WELCOME!

PNW Prostate Cancer SPORE Retreat 2013

Friday, July 12, 2013
Fred Hutchinson Cancer Research Center,
M1-A303-307 (Arnold Building)
1100 Fairview Avenue North, Seattle, WA 98109
retreat [rɪˈtriːt]

vb (mainly intr)
1. (Military) Military to withdraw or retire in the face of or from action with an enemy, either due to defeat or in order to adopt a more favourable position
2. to retire or withdraw, as to seclusion or shelter
3. (Life Sciences & Allied Applications / Physiology) (of a person's features) to slope back; recede
4. (Group Games / Chess & Draughts) (tr) Chess to move (a piece) back

n
1. the act of retreating or withdrawing
2. (Military) Military
   a. a withdrawal or retirement in the face of the enemy
   b. a bugle call signifying withdrawal, esp to within a defended fortification
3. retirement or seclusion
4. a place, (sanatorium or monastery), to which one may retire for refuge, quiet, etc.
5. a period of seclusion, esp for religious contemplation
6. (Medicine) an institution, esp a private one, for the care and treatment of the mentally ill, infirm, elderly, etc.

[from Old French retret, from retraire to withdraw, from Latin retrahere to pull back; see retract]
Prostate Cancer
Collaborative Links

Canary Prostate Active Surveillance Study (PASS)

Canary Prostate Active Surveillance Study (PASS)
Data and bio-specimens collected at clinical sites

Stanford* UCSF* UBC* UW* UTHSCSA* VAPSHCS* BIDMC UM EVMS

De-identified clinical data and data from assays to DMCC

Data Management and Coordinating Center
Data repository, statistical center, coordinating center

Central Biospecimen Repository
Blood and urine specimens; prostate tissue may remain at sites

Specimens to Central Biospecimen Repository for storage and distribution

Assay data to DMCC for analysis

Third party not-for-profit study site
Collaborating assay site for study
Consortium** Assay site for study

Early Detection Research Network

CANARY FOUNDATION
Stopping Cancer Early - The Best Possible Investment
* Original site

**Any of the sites italics
Collaborative Links

The DOD Prostate Cancer Clinical Trials Consortium
Collaborative Links

SU2C-PCF AACR ‘Dream Teams’

Team 1: Precision Therapy for Advanced Prostate Cancer
- MSKCC
- DFCI/Broad/BIDMCC
- Michigan
- UW/FHCRC
- Royal Marsden/London
- Weill Cornell

Team 2: Targeting Adaptive Pathways in Metastatic CRPC
- UC San Francisco
- UC Los Angeles
- UC Santa Cruz
- UC Davis
- UBC
- OHSU
Purpose and Intent

Communicate ideas....
Purpose and Intent

Communicate ideas....
- *new concepts and next steps*
- *‘test the waters’*
- *needed resources?*
Purpose and Intent

Communicate ideas....
Provide feedback...
- reality check
- re-direct...extend the idea
- provide resources?
Purpose and Intent

Communicate ideas…
Provide feedback…
Develop (extend) collaborations…
10:00am: Welcome, Introductions and Agenda Review  (Pete Nelson)

10:10-11:10: Session I: Surveillance/QOL/ Diet/Risk/Biomarkers  (Discussion Leader: Bruce Montgomery)

1. Ruth Etzioni: Beyond observation: using modeling to understand recent high-profile studies of PC screening and treatment
2. John Gore: Patient-centered outcomes research in prostate cancer
3. Jonathan Wright: Hyperglycemia and prostate cancer outcomes
4. Marian Neuhouser: Update on vitamin D and prostate cancer
5. Alvin Liu: Prostate cancer biomarkers: secreted proteins and RNA signature
6. Janet Stanford: Genome-wide DNA methylation profiling to distinguish aggressive prostate cancer

11:10-12:10: Session II: Genetics/Genomics  (Discussion Leader: Paul Lange)

1. Colin Pritchard: Prostate cancer genomics and precision medicine
2. Colin Collins: Prostate cancer transdifferentiation in 5 minutes
3. Laura Heiser: Exploiting cell line model systems for the study of cancer
4. Jason Bielas: Clinical DNA mutagenesis
5. Robert Bradley: Androgen receptor splicing
6. Min Fang: Evaluation of FISH and methylomic markers of prostate cancer

LUNCH: 12:10 - 1:30pm

1:30-2:30pm: Session III: Androgen Receptor  (Discussion Leader: Martin Gleave)

1. Xuesen Dong: Alternative RNA splicing of the AR gene in prostate cancer
2. Joshi Alumkal: Emergent signaling pathways in enzalutamide-resistant prostate cancer
3. Paul Rennie: Inhibiting constitutively active androgen receptor variants with a new class of small molecules
4. Jennifer Bishop: Rational targeting of Her2/EGFR with Lapatinib to overcome Enzalutamide resistance
5. Elahe Mostaghel: Androgens in CRPC

2:30-3:30pm: Session IV: Therapy and Therapy Resistance-Beyond AR  (Discussion Leader: Tom Beer)

