Combining Target-Based and Phenotypic Discovery Assays for Drug Repurposing

Sharlene Velichko, Ph.D.
Director of Research Biology
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Drug Repurposing

*Finding new life for old drugs*

**Initial drug approval:**
10-20 years

**Secondary indication approval:**
3-5 years

- **Initial drug approval:**
  - Average cost is ~ $2.6B\(^1\)
  - Likelihood of approval for a drug that enters Phase II is ~15%\(^2\)

- **Repurposing programs:**
  - Can extend indication space for approved therapies
  - Can salvage failed drugs
  - Have accelerated approval timeframes based on established safety profiles → lower cost

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\(^1\) DiMasi JA, et al. Tufts Center for the Study of Drug Development Briefing (2014)

\(^2\) www.bio.org
Drug Repurposing

Evidence-based guidance for indication selection

Initial drug approval: 10-20 years
Secondary indication approval: 3-5 years

Repurposing indication selections have been based on:
- Serendipitous discovery
- Highly related diseases

A systems biology-based approach would:
- Provide evidence-guided indication selection
- Systematically evaluate multiple indications
- Prioritize repurposing programs
Disease Biology as a Complex Network

Idiopathic Pulmonary Fibrosis (IPF)

PHENOTYPIC ASSAYS

TARGET-BASED ASSAYS

ER Stress
TGFβ signaling
Matrix Biology (CTGF, LOXL2)
Macrophage Biology

DiscoverX
BioMAP® Phenotypic Platform

Modeling human biology for phenotypic drug development

Key features of the BioMAP platform
- Human primary cell-based models of disease biology
- Clinically relevant biomarkers as endpoints
- Database of 4500+ reference compounds

BioMAP Profile
BioMAP® Tools for Drug Repurposing

Panel Covering Broad Biology

Panels Covering Specific Disease Biology

Large Reference Database

BioMAP Knowledgebase, Tools, and Expertise

Safety & Toxicity Assessment

ComboELECT
Large Drug Reference Database

- **Reference Database Includes:**
  - **Drugs** – Clinical stage, approved, and failed
  - **Experimental Chemicals** - Research tool compounds, environmental chemicals, nanomaterials
  - **Biologics** – Antibodies, cytokines, factors, peptides, soluble receptors

- **Key Benefits:**
  - Establishment of MoA
  - Comparison to gold standard drugs
  - Hypothesis testing
Systematic Evaluation of the IPF Potential of Known Drugs

1. Define broad phenotypic signature of approved IPF drugs
2. Search reference database for similar signatures
3. Confirm hits in BioMAP systems modeling fibrosis
4. Rank hits based on IPF potential
Defining the Phenotypic Profile of Pirfenidone
Anti-fibrotic molecule with unknown target(s)

**Anti-inflammatory**
- Decreased inflammation markers in systems containing immune cells
- PMID: 9877280

**Immunosuppression**
- Inhibition of immune cell proliferation.
- PMID: 19667934

**Tissue Remodeling**
- Inhibition of extracellular matrix production.
- PMID: 24613900
Defining the Phenotypic Profile of Nintedanib

*Multi-targeted tyrosine kinase inhibitor*

**Tissue Remodeling**
- Inhibition of extracellular matrix production

**Anti-inflammatory**
- Decreased inflammation markers in systems containing immune cells.

**Immunosuppression**
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Defining the Phenotypic Profile of Nintedanib

Multi-targeted tyrosine kinase inhibitor

- Inhibition of extracellular matrix production
- Tissue Remodeling
- Anti-inflammatory: Decreased inflammation markers in systems containing immune cells.
- Immunosuppression: Inhibition of immune cell proliferation.
- Cell Cycle Inhibition: Inhibited proliferation of non-immune cells, consistent with its oncology indications.
- Hemiostasis modulation: Modulated hemostasis-related proteins thrombomodulin (TM) and tissue factor (TF).
- Vascular EC Inflammation
- Monocyte Activation
- T cell Activation
- B cell Activation
- Epithelial Inflammation and Matrix Remodeling
- Vascular SM Inflammation
- Matrix-modulation, fibrosis, tissue remodeling responses
- Macrophage Activation

ONCOLOGY POTENTIAL

- Inhibition of extracellular matrix production

DiscoverX
Immune cell proliferation and infiltration is positively correlated to rapid disease progression in IPF patients.
PMID: 27159038, 25580363

TNFα drives TGFβ induced fibrotic processes and has been associated with acute exacerbation events in IPF patients.
PMID 2780987

Active PAI-1 is increased in IPF patients. The fibrinolytic pathway controls scar formation.
PMID: 23440593

