Circulating Biomarkers in Metastatic Castration Resistant Prostate Cancer

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Chair, GU Disease Site Committee, NCIC CTG
Disclosures

• Consultant, Honoraria
  • Amgen, Astellas, Astra Zeneca, Bayer, Janssen, Lily, Millenium, Roche, Sanofi, Oncogenex (uncompensated)

• Grants, Research Support
  • Astellas, Exelixis, Janssen, Lily, Millennium, Novartis, Sanofi, Tokai, Oncogenex
Biomarker

• A characteristic that is objectively measured and evaluated as an indicator of normal biological processes, pathogenic processes, or pharmacologic responses to a therapeutic intervention.
Prognostic vs. Predictive Biomarkers

• Prognostic biomarkers
  – Provides information on course of the disease: survival
  – Many examples for CRPC:
    • Hemoglobin, LDH, alkaline phosphatase, PSA
    • Circulating tumour cell enumeration, bone turnover markers, circulating androgens

• Predictive biomarkers
  – Provides information on outcome to a particular treatment
  – Several candidates – none have been validated for clinical utility in CRPC
Molecular Predictive Biomarkers for CRPC

• Tissue is an issue
  – Primary prostate: Not representative of mCRPC
  – Metastatic biopsies: Invasive and difficult
  – “Liquid” biopsies: Technical issues, representative?

CTC: Veridex Platform

Prognostic

Response Indicator

Cox HR (95% CI) = 2.2 (1.9 - 2.6)
chi-square = 101.09
(p-value < 0.0001)

CTC: AdnaGen Platform

AdnaTest ProstateCancerSelect

AdnaTest ProstateCancerDetect

Base pairs

DNA-Ladder | Sample 1 | Sample 2 | Sample 3 | Sample 4 | Negative control (C-) | Positive Control (C+)
---|---|---|---|---|---|---
1500 - | 1500 - | 1500 - | 1500 - | 1500 - | 1500 - | 1500 -
1000 - | 1000 - | 1000 - | 1000 - | 1000 - | 1000 - | 1000 -
850 -  | 850 -  | 850 -  | 850 -  | 850 -  | 850 -  | 850 -
700 -   | 700 -   | 700 -   | 700 -   | 700 -   | 700 -   | 700 -
500 -   | 500 -   | 500 -   | 500 -   | 500 -   | 500 -   | 500 -
400 -   | 400 -   | 400 -   | 400 -   | 400 -   | 400 -   | 400 -
300 -   | 300 -   | 300 -   | 300 -   | 300 -   | 300 -   | 300 -
200 -   | 200 -   | 200 -   | 200 -   | 200 -   | 200 -   | 200 -
150 -   | 150 -   | 150 -   | 150 -   | 150 -   | 150 -   | 150 -
100 -   | 100 -   | 100 -   | 100 -   | 100 -   | 100 -   | 100 -
50 -    | 50 -    | 50 -    | 50 -    | 50 -    | 50 -    | 50 -

PSMA | PSA | EGFR | Actin
AdnaGen Platform to Detect AR-V7

CTC AR-V7 and Outcomes with ABI and ENZA

CTC AR-V7 and Outcomes with Docetaxel

ARV7 +: PSA RR 65%
ARV7 -: PSA RR 41%

ES Antonarakis et al, JAMA Oncol. 2015;1(5):582-591
CTC: Epic Sciences Platform

**Slide Preparation**

**Cell Staining**

**Scanning**

**Biomarker Analysis & CTC Identification**

**Single CTC Digital Pathology**

<table>
<thead>
<tr>
<th>Nuclear Features</th>
<th>Cytoplasmic Features</th>
<th>Cell Features</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nuclear Area</td>
<td>Cytoplasmic Area</td>
<td>AR Expression</td>
</tr>
<tr>
<td>Nuc. Convex Area</td>
<td>Cytoplasmic Area</td>
<td>CK Expression</td>
</tr>
<tr>
<td>Nuc. Major Axis</td>
<td>Cytoplasmic Area</td>
<td>N/C Ratio</td>
</tr>
<tr>
<td>Nuc. Minor Axis</td>
<td>Cytoplasmic Area</td>
<td></td>
</tr>
<tr>
<td>Nuclear Convexity</td>
<td>Cytoplasmic Convexity</td>
<td></td>
</tr>
<tr>
<td>Nuclear Solidity</td>
<td>Cytoplasmic Solidity</td>
<td></td>
</tr>
<tr>
<td>Nuclear Entropy</td>
<td>Cytoplasmic Entropy</td>
<td></td>
</tr>
<tr>
<td>Nuclear Spreading</td>
<td>Cytoplasmic Spreading</td>
<td></td>
</tr>
<tr>
<td>Nucleoli Presence</td>
<td>Cytoplasmic Presence</td>
<td></td>
</tr>
</tbody>
</table>