1. Chris Ong: Crosstalk between SEMA3C and AR pathways in treatment resistant prostate cancer
2. Evan Yu: Identification of molecular characteristics of PC with 11C-acetate and 18F-FDG PET/CT-directed rapid autopsy
3. Eva Corey: Cabozantinib and prostate cancer
4. Tia Higano: Beyond hormonal therapy
5. Kamal Chatta: The immune response in CRPC
6. David Qian: MDH2 lysine deacetylation mediates Docetaxel resistance

3:30: Break

3:40-4:50pm: Session V: Model Systems and Smorgasbord  (Discussion Leader: Robert Vessella)

1. Ming Lam: Disseminated tumor cell heterogeneity
3. Ralph Buttyan: Stems from non-stems
4. Lawrence True: Pathologic effects of androgen deprivation: volume of cancer & histological changes
5. YZ Wang: A different look at "hallmarks of cancer"
6. George Thomas: Update on circulating tumor cells
7. Colm Morrissey: The emergence and profile of the neuroendocrine phenotype in prostate cancer metastases

4:50-5:00 pm: Summary and Adjourn

5:15-6:15 pm Keynote Speaker – Pelton Auditorium
Steven Balk, MD, PhD, Professor of Medicine, Hematology-Oncology Division
Beth Israel Deaconess Medical Center / Harvard Medical School
"Androgen Receptor Functions in Prostate Cancer Development and Progression"

6:15-8:30pm: Dinner – Rooftop Patio in Arnold Building, 5th Floor
Beyond Observation: Using modeling to understand recent high-profile studies of PC screening and treatment

Leslie Mallinger
Rachel Hunter-Merrill
Ruth Etzioni
Roman Gulati
Jing Xia
A new study shows that prostate cancer surgery, which often leaves men impotent or incontinent, does not appear to save the lives of men with early-stage disease, who account for most cases, and many of these men would do just as well to choose no treatment at all. The findings were based on the largest-ever clinical trial comparing surgical removal of the prostate with a strategy known as “watchful waiting.” They add to growing concerns that prostate cancer detection and treatment efforts over the past 25 years, particularly in the United States, have been woefully misguided, rendering millions of men impotent, incontinent and saddled with fear about a disease that was unlikely ever to kill them in the first place.
PLCO and ERSPC

**PLCO**

- 0% mortality reduction
- 74% of controls screened during trial
- 40% compliance with biopsy
- 0 lives saved

Power: 15%

Chance of more deaths in screened group: 15%

**ERSPC**

- 21% mortality reduction
- Much less contamination
- 86% compliance with biopsy
- 1 life saved per 1000 screened

6 lives saved per 1000 screened in population setting

Gulati et al Cancer 2012
PIVOT and SPCG4

**PIVOT**
- Hazard ratio 0.63
- Absolute risk reduction 2.6%
- Fraction screen detected 75%

**SPCG4**
- Hazard ratio 0.65
- Absolute risk reduction 5.4%
- Fraction screen-detected 5%
Modeling PIVOT

• Start with SPCG4 results
• Add lead time and overdiagnosis to screen-detected fraction in PIVOT

Xia et al. JNCI 2013
Why are we doing this work?

Trials

EVIDENCE

MODELS

Policy

Etzioni et al Medical Care 2013
Patient-Centered Outcomes Research in Prostate Cancer

SPORE Retreat
July 12, 2013
John L. Gore, MD, MS, FACS
jlgor@uw.edu
PCOR in Prostate Cancer

- PCOR helps people and their caregivers communicate and make informed health care decisions.
- Research questions derived from patients or patient-partnered methods
Survivorship Care in Prostate Cancer

Quality of Life TRACKER

FILTER by
- AGES RANGE
  - <50
  - 50-59
  - 60-69
  - 70+

KEY
- Radical Prostatectomy
- External Beam Radiation Therapy
- Brachytherapy

Before Treatment

Urinary health comparison chart
Other PCOR Projects

- Development of patient-centered pathology reports
- Patient access to information about clinical trials in prostate cancer
Glucose Regulation and Prostate Cancer Outcomes

Jonathan Wright, MD
Is Glucose Regulation the link between obesity and adverse prostate cancer outcomes

• Obesity epidemic in US
  – Obesity associated PCa recurrence/progression

• Diabetes commonly co-exists with obesity
  – Mixed results for diabetes and PCa outcomes
  – Hyperglycemia is hallmark of diabetes mellitus
    • Metformin and weight loss both improve glucose levels
    • Mixed results for use of metformin and PCa outcomes

• Glucose and insulin are required for cancer cells
  – Preclinical studies of hyperglycemia and PCa growth
  – PCa cells from RP have greater insulin receptor staining than benign prostate cells cells
  – Other cancers (breast and colon) have reported hyperglycemia associated with higher risk of recurrence
PNW SPORE Funded Work

• **Prostate Cancer Diet Study**
  – Pilot study (19 men) based on Diabetes Prevention Trial
    • Pre-prostatectomy; low risk disease
  – **Intervention associated with**: weight loss, improved dietary constituents, improved insulin/glucose related parameters

