Development of New Treatment for Prostate Cancer

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Disclosure
Dr. Figg has no personal financial interest to disclose.

However, the NCI has licensed one of Dr. Figg’s patents to Celgene. Furthermore, the NCI has several CRADAs with pharmaceutical/biotech companies to support research in Dr. Figg’s laboratory. None of those data will be presented today.

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PESG and the NIH staff have no financial interest to disclose.

Learning Objectives
1. Describe the current treatment options for metastatic prostate cancer
2. Discuss the role of androgens and androgen transport plays in driving castration-resistant prostate cancer
3. Describe the regulation of OATP1B3 and the impact of genetic variations on androgen deprivation therapy
4. Describe the pathway from target identification, to screening for new agents, to preclinical evaluation and early phase clinical trials.

Prostate Cancer
• Leading non-cutaneous cancer for men
• 220,800 men in US will be diagnosed*
• 27,540 men will die of prostate cancer each year*
• 91% are diagnosed with localized disease
• Local treatment: Surgery or XRT
• 15% - 35% will develop biochemical recurrence

SEER estimate for 2015

Journal of Urology 1941
Prostate Cancer is Hormone-Dependent

- Prostate function depends on androgens
- Castration or estrogen led to glandular atrophy which could be reversed with androgens
- Patients with metastatic prostate cancer benefited from castration or estrogens

Clarence Hodges 1914-2001
Medical student in the Huggins Lab and co-author of the J Urology paper

Charles Huggins 1901-1997
Nobel Prize 1966 for that discovery

Disease Continuum in Prostate Cancer

- Time
- Asymptomatic
- Symptoms
- Non-Metastatic
- Metastatic
- Castration Sensitive
- Castration Resistant
- Radical/ XRT
- 2nd-Line Hormonal Therapy
- Abiraterone
- Enzalutamide
- Alpharadin
- Docetaxel
- Death
FDA Approvals - mCRPC Indications

First Line Treatment
- Docetaxel – 2004
  - Plus Prednisone
  - Plus Estramustine
- Sipuleucel-T – 2010
  - Asymptomatic or minimally symptomatic
  - Radium-223 – 2013
  - Symptomatic bone metastasis
- Abiraterone – 2013
  - Plus Prednisone
- Enzalutamide – 2014

Second Line Treatment
- Cabazitaxel – 2010
  - Plus Prednisone
- Abiraterone – 2011
  - Plus Prednisone
- Enzalutamide – 2012

TAX-327 – Docetaxel

Efficacy Endpoints

TAX-327 – Docetaxel

Safety and Tolerability

SWOG 99-16 – Docetaxel + Estramustine

Overall Survival

Overall Survival – Subgroup Analysis

TROPIC – Cabazitaxel + Prednisone

Overall Survival

Overall Survival – Subgroup Analysis
TROPIC – Adverse Events

De Bono JS, et al.
Lancet 2010;376:1147-54

Cholesterol
Pregnenolone
17α-OH-pregnenolone
17α-OH-progesterone
Progesterone
Aldosterone
Cortisol
Androstenedione
DHT
CYP17
Androstenediol
Testosterone
DHEA
HSD17B2/3
HSD3B1/2
SRD5A1/2/3
HSD3B1/2
CYP17
CYP17
CYP17
HSD17B2/3
CYPB1, CYP3A, CYP3A43
metabolites
CYP19
estradiol
CYP19
estone
3β-diol
3α-diol
3α-dG
HSD3B
Androstanedione
HSD17B
SRD5A1
Androsterone
HSD17B3
HSD17B2/3
HSD3B

