OVER SEVENTY YEARS OF TARGETING THE ANDROGEN RECEPTOR IN PROSTATE CANCER

Vivek K. Arora
Division of Medical Oncology
Washington University School of Medicine
Inhibition of androgen receptor (AR) signaling in prostate cancer

AR

Androgen receptor

dihydrotestosterone hormone

Surgical Castration
GNRH analogs

Anti-androgens
Bicalutamide
Flutamide

CoR vs Coactivators
NCoR/HDAC

Pol II

Transcription of AR target genes eg PSA
Activation of TMPRSS/ERG fusion
Foundations of Castration Therapy

W. White reported atrophy of prostate glands and decreased prostate weight in dogs following castration.

Clyde Deming reported that castration of primates decreased size of prostate gland.

Ethel Gutman and Alexander Gutman reported increases in serum phosphatase activity in patients with metastatic prostate cancer.

Huggins found that castration or oestrogen treatment of dogs resulted in shrinkage of prostate and decreased acid phosphatase activity in serum.
Clinical Benefit of Castration Therapy in Metastatic Prostate Cancer

Studies on Prostatic Cancer

I. The Effect of Castration, of Estrogen and of Androgen Injection on Serum Phosphatases in Metastatic Carcinoma of the Prostate*

Charles Huggins, M.D., and Clarence V. Hodges, M.D.

(From the Department of Surgery, the University of Chicago, Chicago, Illinois)

Cancer Res 1941;1:293-297

Charles Huggins

1966 – ½ nobel prize “for his discoveries concerning hormonal treatment of prostatic cancer”

• No trial ever established a survival benefit of castration therapy in prostate cancer
• 90% of patients initially respond to ADT
• Duration of Response 1-2 years on average
Incomplete Androgen Ablation with Castration

“Much proof has been adduced that androgen is produced in extragonadal loci especially in the suprarenal gland. The most ready explanation for variations in serum acid phosphatase levels following Orchiectomy as well as the failure of some values to reach the normal range may be postulated by assuming varying amounts or activity of androgens produced in extragonadal sources in different individuals.”

– 1941 Huggins and Hodges

Nature Reviews Cancer 2015 (Watson, Arora, & Sawyers)
BILATERAL ADRENALECTOMY IN PROSTATIC CANCER

CLINICAL FEATURES AND URINARY EXCRETION
OF 17-KETOSTEROIDS AND ESTROGEN*

CHARLES HUGGINS, M.D., AND WILLIAM WALLACE SCOTT, M.D.
CHICAGO, ILL.

FROM THE DEPARTMENT OF SURGERY, UNIVERSITY OF CHICAGO, CHICAGO, ILL

Fig. 3.—Decrease of total urinary 17-ketosteroids after adrenalectomy, Case 3.

Management post-adrenalectomy proved too difficult for common use.
1981: GnRH analogous used to treat prostate cancer in the clinic.

The Nobel Prize in Physiology or Medicine 1977 was divided, one half jointly to Roger Guillemin and Andrew V. Schally "for their discoveries concerning the peptide hormone production of the brain".

Nature Reviews Cancer 2015 (Watson, Arora, & Sawyers)
Ketoconazole found to block steroidogenesis


*Ketoconazole at adrenal inhibitory doses has high toxicity*
Biochemical and Molecular Identification of AR


First generation non-steroidal anti-androgens

- Flutamide
- Bicalutamide
- Nilutamide

*Nature Reviews Cancer 2015 (Watson, Arora, & Sawyers)*
# Strategy: Combined Androgen Deprivation (CAD)

<table>
<thead>
<tr>
<th>Author</th>
<th>Treatment</th>
<th>n</th>
<th>PFS (months)</th>
<th>OS (months)</th>
<th>p</th>
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<tr>
<td>Crawford,</td>
<td>Leuprolide + placebo</td>
<td>300</td>
<td>13.9</td>
<td>28.3</td>
<td>0.03 (PFS)</td>
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<tr>
<td>1989</td>
<td>Leuprolide + flutamide</td>
<td>303</td>
<td>16.5</td>
<td>35.6</td>
<td>0.03 (OS)</td>
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<tr>
<td>Keuppens,</td>
<td>Orchidectomy</td>
<td>163</td>
<td>Diff (subj) 8.1</td>
<td>Diff (MS) 7</td>
<td>0.009 (PFS)</td>
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<tr>
<td>1993</td>
<td>Goserelin + flutamide</td>
<td>161</td>
<td>Diff (obj) 11.0</td>
<td>Diff (PCa-s) 15</td>
<td>0.05 (OS)</td>
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<td>Tyrrell,</td>
<td>Goserelin</td>
<td>282</td>
<td>NR</td>
<td>37.7</td>
<td>0.08 (PFS)</td>
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<td>1993</td>
<td>Goserelin + flutamide</td>
<td>287</td>
<td>NR</td>
<td>42.4</td>
<td>0.14 (OS)</td>
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<tr>
<td>Eisenberger</td>
<td>Orchidectomy + placebo</td>
<td>687</td>
<td>18.6</td>
<td>29.9</td>
<td>0.26 (PFS)</td>
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<tr>
<td>1998</td>
<td>Orchidectomy + flutamide</td>
<td>700</td>
<td>20.4</td>
<td>33.5</td>
<td>0.16 (OS)</td>
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</table>