• **VA Regional network database study**
  – ~ 1,700 patients treated for localized prostate cancer
  – Abnormal glucose (>100mg/dl) at diagnosis independently associated with risk of recurrence *(HR 1.5, 95% CI: 1.1-2.0)*

• **Tissue based study of insulin signaling in biopsies**
  – 100 men undergoing prostate biopsy at Seattle VA
  – Studing differences in insulin-receptor, IGF-1R, AKT, AMPK based on use of metformin
Prostate Active Lifestyle Study (PALS)

- Low to low-intermediate risk prostate cancer*
  - T1C/T2a, Gleason ≤ 3+4, PSA < 20
- BMI > 25 kg/m²
- Elect for, or presently on, active surveillance

**Randomize**

- 6-month Healthy Lifestyle Intervention (Diabetes Prevention Program)
- Controls: given standard diet/exercise recommendations

**Endpoints**

- Biomarkers of glucose regulation: glucose, HbA1C, insulin, c-peptide, IGF-1, IGF-BP3, adiponectin
- Maintenance of changes in serum biomarkers
- Insulin/IGF-1 receptor activation on prostate epithelial cells
- HRQOL
- Adverse pathologic features on surveillance biopsy

**Collaborators:**

- Marian Neuhouser, Janet Stanford, Dan Lin, John Gore, Steve Plymate, Ruth Etzioni, Jeannette Schenk, Mike Porter, Colm Morrissey, Xiotun Zhang, Fred Hutchinson Prevention Center
Prostate Cancer Biomarkers: Secreted proteins and RNA signature

Alvin Liu, PhD
Cancer AGR2/CD10 phenotypes and clinical outcomes

CD10$^{\text{low}}$ AGR2$^{\text{high}}$  
$n=6$

CD10$^{\text{low}}$ AGR2$^{\text{low}}$  
$n=8$

CD10$^{\text{high}}$ AGR2$^{\text{high}}$  
$n=13$

CD10$^{\text{high}}$ AGR2$^{\text{low}}$  
$n=36$

AGR2  
CD10
PRISM-SRM and ELISA protein biomarker measurement in urine and serum

**Urinary AGR2**

**Urinary cancer-associated proteins 12/14**

**Serum AGR2**
nanoString nCounter digital counts of RNA in urine

amplified urine RNA

CD24

CD75s
## Emerging Precision Targets

<table>
<thead>
<tr>
<th>Mutation(s)</th>
<th>Advanced Prostate Cancer Frequency</th>
<th>Utility</th>
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</thead>
<tbody>
<tr>
<td>DNA Repair Genes</td>
<td>~20%?</td>
<td>PARP inhibitors</td>
</tr>
<tr>
<td>PI3K Pathway</td>
<td>20-60% (PTEN)</td>
<td>PI3K inhibitors</td>
</tr>
<tr>
<td>AURKA/N-MYC</td>
<td>Common in Neuroendocrine</td>
<td>Aurora Kinase Inhibitors</td>
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</tbody>
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UW-OncoPlex Background

- Clinical assay designed to identify actionable alterations in tumors to guide therapy
- >850,000bp sequenced
- Implemented August 2012
  - Almost 300 cases reported to date
# UW-OncoPlex™ v3

<table>
<thead>
<tr>
<th>Tier 1: Currently actionable</th>
<th>Tier 2: Actionable in the near future</th>
<th>Tier 3: Frequently mutated</th>
<th>Germline pharmacogenomics</th>
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</thead>
<tbody>
<tr>
<td><strong>Genes Targeted:</strong> 200</td>
<td><strong>DNA Sequenced:</strong> &gt;900,000 bp</td>
<td><strong>&gt;500X Coverage</strong></td>
<td></td>
</tr>
</tbody>
</table>

**Tier 1:**
- ABL1
- BRAF
- DNMT3A
- IDH1
- MPL
- NNX2-1 ROS1
- ABL2
- CHEK1
- GNA11
- KDR
- MLH1
- PDGFRB
- TSC1
- APC
- EPHA3
- GATA1
- MYCl1
- RICTOR
- ABCB1
- CYP2D6
- FCGR2A
- NRPl
- UMPs

**Tier 2:**
- AURKA
- BAP1
- BCR
- BCL2L11
- AURKB
- BCR
- BCLB
- CBL
- CBOR
- CBL
- BCL
- CHEK1
- CHEK2
- GNAQ
- MAPK2
- MLH1
- PDKFB
- TSC1
- APC
- EPHA3
- GATA1
- MYCl1
- RICTOR
- ABCB1
- CYP2D6
- FCGR2A
- NRPl
- UMPs

**Tier 3:**
- ABL1
- BRAF
- DNMT3A
- IDH1
- MPL
- NNX2-1 ROS1
- ABL2
- CHEK1
- GNA11
- KDR
- MLH1
- PDGFRB
- TSC1
- APC
- EPHA3
- GATA1
- MYCl1
- RICTOR
- ABCB1
- CYP2D6
- FCGR2A
- NRPl
- UMPs

**Genes Targeted:** 200
**DNA Sequenced:** >900,000 bp

Colin Pritchard, UW Lab Medicine
Exploiting cell line model systems for the study of cancer

Laura M. Heiser
Oregon Health and Science University
Acquired resistance is associated with focal genomic and transcriptional changes

Cell line model of acquired resistance

Copy number aberrations

Chromosomal position

Copy number ratio

Transcriptional changes

Genes

with Wang, Grasso, Gray, Schiff
Integration across data types identifies putative drivers of resistance

ALPPL2 expression stratifies HER2+ patients
A network-based approach to understanding resistance mechanisms

Copy Number Changes
Differential Expression

Curated Pathways
KEGG, Biocarta,...

