Discovery of GLPG1690: a first-in-class autotaxin inhibitor in clinical development for the treatment of idiopathic pulmonary fibrosis

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Idiopathic Pulmonary Fibrosis (IPF)
High unmet medical need

- NIH defines IPF as “a condition in which over a period of time the lung tissue becomes thickened, stiff and scarred”
  - Progressive interstitial lung disease
  - Difficulties breathing and reduced ability to oxygenate the blood
  - High mortality: ~20% 5-yr survival rate
    - ~40,000 / yr in the US
  - Onset of the disease between 50 and 70 years old
- No curative treatment available

Idiopathic Pulmonary Fibrosis (IPF)
Etiology and drugs approved recently

- IPF believed to result from inflammatory response to microscopic injury
  - Risk factors: cigarette smoking, environmental factors, infectious agents, genetic factors...

- Current therapies (approved since 2010) only slow down disease progression

<table>
<thead>
<tr>
<th>Drugs approved for IPF</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pirfenidone</td>
</tr>
<tr>
<td>Roche</td>
</tr>
<tr>
<td>Precise mechanism of action unknown</td>
</tr>
<tr>
<td>Nintedanib (BIBF-1120)</td>
</tr>
<tr>
<td>Boehringer Ingelheim</td>
</tr>
<tr>
<td>Inhibition of multiple tyrosine kinases</td>
</tr>
</tbody>
</table>

Thannickal V. et al *Drugs* (2016)
**Autotaxin (ATX)**

Secreted enzyme producing bioactive LPA

- First isolated in 1992 from melanoma cells
- Extracellular enzyme
- Converts lysophosphatidyl choline (LPC) into lysophosphatidic acid (LPA)
  - Family of bioactive lipids with varied fatty acid chain length and saturation

Autotaxin-LPA signaling

- LPA acts through GPCR LPA$_{1-6}$
  - LPA signaling involved in multiple cellular processes and pathological conditions
- Recent studies suggest role of ATX-LPA signaling in IPF
  - Increased levels of ATX in fibrotic lungs and of LPA in BronchoAlveolar Lavage Fluid (BALF, mouse and human data)
  - Efficacy in murine IPF models shown for LPA$_1$ antagonists and ATX inhibitors
    - BMS-986020 (LPA$_1$ antagonist) in phase 2 IPF trial

Qian, Y.; Budd, D. *Future Med. Chem.* (2013)
Structure of Autotaxin

Multidomain glycoprotein (~100 kDa) encompassing
- N-terminal signal sequence with two Somatomedin B (SMB)-like domains
- Catalytic domain with two zinc ions
- Nuclease-like domain (inactive)

Co-crystallization of ATX with LPA

Overlay of LPA (14:0) and LPA (22:6) co-crystallized in Autotaxin

- Phosphate groups bind zinc atoms in a similar way
- Lipophilic chains fill lipophilic pocket
- Presence of LPA in the channel suggests a role of ATX for local LPA delivery

## Autotaxin small molecules inhibitors

### Lipid-like structures

<table>
<thead>
<tr>
<th>Structure</th>
<th>Company</th>
</tr>
</thead>
<tbody>
<tr>
<td><img src="image1" alt="S32826" /></td>
<td>Servier</td>
</tr>
<tr>
<td><img src="image2" alt="Br-LPA" /></td>
<td>Prestwich et al.</td>
</tr>
</tbody>
</table>

### Linear structures with lipophilic tail and polar head

<table>
<thead>
<tr>
<th>Structure</th>
<th>Company</th>
</tr>
</thead>
<tbody>
<tr>
<td><img src="image3" alt="Cpd 7905958" /></td>
<td>Parrill et al.</td>
</tr>
<tr>
<td><img src="image4" alt="PF8380" /></td>
<td>Pfizer / Merck KGaA</td>
</tr>
<tr>
<td><img src="image5" alt="HA 155" /></td>
<td>Moolenaar et al.</td>
</tr>
<tr>
<td><img src="image6" alt="Biogen" /></td>
<td>Hofmann-La Roche</td>
</tr>
</tbody>
</table>

