Success stories of structure-based drug discovery

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11.10.17
**Acetylsalicylic Acid** (ASPIRIN – Bayer 1853, 1899)
- antipyretic
- analgesic
- anticoagulant
- pro-drug
- commercially, the most successful drug ever

**Metformin** (Glucophage – Rona 1922, 1958)
- antidiabetic

- antihypertensive
- inhibits Angiotensin Converting Enzyme (ACE)
- pro-drug
- designed from viper snake venom
Discovery and life expectancy

- **1796**: Smallpox vaccine
- **1932, 1945**: Antibiotics
History of the Food & Drug Administration (FDA)

- **1906 Food and Drugs Act** prohibited adulteration or misbranding of pharmaceuticals. Premarket approval of drugs not required - commercialization of hazardous or useless drugs were not prevented.
- **1937** sulfanilamide formulation with untested solvent killed more than 100 people.
- **1962** - required evidence of **effectiveness** through adequate clinical trials.

Sulfanilamide
Antibacterial agent used widely during WW2

Chemist Lee Geismer looking over an NDA in the 1960s
Summary of FDA New Drug Applications (NDAs)

- Average submitted NDAs (1938 - 2011): 254.3/year
- Average approved NDAs (1938 - 2011): 168.9/year
- Average NMEs (1938 - 2011): 21.2/year
Between 2007-2015 average 30 approved NMEs/year.

NME - New Molecular Entity

BLA - Biologics License Application
Big Pharma: Dramatic Decline in R&D Productivity

Attrition Remains Very High

Drug Discovery
Preclinical
Clinical
Regulatory Review/Marketing Approval

5,000 - 10,000 compounds
250 compounds
Phase I (55% SR)
Phase II (25% SR)
Phase III (75% SR)
IND Application
NDA/BLA Application

10% Cumulative Success Rate
10 - 15 Years

Output Not Keeping Up With R&D Expenditures


Global Sales
Global R&D Expenditures
Global New Molecular Entities (NMEs)

Source: PhRMA, CMR, Genentech, Booz Allen Hamilton: The Global Innovation 1000, 2006
Stages of drug discovery

Preclinical studies:
- Research team formed and objectives set
- Novel chemicals synthesized
- Chemicals tested for efficacy and safety in test tubes and animals. Results used to choose drug candidate.
- Formulation, scale-up synthesis, and chronic safety in animals
- Company files Investigational New Drug (IND) application with FDA

Clinical studies:
- Drug is approved for marketing
- FDA reviews NDA
- Company files New Drug Application (NDA)
- Phase III: large clinical trials in many patients
- Phase II: studies in patients (efficacy)
- Phase I: studies in healthy humans (tolerance)

Source: Nature Reviews | Drug Discovery
Structure-based drug design (SBDD)

- Develop new drug candidates for a disease
- Protein target relevant for the disease
- Relies on knowledge of the protein 3D structure
- Find compounds that block (or enhance) protein activity by binding to:
  - catalytic site
  - allosteric site (better for selectivity)
- Structural information of protein-ligand interaction is used to develop new compounds with increased potency and selectivity
Examples of drugs developed using SBDD

- **Dorzolamide (Merck, 1995)** - first SBDD approved drug (anti-glaucoma agent; carbonic anhydrase inhibitor)

- **Imatinib (Novartis, 2001)** - first anti-cancer drug substantially different from previous anti-cancer drugs (inhibitor of the tyrosine kinase *bcr-abl*)

- **Vemurafenib (Roche, 2011)** - first FBDD approved drug (late stage melanoma; inhibitor of B-Raf (V600E)) - only 6 years from fragment to approval!

