Computer-aided discovery and development of novel androgen receptor inhibitors to combat resistant prostate cancer

Dr. Artem Cherkasov

1. Rational drug discovery. CADD

2. Novel androgen receptor inhibitors developed using CADD

Learning objectives:

- To understand current rational approaches to drug discovery
- To understand general capabilities of computer-aided drug discovery (CADD)
- To learn basic types of CADD software currently available
Rational drug discovery

HOW ONE CAN DISCOVER A NEW DRUG?

Nature provides us lots of compounds with interesting biological activities (the penicillin story is instructive).

Existing drugs can have under-appreciated useful side effects (Viagra had originally been designed as an antihypertensive drug).

High throughput screening (HTS)

The most commonly used method of identifying a lead molecule is experimental high throughput screening (HTS) of compound databases against a target protein.
The most commonly used method of identifying a lead molecule is experimental high-throughput screening (HTS) of compound databases against a target protein.

(i) the numbers of compounds that can be economically screened is small compared with the chemical space available, and

(ii) the theoretical coverage of molecular diversity within the screening set is limited

Drug design strategies

- Ligand - based design
  - Structure – activity relationships for small molecules
- Structure – based design
  - DOCKING in silico
  - high throughput screening
  - De Novo Design
    - molecules growth
Drugs act as ‘key-in-lock’

The Lock & Key Analogon

1894

“Um ein Bild zu gebrauchen, will ich sagen, dass Enzym und Glykosid zueinander passen müssen, wie Schloss und Schlüssel, um eine chemische Wirkung aufeinander ausüben zu können.”

Emil Fischer, Nobel Laureate 1902

In Silico Screening
Each molecule can have up to 100s reasonable conformations. This yields to $100^{200}$ possible candidate chemical structures for a given protein binding site (on 30 binding points with 140,000 variants per point).

Protein active site is also not rigid, making the combinatorial problem of finding suitable 'key' for a 'lock' target non-trivial.
Drugs act as ‘key-in-lock’

Novel androgen receptor inhibitors developed using CADD
Androgen Receptor Inhibitors as Prostate Cancer Drugs

- AR inhibitors are used as androgen deprivation therapy
- They all exhibit similar mode of action (target DHT site)
- They share similar chemical scaffold

Nilutamide

Flutamide

Enzalutamide

Bicalutamide

ARN-509

Factors that Causes Resistance to Anti-AR Drugs

Mutations in the DHT site hampers the efficacy of known anti-androgens

- W741C : Bicalutamide
- T877A : Flutamide
- F876L : Enzalutamide

Treatment with Bicalutamide

Tumor growth suppression

Proliferating tumor cells

Mutations

Saher et al. Endocrine-Related Cancer 2004 11 (3) 459 -476
Identification of AR mutants using liquid biopsy

CRPC Resistance is Driven by AR Mutations

Key resistant mutations in DHT binding site convert Casodex, Enzalutamide, ARN509 and other anti-androgens to AR agonists, promoting PCa progression.
Specific Case: Patient VC-012

VC-012 progressing on bicalutamide

VC-012 progressing on enzalutamide

T878A/S889G-AR inhibition

F877L/T878A-AR inhibition

T878A-AR inhibition
### Agonist effect of anti-androgens on AR mutants

<table>
<thead>
<tr>
<th>Patient</th>
<th>Sample ID</th>
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<th>F877</th>
<th>T886</th>
<th>D880</th>
<th>L892</th>
<th>S899</th>
<th>D901</th>
<th>E904</th>
<th>E908</th>
<th>F910</th>
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### CRPC Resistance Driven by AR Splice Variants

- **WT AR/AR1**
  - f
  - 2-3
  - 8-9
  - NTD
  - 
  - LBD
  - Hu et al., 2009

- **AR-V1/AR4**
  - f
  - 2-3
  - 9-11
  - NTD
  - 
  - U
  - Guo et al., 2009; Hu et al., 2009

- **AR-V3/AR6**
  - f
  - 2-3
  - 9-11
  - NTD
  - 
  - U
  - Hu et al., 2009

- **AR-V7/AR3**
  - f
  - 2-3
  - 9-11
  - NTD
  - 
  - U
  - Guo et al., 2009; Hu et al., 2009

