Combining Target-Based and Phenotypic Assays for Successful Drug Repurposing

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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
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

Biology Modeled
Human Primary Cells
Complex Cultures
BioMAP System

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
Define broad phenotypic signature of approved IPF drugs

Search reference database for similar signatures

Confirm hits in BioMAP® systems modeling fibrosis

Rank hits based on IPF potential
Defining the Phenotypic Profile of Pirfenidone

Anti-fibrotic molecule with unknown target(s)

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
---|---|---|---|---|---|---|---
3C | 4H | LPS | SAq | BT | BF4T | BE3C | CASM3C | HDF3CGF | KF3CT | MyoF | Mphg

**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

Profiles:
- Pirfenidone, 1700 uM
- Pirfenidone, 560 uM
- Pirfenidone, 180 uM
- Pirfenidone, 30 uM
Defining the Phenotypic Profile of Nintedanib

Multi-targeted tyrosine kinase inhibitor

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

- Anti-Inflammatory
  Decreased inflammation markers in systems containing immune cells.

- Immunosuppression
  Inhibition of immune cell proliferation.

- Tissue Remodeling
  Inhibition of extracellular matrix production.
### Defining the Phenotypic Profile of Nintedanib

**Multi-targeted tyrosine kinase inhibitor**

<table>
<thead>
<tr>
<th>Vascular EC Inflammation</th>
<th>Monocyte Activation</th>
<th>T cell Activation</th>
<th>B cell Activation</th>
<th>Epithelial Inflammation and Matrix Remodeling</th>
<th>Vascular SM Inflammation</th>
<th>Matrix-modulation, fibrosis, tissue remodeling responses</th>
<th>Macrophage Activation</th>
</tr>
</thead>
<tbody>
<tr>
<td>3C</td>
<td>4H</td>
<td>LPS</td>
<td>SAg</td>
<td>BF4T</td>
<td>BE3C</td>
<td>CASM3C</td>
<td>HDF3CGF</td>
</tr>
<tr>
<td>TM</td>
<td>TF</td>
<td>M-1</td>
<td>CD69</td>
<td>sTNFα</td>
<td>VCA-M-1</td>
<td>MCP-1</td>
<td>MMPI</td>
</tr>
<tr>
<td>MCP-1</td>
<td>sIL-10</td>
<td>sIL-17A</td>
<td>sIL-12</td>
<td>sIL-2</td>
<td>HLA-DR</td>
<td>MCP-1</td>
<td>Mphg</td>
</tr>
<tr>
<td>Hemostasis Modulation</td>
<td>Anti-Inflammatory</td>
<td>Immunosuppression</td>
<td>Cell Cycle Inhibition</td>
<td>Tissue Remodeling</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Modulated hemostasis-related proteins thrombomodulin (TM) and tissue factor (TF).</td>
<td>Decreased inflammation markers in systems containing immune cells.</td>
<td>Inhibition of immune cell proliferation.</td>
<td>Inhibited proliferation of non-immune cells, consistent with its oncology indications.</td>
<td>Inhibition of extracellular matrix production</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**ONCOLOGY POTENTIAL**
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
Score Reference Database

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>Saracatinib</td>
<td>Src/Abl Inhibitor</td>
<td>27</td>
</tr>
<tr>
<td>Bosutinib</td>
<td>Src/Abl Inhibitor</td>
<td>27</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>Dasatinib</td>
<td>Src/Abl Inhibitor</td>
<td>26</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>Imatinib</td>
<td>Src/Abl Inhibitor</td>
<td>25</td>
</tr>
</tbody>
</table>

Toxicity and stability/reactivity issues
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

IPF Potential Score

Imatinib 25
Dasatinib 26
Bosutinib 27
Sunitinib 20
**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 µM
- Saracatinib, 1.1 µM
- Saracatinib, 370 nM
- Saracatinib, 120 nM
Comparison to Pirfenidone

Context-dependent impact guides indication selection

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<tr>
<th>Pulmonary Fibrosis</th>
<th>General</th>
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<tbody>
<tr>
<td>SAEMoF</td>
<td>MyoF</td>
<td>REMoF</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

- **Bosutinib**
  - Profiles:
    - Bosutinib, 3.3 μM
    - Bosutinib, 1.1 μM
    - Bosutinib, 370 nM
    - Bosutinib, 120 nM

- **Saracatinib**
  - Profiles:
    - Saracatinib, 3.3 μM
    - Saracatinib, 1.1 μM
    - Saracatinib, 370 nM
    - Saracatinib, 120 nM

- **Dasatinib**
  - Profiles:
    - Dasatinib, 10 μM
    - Dasatinib, 3.3 μM
    - Dasatinib, 1.1 μM
    - Dasatinib, 370 nM

- **Imatinib**
  - Profiles:
    - Imatinib, 10 μM
    - Imatinib, 3.3 μM
    - Imatinib, 1.1 μM
    - Imatinib, 370 nM
## 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
- Predictive: MoA | Efficacy | Safety | Tox
- 4500+ Reference compounds in BioMAP database
- Validated: 15+ Years
- Partnering experience with Industry leaders
- 15+ years of experience
- Addressing unmet market needs
- Clear value proposition
- 56+ Validated Disease Models
- Adds significant value to any drug discovery program

Actionable Data for Effective, Efficient & Timely Decision Making
Any Questions?

discoverx.com/biomap

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