### Alternative shape

<table>
<thead>
<tr>
<th>Structure</th>
<th>Company</th>
</tr>
</thead>
<tbody>
<tr>
<td><img src="image7" alt="Merck KGaA" /></td>
<td>Amira</td>
</tr>
<tr>
<td><img src="image8" alt="ONO Pharmaceutical" /></td>
<td>X-RX Discovery</td>
</tr>
<tr>
<td><img src="image9" alt="Novartis" /></td>
<td>Biogen</td>
</tr>
<tr>
<td><img src="image10" alt="Novartis" /></td>
<td>Eli Lilly</td>
</tr>
</tbody>
</table>

Assessment of Autotaxin activity

Using natural substrate(s)

Autotaxin

Choline Oxidase

2 O₂ + H₂O

Betaine + 2 H₂O₂

Peroxidase

TOOS + 4-aminoantipyrine

Quinoneimine dye + 4 H₂O

Plasma assay

LC-MS/MS detection

Multiple LPA species in plasma

Luminescence detection

LPC assay

Using synthetic substrate

Autotaxin

Fluorophore

Quencher

FS-3

Fluorescence detection

FS-3 assay

**Autotaxin inhibitors**

Hit series identified by HTS using FS-3 assay

**Imidazo[1,2-a]pyridine hit series**

![Chemical structure of Imidazo[1,2-a]pyridine hit series](image)

Best compounds:
FS-3 assay $\text{IC}_{50}$~30nM

- Reduction of LPA production in plasma
- Poor metabolic stability
- Non drug-like substituents

**Reduction of LPA production in rat plasma (at 10 µM)**

Method: Compound incubation in plasma for 6h at 37°C

- Mean (Control T0)
- Mean (Control 6h)
- Mean Cpd 1 (6h)
- Mean Cpd 2 (6h)
Autotaxin inhibitors
Hit expansion with FS-3 assay

- Improvement of PK properties led to loss of plasma activity
- Gain in potency required for reduction of LPA production in plasma

<table>
<thead>
<tr>
<th>Plasma activity</th>
<th>in active</th>
</tr>
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<tbody>
<tr>
<td>PK species F (%)</td>
<td>Rat 39</td>
</tr>
<tr>
<td>Cl (L/h/kg)</td>
<td>0.15</td>
</tr>
<tr>
<td>Vss (L/kg)</td>
<td>0.49</td>
</tr>
<tr>
<td>Half-life (iv, h)</td>
<td>2.7</td>
</tr>
</tbody>
</table>

HTS hit series

FS-3 assay IC₅₀: 137 nM

FS-3 assay IC₅₀: 122 nM
Autotaxin inhibitors
Potent inhibitors in FS-3 assay

<table>
<thead>
<tr>
<th>IC&lt;sub&gt;50&lt;/sub&gt;</th>
<th>FS-3 plasma</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;10 nM</td>
<td>&gt;10 µM</td>
</tr>
<tr>
<td>7.9 nM</td>
<td>&gt;10 µM</td>
</tr>
<tr>
<td>11.5 nM</td>
<td>4.8 µM</td>
</tr>
</tbody>
</table>

- No good correlation between FS-3 assay and activity in plasma activity
- FS-3 assay not further used to drive SAR optimization
Alternative biochemical assay
Measuring choline released by cleavage of LPC

- Good correlation between LPC and rat plasma activities
- Biochemical assay using natural LPC substrate used for further SAR exploration

<table>
<thead>
<tr>
<th>IC&lt;sub&gt;50&lt;/sub&gt;</th>
<th>plasma activities</th>
</tr>
</thead>
<tbody>
<tr>
<td>FS-3</td>
<td>&lt;10 nM</td>
</tr>
<tr>
<td></td>
<td>&gt;10 µM</td>
</tr>
<tr>
<td>LPC: &gt;10 µM</td>
<td></td>
</tr>
<tr>
<td>7.9 nM</td>
<td>&gt;10 µM</td>
</tr>
<tr>
<td>LPC: 6.8 µM</td>
<td></td>
</tr>
<tr>
<td>11.5 nM</td>
<td>4.8 µM</td>
</tr>
<tr>
<td>LPC: 2.4 µM</td>
<td></td>
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</tbody>
</table>
SAR optimization with LPC assay
Exploring C6 part

