Next-generation sequencing of germline and somatic DNA in men with CRPC

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Disclosure Information

I am an employee of the University of Washington

I have no financial conflict of interest for any of the content I will discuss
Prostate Cancer

Basic Scientists

Oncologists and Urologists

Laboratory Medicine and Pathology

Industry
Emerging Model For mCRPC

Tumor and germline tissue evaluated

Therapy selected based on tumor and germline findings

Genetic counseling based on tumor and germline findings
Outline

• Tumor Mutations and Treatment

• Germline DNA Repair Gene Mutations

• Clinical Testing Panels
Landscape of Metastatic Disease

Adapted from Robinson, Van Allen et. al. (2015) *Cell*
## Emerging Precision Targets

<table>
<thead>
<tr>
<th>Mutation(s)</th>
<th>Metastatic Prostate Cancer Frequency</th>
<th>Potential Utility</th>
</tr>
</thead>
<tbody>
<tr>
<td>HR DNA Repair (e.g. BRCA1/2)</td>
<td>~20%</td>
<td>Platinum therapy, PARP inhibitors</td>
</tr>
<tr>
<td>Mismatch DNA Repair (e.g. MSH2)</td>
<td>~5%</td>
<td>Immunotherapy</td>
</tr>
<tr>
<td>Androgen Receptor</td>
<td>40-60%</td>
<td>Anti-androgens</td>
</tr>
<tr>
<td>PI3K Pathway</td>
<td>30-60% (PTEN)</td>
<td>PI3K inhibitors</td>
</tr>
<tr>
<td>\textit{BRAF} mutation/rearrangement</td>
<td>~3%</td>
<td>BRAF or MEK inhibitors</td>
</tr>
<tr>
<td>RSPO2 fusions</td>
<td>~3%</td>
<td>WNT inhibitors</td>
</tr>
</tbody>
</table>
HR DNA Repair Mutations in mCRPC

- 20% of mCRPC (30/150) cases with bi-allelic HR DNA repair gene inactivation

- About half 12/30 with germline first hit

Adapted from Robinson, Van Allen et. al. (2015) *Cell*
PARPi Responses: TOPARP-A Phase II Olaparib Trial

14/16 (88%) with bi-allelic DNA repair defects responded
2/33 (6%) without bi-allelic DNA repair defects responded

Mateo et al. (2015) NEJM PMID:26510020
Lynparza™ (olaparib) granted Breakthrough Therapy designation by US FDA for treatment of BRCA1/2 or ATM gene mutated metastatic Castration Resistant Prostate Cancer
Platinum Extreme Responders Have *BRCA2* Mutations

HR DNA Repair Mutations and AR Therapies?

• Conflicting data

• DNA repair deficient (n=18) had prolonged PFS in both arms of Abi vs. Abi+Velaparib
  – Hussain et al. J Clin Oncol 34, 2016 (suppl; abstr 5010)

• DNA repair deficient (n=22) had short PFS (3.3 months) on AR-targeted therapy
MMR Mutations in mCRPC

- UW Autopsy Series: 10/103 (9.7%)
- UW-OncoPlex Series: 6/98 (6.1%)
- SU2C/PCF Series: 4/150 (2.7%)
- Germline (Lynch) ~20% of cases

Pritchard et al. (2014) Nature Communications PMID:25255306
MSI in Prostate Cancer

![Graph showing MSI by BROCA on Prostate Cancer]

- **MSI POS**
- **NEG**

- **Fraction unstable loci by BROCA**

- **Hypermethylated**
- **Not Hypermethylated**
PD-1 Blockade in Tumors with Mismatch-Repair Deficiency

**Figure 1. Clinical Responses to Pembrolizumab Treatment.**

Le et al. (2015) *NEJM* PMID:26028255
• 3/10 mCRPC patients responded to pembrolizumub (PD-1 blockade)

• 1/2 patients with tumor tissue available had MSI
Hypermutilated CRPC Case at UW: Evidence of PD-1 inhibitor Response

Tumor: \textit{MSH6} mutation with LOH, MSI high

Thanks to Bruce Montgomery

Schweizer et al. (2016) Oncotarget PMID:27756888
Outline

• Tumor Mutations and Treatment

• Germline DNA Repair Gene Mutations

• Clinical Testing Panels
Germline Mutations

• *BRCA1*, *BRCA2*, and *HOXB13* mutations in a small proportion of familial cases
  – *BRCA2* mutations in 1.2-1.8% of prostate cancer overall

• Mutation frequency in metastatic disease much higher, not recognized until recently