Androgen Pathway and Genes Involved in Androgen Biosynthesis and Metabolism

COU-AA-301 – Abiraterone
Post-Chemotherapy Efficacy Endpoints

De Bono JS, et al.
NEJM 2011;364:1995-2005

COU-AA-301 – Abiraterone
Adverse Events

De Bono JS, et al.
NEJM 2011;364:1995-2005

COU-AA-302 – Abiraterone
1st Line

Ryan CJ, et al.
NEJM 2013;368(2):138-148

COU-AA-302 – Abiraterone
Adverse Events

Ryan CJ, et al.
NEJM 2013;368(2):138-148
Androgens Prostate Cancer

**AFFIRM – Enzalutamide (Post-Chemo)**

**AFFIRM – Enzalutamide Adverse Events**

<table>
<thead>
<tr>
<th>Common (≥ 25%)</th>
<th>Common (10-24%)</th>
<th>Rare (&lt;10%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fatigue</td>
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</tr>
<tr>
<td>Hot flush</td>
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</tr>
<tr>
<td>Nausea</td>
<td>Nausea</td>
<td>Nausea</td>
</tr>
<tr>
<td>Diarrhea</td>
<td>Diarrhea</td>
<td>Diarrhea</td>
</tr>
<tr>
<td>Infection</td>
<td>Infection</td>
<td>Infection</td>
</tr>
<tr>
<td>Infusion-site</td>
<td>Infusion-site</td>
<td>Infusion-site</td>
</tr>
<tr>
<td>Rash</td>
<td>Rash</td>
<td>Rash</td>
</tr>
<tr>
<td>Blood pressure</td>
<td>Blood pressure</td>
<td>Blood pressure</td>
</tr>
<tr>
<td>Dizziness</td>
<td>Dizziness</td>
<td>Dizziness</td>
</tr>
</tbody>
</table>

**PREVAIL – Enzalutamide 1st Line**

**PREVAIL – Enzalutamide Adverse Events**

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<th>Common (≥ 25%)</th>
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<tr>
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<td>Dizziness</td>
</tr>
</tbody>
</table>

**PREVAIL - Enzalutamide**

**Table 2: Summary of Adverse Events.**

<table>
<thead>
<tr>
<th>Variable</th>
<th>Enzalutamide (N=877)</th>
<th>Plutacid (N=395)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Median safety reporting period – mo</td>
<td>13.3 (13.6)</td>
<td>13.3 (13.6)</td>
</tr>
<tr>
<td>Any adverse event – no (%)</td>
<td>844 (98)</td>
<td>857 (90)</td>
</tr>
<tr>
<td>Any grade 4 adverse event – no (%)</td>
<td>174 (20)</td>
<td>183 (33)</td>
</tr>
<tr>
<td>Median time until grade 4 adverse event – mo</td>
<td>22.3 (13.9)</td>
<td>22.3 (13.9)</td>
</tr>
<tr>
<td>Any serious adverse event – no (%)</td>
<td>179 (20)</td>
<td>226 (33)</td>
</tr>
<tr>
<td>Any adverse event leading to treatment discontinuation – no (%)</td>
<td>49 (6)</td>
<td>52 (12)</td>
</tr>
<tr>
<td>Any adverse event leading to death – no (%)</td>
<td>37 (4)</td>
<td>32 (8)</td>
</tr>
</tbody>
</table>
Journal of Urology 1941
Prostate Cancer is Hormone-Dependent
- Prostate function depends on androgens
- Castration or estrogen led to glandular atrophy which could be reversed with androgens
- Patients with metastatic prostate cancer benefited from castration or estrogens

Clarence Hodges
1914-2001
Medical student in the Huggins Lab and co-author of the J Urology paper

Charles Hodges
1901-1997
Nobel Prize 1966 for that discovery

Charles Sawyers (Nature Med 2004):
Increased androgen receptor confers resistance to anti-androgen therapy

Failure to ADT was Thought to Result in Androgen Independent, Androgen Insensitive and Hormone Refractory Disease

For 80 years androgens were thought to enter cell only by passive diffusion

Passive diffusion

Organic Anion Transporting Polypeptides
- Family of 11 transporters
- Encoded by SLC genes
- Xenobiotics: >100 drugs comprising >15 classes are known to be transported by OATPs
OATP1B3 (formerly known as OATP8) is a Liver Protein Encoded by SLC01B3 (formerly known as SLC21A8)

OATP1B3 is "liver-specific"

Membrane Expression of OATP1B3 in the Liver

OATP1B3 antibody (H-52): sc-98981, which is a rabbit polyclonal antibody raised against amino acids 651 – 702 mapping at the C-terminus of OATP1B3 of human origin. The dilution we used was 1:100 and the antigen retrieval was performed using the steamer with sodium citrate buffer at pH 6.0.