**LPC IC₅₀**
- 2.4 µM
- 710 nM
- 357 nM

Introduction of H-bond acceptor and side chain extension improve activity
Co-crystal structure

- Fluorophenyl part fits in lipophilic pocket
- Piperidine and side chain occupy channel
- No binding to Zinc atoms

Mode of binding could possibly inhibit LPA production and delivery

**LPC IC\textsubscript{50}**

357 nM
Exploring ATX channel
Extending C6 part

IC$_{50}$ = 2.4 µM

Plasma instability

IC$_{50}$ = 480 nM

Ester surrogates

<table>
<thead>
<tr>
<th>Structure</th>
<th>IC$_{50}$</th>
</tr>
</thead>
<tbody>
<tr>
<td><img src="image1.png" alt="Structure 1" /></td>
<td>1.5 µM</td>
</tr>
<tr>
<td><img src="image2.png" alt="Structure 2" /></td>
<td>4.1 µM</td>
</tr>
<tr>
<td><img src="image3.png" alt="Structure 3" /></td>
<td>1.7 µM</td>
</tr>
<tr>
<td><img src="image4.png" alt="Structure 4" /></td>
<td>2.4 µM</td>
</tr>
<tr>
<td><img src="image5.png" alt="Structure 5" /></td>
<td>1.6 µM</td>
</tr>
<tr>
<td><img src="image6.png" alt="Structure 6" /></td>
<td>261 nM</td>
</tr>
<tr>
<td><img src="image7.png" alt="Structure 7" /></td>
<td>86 nM</td>
</tr>
<tr>
<td><img src="image8.png" alt="Structure 8" /></td>
<td>246 nM</td>
</tr>
</tbody>
</table>
Exploring ATX lipophilic pocket

Thiazole part substitution

- Thiazole best for activity
- Several substituents can boost potency

<table>
<thead>
<tr>
<th>LPC IC_{50}</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>710 nM</td>
<td>2.3 µM</td>
<td>138 nM</td>
<td>1.4 µM</td>
</tr>
<tr>
<td>281 nM</td>
<td>226 nM</td>
<td>7.5 µM</td>
<td>126 nM</td>
</tr>
</tbody>
</table>

Thiazole IC_{50} values:
- 710 nM
- 1.2 µM
- 7.5 µM
Exploring ATX lipophilic pocket
Modelling hypothesis for potency improvement

water molecule trapped in hydrophobic environment

LPC IC\textsubscript{50} 710 nM 138 nM

water molecule is released and replaced by nitrile group

Increase in potency by introduction of nitrile on thiazole could be explained by removal of a water molecule from the hydrophobic pocket
Autotaxin inhibitor lead compound

- Potent and orally exposed in rodents
- ADMET properties to be improved:
  - hERG inhibition
  - CYP3A4 time-dependent inhibition (TDI)

<p>| | |</p>
<table>
<thead>
<tr>
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<tbody>
<tr>
<td>LPC IC$_{50}$</td>
<td>27 nM</td>
</tr>
<tr>
<td>Rat plasma IC$_{50}$</td>
<td>101 nM</td>
</tr>
</tbody>
</table>
hERG inhibition
Lowering linker basicity

- Replacement of piperidine linker by piperazine (less basic) decreased hERG inhibition
- ATX activity retained

<table>
<thead>
<tr>
<th>Linker</th>
<th>hERG IC$_{50}$</th>
<th>LPC IC$_{50}$</th>
</tr>
</thead>
<tbody>
<tr>
<td><img src="image" alt="Piperidine structure" /></td>
<td>2.9 µM</td>
<td>27 nM</td>
</tr>
<tr>
<td><img src="image" alt="Piperazine structure" /></td>
<td>&gt;11.1 µM</td>
<td>26 nM</td>
</tr>
</tbody>
</table>

hERG IC$_{50}$ vs linker

IC$_{50}$ = 10 µM

[Graph showing hERG IC$_{50}$ vs linker concentrations with data points for piperidine and piperazine linkers]
CYP3A4 time dependent inhibition
Structure-property relationship

