Drug discovery research for small molecule protein-protein interaction (PPI) modulators with unique \textit{in silico} drug design strategy

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Interprotein Corporation
Interprotein’s targets for small molecule drug discovery

**Problem to be solved in drug discovery**
- Exhaustion of target molecules
- Decline in productivity

**Solution by INTERPROTEIN**
- Opening up potential target molecules
- Raising productivity

- **Non-PPI**
  - Lipid mediator - Receptor
  - Enzyme - substrate etc.

- **PPI**
  - Cytokine/chemokine - Receptor
  - Transcription factor - regulator etc.

- **Intracellular**
  - Transcription factor
  - Transcription regulator etc.

- **Extracellular**
  - Cytokine, Growth factor etc.

**Target selection**
- IL-6
- VEGF
- IL-2
- IL-17 etc.

- TPO-R, EPO-R, G-CSF-R etc.
Points of today’s talk

1. In-house PPI inhibitor programs
   1.1. Small molecule IL-6 inhibitor
   1.2. Small molecule VEGF inhibitor

2. Collaborative research for new drug targets with unique in silico molecular design strategy, INTENDD (INTerprotein’s Engine for New Drug Design)
   2.1. Identification of small molecule binding site and in silico screening by SBSG (Structure-Based Scaffold Generation)
   2.2. Strategic evaluation of small molecule PPI inhibitors, especially cytokine-receptor interaction regulators
Two concepts for cytokine-cytokine receptor interaction inhibitors

<table>
<thead>
<tr>
<th>Binding to cytokine or cytokine receptor</th>
<th>Specific binding</th>
<th>Specific binding</th>
</tr>
</thead>
<tbody>
<tr>
<td>Inhibition of cytokine-cytokine receptor binding</td>
<td>Partial inhibition</td>
<td>Full inhibition</td>
</tr>
<tr>
<td>Inhibition of cellular signaling (upper stream)</td>
<td>Full inhibition</td>
<td>Full inhibition</td>
</tr>
</tbody>
</table>

Partial-antagonist type

Full-antagonist type
Image of inhibition of cytokine-cytokine receptor interaction by small molecule compounds

Pathophysiological situation

Partial-antagonist type
- Impaired binding of cytokine to its receptor
- Cytokine
  Small molecule inhibitor
- Receptor
- Regulated signal
- Occurring simultaneously

Full-antagonist type
- Dissociation of cytokine from its receptor
- Cytokine
  Small molecule inhibitor
- Receptor
- No signal
Two types of small molecule IL-6 inhibitors

Partial-antagonist type compound

- **Binding to IL-6 (SPR)**
- **Inhibition of IL-6 binding to IL-6R (SPR)**
- **Inhibition of p-STAT3 (SureFire)**

Full-antagonist type compound

- **Binding to IL-6 (SPR)**
- **Inhibition of IL-6 binding to IL-6R (SPR)**
- **Inhibition of p-STAT3 (SureFire)**
**<Concept>**

- Replacement/dose reduction of tocilizumab (Actemra), and further expansion of anti-IL-6 therapies for tocilizumab-approved diseases and other autoimmune/inflammatory diseases

**<Present status>**

- Under synthesis and evaluation of compounds for optimization.
- Compound X shows good *in vivo* PK profile in mice (30 mg/kg, p.o.; F value, 26%).