![Image](image-url)
Structure-based drug discovery

Expertise:

NMR Spectroscopy
- Protein construct optimization
- Ligand screening and hit validation
- 3D structure determination

Crystallography
- 3D structure determination

Chemistry

Other techniques
- Biophysical techniques
- Computer modelling and docking of ligands
- SAR (activity assays, binding affinities, competition binding)
The essentials for a SBDD project

- **Protein:**
  - Easily overexpressed to high amounts
  - Stable (ideally can be frozen or lyophilised)
  - Folded
  - Crystallised into robust (compound soaking) and high-symmetry crystals (reduced acquisition time)

- **Chemical library:**
  - High-purity (> 95%)
  - High amounts (up to 50 mg)
  - Highly soluble in DMSO and water
  - Without reactive or unstable molecules

- **Infrastructure and technology:**
  - Wet-lab with biophysical equipment
  - High-field NMR spectrometers
  - Crystallography facility
  - X-ray generator and access to synchrotron

- **Chemistry support**
NMR in drug discovery

- Construct optimization of the target protein
- NMR screening and hit validation
- Map the ligand-binding site
- Characterize the protein-ligand interaction
- Protein-ligand structure determination
Cloning and expression of the target protein

Use of diverse labelling schemes:
- Uniform $^{15}$N labelling
- Uniform $^{15}$N, $^{13}$C labelling
- $^2$H,$^{15}$N,$^{13}$C labelling
Is the protein folded?

Protein A

Nicely folded

Protein B

Largely unfolded

⇒ Improve construct/NMR conditions

~ 200 amino acid residues
Does the protein bind to its natural ligands?

**Affinity chromatography**

The domain does not bind to its natural ligand! ⇒ construct problem?

**2D NMR**

The domain binds to its natural ligand ⇒ the construct is valid!
NMR screening and validation

- NMR detects ligand binding \( \text{mM} \rightarrow \text{nM} \)
- Specific binding can be distinguished from unspecific binding
- False positive identification
- Different pH, salt, buffer or redox conditions can be chosen
Saturation Transfer Difference (STD) experiments

- Fast
- Unlabelled protein
- Low protein concentrations (~20 μM)
- Compound soluble in buffer (maximum DMSO levels 20%)
- Binding epitope can be inferred

Problems:

- STD signals but non-specific interaction
- No STD signals but specific binding
2D screening

- Identifies specific binding epitopes
- Requirements:
  - $^{15}$N-labelled protein
  - Assignment and 3D structure of the protein

![Chemical shift perturbations mapping](image)

**Chemical shift perturbations mapping**
Hit validation

Protein + 4456-0499

IC$_{50}$ = 610 nM

Significant shifts
Positive hit

Protein + 4696-0682

IC$_{50}$ = 300 nM

Protein precipitation
False positive hit
Characterizing protein-ligand interactions

Determining the protein-ligand affinity ($K_D$)

Limitations:
- Simple systems
- Fast exchange
- mM → μM binding
- Higher affinities – other techniques e.g. ITC

Ligand titration by NMR
Determining how the ligand exerts its action

STD effect weakens upon natural ligand addition

Free ligand increases upon natural ligand addition

Addition of natural ligand

Competition with a natural ligand

Compound with target protein

STD

T2-filtered 1D

$\delta^1H$ [ppm]
Determining how the ligand exerts its action

Inhibition of the interaction with biological partners
Crystallography in drug discovery

- 3D structure determination of protein-ligand complexes
- High-resolution - fine details of ligand-protein interactions can be determined and used to improve affinity or selectivity of the compound
- Fast (once you get crystals!)
# CY 2011 New Molecular Entities (NMEs)