Outlined pathway modules could be targeted therapeutically (Drugbank.ca)
High-throughput validation of resistance-associated aberrations

Cells are grown on cell spot microarrays containing siRNAs and assayed for multiple response endpoints.

Rantala et al., BMC Genomics 12:162, 2011

Array elements contain siRNAs targeting pathways and/or microenvironment proteins.

~100 malignant or nonmalignant cells per array element.

Image based assessment of IF defined responses.

20,000 culture elements per array.

Growth inhibition following KD of DNA damage genes.
Cancers Clonally Accumulate Somatic Mutations

Predict speed of progression/resistance to therapy

Adapted from PNAS 105 (2008) 4283
Mutations as Cancer Biomarkers

Adapted from Nat Rev Cancer 5 845

Clonal mutations
Random mutations
CTC and ctDNA
mtDNA Mutation as Cancer Biomarkers

Colm Morrissey

Tumor

LCM-isolated cancer cells

mtDNA sequencing

Identify somatic mutations

mtDNA CTC/ctDNA to predict recurrence prostate and nDNA for early detection ovarian cancer

60% clonal mtDNA mutations in prostate cancer

Colm Morrissey

Jessica Bertout

mtDNA CTC/ctDNA to predict recurrence prostate and nDNA for early detection ovarian cancer

Buffy coat

Mitochondrial separation and mtDNA isolation

Dynabead magnetic mtDNA enrichment

DNA

Plasma

Genotypic-selection (restriction digestion)

Frequency of mutant ctmtDNA

Frequency of CTCs

Nolan Ericson

60% clonal mtDNA mutations in prostate cancer

mtDNA GAG ATC G T G ACC
Alternative splicing of the androgen receptor

Robert Bradley
Computational Biology Program, Public Health Sciences Division
Basic Sciences Division
Transcriptional regulation vs. splicing regulation

AR gene

NTD/DBD  \(\text{cryptic exon} \ (A)_n \ \text{LBD} \ (A)_n\)

Ligand-independent AR isoforms are associated with CRPC.

This association occurs across multiple time scales:
- (rapid) up-regulation following androgen deprivation
- (long-term) stable expression via acquisition of DNA rearrangements

Does regulated alternative splicing contribute to the acute androgen withdrawal response?
If AR splicing is regulated, then targeting the spliceosome may be productive.

Aberrant splicing is an important contributor to many solid and liquid cancers.

A core spliceosomal protein complex is a novel therapeutic target in glioblastoma:

(Hubert et al, 2013)
Splicing regulation may contribute to the acute androgen withdrawal response

Transcriptional up-regulation of AR is common following androgen depletion:

Splicing may be regulated in VCaP, but not 22Rv1:
Evaluation of FISH and methylomic markers of prostate cancer

Min Fang, MD, PhD
July 11th, 2013

Contributors:
Xiaoyu Qu, PhD, Yu Wu, PhD, Jerry Davison, PhD
SCCA FISH Technologists
Claudio Jeldres, MD, Chris Porter, MD
Vessella Lab, Nelson Lab
**FISH: TMPRSS2/ERG, AR/X and PTEN**

- **Cohort** (FFPE TMA- 4 sections per pt (3 cancer + 1 normal)
  - **Group 1**: 100 patients who did not relapse after 5 years following RRP;
  - **Group 2**: 100 patients who relapsed within 5 years following RRP

<table>
<thead>
<tr>
<th><strong>TMPRSS2/ERG</strong></th>
<th># of pts</th>
<th>%</th>
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<tbody>
<tr>
<td>Normal</td>
<td>72</td>
<td>46%</td>
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<tr>
<td>Typical fusion</td>
<td>37</td>
<td>23%</td>
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<tr>
<td>Atypical fusion</td>
<td>21</td>
<td>13%</td>
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<tr>
<td>Rearranged 3'ERG</td>
<td>9</td>
<td>6%</td>
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<tr>
<td>Rearranged 5'TMPRSS2</td>
<td>2</td>
<td>1%</td>
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<tr>
<td>Other Alternative rearrangements</td>
<td>5</td>
<td>3%</td>
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<tr>
<td>CNI**</td>
<td>12</td>
<td>8%</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>158</td>
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<table>
<thead>
<tr>
<th><strong>AR/CEPX</strong></th>
<th># of pts</th>
<th>%</th>
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</thead>
<tbody>
<tr>
<td>Gain of AR and CEPX, but no amplification</td>
<td>8</td>
<td>5%</td>
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<tr>
<td>Normal</td>
<td>163</td>
<td>95%</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>171</td>
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</table>