• Germline mutations associated with more aggressive disease and worse cancer-specific survival
Distribution of Germline Mutations

11.8% (82/692) with deleterious germline mutations in 16 DNA repair genes

59% (36/61) with avail. tumors had second allele affected by loss-of-function mutation or copy loss

## Consistency Between Series

<table>
<thead>
<tr>
<th>Case Series</th>
<th>n</th>
<th>Mutated</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>SU2C/PCF Discovery</td>
<td>150</td>
<td>15</td>
<td>10.0%</td>
</tr>
<tr>
<td>SU2C/PCF Validation</td>
<td>84</td>
<td>9</td>
<td>10.7%</td>
</tr>
<tr>
<td>MSKCC</td>
<td>124</td>
<td>23</td>
<td>18.5%</td>
</tr>
<tr>
<td>Royal Marsden</td>
<td>131</td>
<td>16</td>
<td>12.2%</td>
</tr>
<tr>
<td>University of Washington</td>
<td>91</td>
<td>8</td>
<td>8.8%</td>
</tr>
<tr>
<td>Weill Cornell</td>
<td>69</td>
<td>7</td>
<td>10.1%</td>
</tr>
<tr>
<td>University of Michigan</td>
<td>43</td>
<td>4</td>
<td>9.3%</td>
</tr>
<tr>
<td><strong>Combined</strong></td>
<td>692</td>
<td>82</td>
<td>11.8%</td>
</tr>
</tbody>
</table>
**Vs. Primary PC and Population**

<table>
<thead>
<tr>
<th>Gene</th>
<th>Metastatic PC (n=692)</th>
<th>Primary PC (n=499)</th>
<th>ExAC (without TCGA)</th>
<th>Metastatic vs. Primary PC</th>
<th>Metastatic PC vs. ExAC</th>
</tr>
</thead>
<tbody>
<tr>
<td>ATM</td>
<td>1.59%</td>
<td>1.00%</td>
<td>0.25%</td>
<td>1.6 (0.8-2.8)</td>
<td>6.2 (3.1-11.1)</td>
</tr>
<tr>
<td>ATR</td>
<td>0.29%</td>
<td>0.00%</td>
<td>0.08%</td>
<td>-</td>
<td>3.7 (0.4-13.3)</td>
</tr>
<tr>
<td>BRCA1</td>
<td>0.87%</td>
<td>0.60%</td>
<td>0.27%</td>
<td>1.4 (0.5-3.1)</td>
<td>3.2 (1.2-6.9)</td>
</tr>
<tr>
<td>BRCA2</td>
<td>5.35%</td>
<td>0.20%</td>
<td>0.30%</td>
<td>26.7 (18.9-36.4)</td>
<td>17.6 (12.5-24.0)</td>
</tr>
<tr>
<td>BRIP1*</td>
<td>0.18%</td>
<td>0.20%</td>
<td>0.19%</td>
<td>-</td>
<td>0.9 (0.0-5.1)</td>
</tr>
<tr>
<td>CHEK2*</td>
<td>1.87%</td>
<td>0.40%</td>
<td>0.61%</td>
<td>4.7 (2.2-8.5)</td>
<td>3.1 (1.5-5.6)</td>
</tr>
<tr>
<td>FAM175A*</td>
<td>0.18%</td>
<td>0.00%</td>
<td>0.10%</td>
<td>-</td>
<td>1.8 (0.05-10.1)</td>
</tr>
<tr>
<td>GEN1*</td>
<td>0.46%</td>
<td>0.00%</td>
<td>0.08%</td>
<td>-</td>
<td>5.8 (0.7-20.7)</td>
</tr>
<tr>
<td>MRE11A</td>
<td>0.14%</td>
<td>0.20%</td>
<td>0.07%</td>
<td>0.7 (0.0-4.0)</td>
<td>2.1 (0.1-11.8)</td>
</tr>
<tr>
<td>MSH2</td>
<td>0.14%</td>
<td>0.20%</td>
<td>0.04%</td>
<td>0.7 (0.0-4.0)</td>
<td>3.5 (0.1-19.7)</td>
</tr>
<tr>
<td>MSH6</td>
<td>0.14%</td>
<td>0.20%</td>
<td>0.07%</td>
<td>0.7 (0.0-4.0)</td>
<td>2.0 (0.1-11.2)</td>
</tr>
<tr>
<td>NBN</td>
<td>0.29%</td>
<td>0.20%</td>
<td>0.11%</td>
<td>1.4 (0.2-5.2)</td>
<td>2.5 (0.3-9.1)</td>
</tr>
<tr>
<td>PALB2</td>
<td>0.43%</td>
<td>0.40%</td>
<td>0.11%</td>
<td>1.1 (0.2-3.1)</td>
<td>3.3 (0.7-9.7)</td>
</tr>
<tr>
<td>PMS2</td>
<td>0.29%</td>
<td>0.20%</td>
<td>0.11%</td>
<td>1.4 (0.2-5.2)</td>
<td>2.6 (0.3-9.4)</td>
</tr>
<tr>
<td>RAD51C</td>
<td>0.14%</td>
<td>0.40%</td>
<td>0.11%</td>
<td>0.4 (0.0-2.0)</td>
<td>1.3 (0.03-7.2)</td>
</tr>
<tr>
<td>RAD51D</td>
<td>0.43%</td>
<td>0.20%</td>
<td>0.08%</td>
<td>2.2 (0.4-6.3)</td>
<td>5.7 (1.2-16.7)</td>
</tr>
</tbody>
</table>