3 meta-analyses: a 3%–5% overall survival advantage of CAD vs ADT that is statistically significant with non steroidal anti-androgens

NR, not recorded; PFS: progression-free survival; MS, median survival

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Presented by: Maha Hussain, MD, FACP, FASCO
First generation non-steroidal anti-androgens

- Flutamide
- Bicalutamide
- Nilutamide

Use in general practice mixed due to small and equivocal benefit

*Nature Reviews Cancer 2015 (Watson, Arora, & Sawyers)*
Time

Disease Burden

Castration-Sensitive

Hormone Independent Prostate Cancer?
Castration Resistant Prostate Cancer?

What are the molecular drivers?
Androgen receptor is consistently overexpressed in castration-resistant tumors

<table>
<thead>
<tr>
<th>Gene Name</th>
<th>Acc. No.</th>
<th>CWR22</th>
<th>LUCaP23</th>
<th>LUCaP35</th>
<th>LUCaP41</th>
<th>LNCaP</th>
<th>LAPC4</th>
<th>LAPC9</th>
<th>Score</th>
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<tr>
<td>Androgen receptor</td>
<td>M23263</td>
<td>2.5 (I)</td>
<td>3.1 (I)</td>
<td>3.1 (I)</td>
<td>3.6 (I)</td>
<td>2.7 (I)</td>
<td>2.3 (I)</td>
<td>~2.2 (I)</td>
<td>7</td>
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<tr>
<td>Androgen receptor</td>
<td>M23263</td>
<td>2 (I)</td>
<td>3.1 (I)</td>
<td>2.7 (I)</td>
<td>6 (I)</td>
<td>2 (I)</td>
<td>1.3 (NC)</td>
<td>~1.4 (NC)</td>
<td>5</td>
</tr>
<tr>
<td>Tetraspan NET-1</td>
<td>AF065388</td>
<td>2.7 (I)</td>
<td>-1.1 (NC)</td>
<td>-1.3 (NC)</td>
<td>1.4 (MI)</td>
<td>~8.7 (D)</td>
<td>3.1 (I)</td>
<td>1.4 (I)</td>
<td>4</td>
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<tr>
<td>carbonic anhydrase precursor (CA 12)</td>
<td>AF037335</td>
<td>~1.8* (I)</td>
<td>-1.4 (NC)</td>
<td>-1.2 (NC)</td>
<td>4.7 (I)</td>
<td>~3.7 (I)</td>
<td>~1.1 (NC)</td>
<td>~1.5 (I)</td>
<td>4</td>
</tr>
<tr>
<td>MAR/SAR DNA binding protein (SATB1)</td>
<td>M97287</td>
<td>~2.0 (D)</td>
<td>3 (I)</td>
<td>~7.0 (I)</td>
<td>~6.1 (I)</td>
<td>~1.2 (NC)</td>
<td>~1.4 (NC)</td>
<td>~1.7 (I)</td>
<td>4</td>
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<tr>
<td>Elongation factor 1 alpha-2</td>
<td>X70940</td>
<td>-1.1 (I)</td>
<td>-1.2 (NC)</td>
<td>1.7 (I)</td>
<td>1.1 (I)</td>
<td>1 (NC)</td>
<td>1.3 (I)</td>
<td>-1.3 (NC)</td>
<td>4</td>
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<tr>
<td>HLA class I locus C heavy chain</td>
<td>X58536</td>
<td>1.9 (I)</td>
<td>1.1 (NC)</td>
<td>1.1 (NC)</td>
<td>2.5 (I)</td>
<td>1.7 (I)</td>
<td>1.1 (NC)</td>
<td>~4.4 (I)</td>
<td>4</td>
</tr>
</tbody>
</table>