## THE NMEs OF 2011

<table>
<thead>
<tr>
<th>Drug Name</th>
<th>Active Ingredient</th>
<th>Date</th>
<th>What it’s used for</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Eleke</strong></td>
<td>abiciprecept</td>
<td>11/18</td>
<td>To treat wet (subvascular) age-related macular degeneration (AMD), a leading cause of vision loss and blindness in American ages 60 and older.</td>
</tr>
<tr>
<td><strong>Evrintace</strong></td>
<td>apragatide thymic derivatients</td>
<td>11/18</td>
<td>For patients with acute lymphoblastic leukemia (ALL), who have developed an allergy (hypersensitivity) to E. coli derived epirubicin and palonosetron chemotherapy drugs used to treat ALL.</td>
</tr>
<tr>
<td><strong>Jalke</strong></td>
<td>ramotinib</td>
<td>11/16</td>
<td>To treat patients with the bone marrow disease myelodysplasia.</td>
</tr>
<tr>
<td><strong>Ocal</strong></td>
<td>clofibazam</td>
<td>10/24</td>
<td>For use as an adjunctive (add-on) treatment for seizures associated with Lennox-Gastaut syndrome in adults and children 2 years of age and older.</td>
</tr>
<tr>
<td><strong>Ferricopace</strong></td>
<td>defeciprace</td>
<td>10/14</td>
<td>Iron overload from blood transfusions in patients with thalassemia (genetic disorder causing anemia), who had an inadequate response to chelation therapy.</td>
</tr>
<tr>
<td><strong>Xloroni</strong></td>
<td>chrozisinib</td>
<td>08/26</td>
<td>Certain patients with late-stage (locally advanced or metastatic), non-small cell lung cancers who express the abnormal epidermal growth factor receptor.</td>
</tr>
<tr>
<td><strong>Hizaxie</strong></td>
<td>celtibact</td>
<td>08/25</td>
<td>For the treatment of acute attacks of a rare condition called hereditary angioedema (HAE) in people aged 18 years and older.</td>
</tr>
<tr>
<td><strong>Ad servicis</strong></td>
<td>brentroninab vedotin</td>
<td>08/19</td>
<td>Hodgkin lymphoma and ALCCL (systemic anaplastic large cell lymphomas).</td>
</tr>
<tr>
<td><strong>Zelboraz</strong></td>
<td>venurafinib</td>
<td>08/17</td>
<td>To treat patients with late-stage (metastatic) or unresectable (cannot be removed by surgery) melanoma, the most dangerous type of skin cancer.</td>
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<td>Dificid</td>
<td>fidaxomycin</td>
<td>05/27</td>
<td>For the treatment of <em>Clostridium difficile</em>-associated diarrhea (CDAD).</td>
</tr>
<tr>
<td>Luceneb</td>
<td>telagrevir</td>
<td>05/23</td>
<td>To treat certain adults with chronic hepatitis C infection.</td>
</tr>
<tr>
<td>Edmund</td>
<td>rilpivirine</td>
<td>05/20</td>
<td>Treatment of HIV-1 infection in adults who have never taken HIV therapy.</td>
</tr>
<tr>
<td>Vietusee</td>
<td>boceprevir</td>
<td>05/13</td>
<td>To treat certain adults with chronic hepatitis C.</td>
</tr>
<tr>
<td>Tzaphilita</td>
<td>imagliptin</td>
<td>05/02</td>
<td>Addition to diet and exercise to improve glycemic control in adults with type 2 diabetes mellitus.</td>
</tr>
<tr>
<td>Zyncia</td>
<td>abaterone acetate</td>
<td>04/28</td>
<td>In combination with prednisone to treat patients with late-stage (metastatic) castration-resistant prostate cancer who have received docetaxel (chemotherapy).</td>
</tr>
<tr>
<td>Carciplas</td>
<td>vandetamb</td>
<td>04/06</td>
<td>To treat adult patients with late-stage (metastatic) mediastinal thymic cancer, ineliglible for surgery who have disease that is growing or causing symptoms.</td>
</tr>
<tr>
<td>Horizant</td>
<td>gabanepin encarbel</td>
<td>04/06</td>
<td>A once-daily treatment for moderate-to-severe restless legs syndrome (RLS).</td>
</tr>
<tr>
<td>Vervor</td>
<td>ipilimumab</td>
<td>03/25</td>
<td>Late-stage (metastatic) melanoma, the most dangerous type of skin cancer.</td>
</tr>
<tr>
<td>Gadoast</td>
<td>gadobrocol</td>
<td>03/14</td>
<td>Magnetic resonance imaging (MRI) of the central nervous system.</td>
</tr>
<tr>
<td>Benlytza</td>
<td>belimumab</td>
<td>03/10</td>
<td>To treat patients with active, autoantibody-positive lupus (systemic lupus erythematosus) who are receiving standard therapy, including corticosteroids, immunosuppressives, and nonsteroidal anti-inflammatory drugs.</td>
</tr>
<tr>
<td>Dalireep</td>
<td>rofutilast</td>
<td>02/28</td>
<td>To decrease the frequency of flare-ups (exacerbations) or worsening of symptoms from severe chronic obstructive pulmonary disease (COPD).</td>
</tr>
<tr>
<td>Edafel</td>
<td>azithromycin methenemethyl</td>
<td>02/25</td>
<td>To treat high blood pressure (hypertension) in adults.</td>
</tr>
<tr>
<td>Visbyrd</td>
<td>vilazodone HCI</td>
<td>01/21</td>
<td>To treat major depressive disorder in adults.</td>
</tr>
<tr>
<td>Naroobsa</td>
<td>spinocoid</td>
<td>01/18</td>
<td>For the treatment of head lice infestation in patients ages 4 years and older.</td>
</tr>
<tr>
<td>Datonec</td>
<td>iodipano 1-123</td>
<td>01/14</td>
<td>An imaging drug used to assist in the evaluation of adult patients with suspected Parkinsonian syndromes (PS).</td>
</tr>
</tbody>
</table>