**Nucleus**

**CK**

**Biomarker**
CTC: Single Cell Genomics

- CTC Identified
- Coordinates recorded
- Slide treated
- Coordinates transferred Slide Mounted
- DNA Purified
- WGA Amplification
- Cells Lysed
- CTC transferred to PCR Plate
- CTC relocated & Picked
- DNA Product QC
- DNA Quantitation
- DNA Library Prep
- Library QC
- Illumina Sequencing

DNA yield QC report
Library yield QC Report

EPIC SCIENCES™
CTC: Single Cell Genomics

M. Landers et al, J Clin Oncol 33, 2015 (suppl; abstr 11035)
CTC AR-V7 and Treatment Outcomes

<table>
<thead>
<tr>
<th>AR Therapy (N=123)</th>
<th>Taxane Therapy (N=56)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>PSA Responder</td>
</tr>
<tr>
<td>AR-v7 Positive</td>
<td>0</td>
</tr>
<tr>
<td>AR-v7 Negative</td>
<td>57</td>
</tr>
<tr>
<td>Total</td>
<td>57</td>
</tr>
</tbody>
</table>

HR: 2.92 (95% CI: 1.63, 5.22)

HR: 11.44 (95% CI: 5.59, 23.44)

H. Scher et al, ESMO, 2015
Galeterone

**CYP17 Lyase Inhibitor**
- No mandatory steroids
- Fasting not required
- Preclinical activity in mutation T878A

**AR Antagonist**
- Not a GABA$_A$ antagonist
- No seizures
- Preclinical activity in mutation F876L

**AR Degrader**
- Active in C-terminal loss AR splice variants

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**Maximal PSA, %**

**M0 and M1 Treatment Naïve Arms**
Patients C-terminal loss on CTC (AR-V+)

M-E Taplin et al, ESMO, 2014
Galeterone: ARMOR-3 Study

- **mCRPC**
  - Treatment naïve
  - *Screen 1500 patients for AR-V7*
  - 148 to randomize

**Randomize**

- **n = 74**
- **Galeterone**
- **Enzalutamide**

**Primary:**
- rPFS

**Secondary:**
- OS
- Time to chemotherapy
- Time to 1st SRE
- PSA50 response
- Objective response
- ECOG PS Change
- CTC enumeration
- Safety
- QOL
- Pain

ClinicalTrials.gov: NCT02438007
EPI-001 and Analogues
(ESSA Pharmaceuticals)

RJ Andersen, Cancer Cell 17:535, 2010; M. Sadar, Cancer Res, 71:1208, 2011; ClinicalTrials.gov: NCT02606123
Targeting the AR DNA Binding Domain

Cell free DNA (cfDNA) and circulating tumour DNA (ctDNA)
- cfDNA: DNA found freely in the circulation
- Higher cfDNA concentration in cancer patients vs. healthy controls
- ctDNA
  - Highly degraded and present in only small amounts
  - Diluted: can constitute <1% - 90% of total cfDNA
- Genomic changes in ctDNA are detectable in mCRPC patients
  - CN gains/losses, mutations, rearrangements
  - Digital PCR, NGS

Plasma AR Sequencing and Outcomes with Abiraterone

Plasma AR Sequencing and Outcomes with Abiraterone

Overall survival

- Hazard ratio (log-rank): 7.33
- $P$ value: $1.2 \times 10^{-7}$
- 95% CI: 3.51–15.34
- Log-rank test: $P$ value $1.3 \times 10^{-9}$

Progression-free survival

- Hazard ratio (log-rank): 3.73
- $P$ value: $2 \times 10^{-6}$
- 95% CI: 2.17–6.41
- Log-rank test: $P$ value $5.6 \times 10^{-7}$

Patients with mCRPC commencing Enzalutamide (n=65) → Plasma collected at baseline, 12-weeks and/or progression → DNA extracted from plasma and WBC → cfDNA profiled with:
- aCGH
- Illumina MiSeq: AR exons 2-8
- Ion Ampliseq Custom Panel
→ AR and non-AR gene aberrations correlated with:
- PSA response (decline ≥50%)
- PFS (clinical +/- radiographic)

Extraction of cfDNA: 122/126 samples (97%)
aCGH: 117/122 samples (96%)
AR deep sequencing (mean 31,000X): 120/122 samples (98%)

KN Chi et al, Eur J Cancer, 51(S3):Abstract 2504, 2015
Genomic Profile of Baseline cfDNA: aCGH