<table>
<thead>
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<th><strong>PTEN/CEP10</strong></th>
<th># of pts</th>
<th>%</th>
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<tr>
<td>Normal</td>
<td>121</td>
<td>74%</td>
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<tr>
<td>Heterozygous deletion**</td>
<td>19</td>
<td>12%</td>
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<tr>
<td>Homozygous deletion</td>
<td>7</td>
<td>4%</td>
</tr>
<tr>
<td>Monosomy 10</td>
<td>2</td>
<td>1%</td>
</tr>
<tr>
<td>Trisomy 10</td>
<td>15</td>
<td>9%</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>164</td>
<td></td>
</tr>
</tbody>
</table>

**HR: 0.13 P=0.022**
Identification of methylomic prognostic markers based on Gleason grade

CHARM: comprehensive high-throughput array-based relative methylation analysis
• 4.6 million total CpG sites across genome; highly quantitative for 100,000
• 43,982 gene loci, 4,500 control probes (30 genomic regions) for standardization
• Comprehensive statistical program built in R

Cohort: Age-matched; tumor cell content ≥75%.
• Group 1: low-risk with Gleason 3+3 and below. (n=8)
• Group 2: high-risk with Gleason 3+4 and above. (n=8)
• Group 3: normal prostate tissue or benign prostate lesions. (n=10)

Validation: by pyrosequencing quantitative analysis
• GS6 (n=20); GS7 (n=19); GS8/9 (n=14) Total n=53
• All 6 selected gene loci significant for Cancer vs. Normal
• 4 of 6 significant for GS6 vs GS7/8/9 adjusted for age
• ROC curve using the best marker is 0.86; adding the second best is 0.89.
Alternative RNA Splicing of the AR gene in Prostate Cancer Cells

Xuesen Dong

Assistant Professor, Vancouver Prostate Centre, Department of Urologic Sciences, University of British Columbia

I.  PCa cell models & the AR Gene Profiles
II. Alternative RNA splicing of AR-v7 is coupled with AR gene transcription & requires recruitments of hnRNP I, U2AF65 and ASF/SF2 to AR pre-mRNA

1) AR/DHT vs AR/MDV
2) Reversible regulation by AR signaling
3) ActD/DRB/TSA
4) Co-recruitment of pol II and splicing factors
5) Cells vs castration resistant VCaP tumors

6) AR-v7 minigenes locate ISE and ESE
7) RNA ChIP and RNA pulldown demonstrate pre-mRNA - splicing factor interactions
8) hnRNP I vs U2AF65 and ASF/SF2
**Seattle:**
Shihua Sun
Elahe Mostahel
Stephen Plymait

**Vancouver:**
Liangliang Liu (postdoc)
Ning Xie (technician)

**Research Funds:**
Prostate SPORE
CIHR
Prostate Cancer Canada

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### Diagram Description

**Seattle:**

**Vancouver:**

**Research Funds:**

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**Legend:**
- AD (Androgen-dependent)
- ADT (Androgen-deprived)
- CRPC (Castration-resistant prostate cancer)

**Graphs:**
- DHT and MDV effects over 6 days:
  - DHT: Increase in Fold change in OD 490nm.
  - MDV: Increase in Fold change in OD 490nm.

**Bar Charts:**
- PSA and UGT2B17 levels under different conditions:
  - Veh, DHT, MDV treatments.

**Gene Expression:**
- AR gene transcription
- AR function
- AR-v7 expression/function

**Additional Notes:**
- Adaptive survival
- Differentiation
- EMT
- Androgen phobic?
Emergent signaling pathways in enzalutamide-resistant prostate cancer

Joshi Alumkal

OHSU
Activitome: Comprehensive cellular pathway activity description

Network associated with resistance

Copy Number Changes

Differential Expression

Curated Pathways

KEGG
Biocarta

Slide courtesy of Laura Heiser
Enzalutamide Response in a Prostate Cancer Cell Line Panel

All cell lines have undergone RNA-seq, whole exome-seq

Jim Korkola

DOD Synergistic Idea Award (Alumkal-Korkola-Heiser)
“Co-Clinical” Trial

Functional RNAi and drug screening
Inhibiting constitutively active androgen receptor variants with a new class of small molecules

Paul Rennie, Ph.D.
Director, Laboratory Research, Vancouver Prostate Centre
Professor, Department of Urologic Sciences, UBC
**Inhibiting Constitutively Active Androgen Receptor Variants with a New Class of Small Molecules**

- AR-Variants lacking the C-terminal ligand binding domain (LBD) provide a strong rationale for the pursuit of new avenues of therapeutic intervention distinct from current antiandrogens targeting the LBD.

- Suitable binding sites on the AR DNA Binding Domain (DBD) subunit were evaluated using computer-aided screening analysis. Potential compounds (>300) that could disrupt the interaction between the AR DBD and DNA were identified and evaluated using *in-vitro* biological assays.