<table>
<thead>
<tr>
<th>Parameters tested</th>
<th>Criteria</th>
<th>Compound X</th>
<th>Positive compounds</th>
</tr>
</thead>
<tbody>
<tr>
<td>clogP</td>
<td>2.0&lt;clogP&lt;6.0</td>
<td>Clear</td>
<td>-</td>
</tr>
<tr>
<td>tPSA (A²)</td>
<td>&lt;140</td>
<td>Clear</td>
<td>-</td>
</tr>
<tr>
<td>pSTAT3 (IC₅₀, μmol/L)</td>
<td>&lt;50</td>
<td>Clear (&lt;1)</td>
<td>39/136 (28.7%)</td>
</tr>
<tr>
<td>IC₅₀ ratio vs. tocilizumab</td>
<td>&lt;1/1000</td>
<td>Clear (1/50-1/200)</td>
<td>-</td>
</tr>
<tr>
<td>Binding to IL-6 (SRP, RU)</td>
<td>&gt;5</td>
<td>Clear</td>
<td>70/88 (79.5%)</td>
</tr>
<tr>
<td>Inhibition of IL-6/IL-6R binding</td>
<td>&gt;5</td>
<td>Clear</td>
<td>18/122 (14.8%)</td>
</tr>
<tr>
<td>interaction (SPR, % inhi.)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Interaction with IL-6 (¹⁵N-NMR)</td>
<td></td>
<td>Clear (change at</td>
<td>34/54 (62.9%)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>7/10 residues)</td>
<td></td>
</tr>
</tbody>
</table>
**Small molecule VEGF inhibitor** (*VEGF/VEGFR interaction inhibitor*)

### <Concept>
- Replacement and/or dose reduction of bevacizumab (Avastin), and further penetration of anti-VEGF therapies (acquisition of indications for which bevacizumab has been not approved; diffusion of combination therapy with small molecule VEGF inhibitor and current standard chemotherapies → for distinction from tyrosine kinase inhibitors, TKIs)

### <Present status>
- Under synthesis and evaluation of compounds aiming for lead optimization.
- Compound Y shows great *in vivo* efficacy in mouse xenograft model (40 mg/kg, p.o.).

<table>
<thead>
<tr>
<th>Parameters tested</th>
<th>Criteria</th>
<th>Compound Y</th>
</tr>
</thead>
<tbody>
<tr>
<td>VEGF-stimulated HUVEC growth</td>
<td>$IC_{50} &lt; 100$ nM</td>
<td>Clear</td>
</tr>
<tr>
<td>Selectivity</td>
<td>10-fold $IC_{50}$ or more for:</td>
<td>Clear</td>
</tr>
<tr>
<td></td>
<td>EGM2-stimulated HUVEC growth, LS174T/fibroblast growth</td>
<td></td>
</tr>
<tr>
<td>In vivo efficacy</td>
<td>Comparable to bevacizumab</td>
<td>Clear</td>
</tr>
<tr>
<td>Binding to VEGF (SRP)</td>
<td>$&gt; 5$ RU</td>
<td>Under assessment</td>
</tr>
<tr>
<td>Inhibition of VEGF/VEGFR binding (SPR)</td>
<td>$&gt; 5%$</td>
<td>Under assessment</td>
</tr>
<tr>
<td>Interaction with VEGF ($^{15}$N-NMR)</td>
<td>Chemical shift change at proposed binding site</td>
<td>Under assessment</td>
</tr>
</tbody>
</table>
Pharmacological profile of Small molecule VEGF inhibitor  
(Preceding compound, Compound Y)

Summary of *in vitro* evaluation

<table>
<thead>
<tr>
<th>Test</th>
<th>IC50 (nM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>VEGF-HUVEC</td>
<td>&lt; 15.6</td>
</tr>
<tr>
<td>EGM2-HUVEC</td>
<td>304</td>
</tr>
<tr>
<td>LS174T</td>
<td>&gt; 1000</td>
</tr>
<tr>
<td>Fibroblast</td>
<td>&gt; 1000</td>
</tr>
</tbody>
</table>

LS174T-inoculated xenograft model in nude mice (established tumor model)

Experimental group:
1. Control (PBS, days 0 - 8, i.p.)
2. Avastin 5 mg/kg day 0, 3, 7, i.p.
3. Compound Y 40 mg/kg days 0 - 8, p.o.

