Polypharmacology: A Brief History of Drug Design Philosophy
Outline

- What is polypharmacology?

- Historical context:
  - classical drug discovery
  - rational drug design
  - the modern era

- Case studies
  - PARP inhibitors
  - fragment-based drug design

- Lessons learned and looking forward
How do we define polypharmacology?

polypharmacology

the binding of a molecule to more than one target
at therapeutically relevant concentrations

polypharmacy

the regular use of more than 5 medications
at once in a single patient

What kinds of molecules qualify as exhibiting polypharmacology?

How do we define polypharmacology?

Ibuprofen

NSAID - Non-steroidal anti-inflammatory drug

NSAIDs mechanism of action

Prostaglandin G2
mediator of pain/inflammation

arachidonic acid

COX
cyclooxygenase

Ibuprofen

NSAIDs mechanism of action

Prostaglandin G2
mediator of pain/inflammation

inhibition of prostaglandin synthesis

Ibuprofen

COX
cyclooxygenase

arachidonic acid

NSAIDs mechanism of action

Two distinct COX enzymes exist. Both are targets of NSAIDs. ...so what's the difference?

COX-1

cyclooxygenase-1

COX-2

cyclooxygenase-2

Ibuprofen

\[
\text{Me} - \text{Me} - \text{C} - \text{CO}_2\text{H}
\]
NSAIDs mechanism of action

COX-1
- cyclooxygenase-1
- gastrointestinal prostaglandins
  - protect gastrointestinal mucosa

COX-2
- cyclooxygenase-2
- inflammation/pain related prostaglandins
  - anti-inflammatory/pain relieving effect

Ibuprofen
- "nonselective COX inhibitor"
  - exhibits promiscuity

"off-target"
"anti-target"

How do we define polypharmacology?

1. Multiple structurally similar targets

Designer multi-targeting molecules

Designer multi-targeting molecules

**ruxolitinib**  
JAK 1/2 kinase inhibitor  
*anti proliferative, anti-inflammatory effects*  
$IC_{50} = 3 \text{ nM}$

**vorinostat**  
pan-HDAC inhibitor  
*blocks de-acetylation of histones - epigenetic effects*  
$IC_{50} = 10 \text{ nM}$

Designer multi-targeting molecules

Dual JAK-HDAC Inhibitor

excellent activity for both JAK and HDAC proteins

\[ \text{JAK } IC_{50} = 75 \text{ nM} \]

\[ \text{HDAC1 } IC_{50} = 6.9 \text{ nM}, \text{ HDAC6 } IC_{50} = 1.4 \text{ nm} \]

Designer multi-targeting molecules

Dual JAK-HDAC Inhibitor

excellent activity for both JAK and HDAC proteins

**JAK** $IC_{50} = 75 \text{ nM}

**HDAC1** $IC_{50} = 6.9 \text{ nM}$, **HDAC6** $IC_{50} = 1.4 \text{ nm}$


**How do we define polypharmacology?**

1. **Multiple structurally similar targets**
   - One drug, multiple highly similar targets

2. **Multi-targeted by design**
   - One drug, multiple targeting motifs

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Further targets of NSAIDs

1990s - it was noticed that users of NSAIDs showed reduced instances of Alzheimer's disease.

Early 2000s - efforts to study why this trend was observed.

Ibuprofen

\[ \text{Ibuprofen} \]

- COX1, COX2 inhibitor
- anti-inflammatory, anti-pain


Further targets of NSAIDs

Ibuprofen

COX1, COX2 inhibitor

anti-inflammatory, anti-pain

Nature, 2001 - Effect of NSAIDs on Amyloid Beta Levels

ibuprofen and other NSAIDs reduce amyloid-beta 42 levels


Further targets of NSAIDs

Nature, 2001 - Effect of NSAIDs on Amyloid Beta Levels

reduction in Aβ42 is independent of COX inhibition activity

performed in COX1-/COX2- cell line

Further targets of NSAIDs

**Ibuprofen**

- COX1, COX2 inhibitor
- anti-inflammatory, anti-pain
- additionally targets Rho protein

**Science, 2003 - Mechanism of NSAID Amyloid Beta Reduction**

Further targets of NSAIDs

Ibuprofen

- **COX1, COX2 inhibitor**
- **anti-inflammatory, anti-pain**
- **additionally targets Rho protein**

2007 - Ibuprofen, via RhoA inhibition, boosts neuronal growth

*neuronal outgrowth model*

How do we define polypharmacology?