- Core modification improved CYP3A4 TDI
  - Degradation of DMSO solutions of imidazo[1,2-a]pyrazine compounds observed
CYP3A4 time dependent inhibition
Structure-property relationship

Formation of reactive metabolite from imidazo[1,2-a]pyridine

- Imidazo[1,2-a]pyridine known to form reactive metabolite upon CYP activation
- Metabolite can react with nucleophiles (GSH, CYP side-chain residues)

CYP3A4 time dependent inhibition
Introducing soft spot/steric hindrance

- Introduction of methyl in position 7 or 8 of imidazo[1,2-a]pyridine removed CYP3A4 TDI
- Slight loss of potency with additional methyl group on core

| LPC IC$_{50}$ | 811 nM (non-methylated analog: 246 nM) | 103 nM (non-methylated analog: 27 nM) |

Combining structural features
GLPG1690

<table>
<thead>
<tr>
<th></th>
<th>LPC IC$_{50}$ 131 nM (K$_i$ = 15 nM, competitive inhibitor)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plasma IC$_{50}$</td>
<td>Mouse: 417 nM Rat: 542 nM Human: 221 nM</td>
</tr>
<tr>
<td>hERG IC$_{50}$</td>
<td>&gt;10 µM</td>
</tr>
<tr>
<td>CYPs IC$_{50}$</td>
<td>3A4, 2D6, 2C9, 2C19, 1A2 &gt;10 µM</td>
</tr>
<tr>
<td>CYP3A4 TDI</td>
<td>negative</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>PK species</th>
<th>F (%)</th>
<th>Cl (L/h/kg)</th>
<th>Vss (L/kg)</th>
<th>Half-life (iv, h)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mouse</td>
<td>29</td>
<td>0.23</td>
<td>0.31</td>
<td>1.4</td>
</tr>
<tr>
<td>Rat</td>
<td>37</td>
<td>0.51</td>
<td>0.61</td>
<td>1.5</td>
</tr>
<tr>
<td>Dog</td>
<td>63</td>
<td>0.12</td>
<td>0.43</td>
<td>3.5</td>
</tr>
</tbody>
</table>

- Potent, orally exposed Autotaxin inhibitor with optimized in vitro ADMET profile
- Good safety margins from preliminary rat and dog toxicity studies to progress as preclinical candidate

WO2014139882, WO2014202458
Combining structural features
GLPG1690

<table>
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<tr>
<th></th>
<th>LPC IC$_{50}$</th>
<th>Plasma IC$_{50}$</th>
<th>hERG IC$_{50}$</th>
<th>CYPs IC$_{50}$</th>
<th>CYP3A4 TDI</th>
<th>PK species</th>
</tr>
</thead>
</table>
|                | 131 nM (K$_i$ = 15 nM, competitive inhibitor) | Mouse: 417 nM  
Rat: 542 nM  
Human: 221 nM | >10 µM | 3A4, 2D6, 2C9, 2C19, 1A2 >10 µM | negative | Mouse  
F (%)  
Cl (L/h/kg)  
Vss (L/kg)  
Half-life (iv, h)  
Rat  
Dog |
|                |               |                  |                |                |          | Mouse: 29  
0.23  
0.31  
1.4  
37  
0.12 |
|                |               |                  |                |                |          | Rat: 37  
0.51  
0.61  
1.5  
63  
0.43 |
|                |               |                  |                |                |          | Dog: 63  
0.12  
0.43  
3.5  
63  
0.43 |

- Potent, orally exposed Autotaxin inhibitor with optimized *in vitro* ADMET profile
- Good safety margins from preliminary rat and dog toxicity studies to progress as preclinical candidate