OATP1B3 is a Liver Transporter

Expressed on basolateral membrane of hepatocyte Involved in "uptake" of substrates

SLCO1B3 Gene Coding Region Variants

<table>
<thead>
<tr>
<th>Nucleotide Change</th>
<th>AA Change</th>
<th>Exon</th>
</tr>
</thead>
<tbody>
<tr>
<td>69C&gt;T</td>
<td>Arg23Arg</td>
<td>1</td>
</tr>
<tr>
<td>334T&gt;G</td>
<td>Ser112Ala</td>
<td>3</td>
</tr>
<tr>
<td>439A&gt;G</td>
<td>Thr147Ala</td>
<td>4</td>
</tr>
<tr>
<td>699G&gt;A</td>
<td>Met233Ile</td>
<td>6</td>
</tr>
<tr>
<td>787T&gt;A</td>
<td>Arg253Arg</td>
<td>7</td>
</tr>
<tr>
<td>924A&gt;T</td>
<td>Thr308Thr</td>
<td>7</td>
</tr>
<tr>
<td>1587A&gt;G</td>
<td>Ala519Ala</td>
<td>11</td>
</tr>
<tr>
<td>1599A&gt;C</td>
<td>His520Pro</td>
<td>11</td>
</tr>
<tr>
<td>1593A&gt;G</td>
<td>Thr531Thr</td>
<td>11</td>
</tr>
<tr>
<td>1835T&gt;C</td>
<td>Val560Val</td>
<td>11</td>
</tr>
<tr>
<td>1832G&gt;A</td>
<td>Gly561Gly</td>
<td>13</td>
</tr>
<tr>
<td>1997G&gt;A</td>
<td>Ser659Ser</td>
<td>14</td>
</tr>
</tbody>
</table>

Polymorphisms in the OATP1B3 Protein

Two Major SNPs are in Complete Linkage Disequilibrium of 334T>G (exon 3 - Ser112Ala) and 699G>A (exon 6 - Met233Ile)

<table>
<thead>
<tr>
<th>Ethnic Groups</th>
<th>Haplotype</th>
<th>334T&gt;G</th>
<th>699G&gt;A</th>
<th>Frequency</th>
</tr>
</thead>
<tbody>
<tr>
<td>European Caucasian</td>
<td>1 G A</td>
<td>0.804</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>2 T G</td>
<td>0.159</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>3 T A</td>
<td>0.021</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>4 G G</td>
<td>0.005</td>
<td></td>
<td></td>
</tr>
<tr>
<td>American Caucasian</td>
<td>1 G G</td>
<td>0.875</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>2 T G</td>
<td>0.122</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Caucasians 97% to 99% linkage

Smith N et al. (Figg) Clin Pharmcol Ther 2007
Substrates of OATP1B3
- Bilirubin
- BQ-123
- Bromosulphophthalein
- Cholylcystokinin 8
- Dalterpin II
- Demethylhalothane
- Dextrorotatory sulfoxide
- Deltorphin II
- Demethylphalloin
- Dehydroepiandrosterone Sulfate
- Docetaxel
- Digoxin
- Monoglucuronosyl bilirubin
- Estradiol-17β-Glucuronide
- Estrone-3-sulfate
- Ouabain
- Paclitaxel
- Pitavastatin
- Rifampin
- Taurocholate
- T3 (Triiodothyronine)
- T4 (Thyroxin)

SNPs in SLC01B3 Effect on Pharmacokinetics
- Is there a difference in the PK of docetaxel between wild-type and variant? **NO**
- But we did see a difference in overall survival between the two groups (wild-type and variant)

**Is OATP1B3 Expressed in Prostate Tissue by Immunofluorescence?**

**Goat polyclonal OATP1B3 primary antibody**
Is there a difference in OATP1B3 SNP frequency between patients with cancer and normal volunteers? **NO**

In patients with prostate cancer, is there a difference in outcome between those with wild-type and those with variant?

**Clinically OATP1B3 Polymorphisms Affect Outcome**

Hamada et al. (Figg) Clin Cancer Res 2008

**Clinically OATP1B3 Polymorphisms Affect Outcome**

Hamada et al. (Figg) Clin Cancer Res 2008

Hamada et al. (Figg) Clin Cancer Res 2008

We next asked “Does OATP1B3 Transport Testosterone?”

- OATP1B3 polymorphisms are related to nearly two-fold increased uptake of testosterone with the 334T-allele vs. 334G-allele.
- Transient transfection cells

Sharifi et al. (Figg) Br J Urology 2008

Sharifi et al. (Figg) Br J Urology 2008
Korean Group replicated our initial findings in 2012 for Testosterone Uptake.

OATP1B3 in Prostate Cancer

Which Normal Tissues/Cancers Express SLCO1B3?