Genes up-regulated in AI tumors

Chen et al Nature Med, 2004
Androgen receptor is consistently overexpressed in castration-resistant tumors

Chen et al, Nature Med, 2004
AR over-expression is sufficient to drive tumor growth in castrate settings

LAPC4 in castrate mice

LnCAP in castrate mice

Chen et al. Nature Med, 2004
AR expression is necessary for optimal growth of castration resistant tumors
AR over-expression reveals agonism of bicalutamide: evidence for cross-resistance

Chen et al. Nature Med, 2004
AR amplification and over-expression in >50% of human CRPC

**AR Gene Quantification (FISH)**
- ● Primary Ca
- ○ CRPC (local recurrence)

16/23 CRPC samples with AR copy gain

**AR mRNA (Expression Array)**

**Data Mined from Grasso et. al. Nature 2012**

Adapted from Visakorpi et. al. Nature 1995
Castration or bicalutamide resistance = AR independence

1. AR amplification
Persistent Intraprostatic Androgen Concentrations after Medical Castration in Healthy Men

The Journal of Clinical Endocrinology & Metabolism 91(10):3850 – 3856
Castration or bicalutamide resistance = AR independence

1. AR amplification
2. Increased intratumoral DHT?
A mutation/SNP in HSD3B1 increases intraprostatic DHT generation.
Castration or bicalutamide resistance = AR independence

1. AR amplification
2. Increased intratumoral DHT
3. AR point mutation/splice variants
2nd Generation AR targeting Agents

Nature Reviews Cancer 2015 (Watson, Arora, & Sawyers)
Abiraterone Acetate: a better CYP17 inhibitor built from ketoconazole

Attard et al 2008 JCO
FDA approval of 2 agents based on increased survival in mCRPC

Nature Reviews Cancer 2015 (Watson, Arora, & Sawyers)
Why is AR a good drug target?
Candidate Oncogenic Signals Activated by RTKs

Figure 5.1 The Biology of Cancer (© Garland Science 2014)
Candidate Oncogenic Signals Activated by AR
Oncogenic Activation of Receptors: Is AR an oncogene?
AR gene alterations associated with treatment resistance, but not primary disease

63% of mCRPC cases have AR amp or mutation

<1% of primary prostate cancer cases have AR amp or mutation
Over-expression of AR co-operates with oncogenes to drive prostate tumorigenesis, but alone drives differentiation
The Most Frequently Mutated Gene in Prostate Cancer (SPOP ~15%) Regulates AR Protein Stability

Is prostate epithelium intrinsically AR dependent?

AR in stroma may explain some of normal prostate dependence

Culture of epithelial cells in absence of stroma however confirm intrinsic dependence of luminal cell on androgens.
Resistance to 2nd generation AR inhibitors

Nature Reviews Cancer 2015 (Watson, Arora, & Sawyers)
Summary I

• AR can be targeted in multiple, possibly synergistic ways
  • Castration
  • Direct AR inhibition
  • Inhibiting adrenal androgen synthesis
• Progression to castration resistant disease is driven by re-activation of AR due to amplification, mutation, or increased ligand production
• AR is not a classical oncogene, but may be hyper-activated by gene alterations associated with prostate cancer
• AR is drives multiple pathways that may contribute to tumor maintenance and is also necessary for maintenance of luminal epithelial tissue in the prostate
Basis of Nuclear Receptor Activation and Antagonism
NRs Bind to DNA as Homo and Heterodimers
Stabilization of Helix 12 and co-activator binding defines NR activation
Point mutations can convert antagonists to agonists: Using modeling to guide rational anti-androgen design
F876L mutation-based resistance
Proposed 3rd generation antiandrogens to restore antagonism for AR F876L

Enzalutamide

(±)-DR103

“D” ring

Yang Shen, Minna Balbas, Mike Evans
3rd generation antiandrogen DR103 can restore antagonism against AR F876L
Overlay of predicted structures for AR F876L

Yang Shen, David Hosfield
Derivation of Tissues Resistant to 2\textsuperscript{nd} Generation Anti-Androgens

1. Pilot Cohort (Affymetrix)
2. Validation Cohort (Illumina)

Expression Array

Diagram:
- LnCAP/AR
- Vehicle or Anti-Androgen
- Expression Array
Summary II

- Nuclear Receptor Antagonists typically fail to stabilize helix 12 in an ‘active’ conformation and block agonist binding
- Point mutations in the ligand binding region can convert antagonists to agonists
- Pharmacokinetic properties are crucial determinants of an antagonist’s in vivo activity
Emerging mechanisms of resistance to 2nd generation AR inhibitors