IL-10 is increased in IPF patients. IL-10 is produced by macrophages that promote wound healing/fibrosis.
PMID: 9316504
1. Develop scorecard based on shared activities of approved drugs

2. Score drug profiles in reference database

<table>
<thead>
<tr>
<th>Rank</th>
<th>ID</th>
<th>Score</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Drug A</td>
<td>30</td>
</tr>
<tr>
<td>2</td>
<td>Drug B</td>
<td>28</td>
</tr>
<tr>
<td>3</td>
<td>Drug C</td>
<td>27</td>
</tr>
<tr>
<td>4</td>
<td>Drug D</td>
<td>25</td>
</tr>
<tr>
<td>5</td>
<td>Drug E</td>
<td>22</td>
</tr>
</tbody>
</table>

3. Rank drugs by score
### Signature Search Reveals Multiple Src/Abl Inhibitors

<table>
<thead>
<tr>
<th>Compound</th>
<th>Mechanism</th>
<th>IPF Potential Score (out of 30)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Geldanamycin</td>
<td>Hsp90 Inhibitor</td>
<td>30</td>
</tr>
<tr>
<td>Radicicol</td>
<td>Hsp90 Inhibitor</td>
<td>30</td>
</tr>
<tr>
<td>Tunicamycin</td>
<td>Antibiotic</td>
<td>30</td>
</tr>
<tr>
<td>Afatinib</td>
<td>EGFR/HER2 Inhibitor</td>
<td>29</td>
</tr>
<tr>
<td>Spebrutinib</td>
<td>BTK Inhibitor</td>
<td>28</td>
</tr>
<tr>
<td><strong>Saracatinib</strong></td>
<td><strong>Src/Abl Inhibitor</strong></td>
<td><strong>27</strong></td>
</tr>
<tr>
<td><strong>Bosutinib</strong></td>
<td><strong>Src/Abl Inhibitor</strong></td>
<td><strong>27</strong></td>
</tr>
<tr>
<td>Dabrafenib</td>
<td>BRAF Inhibitor</td>
<td>27</td>
</tr>
<tr>
<td>ICG-001</td>
<td>Beta Catenin Inhibitor</td>
<td>27</td>
</tr>
<tr>
<td>Cycloheximide</td>
<td>Antibiotic</td>
<td>26</td>
</tr>
<tr>
<td><strong>Dasatinib</strong></td>
<td><strong>Src/Abl Inhibitor</strong></td>
<td><strong>26</strong></td>
</tr>
<tr>
<td>OTX015</td>
<td>BET Inhibitor</td>
<td>26</td>
</tr>
<tr>
<td>Pazopanib HCl</td>
<td>VEGFR Inhibitor</td>
<td>26</td>
</tr>
<tr>
<td>Rosiglitazone</td>
<td>PPARg Agonist</td>
<td>26</td>
</tr>
<tr>
<td>Vorinostat</td>
<td>HDAC Inhibitor</td>
<td>26</td>
</tr>
<tr>
<td>VX-809</td>
<td>CFTR Corrector</td>
<td>26</td>
</tr>
<tr>
<td>AM-966</td>
<td>LPA1 Inhibitor</td>
<td>25</td>
</tr>
<tr>
<td><strong>Imatinib</strong></td>
<td><strong>Src/Abl Inhibitor</strong></td>
<td><strong>25</strong></td>
</tr>
</tbody>
</table>

**Toxicity and stability/reactivity issues**

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*Note: The table entries and their corresponding scores are for illustrative purposes only.*
Src/Abl Inhibitors

- Src family kinases include 9 members:
  - SrcA subfamily: c-Src, Yes, Fyn, Fgr
  - SrcB subfamily: Lck, Hck, Blk, Lyn
  - Frk

- c-Src and Abl are closely related cytoplasmic tyrosine kinases that respond to external stimuli by activating multiple signaling pathways
  - several Src-Abl inhibitors approved for the treatment of Ph+ CML

- Src activity has been associated with the pathogenesis of solid tumors

- Src activity has also been shown to play an important role in myofibroblast activation and differentiation
Polypharmacology of Src/Abl Related Inhibitors

*Is selective polypharmacology the key to efficacy?*

Opportunity to tailor target selectivity for optimal efficacy/safety in IPF
### Evaluation of Organ Specific Fibrosis