<table>
<thead>
<tr>
<th>CN change</th>
<th>Frequency</th>
</tr>
</thead>
<tbody>
<tr>
<td>8p loss</td>
<td>24%</td>
</tr>
<tr>
<td>8q gain</td>
<td>33%</td>
</tr>
<tr>
<td>AR gain/amp</td>
<td>30%</td>
</tr>
<tr>
<td>MYC gain/amp</td>
<td>29%</td>
</tr>
<tr>
<td>RB1 loss</td>
<td>21%</td>
</tr>
<tr>
<td>MET gain/amp</td>
<td>13%</td>
</tr>
<tr>
<td>CCND1 gain/amp</td>
<td>10%</td>
</tr>
<tr>
<td>CCNE1 gain/amp</td>
<td>6%</td>
</tr>
</tbody>
</table>

KN Chi et al, Eur J Cancer, 51(S3):Abstract 2504, 2015
Genomic Profile of Baseline cfDNA: AR Sequencing

<table>
<thead>
<tr>
<th>AR mutation</th>
<th>Frequency</th>
</tr>
</thead>
<tbody>
<tr>
<td>Any</td>
<td>22%</td>
</tr>
<tr>
<td>L702H</td>
<td>10%</td>
</tr>
<tr>
<td>H875Y</td>
<td>10%</td>
</tr>
<tr>
<td>T878A</td>
<td>8%</td>
</tr>
<tr>
<td>W741L/C</td>
<td>6%</td>
</tr>
<tr>
<td>Other</td>
<td>2%</td>
</tr>
<tr>
<td>Multiple (≥ 2)</td>
<td>10%</td>
</tr>
</tbody>
</table>

All patients with L702H (glucocorticoid activated) and T878A (progesterone activated) mutations had received prior abiraterone acetate
Genomic Aberrations in Baseline cfDNA: Outcomes on Enzalutamide

<table>
<thead>
<tr>
<th>Variable</th>
<th>PSA Decline ≥ 50%</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>RB1 loss (yes vs. no)</td>
<td>8% vs. 46%</td>
<td>0.011</td>
</tr>
<tr>
<td>AR gain/amp (yes vs. no)</td>
<td>16% vs. 48%</td>
<td>0.017</td>
</tr>
<tr>
<td>MET gain/amp (yes vs. no)</td>
<td>0% vs. 44%</td>
<td>0.018</td>
</tr>
<tr>
<td>MYC gain/amp (yes vs. no)</td>
<td>22% vs. 44%</td>
<td>0.101</td>
</tr>
<tr>
<td>AR mutation (yes vs. no)</td>
<td>23% vs. 41%</td>
<td>0.239</td>
</tr>
</tbody>
</table>

KN Chi et al, Eur J Cancer, 51(S3):Abstract 2504, 2015
cfDNA at Progression

AR GAIN

AR MUTATIONS

PI3K/AKT

PARP Inhibitor

WNT PATHWAY

KN Chi et al, Eur J Cancer, 51(S3):Abstract 2504, 2015
Abiraterone vs. Enzalutamide Sequencing Study

A phase 2, randomized, multicenter study

**Whole Blood:**
- cfDNA Collection
- RNA Collection

**1ST LINE THERAPY**
- ARM A
  - ABIRATERONE + PREDNISONE
- ARM B
  - ENZALUTAMIDE

**2ND LINE THERAPY**
- ARM A
  - CROSS-OVER TO ENZALUTAMIDE
- ARM B
  - CROSS-OVER TO ABIRATERONE + PREDNISONE

CONTINUE UNTIL CLINICAL PROGRESSION

ClinicalTrials.gov: NCT02125357
Cabazitaxel vs. Abiraterone or Enzalutamide in Poor-Prospective mCRPC

OZM-054: A phase 2, randomized, multicenter study

CBZP: cabazitaxel; ENZA: enzalutamide
ClinicalTrials.gov: NCT02254785
Precision Medicine for CRPC

Plasma and whole blood from pts progressing on ABI and/or ENZA

- Gene copy number profiling and sequencing of cfDNA
- RT-PCR for AR splice variants

- RB1 loss → Docetaxel
- CCND1 gain/amp → CDK 4/6 inhibitor
- MET gain/amp → MET inhibitor
- MYC gain/amp → BET inhibitor
- Homologous Repair Pathway Defect → WEE1 inhibitor
- BRCA1/2 mutation → PARP inhibitor
- PIK3CA mutation → AKT/PI3K inhibitor
- AR splice variants AR mutants → Non-ligand binding domain AR inhibitors
Summary

• Analyses of CTC and cfDNA are promising, minimally-invasive means to molecularly characterize CRPC for predictive biomarkers
  – AR copy number gains, mutations and splice variants have been associated with poor clinical outcomes and resistance with our current AR targeted agents
  – Other informative genomic aberrations are detectable and potentially actionable
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