SLCO1B3 RNA is frequently expressed in normal hepatic tissues and testis.

SLCO1B3 RNA is frequently expressed in liver, pancreas, prostate and testicular cancer.
Which Normal Tissues/Cancers Express SLCO1B3?

SLCO1B3 RNA is occasionally expressed (less than 20%) in esophagus, lung, ovarian and bladder cancer.

Which Normal Tissues/Cancers Express SLCO1B3?

But prostate goes from no expression in normal tissue to more than 50% of the tumors expressing SLCO1B3.

What Regulates Expression?

Putative Hypoxia and Androgen Regulatory Elements located upstream of SLCO1B3.

SLCO1B3 Expression in Prostate Cancer Cell Lines

CRPC cell lines express ~100- to 1,000-fold more SLCO1B3 than do hormone sensitive prostate cancer cell lines.

SLCO1B3 Expression in Prostate Cancer Cell Lines

Cobalt chloride is a chemical inducer of hypoxia-like response.
Cobalt chloride had no affect on SLCO1B3.

Chetomin (CTM) has been shown by our group to block the interaction of HIF and p300 [Cook et al. (Figg) J Biol Chem 2009].
We hypothesized it would block the effect of hypoxia.
**SLCO1B3 Expression in Prostate Cancer Cell Lines**

Chetomin increased the expression of SLCO1B3 in the two hormone sensitive cell lines (LNCaP and 22Rv1)

Chetomin slightly decreased the expression of SLCO1B3 in the two CRPC cell lines (PC3 and DU145)

---

**Is OATP1B3 and HIF1α Colocalized in Prostate Tissue?**

The answer is yes based on IHC staining

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**What Regulates Expression?**

So we are still trying to understand what regulates OATP1B3 - androgens don’t seem to play a role; however, p300 seems to play a role (and HIF to a lesser extent)

---

**Androgen Pathway and Genes Involved in Androgen Biosynthesis and Metabolism**

- Cholesterol
- Pregnenolone
- 17α-OH-pregnenolone
- 17α-OH-progesterone
- Progesterone
- Aldosterone
- Cortisol
- Androstenedione
- DHT
- Androgen Pathway and Genes Involved in Androgen Biosynthesis and Metabolism

- CYP17
- Androstenediol
- Testosterone
- DHEA
- HSD17B2/3
- HSD3B1/2
- SRD5A1/2/3
- CYP3A, CYP19
- estradiol
- 3β-diol
- 3α-dG
- Androgen Pathway and Genes Involved in Androgen Biosynthesis and Metabolism
Androgen Uptake in OATP1B3 Expressing CHO Cells

<table>
<thead>
<tr>
<th>Androgen</th>
<th>Km (mM)</th>
<th>Vmax (pmol/min/mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Testosterone</td>
<td>36.0 ± 8.586</td>
<td>166.8 ± 12.11</td>
</tr>
<tr>
<td>Dihydrotestosterone</td>
<td>31.1 ± 4.996</td>
<td>108.8 ± 26.53</td>
</tr>
<tr>
<td>Androstenedione</td>
<td>58.2 ± 10.04</td>
<td>108.8 ± 26.53</td>
</tr>
</tbody>
</table>

Km is the concentration that yields half-maximal velocity and Vmax is the maximum velocity in Chinese hamster ovary cells.

Using the Knowledge that Chetomin Upregulates OATP1B3 in LNCaP Cells

3H-Testosterone Uptake in LNCaP Cells

Chetomin increased Testosterone uptake at 30 min and 60 min.

Does Testosterone Affect Cell Proliferation in OATP1B3 Expressing Cells?

Testosterone Plasma Concentration in OATP1B3 Knock-Out/Knock-In Mice

Higher AUC in KO: 112 vs 96 hr*nmol/L; p=0.00048
Liver Testosterone Concentration is Higher in OATP1B3 Knock-In Mice

Preliminary

Ursolic Acid

- Steroid hormone backbone structure (triterpene)
- Peels of fruits (e.g., apples) and in herbs (e.g., rosemary)
- 1449 PubMed cites, many describing biochemical effects:
  - May have a role in the PK interactions with grapefruit juice and CYP3A4 substrates. CYP3A4 inhibition <10μM
  - Tumor apoptosis
  - Aromatase inhibition
  - Inhibition of cell signaling