1. Multiple structurally similar targets

2. Multi-targeted by design

3. Multiple completely orthogonal targets

**Historical context of polypharmacology in drug discovery**

**The Phenotypic Approach (????-late 1980s)**

“observational”  “anecdotal”

Willow tree bark

Salicylic acid

Acetylsalicylic acid (Aspirin)

*first synthesized/registered by Bayer in 1899*

*mechanism wasn’t elucidated until 1970s*

**Historical context of polypharmacology in drug discovery**

**The Phenotypic Approach (????-late 1980s)**

- "observational"
- "anecdotal"

Willow tree bark

[![Willow tree bark image](image)]

Salicylic acid

[![Salicylic acid structure](image)]

**Acetylsalicylic acid (Aspirin)**

[![Acetylsalicylic acid structure](image)]

**1982 Nobel Prize in Physiology/Medicine**

"for their discoveries concerning prostaglandins and related biologically active substances"

[![Nobel Prize laureates](image)]

**COX polypharmacology wasn’t discovered until 1991**

**The Phenotypic Approach (????-late 1980s)**

- **Advantages**
  - lead compound, direct effect readout
  - chemistry, biology are “in-sync”
  - in retrospect: favors potential polypharmacology

- **Disadvantages**
  - phenotype $\neq$ mechanistic insight
  - no structural knowledge to guide optimization
  - off-targets, toxicity hard to predict/measure

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**Why was this approach taken?**

- observational
- anecdotal

**limitations in biochemical understanding**

- chemistry
- biology

- laborious
- “black-box”

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**Historical context of polypharmacology in drug discovery**

-Brown, D. Drug Discovery Today. 2007, 12, 23.
Historical context of polypharmacology in drug discovery

"target based"  
Rational Drug Design (Late 1980s and onward)  
"hypothesis based"

enabled by two major advances in the late 1980s:

- recombinant DNA technologies
- fast-protein liquid chromatography

protein accessibility led to:

- quantitative assay data
- high-throughput screening
- metabolism/PK studies

Brown, D. Drug Discovery Today. 2007, 12, 23.
Historical context of polypharmacology in drug discovery

“target based”  \hspace{2cm} Rational Drug Design (Late 1980s and onward)  \hspace{2cm} “hypothesis based”

**BCR-ABL**

- tyrosine-protein kinase
- breakpoint-cluster region protein

**ABL**

**BCR**

fusion-product of BCR and ABL genes

known as the Philadelphia Chromosome (Ph)

common in chronic myelogenous leukemia (CML)

early example of a specific protein-product that was directly linked to a cancer type

Historical context of polypharmacology in drug discovery

“target based”  
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**BCR-ABL**
- **ABL**
  - tyrosine-protein kinase
- **BCR**
  - breakpoint-cluster region protein

> fusion-product of BCR and ABL genes

**late 1990s - campaign to screen for BCR-ABL inhibitors**
**Historical context of polypharmacology in drug discovery**

“target based”

**Rational Drug Design (Late 1980s and onward)**

“hypothesis based”

**BCR-ABL**

fusion-product of

**BCR and ABL genes**

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Nicholas Lydon performed high-throughput screening of molecules against BCR-ABL.

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Survival statistics for chronic myeloid leukemia. *Canadian Cancer Society*, **2022**.
Historical context of polypharmacology in drug discovery

“target based”  

Rational Drug Design (Late 1980s and onward)  

“hypothesis based”

2-phenylaminopyrimidine showed BCR-ABL inhibition

imatinib

final optimized compound

BCR-ABL fusion-product of BCR and ABL genes

Historical context of polypharmacology in drug discovery

“target based”  
Rational Drug Design (Late 1980s and onward)  
“hypothesis based”

Inactive ABL  
Active ABL

auto-inhibitory region

imatinib

final optimized compound

Historical context of polypharmacology in drug discovery

“target based” Rational Drug Design (Late 1980s and onward) “hypothesis based”

BCR-ABL Fusion Protein

Imatinib

Final optimized compound

Historical context of polypharmacology in drug discovery

“target based”

*Rational Drug Design (Late 1980s and onward)*

“hypothesis based”

imatinib locks ABL into its inactive conformation

**imatinib**

final optimized compound

FDA approved in May 2001

$4.6$ billion peak sales in 2012

as of 2023, CML 5 year survival rate has surpassed 90%


Historical context of polypharmacology in drug discovery

“target based”  Rational Drug Design (Late 1980s and onward)  “hypothesis based”

`imidn

FDA approved in May 2001

$4.6 billion peak sales in 2012

as of 2023, CML 5 year survival rate has surpassed 90%

first “magic bullet”, beginning of a new era


Historical context of polypharmacology in drug discovery

“target based”  
Rational Drug Design (Late 1980s and onward)  
“hypothesis based”

c-KIT  
tyrosine protein kinase  
close structural similarity to ABL

imatib  
final optimized compound

2002 - FDA approved for GIST (gastrointestinal stromal tumors)

imatib also inhibited c-KIT  
coincidental polypharmacology allowed for multiple oncology use-cases