<table>
<thead>
<tr>
<th>Restored AR signalling</th>
<th>AR bypass signalling</th>
<th>Complete AR independence</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Clinical relapse profile</strong></td>
<td>• AR⁺</td>
<td>• AR⁺</td>
</tr>
<tr>
<td>• Rising PSA</td>
<td>• Rising PSA</td>
<td>• Low PSA</td>
</tr>
<tr>
<td><strong>Histological features</strong></td>
<td>Adenocarcinoma</td>
<td>Adenocarcinoma</td>
</tr>
<tr>
<td></td>
<td></td>
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</tr>
<tr>
<td><strong>Molecular features</strong></td>
<td>• AR-activating mutations</td>
<td>GR upregulation</td>
</tr>
<tr>
<td>• AR active splice variants</td>
<td></td>
<td></td>
</tr>
<tr>
<td>• Intratumoural DHT synthesis from adrenal precursors</td>
<td></td>
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</table>

*Nature Reviews Cancer 2015 (Watson, Arora, & Sawyers)*
Development of a Second-Generation Antiandrogen for Treatment of Advanced Prostate Cancer


*To whom correspondence should be addressed. E-mail: sawyersc@mskcc.org; jung@chem.ucla.edu

Published 9 April 2009 on Science Express
DOI: 10.1126/science.1168175
Cell-based screen for compounds with greater antagonism and no agonism ("pure antagonists") in LnCaP/AR

**Design tools:**
- Crystal structure
- Homology modeling
- Binding affinity

High AR binding affinity
($K_a = 20$ nM for human AR)
But with agonistic activity

Hydrophobic interactions with AR

Samedy Ouk, Michael Jung (UCLA Department of Chemistry)
Fig. 1 Effect of RD162 and MDV3100 in human prostate cancer cells in vitro.

Chris Tran et al. Science 2009;324:787-790
Figure S1. Effect of bicalutamide, RD162, and MDV3100 on PSA and TMPRSS2 transcription qRT-PCR analysis of the AR-dependent genes PSA and TMPRSS2 in LNCaP cells cultured in androgen-depleted media with 5% charcoal-stripped serum (CSS). Cells were treated for 8 hours with or without 1nM R1881 combined with DMSO (Vehicle), bicalutamide (Bic, 1 and 10 μM), RD162 (1 and 10 μM) and MDV3100 (1 and 10 μM) (normalized to actin mRNA, Mean ± SD, n=3).
Fig. 2 Activity of RD162 in mice.

A. RD162 - Pharmacokinetics

B. Vehicle vs. RD162

C. Individual LNCaP/AR-luc Xenograft Tumors, Day 28 (castrate males)

D. % tumors with vol > 50% from baseline

Chris Tran et al. Science 2009;324:787-790

Published by AAAS
Fig. 3 RD162 and MDV3100 impair AR nuclear translocation, DNA binding, and coactivator peptide recruitment.

Chris Tran et al. Science 2009;324:787-790
Figure S10. Effect of bicalutamide, RD162, and MDV3100 on PSA and TMPRSS2 AR recruitment to PSA and TMPRSS2 enhancers
LNCaP cells grown in androgen-depleted media with 5% charcoal-stripped serum were treated for 8 hours with or without 1nM R1881 combined with DMSO (Veh), bicalutamide (Bic, 1 and 10 μM), RD162 (1 and 10 μM) and MDV3100 (1 and 10 μM). Cells were crosslinked and processed for ChIP using AR antibody (PG21). Real-time PCR quantification of immunoprecipitated PSA enhancer and TMPRSS2 enhancer is shown (% input Mean ± SD, n=3).
Figure S3. Activation of an androgen-regulated reporter gene by W741C mutant AR
Cos-7 cells were co-transfected with ARE(4x)-Luc and AR W741C plasmids and treated for 24 hours with or without 1nM R1881 combined with DMSO (Veh), bicalutamide (Bic, 1 and 10 μM), RD162 (1 and 10 μM) and MDV3100 (1 and 10 μM) for 24 hours. A luciferase assay was conducted using cell lysates and relative light units shown (n=3, mean ± SEM).
Fig. 4 PSA response data in the first 30 patients receiving MDV3100 in Phase I/II trial.

Chris Tran et al. Science 2009;324:787-790

Published by AAAS