<table>
<thead>
<tr>
<th>ID</th>
<th>eGFP IC₅₀ (µM)</th>
<th>PSA IC₅₀ (µM)</th>
<th>DHT Displacement (at 50 µM)</th>
<th>BLI (LBD)</th>
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<tr>
<td>14257</td>
<td>0.112</td>
<td>0.079</td>
<td>weak</td>
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<td>0.223</td>
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<td>14103</td>
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<td>0.585</td>
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<td>14150</td>
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Inhibition of cell proliferation using compound VPC14228. LNCaP (Androgen sensitive cells); MR49F (enzalutamide-resistant cells); PC3 (no AR control cells).

Wild type AR and the AR V7 variant were transiently transfected into PC3 cells and treated with serial dilutions of compound VPC-14228 for 24 hours. The activity of each AR protein was then measured using a luciferase reporter under the control of an ARE promoter.
Point mutations can validate drug binding site

Point mutations were introduced in the AR DBD constructs at locations predicted to affect VPC-14228 binding. The mutated AR DBDs were transiently transfected in PC3 cells. After treatment with 10 µM 14228, the activity of each mutated AR DBD was measured using a luciferase reporter under the control of an ARE promoter. Mutations #2 and #4 affect the capability of 14228 to bind to the DBD as predicted.
Rational targeting of Her2/EGFR with Lapatinib to overcome Enzalutamide resistance

Jennifer Bishop, PhD
Vancouver Prostate Centre
EGFR/HER2 are targets for combination therapy
Lapatinib delays Enzalutamide resistance
Effective therapies target pathways of resistance & CRPC

- Combination therapies may target a variety of AR activators
  - Oncogenic pathways (AKT-ERK)
  - Molecular chaperones (Clusterin)
Androgens in CRPC

Elahe Mostaghel, M.D., Ph.D.
Associate Member, Clinical Research
Fred Hutchinson Cancer Research Center
Modeling Androgens in CRPC

1. Are there androgens in CRPC? Are they driving recurrence? Can we use them to predict response to therapy?

- **Delayed recurrence**
  - Recover basal DHT levels

- **More rapid recurrence**
  - Higher than basal DHT levels

- **Recurrence with very low DHT levels**

---

**Key:***
- T intact
- DHT intact
- T Cx
- DHT Cx
2. How do androgens in recurrent tumors influence other driver pathways? 

- AR splice variants, PTEN/PI3K, TMPRSS2:ERG

3. How will tumor androgens influence co-targeting strategies?

- Minimal castration response
  - Low DHT levels
  - AR splice variants
  - PTEN neg
  - ERG Fusion

- Minimal castration response
  - Persistent or higher DHT levels
  - AR H874Y

- Moderate castration response
  - Recover basal DHT levels
  - AR T877A, V715M
  - PTEN neg

Graphs showing changes in DHT levels over time with different genotypes and treatments.
Modeling Androgens in CRPC

4. How do other therapies influence tumor androgens? Is this relevant to their mechanisms of activity?

→ IGFR inhibitors, MDV, Docetaxel may impact tumor androgens

5. What will measuring androgens in patient tumor biopsies tell us?
Are they driving recurrence? Can we use them to predict response to therapy?
AR-splice variant transgenic mouse - epithelial and stromal interactions

Stephen R. Plymate, M.D.

University of Washington
AR variant ARv567es induces carcinogenesis in a novel transgenic mouse model of prostate cancer

Gang Liu, Cynthia Sprenger, Shihua Sun, Kathryn Soriano Epilepsia, Kathleen Haugk, Xiaotun Zhang, Ilsa Coleman, Peter S. Nelson, Stephen Plymate

Foci of hyperplasia could be seen in young adults (16 weeks), with a gradual progression to PIN lesions by 40 weeks.
Castration and sham operations performed on 50-week old mice. Three weeks post-castration, well-differentiated adenocarcinoma is seen in the sham-operated mice. More advanced progression to invasive adenocarcinoma is evident in the castrated group.
EMT markers were also examined. Loss of E-cadherin, and higher expression of Twist and Vimentin suggested an epithelial to mesenchymal transition (EMT).
Gene set enrichment analysis (GSEA) (Figure 5A and 5B) showed differentially regulated gene sets that included inflammatory related cytokines, transcriptional factors, and tumorigenesis-associated factors.

Our *Pb-ARv567es* mouse demonstrates that the distinct AR variant transcriptome is a common and direct molecular consequence when AR-Vs occur.
Identification of molecular characteristics of PC with 11C-acetate and 18F-FDG PET/CT-directed rapid autopsy

Evan Y. Yu, M.D.
TAN program, acknowledging Bob Vessella and many others in the room, and how it emphasizes difference in Gleason, PSA and CGA expression in bone mets. Thus, heterogeneity is proven and could have major impact.

Does it impact sensitivity and resistance to systemic therapy?

Are we really capturing all the heterogeneity? We know that PET can show it in bone mets.

And what exactly does PET tell us. Well it depends on the tracer. We believe FDG is glycolytic activity and uptakes better in lytic lesions due to more flow, fluoride is like a fancy bone scan for blastic bone remodeling and acetate/choline image lipid metabolism. But why are some lesions hot and others completely cold? And when uptake increases or decreases, what exactly is that telling us about the biologic manipulation of the tumor.
Case #1

PSA baseline when dasatinib added to nilutamide 316 rising to 954 after 6 cycles of therapy
CT and bone scan stable although bone scan was basically superscan at baseline.