**Historical context of polypharmacology in drug discovery**

“target based”  |  **Rational Drug Design (Late 1980s and onward)**  |  “hypothesis based”

**Advantages**
- high-throughput screening possible
- direct structure-binding optimization possible
- allows high selectivity, mitigation of promiscuity

**Disadvantages**
- reductionist system, not 1:1 transferable findings
- relied upon single-target amenable diseases
- single-target compounds can lead to resistance

*in acute lymphoblastic leukemia (ALL) - imatinib resistance within 6 months is as high as 70%*

*point mutations in ABL gene lead to failure of response*

*highly selective drugs are also more reliant on single-target efficacy*

Recognition of Polypharmacology as a Strategy (early 2000s)

until this time, was mostly viewed as something to be avoided or coincidental

Magic shotguns versus magic bullets: selectively non-selective drugs for mood disorders and schizophrenia

Bryan L. Roth, Douglas J. Sheffler and Wesley K. Kroeze

the idea that “dirty” drugs may actually be better

highly relevant in CNS contexts

Recognition of Polypharmacology as a Strategy (early 2000s)

majority of CNS drugs exhibit polypharmacology

literature outcome analysis of drugs for:

Schizophrenia  
Depression

in both cases, “non-selective” drugs have better outcomes

"Clearly, conventional approaches relying on high-throughput screening (HTS) of cloned human molecular targets and the subsequent optimization of these ‘single-target agents’ is not likely to yield selectively non-selective agents, except, perhaps, by chance."

**Historical context of polypharmacology in drug discovery**

**The Modern Era (2012 onwards)**

new, powerful technologies for studying drug mechanism:

- genetic screening/CRISPR
- modern high-fidelity chemoproteomics

Historical context of polypharmacology in drug discovery

The Modern Era (2012 onwards)

new, powerful technologies for studying drug mechanism:

- genetic screening/CRISPR
  - high throughput gene profiling
  - Which genes affect this drug's activity?

- modern high-fidelity chemoproteomics
  - protein-level interaction data
  - What proteins is this drug interacting with?

"How specific are these drugs really?"

Case study: polypharmacology in PARP inhibitors

PARP
poly-(ADP) ribose polymerase
DNA repair, genomic stability

recognizes single strands breaks (SSBs) in DNA and recruits repair machinery

PARP inhibition is a powerful anti-cancer strategy (BRCA ovarian/breast cancer)

olaparib
FDA approved 2014

$2.8 billion in sales in 2022

Case study: polypharmacology in PARP inhibitors

**Comparison of PARP inhibitors**

- **rucaparib**
  - FDA approved 2016

- **olaparib**
  - FDA approved 2014

- **niraparib**
  - FDA approved 2017

- **talazoparib**
  - FDA approved 2018

**Conserved benzamide motif - PARP binding**

**Significant variability on rest of scaffold**
Comparison of PARP inhibitors

Clinically, there is no strong rationale for selecting one PARP inhibitor over another

...but they have different structures

So are there differences?

Case study: polypharmacology in PARP inhibitors

Clues to promiscuity

PJ34 has unique properties, unexplainable solely by PARP binding.

PJ34 leads to mitotic arrest (cell cycle stoppage)

PARP1 knockout does not affect mitotic arrest activity

- “test” molecule, known PARP inhibitor
- discovered in 2001
- used in 100s of studies for PARP biology

Assumed to be highly selective PARP1 inhibitor

IC$_{50}$ = 20 nM

Case study: polypharmacology in PARP inhibitors

PJ34 had unique properties, unexplainable solely by PARP binding

PJ34 binding mode shown in white

Contrasted with other kinase inhibitors

No similarity in PARP1, PIM1 binding motifs/rationales

PJ34 had unique properties, unexplainable solely by PARP binding

In-silico screen of potential targets reveals PIM1, PIM2 as predicted off-targets

- "test" molecule, known PARP inhibitor
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- used in 100s of studies for PARP biology

Assumed to be highly selective PARP1 inhibitor

$IC_{50} = 20 \text{ nM}$

PIM1 inhibition and its implications

**PIM** - proto-oncogene serine/threonine kinase

overexpressed in many cancers, target for cancer therapy

Does this activity extend to FDA approved PARP inhibitors?