- **AR-V12/AR5**
  - f
  - 2-3
  - 9-11
  - NTD
  - 
  - U
  - Sun et al., 2010; Hu et al., 2011

- **AR-V45/AR2**
  - f
  - 2-3
  - 9-11
  - NTD
  - 
  - U
  - Ahmed-Fath et al., 2005

Other known factors that hamper the efficacy of the current AR drugs:
- Amplification or over-expression of AR
- AR activation by residual androgens (adrenal or intra-tumoral)
- Over-expression of AR coactivators
- NEED FOR DRUGS WITH NOVEL MOA
**Hypothesis:** Targeting alternative functional sites on AR should provide a promising strategy for treatment of PCa including its resistant forms where known mutations and splice variants hamper efficacy of the current drugs.

**Novel Strategy to Target AR**

*In Silico Screening Workflow*

- Protein structure
- Molecular docking (~10,000,000 mol)
- Computational protocols (~1,000,000)
- Small Molecule Database (50,000,000)
- Virtual Hits (100 to 200)

*In silico screening protocol used for the discovery of novel AR inhibitors. The numbers indicate compounds considered at each screening step.*
Experimental Screening Workflow

Initial in silico hits

<table>
<thead>
<tr>
<th>VPC-ID</th>
<th>Structure</th>
<th>eGFP IC50 (µM)</th>
<th>PSA IC50(µM)</th>
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<td>14203</td>
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<td>3.17 ± 0.3</td>
<td>3.91</td>
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<td>14320</td>
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<td>4.20 ± 0.6</td>
<td>2.26</td>
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<td>14378</td>
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<td>7.41 ± 0.4</td>
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<tr>
<td>14204</td>
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<td>9.16 ± 0.5</td>
<td>10.6</td>
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<tr>
<td>14410</td>
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<td>9.84 ± 3</td>
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### MedChem derived analogues, 1st round

<table>
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<tr>
<th>VPC-ID</th>
<th>A ring</th>
<th>B ring</th>
<th>C ring</th>
<th>eGFP IC50 (µM)</th>
<th>PSA IC50 (µM)</th>
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</thead>
<tbody>
<tr>
<td>14228</td>
<td></td>
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<td>0.33 ± 0.12</td>
<td>0.28</td>
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<tr>
<td>14103</td>
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<td>0.52 ± 0.03</td>
<td>0.51</td>
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<tr>
<td>14385</td>
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<td></td>
<td></td>
<td>0.62 ± 0.06</td>
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<tr>
<td>14292</td>
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<td></td>
<td>0.61 ± 0.02</td>
<td>0.58</td>
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<tr>
<td>14293</td>
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<td>0.62 ± 0.06</td>
<td>0.52</td>
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<tr>
<td>14255</td>
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<td>0.65 ± 0.06</td>
<td>0.41</td>
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</table>

2nd round of MedChem resulted in >100 Derivatives

<table>
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<tr>
<th>ID</th>
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<th>eGFP IC50 (µM)</th>
<th>PSA IC50(µM)</th>
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<td>14449</td>
<td></td>
<td>0.10 ± 0.05</td>
<td>0.17</td>
</tr>
<tr>
<td>14370</td>
<td></td>
<td>0.18 ± 0.01</td>
<td>0.25</td>
</tr>
<tr>
<td>14408</td>
<td></td>
<td>0.25 ± 0.05</td>
<td>0.43</td>
</tr>
<tr>
<td>14404</td>
<td></td>
<td>0.26 ± 0.02</td>
<td>0.22</td>
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<tr>
<td>14365</td>
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<td>0.27 ± 0.04</td>
<td>0.15</td>
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<tr>
<td>14367</td>
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<td>0.30 ± 0.02</td>
<td>0.23</td>
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<tr>
<td>14450</td>
<td></td>
<td>0.33 ± 0.01</td>
<td>0.44</td>
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<tr>
<td>14402</td>
<td></td>
<td>0.68 ± 0.01</td>
<td>0.57</td>
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</table>
Activity Profile of the Current Lead VPC-14449

(Left)  Dose-response curve illustrating the inhibiting effect of 14449 (IC$_{50}$ = 0.10µM) Enzalutamide (IC$_{50}$ = 0.08µM) on the AR transcriptional activity in LNCaP cells.