There is a disconnect between PSA and fluoride PET imaging changes and this patient fell in the middle of the group in terms of response. But I want to emphasize the heterogeneity. Not all bone mets respond, so what is important? Currently, we have analyzed only a 15 cm FOV and we need to do entire torso analyses, but the strength of PET is that we can see what is happening at independent sites.

And I have other examples from my earlier acetate and FDG studies, but not enough time for the nuc med guys to pull pretty images.
The Proposal

**Methods**

- $^{11}$C-acetate (lipids), $^{18}$F-FDG (glucose and lytic) and $^{18}$F-fluoride (blastic) PET/CT obtained with last line of therapy in 5 patients with ≤2 months anticipated survival
  - Earmark various combinations on $^{11}$C-acetate and $^{18}$F-FDG PET e.g. hot/hot, hot/cold, cold/hot, cold/cold
- Consent patients for TAN protocol
- Each lesion studied in triplicate for:
  - IHC: PSA, CGA, SYN, AR, ARv
  - cDNA microarray
- Attempt xenograft formation with “hottest” lesion and “coldest” lesion

**Potential Gains**

- Better understanding of biology behind various PET modalities
  - May offer hints on best situations to utilize various PET radiotracer modalities
- PET offers “in vivo” insights on tumor heterogeneity that are difficult to study with metastatic biopsies or blood/urine biomarkers
- Goal for future to utilize PET for patient treatment selection, response and precision medicine
- Animal model formation from uniquely selected metastases to perform future drug testing

PET acetate/fluoride from IND going in now and protocol going into IRB and FDG 3rd party payor

The hottest lesion may be the most metabolically active and drug resistant lesions (will have all prior known therapies) and a completely cold lesion on PET should be studied to understand why

For instance, if we find out that FDG hot lesions don’t express as much AR and have AR gene signatures that are low, we might pair it’s use more with chemotherapy or visa versa

Metastatic biopsy is one lesion, blood/urine markers are whole body sum

It would be great if we could use PET for selection of treatment or pairing with specific drugs in the future
Cabozantinib and prostate cancer

Eva Corey, Ph.D.
University of Washington, Urology
**Cabozantinib and Prostate Cancer**

- Encouraging anti-tumor activity in metastatic castration-resistant patients with progressive disease
- Complete or partial resolution of lesions on bone scan in majority of subjects
- Evidence of tumor regression in soft tissue lesions in majority of subjects
**Cabozantinib Effects on PCa Growth in Bone**

LuCaP 23.1 (60 mg/kg)

C4-2B (60 mg/kg)

LuCaP 23.1 (30 mg/kg)

C4-2B Subcutaneous Tumors (60 mg/kg)

TuV

PSA

Weeks

Weeks

Weeks

Weeks
Prostate Immunology

- Antigen
- Optimal Dendritic cells – polarized DC
- Reversing immunosuppression (Tregs/Reg DCs)
- Effector CTL/NK cells

Gurkamal Chatta
Virginia Mason Medical Center
CRITIQUE/QUESTIONS (PROVENGE)

- STUDY DESIGN – Placebo arm and role of GM-CSF
- Dichotomy between overall survival and anti-tumor response
- Lack of correlation between survival and T cell response
- Higher antibody response to PAP-GMCSF vs PAP
- Nature of vaccine – Adoptive therapy vs Dendritic cell based
- Patient to patient variation: Each dose - 40 million cells expressing the costimulatory molecule CD54.
- COST – $100,000 for 4 months
Signal 1. (antigen): Specificity of response

Signal 2. (costimulatory molecules): Magnitude

Signal 3. (IL-12, cytokines, other): Effector functions

Signal 4. (IL-12, Vitamins A&D, other): Peripheral homing
Tumor

Th2 (undesirable; tumor-promoting)

Th1/CTL/NK cells (desirable; promote tumor elimination)

Signal 1. (antigen)
Signal 2. (costimulation)
Signal 3. (IL-12 family, IFNs, other factors)
Signal 4. (IL-12, Vit A, Vit D, other factors)

Th1

CTL

NK

1. high specificity
2. high magnitude
3. killer functions
4. homing to tumors

Th17 (unclear role; may promote tumor progression)

Tregs (undesirable; tumor-promoting)

Th2 (undesirable; tumor-promoting)

Ag-loaded DCs

1. high specificity
2. high magnitude
3. killer functions
4. homing to tumors
Maturation Status and Function of αDC1 is Not Affected by Loading with Apoptotic PCa Cells

Exposure of maturing DCs to apoptotic LNCap cells does not impair their mature status and high IL-12 producing capacity of αDC1s. **Left:** Phenotype. **Right:** High IL-12p70 producing capacity αDC1. αDC1s, sDCs (IL-1β/TNFα/IL-6/PGE2-matured DC), or immature DCs were stimulated with CD40L-transfected J558 cells for 24 h.
Disseminated Tumor Cell Heterogeneity

Hung-Ming Lam, PhD
GU Cancer Lab
University of Washington
PNW Prostate Cancer SPORE Retreat 2013

