Innovations in Drug Discovery:
Fragment-Based Drug Discovery & Activity-Based Protein Profiling

Literature presentation
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April 21st, 2020
Chemical technologies impacted drug discovery

1960s: natural product-derived leads

**taxol**

**pacific yew**

**rapamycin**

Contemporary Drug Discovery

Chemical technologies impacted drug discovery

1960s: natural product-derived leads

1970s: quantitative structure-activity relationships (QSAR)

Molecular structure

Molecular descriptor
- Electronics
- Sterics
- Lipophilicity
- Hydrophobicity
- Solubility
...

Multivariate analysis & activity prediction

Contemporary Drug Discovery

Chemical technologies impacted drug discovery

1960s: natural product-derived leads

1970s: quantitative structure-activity relationships (QSAR)

1980s: structure-based drug discovery (SBDD)


Abl in complex with imatinib (PDB: 2HYY)

**Contemporary Drug Discovery**

**Chemical technologies impacted drug discovery**

<table>
<thead>
<tr>
<th>Year</th>
<th>Technology Description</th>
</tr>
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<tbody>
<tr>
<td>1960s</td>
<td>natural product-derived leads</td>
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<td>1970s</td>
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</tr>
<tr>
<td>1980s</td>
<td>structure-based drug discovery (SBDD)</td>
</tr>
<tr>
<td>1990s</td>
<td>combinatorial chemistry and high-throughput screening (HTS)</td>
</tr>
</tbody>
</table>

- microarray
- DNA-encoded library

https://cen.acs.org/articles/95/i25/DNA-encoded-libraries-revolutionizing-drug.html
Drug Discovery in 20th Century

Advanced analytical techniques for HTS

NMR

X-ray

Surface plasmon resonance (SPR)

Systems biology

Genomics

Transcriptomics

Proteomics

Metabolomics

Targets & Biomarkers

Personalized therapy

Mechanism & Interactions

Drug repurposing

Fragment-Based Drug Discovery

**Traditional HTS**

- Ligand design and screening
- Lead compound identification
- Ligand optimization

**Fragment-Based Drug Discovery**

- Ligand screening
- Lead fragment identification
- Fragment linking and growing

\[ \text{Kd} \approx 100 \, \mu\text{M} \]
\[ \Delta G \approx 9-10 \, \text{kcal/mol} \]
\[ \text{Kd} \approx 3 \, \text{nM} \]
\[ \Delta G \approx \Delta G_1 + \Delta G_2 \approx 15-16 \, \text{kcal/mol} \]

Fragment-Based Drug Discovery

High-Throughput Screening (HTS)

- Library size > 100000
- Molecular weight > 300 Da

Fragment-Based Drug Discovery (FBDD)

- Library size < 5000
- Molecular weight < 300 Da

- Reduced synthetic resources
- High throughput 2D-NMR method
- Successful drug development cases

Case Study: Navitoclax

Bcl-X\textsubscript{L} (antiapoptotic protein)

\[
\begin{align*}
F-\begin{array}{c}
\text{aryl} \\
\text{aryl}
\end{array}-\text{CO}_2\text{H} & \quad K_D = 0.3 \text{ mM} \\
\text{aryl} & \quad K_D = 4.3 \text{ mM}
\end{align*}
\]

Bcl-X\textsubscript{L} binds to Bax and inhibits apoptotic cell death.

Mutated Bcl-X\textsubscript{L} turns off apoptotic pathway of cancer cells.

Targeting PPI between Bcl-X\textsubscript{L} & Bax

Case Study: Navitoclax

Bcl-X\(_L\) (antiapoptotic protein)

\[
\begin{align*}
F-\text{aryl}-\text{aryl}-\text{CO}_2\text{H} & \quad K_D = 0.3 \text{ mM} \\
\text{aryl} & \quad K_D = 4.3 \text{ mM} \\
\text{aryl} & \quad K_i = 1.4 \mu\text{M}
\end{align*}
\]

Case Study: Navitoclax

$K_i = 36 \text{nM}$

$K_i = 1.4 \mu\text{M}$

Case Study: Navitoclax

Navitoclax

$K_i = 36 \text{ nM}$

$K_i < 0.5 \text{ nM}$

Case Study: Navitoclax

Dasatinib bound to ABL kinase (PDB: 2GQG)

Palbociclib bound to CDK6 (PDB: 5L2I)