**First fragment-based drug approved 17/08/2011**
The story of vemurafenib (ZELBORAF)

**Vemurafenib=PLX4032**

- Drug discovered at Plexxikon in partnership with Roche; Plexxikon acquired by Daiichi Sankyo
- 6 years from fragment to approval!

- Treatment of late stage melanoma
- Targets B-Raf (V600E), a Ser-Thr protein kinase
- 50% melanomas carry this mutation
- B-Raf most frequently mutated kinase in human cancers
- Increases survival by approximately 5 months longer
- $9400 /month
**Compound evolution**

- Initial screen of a 20000 compound library against the ATP-binding site of 3 kinases (Pim-1, CSK, p38)

Zelboraf (PLX4032) has better pharmacokinetic properties in dogs and monkeys than PLX4720

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**Fig. 1.** Structures of individual compounds leading to the discovery of PLX4720 are shown. (A) The chemical structure of 3-aminophenyl-7-azaindole (compound 1) is shown beneath its costructure with Pim-1 kinase. (B) The chemical structure of 3-(3-methoxybenzyl)-7-azaindole (compound 2) is shown beneath its costructure with the kinase domain of FGFR1. (C) The chemical structure of PLX4720 is shown beneath its costructure with B-Raf kinase.

Discovery of a selective inhibitor of oncogenic B-Raf kinase with potent antimelanoma activity.
Discovery of a selective inhibitor of oncogenic B-Raf kinase with potent antimelanoma activity.
Second fragment-based drug approved 11/04/2016
The story of venetoclax (VENCLEXTA)

- Drug discovered at AbbVie and Genentech; Initial work done at Abbott
- Two decades from initial 3D structure to approval!
- Second generation drug for the treatment of chronic lymphocytic leukemia (CLL)
- Targets Bcl-2, a protein regulator of apoptosis
- Orphan drug for the thousands of patients with relapsed CLL who have 17p deletion
- In the registration trial, 80% of patients showed a partial or complete remission
Compound evolution until ABT-263

Fragments

Fragment-linking + optimization

Lead optimization
remove binding to albumin and increase affinity to Bcl-2

Improvement oral pharmacokinetics

Compound evolution until drug

- Power of SBDD and FBDD to tackle difficult targets
- Violation of the Lipinsky rule of 5
- Contains a nitro group, a moiety red-flagged due to its potential for forming toxic metabolites
Summary

- Structure-based drug discovery is a powerful method for delivering new drugs
- Strategy for screening, hit validation and optimization - lead compound
- Expertise at STB- HMGU (NMR spectroscopy, X-ray crystallography, SBDD, Chemoinformatics)
Lipinski's rules

Lipinski's rule states that, in general, an orally active drug has no more than one violation of the following criteria:

- No more than 5 hydrogen bond donors (the total number of nitrogen-hydrogen and oxygen-hydrogen bonds)
- No more than 10 hydrogen bond acceptors (all nitrogen or oxygen atoms)
- A molecular mass less than 500 daltons
- An octanol-water partition coefficient log $P$ not greater than 5
Ligand efficiency

Figure 1.2 Ligand efficiency (LE) shows a precipitous decline between 10 and 25 nonhydrogen atoms (HA). We have extracted the affinity data used in this plot from the BindingDB database developed at the University of Maryland Biotechnology Institute (Lin et al., 2007).

Important arbiter of progress  Ligand Efficiency (LE) - free energy of binding per heavy atom

LE=ΔG/HA

where ΔG=-RTln K_d, -RTln K_i, -RTln IC_{50}