Funding: PCF Young Investigator Award, PNW Prostate Cancer SPORE Career Development Award
Single Disseminated Tumor Cell (DTC)

Prostate epithelial cell
Tumor epithelial cell
Motile tumor epithelial cell
Metastatic tumor cell

DTC Single Cell NED vs. ADV

EpCAM+/CD45- cell

NED: No evidence of disease, after >5 years of radical prostatectomy with an undetectable PSA
ADV: Advanced disease, radiographic bone metastasis or PSA relapse after primary curative therapy
Removing cells with Erythroid progenitor-like signature

<table>
<thead>
<tr>
<th>EpCAM intensity 2</th>
<th>3</th>
<th>4</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>N=95</strong></td>
<td><strong># DTC</strong></td>
<td><strong># patients</strong></td>
</tr>
<tr>
<td>NED</td>
<td>42</td>
<td>5</td>
</tr>
<tr>
<td>ADV</td>
<td>53</td>
<td>6</td>
</tr>
</tbody>
</table>

**Gene Expression Matrix**

- **EpCAM Intensity**
  - 2: Low
  - 3: Intermediate
  - 4: High

- **Groups**
  - ADV
  - NED

- **Patients**
  - 2613
  - 2665
  - 2675
  - 2676
  - 2677
  - 2678
  - 2679
  - 2680
  - 2687
  - 2690
  - 2695

- **EpCAM Intensity**
  - 2: included 45 cells
  - 3: included 45 cells
  - 4: omitted 50 cells

**Legend**
- **AHSP**
- **CA1**
- **HBA1**
- **HBA2**
- **HBB**
- **HBD**
- **LMO2**
- **MYB**
- **AR**
- **CD63**
- **FOLH1**
- **HOXB13**
- **ID1**
- **NKK3-1**
- **RELB**
- **XAGE1A**
Two populations of DTC in ADV patients

<table>
<thead>
<tr>
<th></th>
<th>NED</th>
<th>ADV_1</th>
<th>ADV_2</th>
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<tbody>
<tr>
<td>N=44</td>
<td></td>
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</tr>
<tr>
<td># DTC</td>
<td>7/42 (16%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td># patients</td>
<td>4/5</td>
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<td></td>
</tr>
<tr>
<td>NED</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ADV</td>
<td>37/53 (69%)</td>
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<tr>
<td></td>
<td>6/6</td>
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</table>

NED $\rightarrow$ ADV?
### Pathologic effects of androgen deprivation: Histological changes

#### Volume of cancer

<table>
<thead>
<tr>
<th>Benign</th>
<th>Benign</th>
<th>Benign</th>
</tr>
</thead>
<tbody>
<tr>
<td>Atrophy</td>
<td>Basal cell hyperplasia</td>
<td>Inflammation</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Coalescent corpora amylacea</td>
</tr>
</tbody>
</table>

- Basal cells
- Corpora amylacea

<table>
<thead>
<tr>
<th>Cancer</th>
<th>Cancer</th>
<th>Cancer</th>
</tr>
</thead>
<tbody>
<tr>
<td>Single inconspicuous cancer cells</td>
<td>Intraductal cancer</td>
<td>Spaces w/o cells</td>
</tr>
</tbody>
</table>

- duct
- Intraductal CA
- nerve
## Estimating volume of cancer
### Similar volume, different tumor cellularity

<table>
<thead>
<tr>
<th>No neoadjuvant</th>
<th>Abiraterone</th>
</tr>
</thead>
<tbody>
<tr>
<td><img src="image1" alt="Cancer region" /></td>
<td><img src="image2" alt="Cancer region" /></td>
</tr>
</tbody>
</table>

### Ways to estimate cellularity
- Visual estimate (as %)
- Stereological
- Image analysis (% AMACR)
- Count the cells

### Caveats (precision, accuracy, effort)
- **Quickest, low precision**
  - Novel (not tried)
  - ? effort, reproducibility (threshold, ROI)
  - Hi effort, Holmes effect (gold standard)
Estimates of tumor cellularity
Abiraterone trial, 4 pathologists

We are good at estimating low (<5%) cellularity cases.
We aren’t very precise at estimating higher (>10%) cellularity cases.
How precise should we be?

Holmes effect
The number of translucent objects (nuclei) increases with section thickness.
A Different Look at “Hallmarks of Cancer”

YZ Wang, Ph.D.
University of British Columbia
Vancouver Prostate Centre
Hallmarks of cancer:

- Considered fundamental to understanding cancer biology
- Number increased as our knowledge expanded

(Hanahan & Weinberg, 2011)
A proposed model for the central, regulatory immunosuppressive role of cancer-generated lactic acid

Cancer-generated lactic acid: a regulatory, immunosuppressive metabolite?

Stephen Yiu Chuen Choi, Colin C. Collins, Peter W. Gout, and Yuzhuo Wang
• **A Different Perspective:**
  
  – A hierarchy of hallmarks?
  – Cancer cells would actively generate fundamental hallmarks, from which the other hallmarks would arise
See the entire presentation of Steven Balk’s talk on the PNW Prostate Cancer SPORE website:

prostatespore.fhcrc.org/resources/video/#Balk