Liver &amp; intrahepatic bile duct</td>
<td>24,600</td>
</tr>
<tr>
<td><strong>All Sites</strong></td>
<td><strong>855,220</strong></td>
</tr>
<tr>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Prostate</strong></td>
<td><strong>29,480</strong></td>
</tr>
<tr>
<td>Lung &amp; bronchus</td>
<td>26,270</td>
</tr>
<tr>
<td>Colorectum</td>
<td>20,170</td>
</tr>
<tr>
<td>Pancreas</td>
<td>15,870</td>
</tr>
<tr>
<td>Leukemia</td>
<td>14,040</td>
</tr>
<tr>
<td>Esophagus</td>
<td>12,450</td>
</tr>
<tr>
<td>Urinary bladder</td>
<td>11,170</td>
</tr>
<tr>
<td>Non-Hodgkin lymphoma</td>
<td>10,470</td>
</tr>
<tr>
<td>Kidney &amp; renal pelvis</td>
<td>8,900</td>
</tr>
<tr>
<td><strong>All Sites</strong></td>
<td><strong>310,010</strong></td>
</tr>
</tbody>
</table>
Our PATHOLOGIC description of prostate cancer has NOT evolved
Prostate cancer is classified relative to therapy: Clinical states model (2000)

- Localized
- Rising PSA
- Clinical Metastases, Non-Castrate
- Clinical Metastases, Castrate

Death from prostate cancer

Adapted from HI Scher, Urology 2000
New targets and approaches in prostate cancer
Prostate cancer is STILL classified by therapy: Clinical states model (2014)

- Localized
  - Non-metastatic, castration-sensitive
  - Non-metastatic, castration-resistant

- Clinical metastases, castration-sensitive

- Clinical metastases, castration-resistant

- Clinical metastases docetaxel-resistant

- Clinical metastases cabazitaxel-resistant

- Clinical metastases abiraterone-resistant

- Clinical metastases enzalutamide-resistant

- Clinical metastases sipuleucel-T-resistant

- Clinical metastases Radium-223-resistant

Death from prostate cancer
Tissue is difficult to obtain in prostate cancer.
Circulating tumor cells (CTCs)- easily accessible prostate cancer cells
CTC Technologies

CTC Technologies: CellSearch (Janssen Diagnostics, LLC)

Frequency of CTCs in Healthy Controls vs Patients with Metastatic Breast (mBC), Colorectal (mCRC), and Prostate* (mPC) Cancers²

<table>
<thead>
<tr>
<th># of Cases</th>
<th>Healthy Controls</th>
<th>Benign Disease</th>
<th>Baseline 3-5 Weeks mBC Patients</th>
<th>Baseline 3-5 Weeks mCRC Patients</th>
<th>Baseline 3-5 Weeks mPC Patients</th>
</tr>
</thead>
<tbody>
<tr>
<td>N (%) ≥ 1 CTC</td>
<td>265</td>
<td>255</td>
<td>177</td>
<td>132</td>
<td>413</td>
</tr>
<tr>
<td>N (%) ≥ 2 CTC</td>
<td>10 (3%)</td>
<td>19 (7%)</td>
<td>125 (71%)</td>
<td>73 (55%)</td>
<td>196 (47%)</td>
</tr>
<tr>
<td>N (%) ≥ 3 CTC</td>
<td>0 (0%)</td>
<td>2 (0%)</td>
<td>108 (61%)</td>
<td>53 (40%)</td>
<td>138 (33%)</td>
</tr>
<tr>
<td>N (%) ≥ 4 CTC</td>
<td>0 (0%)</td>
<td>0 (0%)</td>
<td>102 (59%)</td>
<td>47 (36%)</td>
<td>108 (26%)</td>
</tr>
<tr>
<td>N (%) ≥ 5 CTC</td>
<td>0 (0%)</td>
<td>0 (0%)</td>
<td>94 (53%)</td>
<td>42 (32%)</td>
<td>90 (22%)</td>
</tr>
</tbody>
</table>
CTCs as a prognostic tool

Breast cancer

Colon cancer

Prostate cancer


Cohen, SJ. et al., *J Clin Oncol* 2008

de Bono, JS. et al., *Clin Cancer Res* 2008
Beyond Enumeration of CTCs
Could CTCs serve as a liquid biopsy?