Data: mean ± S.E.M. (n = 5)
LS174T inoculation: s.c.
Tumor volume at the start of administration: 276 mm³
**Unique SBDD strategy, INTENDD**

**Essential Principle for Drug Design:** Shape” and “Color” have to be matched (“Shape” means 3D surface structure, and “color” means H-bond and so on)

**Components of INTENDD (INTerprotein’s Engine for New Drug Design)**

1) Identification of target cavity [1 month] → Identification of Target Cavity with Precise 3D Model

2) *In silico* screening by Structure-Based Scaffold Generation (SBSG) method (proposal of around 200 compounds) [3 months]

(3D models can be produced easily in 2 hours)

**Value & Advantage of INTENDD**

A) Discover real hits compounds in high-ranked 200 comp.

B) Create new & druggable scaffolds with wide diversity at one try (also applicable to back-up compounds)

C) Acceptable structures for medicinal chemists

D) Proven MOA by SPR, NMR, X-ray, HDx in many projects

E) Not restricted by types of targets (PPI, enzyme, receptor and so on)

F) Applicable not only to hit identification but also lead generation and optimization
**Procedure of SBSG method**

**SBSG:** Structure-Based Scaffold Generation

- Commercially available chemical libraries (ca. 10 millions)
- Search of ring positions at the cavity
- Formation of ligand skeletons (ca. 50 structures manually)
- Search of skeletons in available compound data bases
- Collision check with protein atoms
- Selection of promising compounds by clustering and filtering by **binding structure-based mechanisms**

Proposal of compounds for wet screening (ca. 2 hundreds) — **Wet Screening**

*<not require large cost and HTS>*
## Comparison between SBSG and current methods

<table>
<thead>
<tr>
<th>Item</th>
<th>SBSG</th>
<th>Docking</th>
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<tbody>
<tr>
<td>Coverage of search space</td>
<td>Complete with novel skeletons</td>
<td>Limited to data base cmps.</td>
</tr>
<tr>
<td>Examined compounds</td>
<td>Ca. $10^6$ data base cmps.</td>
<td>Ca. $10^6$ data base cmps.</td>
</tr>
<tr>
<td>Major driving force in search</td>
<td>Binding structure-based mechanism</td>
<td>Calculated binding energy-based</td>
</tr>
<tr>
<td>Scoring of cmps.</td>
<td>Filtering on assumed mechanism</td>
<td>Calculated binding energy</td>
</tr>
<tr>
<td>Time frame for design</td>
<td>1 month x 3 iterations</td>
<td>1 – 3 months</td>
</tr>
</tbody>
</table>
| Required number of cmps. to obtain hit cmps. in wet screening systems | 200 – 300  
  ◆ Low purchase cost of cmps.  
  ◆ Easily assayed by manual screening system (no need for HTS) | A few thousands or more                        |
| Number of hits & diversity                       | 10 - 30 with broad diversity                   | 0 – 5 with poor diversity                    |
### Example of collaborative research
- the case where information on X-ray crystal structure of target protein is existing -

#### Schedule

<table>
<thead>
<tr>
<th></th>
<th>1st year</th>
<th>2nd year</th>
<th>3rd year</th>
<th>4th year &amp; later</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td>5</td>
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<td>6</td>
<td>7</td>
<td>8</td>
<td>9</td>
<td>10</td>
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<tr>
<td>11</td>
<td>12</td>
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#### Events
- Research collaboration agreement
- Primary hits identified
- Secondary hits identified
- Leads identified
- Candidate identified
- Pre-clinical & clinical studies
- Milestone/Royalty (or sharing of profit from 3rd party)

#### Payments
- Up-front fee
- Contingency fee (primary, secondary, or both)
- Leads identified
- Lead optimization
- Pre-clinical & Clinical studies

#### Role of each side
**Interprotein**
- Identification of binding site
- In silico screening
- Supports of Purchase and screening
- Supports of Purchase and screening
- Supports from the view of SBDD

**Pharmaceutical company**
- Proposal of target protein
- Purchase of compounds + Wet screening
- Purchase of compounds + Wet screening
- Lead generation
- Pre-clinical & Clinical studies
Functions and expertise of Interprotein

1. Proposal of hit candidates by INTENDD
   ◆ Real 3D model-based identification of binding site
   ◆ *In silico* screening by SBSG method