*Metastatic cases with inadequate sequencing for this gene are censored
Key Findings

• >> Frequency in mPC (11.8%) compared to TCGA (4.7%) and ExAC-TCGA (2.7%)

• No association with age >60 or race

• Association with family history of other cancers

• Marginal significant association with Gleason ≥8
# BRCA2, BRCA1, and ATM Germline Prevalence

<table>
<thead>
<tr>
<th>Gene</th>
<th>Pritchard 2016</th>
<th>Na 2016</th>
<th>Annala 2017</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>mPC (n=692)</td>
<td>mCRPC (n=313)</td>
<td>mCRPC (n=319)</td>
</tr>
<tr>
<td><strong>BRCA2</strong></td>
<td>5.3%</td>
<td>3.5%</td>
<td>5.0%</td>
</tr>
<tr>
<td><strong>BRCA1</strong></td>
<td>0.9%</td>
<td>0.6%</td>
<td>0.3%</td>
</tr>
<tr>
<td><strong>ATM</strong></td>
<td>1.6%</td>
<td>1.9%</td>
<td>0.3%</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Gene</th>
<th>Pritchard 2016</th>
<th>Na 2016</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>TCGA Localized* (n=499)</td>
<td>Low Risk Localized (n=486)</td>
</tr>
<tr>
<td><strong>BRCA2</strong></td>
<td>0.3%</td>
<td>0.8%</td>
</tr>
<tr>
<td><strong>BRCA1</strong></td>
<td>0.6%</td>
<td>0.4%</td>
</tr>
<tr>
<td><strong>ATM</strong></td>
<td>1.0%</td>
<td>0.4%</td>
</tr>
</tbody>
</table>

*TCGA is enriched for high-risk localized disease. 0 of 45 Gleason 6 patients in TCGA had a germline mutation in these 3 genes.
Risk Stratification

- Castro 2013 JCO (HR 1.8), Na 2016 Eur. Urol.: Worse OS (HR 2.1)

Castro et al. 2015 *Eur Urol*  
PMID:25454609

Na et al. 2016 *Eur Urol*  
PMID:27989354
Histologic Predictors?


- Germline HR DNA repair mutations in 2 of 9 patients with *ductal* histology (Schweizer 2016 Oncotarget)

![Papillary pattern of Ductal Adenocarcinoma]

Thanks to Larry True
Implications

- The frequency of germline DNA repair gene mutations in men with metastatic prostate cancer is prob. >10%.

- Men with mPC should be considered for genetic testing – regardless of age of onset or prostate cancer family history.

- Germline mutations may help identify families with dominant cancer predisposition syndromes.

- Germline mutations have implications for treatment selection (PARP inhibitors and platinum chemotherapy).
NCCN Guidelines 2.2017

• When To Test BRCA1/2: Genetics High Risk Committee

Personal history of prostate cancer (Gleason score ≥7) at any age with ≥1 close blood relative with ovarian carcinoma at any age or breast cancer ≤50 y or two relatives with breast, pancreatic, or prostate cancer (Gleason score ≥7) at any age

• How to Screen of BRCA1/2 Carriers Prostate Screening
  – At 45 begin screening BRCA2 carriers (previously rec. was 40)
  – Consider screening BRCA1 carriers
Outline

• Tumor Mutations and Treatment

• Germline DNA Repair Gene Mutations

• Clinical Testing Panels
Questions Every Clinician Should Ask the Genetics Lab

- Is there a lab director I can work with?
- Does the clinical lab have expertise in **both** germline and somatic cancer genetics?
- Whole genes captured?
- Copy number changes detected?
- LOH accurately detected and reported?
- MSI and hypermutation detected and reported?
- How are variants interpreted?
- Published validation study?
Cancer Mutation Panels