WO2014139882, WO2014202458
GLPG1690 synthesis

8 linear steps, 37% overall yield
X-ray structure
GLPG1690 in human Autotaxin

- Compound co-crystallized with human Autotaxin
  - Resolution: 2.4Å
- Hydrophobic pocket and channel occupancy by GLPG1690
Inhibition of LPA species production

*Ex vivo* human plasma assay (LC/MS)

- Method: Compound incubation in plasma for 2h at 37°C. LC-MS/MS

<table>
<thead>
<tr>
<th>LPA species</th>
<th>IC₅₀ (nM)</th>
</tr>
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<tbody>
<tr>
<td>14:0</td>
<td>96</td>
</tr>
<tr>
<td>16:0</td>
<td>117</td>
</tr>
<tr>
<td>18:1</td>
<td>115</td>
</tr>
<tr>
<td>18:2</td>
<td>112</td>
</tr>
<tr>
<td>18:3</td>
<td>102</td>
</tr>
<tr>
<td>22:6</td>
<td>94</td>
</tr>
<tr>
<td>20:4</td>
<td>93</td>
</tr>
</tbody>
</table>

- GLPG1690 inhibits *ex vivo* LPA production, similar IC₅₀ for all LPA species
- GLPG1690 also inhibits LPA production (LPA 18:2) in mouse and rat plasma (not shown)
GLPG1690: mouse PK/PD properties
Reduction of plasma LPA 18:2 as biomarker

Administration of GLPG1690 to mice causes a sustained reduction in plasma LPA levels from a dose of 3 mg/kg onwards which demonstrates target engagement.
GLPG1690 *in vivo* activity

Lung fibrosis in mouse bleomycin (BLM) model

**Ashcroft fibrosis score**

**Collagen content**

*0.01<p<0.05 vs BLM vehicle*

*0.01<p<0.05 vs pirfenidone*

GLPG1690 at 30 mg/kg bid significantly superior to pirfenidone
Mouse BLM lung fibrosis model
Impact on LPA levels in BALF

- Significant increase of various LPA species in the disease group
- Short LPA species reduced by GLPG1690 show target engagement in a relevant matrix for lung fibrosis
GLPG1690 First-in-Human
Objectives/design

• Part 1: single ascending dose (SAD)
  ➢ 16 healthy volunteers; 6 active/2 placebo per dose level
  ➢ dose levels: 20 mg up to 1500 mg (as oral suspension)

• Part 2: multiple ascending dose (MAD)
  ➢ 24 healthy volunteers; 8/cohort; 6 active/2 placebo
  ➢ dose levels: 150 mg bid - 600 mg qd - 1000 mg qd for 14 days (as oral suspension)

• Objectives
  ➢ safety and tolerability
  ➢ pharmacokinetic profile
  ➢ pharmacodynamics: effect on LPA 18:2 plasma level
GLPG1690 SAD
LPA 18:2 reduction

- Dose-dependent reduction observed in plasma LPA 18:2
- GLPG1690 plasma levels above *ex vivo* IC$_{50}$ for LPA 18:2 reduction as of dose of 60 mg, in line with PD marker
GLPG1690 SAD
Plasma PK/PD relationship

In vivo IC\textsubscript{50} for inhibition of LPA 18:2 is in accordance with ex vivo IC\textsubscript{50}
Conclusions

- Imidazo[1,2-a]pyridine series as new chemotype of Autotaxin inhibitors
  - Series identified by HTS using FS-3 assay
    - SAR further developed with alternative assay using natural LPC substrate
  - CYP TDI and hERG properties improved through modulation of physicochemical and structural properties
- Unprecedented binding mode of series in Autotaxin with both lipophilic pocket and channel occupancy
  - Possible inhibition of LPA production and delivery
- Attractive profile for clinical candidate GLPG1690
  - Good correlation between PK and PD (LPA reduction)
  - High efficacy in mouse IPF model
- GLPG1690 successfully completed Phase 1 evaluation
- GLPG1690 moving to phase 2 in IPF in 2016
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