2. Synthesis of compounds
   ◆ Know-how of lead generation/optimization of small molecule PPI inhibitors

3. Evaluation of compounds
   ◆ Know-how of strategic assessment of small molecule PPI inhibitors
   ◆ Close collaboration with experts of protein/drug discovery research (NB Health Laboratory, Kyoto Sangyo Univ., Osaka Univ., MARUWA Foods & Biosciences, Japan Aerospace Exploration Agency (JAXA), RIKEN, etc.)
Strategic evaluation of small molecule PPI inhibitors

- Inhibition of binding of target protein to its binding partner by compound (SPR, AlphaScreen, FRET, etc.)
- Binding of compound to target protein (SPR)
- Inhibition of target protein-related signal by compound (upper-stream; SureFire, cell-based)

Decision of hit compounds

- Interaction of compound with target protein (NMR)
- Inhibition of cellular response (lower-stream; including cytotoxicity)
- Examination on selectivity (protein-based and cell-based)
- Co-crystallization of compound and target protein (X-ray)

Comprehensive decision of hit/lead compounds

- In vivo efficacy pharmacology
- In vitro & in vivo eADMET

Cycle of synthesis and evaluation

Selection of candidate for pre-clinical studies
Outlines of R&D activities of Interprotein

1. In-house programs (→ searching for license/collaboration partners)

<table>
<thead>
<tr>
<th>Program</th>
<th>Stage</th>
<th>Mechanism of action</th>
</tr>
</thead>
<tbody>
<tr>
<td>VEGF inhibitor</td>
<td>Lead optimization</td>
<td>Inhibition of VEGF/VEGFR2 interaction; not TKI</td>
</tr>
<tr>
<td>IL-6 inhibitor</td>
<td>Lead optimization</td>
<td>Inhibition of IL-6/IL-6R interaction</td>
</tr>
<tr>
<td>Tubulin inhibitor</td>
<td>Lead optimization</td>
<td>Inhibition of tubulin polymerization</td>
</tr>
<tr>
<td>Notch 1 inhibitor</td>
<td>Lead optimization</td>
<td>Inhibition of NICD/RBP-Jk/MAM interaction</td>
</tr>
<tr>
<td>IgE inhibitor</td>
<td>Lead generation</td>
<td>Inhibition of IgE/FcεRI interaction</td>
</tr>
</tbody>
</table>

2. Drug discovery research for new targets (→ searching for collaborative research partners)

<table>
<thead>
<tr>
<th>Target</th>
<th>Main role</th>
</tr>
</thead>
</table>
| Proposed by the Partners PPIs/non-PPIs inhibitors/agonists | Interprotein

*In silico* screening by INTENDD (support of wet screening)

<table>
<thead>
<tr>
<th>Pharmaceutical Company</th>
<th>Wet screening by protein/cell-based assay systems</th>
</tr>
</thead>
</table>

Ajinomoto Pharmaceuticals Co. Ltd.
Takeda Pharmaceutical Co. Ltd.,
etc.

Ex. of collaboration
Interprotein said partner Takeda will continue to evaluate small molecule inhibitors of protein-protein interactions under a 2011 deal after **about 11% of the compounds tested were found to bind to the target protein**. Interprotein said it is "generally recognized" that hit rates against protein targets through computational drug design are "well below 11%." The companies partnered last December to develop the compounds using Interprotein's Engine for New Drug Design (INTENDD) technology. The companies could not be reached for comment (see BioCentury, Jan. 9).
We are searching alliance partners for:

1. Small molecule VEGF inhibitor
2. Small molecule IL-6 inhibitor
3. Small molecule Notch1 inhibitor
4. Small molecule IgE inhibitor
5. Tubulin polymerization inhibitor
6. Inhibitors/agonists for new drug targets (PPI/non-PPI)

License and/or research collaboration

Collaborative research

Contribution to raising the productivity of drug discovery research for all types of drug targets with 3D structure information

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