Inhibition of Uptake with Ursolic Acid in OATP1B3 Expressing CHO Cells

Testosterone Dihydrotestosterone Androstenedione

K_i (μM)

Androstenedione 8.60±0.1
Dihydrotestosterone 8.40±0.1
Testosterone 8.40±0.1

K_i is the inhibition dissociation constant in Chinese hamster ovary cells

3H-Testosterone Uptake in LNCaP Cells

Ursolic Acid inhibited Testosterone uptake at 30 min and 60 min

siRNA for SLC01B3 decreased 3H-Testosterone Uptake in DU145 Cells
Eovist

- Gadoxetate disodium (Eovist, Bayer) is an MRI imaging agent which is FDA-approved for detecting hepatocellular carcinoma (HCC)
- Normal hepatocytes express OATP1B3 while some HCC lesions do not
- Those HCCs that do take up Eovist have been shown to express OATP1B3
We asked Could Eovist help elucidate OATP1B3 clinical function in patients with prostate cancer?

Eovist imaging trial is currently accruing (n=19 enrolled)
- Image 10 patients with localized disease
- Image 10 patients with metastatic CRPC patients
- All patients required biopsy of tumor and OATP1B3 IHC staining

Eovist Patient #2: 72 y/o male with localized prostate cancer
PSA 18.41, Gleason 4+4=8, clinical T2a

Numerous blastic bony metastases
Left iliac bone lesion shows no 18F-NaF uptake by PET/CT

Baseline T1W MRI
60 minute post-Eovist injection T1W MRI
Left iliac lesion shows delayed phase Eovist uptake
Is that lesion a lytic lesion rather than a blastic lesion?

Left iliac bony lesion was biopsied under CT guidance
- result was metastatic poorly-differentiated carcinoma consistent with prostatic adenocarcinoma

Eovist Patient #15 - 70yo with CRPC
Baseline T1W MRI
60 minute post-Eovist injection T1W MRI
Left iliac lesion shows delayed phase Eovist uptake
Is that lesion a lytic lesion rather than a blastic lesion?

Left iliac bony lesion was biopsied under CT guidance
- result was metastatic poorly-differentiated carcinoma consistent with prostatic adenocarcinoma

These data support that OATP1B3 is expressed clinically in prostate cancer and is a functional transporter in patients
Why has our Triple Therapy Regimen Been More Active than some Combination Regimens in CRPC

<table>
<thead>
<tr>
<th>Trial</th>
<th>Treatment</th>
<th>OS (mo)</th>
</tr>
</thead>
<tbody>
<tr>
<td>NCI</td>
<td>Thalidomide</td>
<td>18.0</td>
</tr>
<tr>
<td>Tannock</td>
<td>Docetaxel-P</td>
<td>18.9</td>
</tr>
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</tr>
</thead>
<tbody>
<tr>
<td>MAINSAIL</td>
<td>Docetaxel-P/Lenalidomide</td>
<td>17.7</td>
</tr>
<tr>
<td>NCI</td>
<td>Thalidomide</td>
<td>18.0</td>
</tr>
<tr>
<td>Tannock</td>
<td>Docetaxel-P</td>
<td>18.9</td>
</tr>
<tr>
<td>VENICE</td>
<td>Docetaxel-P/Aflibercept</td>
<td>22.1</td>
</tr>
<tr>
<td>CALGB</td>
<td>Docetaxel-P/Bevacizumab</td>
<td>22.6</td>
</tr>
</tbody>
</table>

Selected testosterone-related mechanisms for maintaining AR signaling during ADT

A. T (from testes) → AR
B. T (low conc.) → AR
C. T (low conc.) → AR

AR-mutation confers androgen promiscuity
AR-overexpression confers hyperactivity during ADT

D. T (from tumor) → AR
E. Increased intratumoral androgen production

Testosterone scavenging mechanisms (e.g., thru OATP1B3 and OATP2B1)

Acknowledgements for OATP Project

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- Wooin Lee

Univ. of Pisa
- Romano Danesi

VCU
- Akinobu Hamada

Univ. of Pisa
- Romano Danesi

* Left the NCI

Lab of Cell Biology, NCI
- Lab of Cell Biology, NCI
- Lab of Cell Biology, NCI
- Lab of Cell Biology, NCI
- Lab of Cell Biology, NCI

* Left the NCI
Disclosure

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Thank You!

Questions

[Image of a chimpanzee]