- **Hotspot Panels**
  - 1kb-200kb typical
  - Partial gene sequencing
  - Multiplex PCR-based enrichment
  - CNV/fusion detection uncommon

- **Comprehensive Sequencing Panels**
  - 200kb-2,000kb typical
  - Full gene sequencing
  - Capture-based enrichment
  - CNV/fusion detection common
## Sample Issues

<table>
<thead>
<tr>
<th></th>
<th>Fresh Tumor Tissue</th>
<th>Fixed Tumor Tissue</th>
<th>Plasma ctDNA</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Quality</strong></td>
<td>High</td>
<td>Moderate</td>
<td>Low</td>
</tr>
<tr>
<td><strong>Quantity</strong></td>
<td>High</td>
<td>Moderate</td>
<td>Very Low</td>
</tr>
<tr>
<td><strong>Tumor Content</strong></td>
<td>High (usually)</td>
<td>High (usually)</td>
<td>Low (usually)</td>
</tr>
<tr>
<td><strong>False Negatives</strong></td>
<td>Less common</td>
<td>Less common</td>
<td>More common</td>
</tr>
<tr>
<td><strong>False Positives</strong></td>
<td>Less common</td>
<td>Fixation artifact</td>
<td>Somatic clones in blood misinterpreted as cancer-derived</td>
</tr>
</tbody>
</table>
Clonal Hematopoiesis Interferes with Germline and ctDNA Testing

TP53, ASXL1, TET2, Many others

Treatment-Related Clones?

**JAMA Oncology**

**Somatic Mosaic Mutations in PPM1D and TP53 in the Blood of Women With Ovarian Carcinoma**

Elizabeth M. Swisher, MD; Maria I. Harrell, PhD; Barbara M. Norquist, MD; Tom Walsh, PhD; Mark Brady, PhD; Ming Lee, PhD; Robert Hershberg, MD, PhD; Kimberly R. Kalli, PhD; Heather Lankes, PhD; Eric Q. Konnick, MD, MS; Colin C. Pritchard, MD; Bradley J. Monk, MD; John K. Chan, MD; Robert Burger, MD; Scott H. Kaufmann, MD, PhD; Michael J. Birrer, MD, PhD

**JAMA Oncol. 2016;2(3):370-372**

**PPM1D Mosaic Truncating Variants in Ovarian Cancer Cases May Be Treatment-Related Somatic Mutations**


**JNCI J Natl Cancer Inst (2016) 108(3):**
### Reporting Considerations

#### ANALYTICAL
1. Types of mutations validated
2. Limits of detection
3. Pseudogenes
4. Platform-specific considerations

#### CLINICAL
1. Clinical context
2. Strategy for poorly characterized variants
3. Decision Support
4. Incidental findings
Somatic Variant Interpretation: AMP/ASCO/CAP

Li et al. JMD (2017) PMID:27993330
Germline Interpretation ≠ Somatic Interpretation

• 5-tiered schema (ACMG and IARC)

• Labs should have expertise in both germline and somatic variants

• Genetic counseling critical
Q: Is reporting a genomic sequencing assay more like making a histologic diagnosis (practice of medicine) or more like a reporting a sodium value (medical device)?
Clinical History Is Critical

• Similar diagnostic challenge to complex radiology or anatomic pathology testing

• Accurate interpretation and reporting requires specialized expertise and is the practice of medicine

• Clinical scenario determines how the data is interpreted and a diagnosis reached
Negative vs. Indeterminate

• Assessment of tumor content critical

• A ctDNA or tissue study without confirmed tumor is indeterminate, not negative
Summary

• DNA repair gene mutations are common in metastatic prostate cancer
  – ~20% HR DNA repair deficiency (1 in 2 germline)
  – ~2-10% MMR DNA repair deficiency (1 in 5 germline)
  – Strong implications for PARPi/platinum (HR) and checkpoint blockade immunotherapy (MMR)

• Germline mutations far more common than we thought in metastatic disease (10%+)
  – Implications for screening and risk stratification
Discussion Questions

• **Who to test?** Ethnicity? Family History? Age? Histology?

• **Tumor only, germline only, or both up-front?**
  Appropriate Counseling? Sample Source? Who orders?

• **Which genes?** (e.g. panel vs. \textit{BRCA1/2 ATM} only)

• **MSI/MMR screening?**

• **Counseling:** gene-specific estimates of cancer risk?
  When/how to start cancer screening of unaffected carriers?
  (Dr. Eeles, IMPACT coming up next…. )