(Right) IC$_{50}$ curve illustrating the inhibiting effect of 14449 (IC$_{50}$ = 0.17µM) and Enzalutamide (IC$_{50}$ = 0.08µM) on the PSA levels in LNCaP cells.

14449 Effect on MR49F(Enzalutamide Resistant) Cell Line

The effect of 14449 on cell viability in an Enzalutamide resistant cell line (MR49F) where the compound demonstrated IC$_{50}$ of 0.21µM.
**14449 Inhibits the Activity of AR Splice Variant, V7**

14449 inhibits the transcriptional activity of wild type AR splice variant, V7 in luciferase reporter assay. Enzalutamide has no effect on V7.

**14449 is Selectively Toward the AR**

(Left) Enzalutamide and (Right) 14449 inhibits AR but not ER, GR and PR in luciferase assays against transiently expressed AR, GR, and PR or against endogenous ER in MCF-7 cells. AR, GR and PR activity was assessed with the ARR3tk-luciferase reporter.
Molecular dynamics simulations were performed using explicit solvent model. The total run time 3µs. The MD simulation study supports that 14449 binds to DBD site stably.

14449 finds its binding spot from various starting positions on the DBD.

CONSENSUS validation of the binding site and poses

1. Multiple MD simulations  2. Sampled binding poses  3. Cluster analysis & Binding energy calc.  4. Representative pose

Verification #1
- MD simulation starting from the representative pose

Verification #2
- Comparison with the mutation experiment
14449 Demonstrated poor stability

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<th>Time (hr)</th>
<th>Serum conc (µM)</th>
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<tbody>
<tr>
<td>0</td>
<td>1.0E+01</td>
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<tr>
<td>10</td>
<td>9.12E+02</td>
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<td>6.99E+02</td>
</tr>
<tr>
<td>40</td>
<td>5.27E+02</td>
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<table>
<thead>
<tr>
<th>Compound</th>
<th>14449</th>
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<tbody>
<tr>
<td>eGFP IC50 (µM)</td>
<td>0.38</td>
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<tr>
<td>PSA IC50 (µM)</td>
<td>0.17</td>
</tr>
<tr>
<td>T1/2 Microsomes (min)</td>
<td>40</td>
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9 CD1 mice 8-10 weeks old were divided into 3 groups, 3 mice each

Route of administration: Intravenous (IV), intraperitoneal (IP) or Oral (PO)

Dose: 100 mg/kg of 14449 formulated using 1:10 hydroxypropyl cyclodextrin: dd H2O

To measure serum drug levels, tail blood samples were taken following the administration, at time points corresponding to 0.0, 0.5, 1, 2, 4, 6, 8, 24hr
(Left) The effect of **14449** on the tumor volume of the Enzalutamide-resistant (MR49F) xenograft model.

(Right) The effect on tumor volume of castration resistant (androgen insensitive) C4-2 xenograft model.

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**Screening Workflow**

1. **Hits**
   - Activity in AR-eGFP/PSA assay
   - Activity in v7 assay
   - Cell proliferation assay
   - AR DBD mutations confirmed
   - BLI binding detection to AR DBD

2. **Lead optimization**

3. **In vivo studies**

Experimental pipeline used for the evaluation of virtual hits
Experimental pipeline used for the evaluation of virtual hits

Modelled metabolic liabilities of 14449
Second generation compounds: 14512, 14513 – better stability and potency

![Chemical structure of compound 14512]

<table>
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<th>Compound</th>
<th>14449 (µM)</th>
<th>14512 (µM)</th>
<th>PSA IC50 (µM)</th>
<th>T1/2 Micromes (min)</th>
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<td>0.38</td>
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<td>PSA IC50</td>
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<td>58</td>
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Second generation compounds: 14518 – much better stability and potency

![Chemical structure of compound 14518]

<table>
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<th>14512 (µM)</th>
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<td>PSA IC50</td>
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Selecting a clinical candidate (14518)

22rv1 MTS

% Cell Viability

[-150, 150] vs

[0.01, 100] [Compound] (μM)

Enz 14518

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Fariba Ghaidi

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Hans Adomat
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