**Case study: polypharmacology in PARP inhibitors**

*in-vitro kinome screen*

screening of PARP inhibitors against 392 human kinases (76% of all known human kinases)

**olaparib**

*FDA approved 2014*

![olaparib structure](image)

**rucaparib**

*FDA approved 2016*

![rucaparib structure](image)

**niraparib**

*FDA approved 2017*

![niraparib structure](image)

**talazoparib**

*FDA approved 2018*

![talazoparib structure](image)

Case study: polypharmacology in PARP inhibitors

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unique differences in kinase off-targets observed

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Case study: polypharmacology in PARP inhibitors

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**Case study: polypharmacology in PARP inhibitors**

**in-vitro kinome screen**

screening of PARP inhibitors against 392 human kinases (76% of all known human kinases)

unique differences in kinase off-targets observed

- **rucaparib**
  - FDA approved 2016
  - inhibits CDK16 ($IC_{50} = 381$ nM)
  - cell cycle control protein
  - involved in cancer proliferation

- **niraparib**
  - FDA approved 2017
  - inhibits DYRK1B ($IC_{50} = 254$ nM)
  - cell cycle transition regulator
  - involved in cancer proliferation

  both bind PIM1/PIM2
  - relatively weak ($\mu$M)

### Case study: polypharmacology in PARP inhibitors

**Meta-analysis based upon differing polypharmacology**

<table>
<thead>
<tr>
<th>Differential adverse reactions between FDA-approved clinical PARP inhibitors</th>
</tr>
</thead>
<tbody>
<tr>
<td>Side-effect</td>
</tr>
<tr>
<td>------------</td>
</tr>
<tr>
<td>Dry mouth</td>
</tr>
<tr>
<td>Anxiety</td>
</tr>
<tr>
<td>Insomnia</td>
</tr>
<tr>
<td>Hypertension</td>
</tr>
<tr>
<td>Palpitations</td>
</tr>
<tr>
<td>Increase in mean corpuscular volume</td>
</tr>
<tr>
<td>Decrease in lymphocytes</td>
</tr>
</tbody>
</table>

*Side-effects may be patient-dependent based upon expression profile of off-targets*

Case study: polypharmacology in PARP inhibitors

2024 clinical trial comparison of PARP inhibitors

- **Olaparib**: FDA approved 2014
  - Few kinase off-targets

- **Niraparib**: FDA approved 2017
  - Significant kinase off-targets

Advanced ovarian cancer patients after 3:1 matching

Case study: polypharmacology in PARP inhibitors

2024 clinical trial comparison of PARP inhibitors

olaparib  
FDA approved 2014

niraparib  
FDA approved 2017

advanced ovarian cancer patients

no statistically significant survival difference

but:

niraparib patients showed increased frequency of:

- thrombocytopenia  
  $p = 0.021$

- neutropenia  
  $p = 0.011$

hematological toxicity

few kinase off-targets  

significant kinase off-targets

Case study: large scale proteomics for ligand discover/prediction

April 26, 2024 - Pfizer, Georg E. Winter

- 407 drug fragments encompassing a variety of chemical space
- each fragment linked to diazirine-azide moiety for PAL
- tested against HEK293T cells at 50 µM

What if we could do FBLD in cells?

Case study: large scale proteomics for ligand discover/prediction

Case study: large scale proteomics for ligand discover/prediction

promiscuity ranking of drug fragments

localization of ligand targets

Case study: large scale proteomics for ligand discover/prediction

list of most commonly enriched proteins by fragments

Case study: large scale proteomics for ligand discover/prediction

utilizing fragment data to predict promiscuity

generate predicted promiscuity parameters
utilizing of fragment data as a training set for machine learning

utilize 100 physiochemical properties as predictors for promiscuity

analysis of most important molecular parameters

Case study: large scale proteomics for ligand discover/prediction

validation of promiscuity readouts

more promiscuous

less promiscuous

Case study: large scale proteomics for ligand discover/prediction

Validation of promiscuity readouts

Test compounds followed trend of promiscuity

Polypharmacology takeaways

1. Polypharmacology isn't inherently good or bad
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2. As our understanding of disease biology grows, so does the potential of polypharmacological approaches
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1. Polypharmacology isn't inherently good or bad

2. As our understanding of disease biology grows, so does the potential of polypharmacological approaches

3. Revisiting of already approved drugs can uncover useful polypharmacology that can lead to drug repurposing

\[
\text{celecoxib} \quad \text{thalidomide}
\]
Polypharmacology takeaways

1. Polypharmacology isn't inherently good or bad

2. As our understanding of disease biology grows, so does the potential of polypharmacological approaches

3. Revisiting of already approved drugs can uncover useful polypharmacology that can lead to drug repurposing

4. We are in a golden age for drug repurposing with new methodologies and opportunities
Questions?