Navitoclax

$K_i < 0.5 \text{ nM}$

$MW = 975$

**Resurgence of Covalent Drugs**

- **Aspirin**
  - Bayer synthesized and distributes aspirin to patients

- **Penicillin**
  - Discovery of penicillin

- **Omeprazole**
  - Blockbuster proton pump inhibitor (omeprazole) approved

- **Clopidogrel**
  - Blockbuster antiplatelet drug (clopidogrel) approved

**Timeline**
- 1899
- 1928
- 1980s
- 1990s

**Development of targeted covalent drugs**

**Acetaminophen-induced hepatotoxicity**

- **Tylenol**
  - Reactive quinone intermediate readily reacts with cysteine residues

**Nonselective covalent modification of proteins**

**Acute tissue injury**

**Immune system activation through haptenization**

Features of Covalent Drugs

Traditional ligand efficiency (~0.3 kcal/mol per heavy atom)
- Covalent interactions exceed these ligand efficiency limits

- Shorter exposure, longer effect

Pharmacokinetic half life = 1–2 hr
ATPase resynthesis half life = 54 hr
Duration of inhibition = 28 hr

Features of Covalent Drugs

- High degrees of discrimination between closely related proteins

![Chemical structures]

<table>
<thead>
<tr>
<th></th>
<th>IC50 (µM)</th>
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<tbody>
<tr>
<td>EGFR</td>
<td>0.09</td>
</tr>
<tr>
<td>HER-2</td>
<td>0.18</td>
</tr>
<tr>
<td>A431</td>
<td>0.11</td>
</tr>
<tr>
<td>SKBR3</td>
<td>0.12</td>
</tr>
<tr>
<td>SW620</td>
<td>0.30</td>
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</table>

- Less susceptible to resistance mutants

![Graph showing IC50 values for different drugs]

Activity-Based Protein Profiling

**Chemical genetics-based drug discovery**

- CRISPR-Cas9 or RNAi
- Genetic perturbation
- Small molecule HTS

**Chemical proteomics-based drug discovery**

- Probe-labeled proteome
- CuAAC
- Reporter-tagged proteome

**Target ID & lead optimization**

Cannot address post-translational modifications

Not applicable for HTS
Activity-Based Protein Profiling

**Serine hydrolase**

**Cysteine protease**

Inhibitor discovery by competitive ABPP

Cysteine proteome → Inhibited proteins loose activity

No modification of protein & small molecules

Qualitative measurement of activity inhibition

activity-based protein profiling

Carvatt, B.F.; Wright, A.T.; Kazarich, J.W.


reactivity-based probe

Nimbolide

vs.

active against triple negative breast cancer cell 231MFP


Activity-Based Protein Profiling

1. Mix
2. Avidin enrichment
3. Tryptic digestion
4. TEV digestion

Protein 1
YWKDAC*SHR

Protein 2
SYC*WHIL

Light/heavy: 10 not inhibited

no dose dependent response to nimbolide

Targeting “Undruggable” Proteins

Protein–Protein interaction

No dominant Hot Segment
Surface area < 2500 Å²
K_d < 200 nM

Main drivers of cancer

- **p53**: Guardian of genome
- **Myc**: Master regulator
- **Ras**: Beating heart of cancer
- **β-catenin**:
Targeting KRAS Directly

- Three isoforms: KRAS, NRAS, HRAS
- Mutated in approximately 25 percent of all human cancers
  - 90 percent of pancreatic cancers
  - 35–45 percent of colorectal cancers
  - 25 percent of lung cancers
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KRAS mutation frequency

pM binding affinity w/ GTP
multiple downstream signals

G12C – Smoking-associated lung cancer

Seminal Report by Shokat and Coworkers

Initial hits

$\text{R} = \text{Me}_{2}N\text{H}$

Relative potency

$\text{R} = \text{Me}_{2}N\text{H}$

Modification (%)

- K-Ras(G12C)
- K-Ras WT
- H-Ras(G12C)
- K-Ras(G12C) +1 mM GDP
- K-Ras(G12C) (GTP state)

Seminal Report by Shokat and Coworkers

Irreversible covalent binder

Shift of switch II and partial disordering of switch I

<table>
<thead>
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<th>100 µM compound 12</th>
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<tbody>
<tr>
<td><strong>Time (h)</strong></td>
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<tr>
<td><strong>IP: Ras</strong></td>
</tr>
<tr>
<td><strong>WCL</strong></td>
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Raf protein did not co-immunoprecipitate after treatment

KRAS (G12C) mutant cell lines

Lead Optimization

Shokat's covalent binder

ARS-1620
Arazex Pharma/Wellspring Biosciences

AMG-510
Amgen

MRTX-1257
Mirati Therapeutics/Array Biopharma

AMG-510

Chemical structure of AMG-510

**Cystein proteomic profiling**

**AMG-510**

AMG-510

Amgen


AMG-510

**Cystein proteomic profiling**

Clinical Activity of AMG-510 in Patients

**KRAS^{G12C} lung carcinoma patients**

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