<table>
<thead>
<tr>
<th>Pulmonary Fibrosis</th>
<th>General</th>
<th>Renal Fibrosis</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Decreased Inflammation</strong></td>
<td><strong>Inhibition of myofibroblast activation</strong></td>
<td><strong>Decreased ECM production</strong></td>
</tr>
<tr>
<td>Decreased expression of IL-6, IL-8</td>
<td>Decreased expression of α-smooth muscle actin in MyoF, REMyoF but not SAEMyoF</td>
<td>Decreased expression of collagens –I, -III and –IV in MyoF, REMyoF but not SAEMyoF</td>
</tr>
</tbody>
</table>

**Profiles**
- Saracatinib, 3.3 uM
- Saracatinib, 1.1 uM
- Saracatinib, 370 nM
- Saracatinib, 120 nM
Comparison to Pirfenidone

Context-dependent impact guides indication selection

<table>
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<tr>
<th>Pulmonary Fibrosis</th>
<th>General</th>
<th>Renal Fibrosis</th>
</tr>
</thead>
<tbody>
<tr>
<td>SAEMyoF</td>
<td>MyoF</td>
<td>REMyoF</td>
</tr>
</tbody>
</table>

Profiles
- Saracatinib, 3.3 uM
- Pirfenidone, 5000 uM

Less similar

More similar
Fibrosis Panel Profiles of Src/Abl Related Inhibitors

Overall target profile leads to unique phenotypic signatures
## Ranking of Src/Abl Inhibitors for IPF Potential

*Combine broad and lung-specific activities into composite score*

<table>
<thead>
<tr>
<th>Agent Name</th>
<th>Broad Score</th>
<th>Lung Specific Score</th>
<th>Composite Score</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dasatinib</td>
<td>26</td>
<td>5</td>
<td>31</td>
</tr>
<tr>
<td>Saracatinib</td>
<td>27</td>
<td>2</td>
<td>29</td>
</tr>
<tr>
<td>Bosutinib</td>
<td>27</td>
<td>1</td>
<td>28</td>
</tr>
<tr>
<td>Imatinib</td>
<td>25</td>
<td>0</td>
<td>25</td>
</tr>
</tbody>
</table>

- Dasatinib has the highest composite score amongst tested Src/Abl related inhibitors based on overall similarity to approved IPF drugs.
- Dasatinib has recently been shown to be effective in a mouse model of pulmonary fibrosis (PMID 26607773), as well as block myofibroblast differentiation through the Src-SRF pathway (PMID: 26548624)
Comparison of Target Profiles

Src& Abl Kinases
- Common targets of all 3 drugs

Targets common to dasatinib and nintedanib include c-Kit, DDR1, DDR2.
- DDRs: collagen receptors that control MMP expression (PMID: 22336030)
- c-Kit: c-Kit\(^+\) cells in lung promote myofibroblast differentiation (PMID 23401096)
Evaluation of Drugs for IPF

Combining Phenotypic and Target-based Approaches for Prioritization

Define shared phenotypic signature of approved IPF drugs

- Despite different target profiles, pirfenidone and nintedanib have a shared phenotypic impact in human primary cells

Search reference database for similar signatures

- Compound profiles in reference database were scored based on similarity to shared activities with approved IPF drugs to identify novel compounds with IPF potential
- Drugs in the Src/Abl inhibitor class were among the top scoring compounds

Confirm hits in BioMAP systems modeling fibrosis

- Src-Abl inhibitors have unique activities in assays modeling fibrosis
- Simultaneous evaluation of context-specific effects can further refine indication selection

Rank hits based on anti-fibrotic potential

- Src-Abl inhibitors were ranked by IPF repurposing potential
- Next steps: refine scoring algorithm to include phenotypic signatures of safety
- Expand scoring algorithm to other disease indications
Meeting the Challenges of Drug Repurposing

Identifying potential indications

• Combining phenotypic and target-based information can streamline hypothesis testing, expand the number of potential therapeutic areas and increase the chances of clinical success for repurposing programs

Prioritizing drugs

• Side-by-side evaluation of drugs in a systematic manner on experimentally validated systems can rank candidates for particular indications
• The same approach can be used to evaluate drug combinations
BioMAP®: The Leading Phenotypic Screening Platform

Drug discovery & development using human primary cell-based disease models

30+ Human Primary Cell Types

200+ Clinically relevant biomarker readouts

20+ core patents

200+ clinically relevant biomarker readouts

4500+ Reference compounds in BioMAP database

56+ Validated Disease Models

Addsg significant value to any drug discovery program

Validated 15+ Years

Partnering experience with Industry leaders

Predictive MoA | Efficacy | Safety | Tox

15+ years of experience

Addressing unmet market needs

Clear value proposition

Actionable Data for Effective, Efficient & Timely Decision Making