**Androgen Receptor Signaling in Circulating Tumor Cells as a Marker of Hormonally Responsive Prostate Cancer**


**The Potential of Circulating Tumor Cells as a Liquid Biopsy to Guide Therapy in Prostate Cancer**

Klaus Pantel and Catherine Alix-Panabières

**Summary:** Miyamoto and colleagues present data that prostate-specific antigen/prostate-specific membrane antigen (PSA/PSMA)-based measurements of androgen receptor (AR) signaling in circulating tumor cells (CTC) enable real-time quantitative monitoring of intratumoral AR signaling. This finding indicates that measuring AR signaling within CTCs may help to guide therapy in metastatic prostate cancer and highlights the use of CTCs as liquid biopsy. *Cancer Discov.* 2(11): 974–5. © 2012 AACR.

Commentary on Miyamoto et al., p. 995 (6).
PSA/PSMA expression of CTCs relates to castration sensitivity
NanoVelcro CTC isolation technology
Automatic microscopic scanning for CTCs
NanoVelcro chip for enumeration in localized PCa patients

mCRPC patients

RP patients
NanoVelcro (NV) vs. CellSearch (CS): advanced mCRPC

CTC count (NV) vs. Study Day

CTC-NV (per 7.5 mL) vs. CTC-CS (per 7.5 mL)
Next generation sequencing in CTCs

Polymer-NanoVelcro CTC Chip
Laser Capture Microdissection
Multiple Displacement Amplification
Whole Genome Sequencing
DNA extraction and sequencing quality assessment

GC Content

Regional Depth
CTC genomic alteration landscape

- Confident mutations
- Mutations of low allele frequency
- No mutation
- Founder mutations
- Private mutations of metastasis

Confident mutations

No mutation

Mutations of low allele frequency

Private mutations of metastasis

CTC-A16
CTC-A9
CTC-U15
CTC-U17

Metastasis
Primary

OLIG2
BANP
FCGR1A
Shared rearrangements between primary, metastasis, and CTCs

Intergenic region

TMEM207

Intergenic region

TMEM207

Metastasis

Primary

CTC-A9

CTC-U15

CTC-U17
Structural variations in cancer-related genes: tumors and CTCs

Primary and Metastasis

CTCs

BRCA2

RB1

PTEN

WBC
RNA assessment from CTCs-qPCR

Cytokeratin

Expression (FPKM)

CD45

Expression (FPKM)
Prostate cancer detection and evaluation in 2014 has not evolved significantly

- Prostate cancer is only pathologically characterized at diagnosis
- The only characterizations used are morphologic (Gleason score) and clinic- NO MOLECULAR FEATURES
- Current experimental approaches involve painful tissue extractions such as bone marrow biopsies
Modernizing personalized medicine using circulating tumor cells

Detect (Relevant) Disease

Systemic Therapy

Molecular Analysis

Ex vivo modeling

Risk stratification & molecular tumor board

CTC “Liquid” Biopsy
Thank you

Posadas Lab
Murali Gururajan, PhD
Yi-Tsung “John” Lu, MD
Margaret Sievert, MS
Challen Lu, MS
Jake Licherterman
Andrew Hallum

Hsian-Rong Tseng, PhD (UCLA)
Bo Li, PhD

Leland W K Chung, PhD (CSMC)
Ruoxiang Wang, PhD
Haiyen Zhua, PhD

CSMC UOP Research Team
Amy Oppenheim
Jessica Hamman
Nancy Moldawer, RN, MSN
James MacDonald, RN, MSN

Edwin M. Posadas, MD FACP KM: 310-423-7600 Edwin.Posadas@csmc.edu