STRATEGY AND TACTICS FOR THE DISCOVERY OF KINASE INHIBITORS FOR CNS TARGETS. A LUNDBECK PERSPECTIVE

Klaus Baek Simonsen, Ph.D.
Molecular Discovery and Innovation
Outline

- Introduction (to Lundbeck)
- CNS challenges in general
- (CNS) Kinase inhibitor challenges
- Kinase inhibitors programs at Lundbeck
  - Past and current
  - Drivers for success
  - LRRK2 - H2L and Lead optimization
  - TTBK1 – tool finding exercise
- Outlook
The Pharma Industry

How are we doing

- R&D spending's increases
- Success rate (preclinical to launch) still very low
- Biotech delivers more and more NCEs, via Pharma
- Everyone are trying to fix it and propose new models

Why are we in this business?

Paul et al, Nat rev. Drug. Dis. 2010 (R&D productivity)
Abou-Gharbia and Childers, J. med Chem 2014 (Pharma challenges)

Pieter Breugel the Elder; 1568
LUNDBECK

We want to improve the quality of life of people suffering from psychiatric and neurological disorders
ABOUT LUNDBECK

Vision
We strive for global leadership in psychiatry and neurology by improving the lives of patients.

Principles
We are focused, passionate and responsible.

Key disease areas
- Depression
- Schizophrenia
- Alzheimer’s disease
- Parkinson’s disease

For more than 70 years, we have been at the forefront of neuroscience research and our development of pioneering treatments makes a difference to patients worldwide.
**Revenue**
Our 2017 revenue reached DKK 17,234 million

17.2bn

**Employees**
We are approximately 5,000 employees.

5,000

**History**
Lundbeck was founded by Hans Lundbeck more than 100 years ago in 1915 in Copenhagen.

1915

**Global presence**
We are headquartered in Denmark and located in more than 50 countries.

50

**Ownership**
Our largest shareholder is the Lundbeck Foundation, which holds approximately 70% of the shares.

70%

**Research and development**
Within our four key disease areas we have more than 20 active research and development projects.

20
Lundbeck Key Product

Products in all four disease areas
Molecular Discovery and Innovation

Highly experienced CNS Drug Discovery organization with external arm

- Strong knowledge and ability to deliver high quality CNS development candidates
- Blood brain barrier understanding delivers molecules with relevant brain exposure
- Diverse target class track record
- GPCRs, transporters, ion channels, enzymes
- Collaborations to leverage internal capacity, competencies and expertise
- Structure/Fragment based Drug Discovery
- CNS biased HTS and fragment collection
- Translational tools (PET, chemical probes)

Linking biology to patients via molecules
CNS Drug discovery challenges

- CNS Diseases are complex (heterogeneous and multifactorial)
  - We need a better understanding of the pathophysiology within the CNS
- Neuroscience and the associated biology is complex
  - Translatability and validity of preclinical models
- Clinical testing paradigm
  - Large trials and time consuming, subjective scales, no early readout (POC)
- Blood brain barrier – “just another bio membrane!"

- Still a Huge unmet need…….
GLOBAL DISEASE BURDEN

300 million people worldwide are living with depression

21 million people worldwide are living with schizophrenia

50 million people worldwide are living with Alzheimer’s disease and other dementias

6 million people worldwide are living with Parkinson’s disease
Protein Kinase inhibitors for the CNS

Consideration and response strategy

Challenges

✦ Pgp and BCRP - Poor brain penetration
✦ Chronic treatments - Tox
✦ Cell shift - Intracellular [ATP]
✦ Substrates for CYP (2D6 and 3A4)

Response strategy

✦ Control Kpuu and phys chem
✦ Very selective inhibitors
✦ Potent and highly permeable (or allosteric)
✦ Start small and control molecular footprint

Imatinib

Sorafenib

MPO: 4.46
LLE: 4.7

MPO: 3.13
LLE: 4.3

Early kinase experience at Lundbeck

CEP-1347 - An MLK inhibitor developed early 2000

- Biological rationale
  - MLK - Mixed-Lienage Kinase 1
  - Blockage of the JNK pathway prevent neuronal death
    - Shown in In vitro and in vivo models
  - Potential DM treatment for Parkinson disease

- Translation to humans failed
  - PRECEPT – assess DM potential in early PD
  - PK in humans (10-50 mg) similar to several in vivo models
  - Big Question - Did we achieve central target engagement?

- CNS challenges - translation and high quality compounds…..

Parkinson study group for
PRECEPT Neurology (2007)
Finding the sweet spot in the sweet spot

Critical to get the right starting points (hits) up front

Increased understanding the last decade (enlightenment)

- Free drug hypothesis (PK/PD)
- “rules”/guiding principles (RO5, 3/75)
  - Efficiency metrics/composite parameters
  - Multi parameter optimization (MPO, QED)
- Structural alerts (PAINS)
- Start small, think big (FBLD)

Biological rationale

- Strong genetic evidence causally associates LRRK2 to familial and sporadic PD.
- Combined genetic and biochemical evidence supports a hypothesis in where the LRRK2 kinase function correlates with disease risk.
- LRRK2 kinase inhibitors would be a new treatment paradigm for PD.
- Expression of LRRK2 is highly enriched in brain, lung, kidney and blood.

Objective: Identify LRRK2 clinical candidate (WT and G2019S)
Parkinson disease - 200 years (2017)

From L-DOPA to (hopefully) disease modifying treatments

- James Parkinson’s clinical description still stands after 200 years
- still a debilitating progressive disease
- L-Dopa has been the one and only revolutionary treatment
- 6 million PD patients are still under treated along their course of the disease
- The major advancement in disease understanding is genetic and neuroimaging plus the emerging importance of the non motor symptoms
- We should be able to do better……
LRRK2 consideration prior to project initiation 2010

Concerns up front

★ Limited Kinase experience
★ Brain exposure of kinase inh.
★ Selectivity and Target related tox
★ No pharmacological relevant model

Strategic collaboration to leverage internal CNS expertise with a company with kinase, Structural Biology and Fragment experience

Strategy and Drivers for success

★ Control your molecular footprint
  ★ Be disciplined
★ FBDD provides best starting point to dial in good CNS properties
★ X-tal structure to guide design
  ★ Dial in selectivity to avoid off target effects
★ Multiple chemical series
  ★ many hit-finding campaigns
★ Target engagement early on
  ★ PET or LRRK2 inhibition in CSF/Blood
Structural Biology

Use of CHK1 mutant as crystallographic surrogate for LRRK2

- Analysis of PDB identified 10 protein kinase structures with >50% ATP binding site similarity to LRRK2
- CHK1 was selected, given Vernalis experience with expression, purification and crystallisation to enable crystal structures for SBDD
- Mutated residues in CHK1 had <4 Å proximity to staurosporine in a published ligand bound X-ray structure, and included areas known for kinase selectivity.
- Key LRRK2 residues in surrogate- Ala2016 (hydrophobic pocket near gate keeper) Ser147Ala in mutant, Leu1949 (hinge at gate keeper+2) Tyr86Leu in mutant
- Correlation between surrogate and LRRK2 binding
Hitfinding – finding the right starting points

Many screening approaches (fragment, LMW, kinase libraries, MTS, HTS)

- Good experience from multiple fragment screens for LRRK2
- LRRK2 transpired to have highly “ligandable” ATP binding site → high LE hits
- Biochemical Lanthascreen could be used
- Hits validated by biophysical methods
- Pyrrolo[2,3-b]pyridines Hits from low molecular hinge binder library and fragment libraries
- Binding mode obtained from X-ray structures using LRRK2 surrogate

*LRRK2 (G2019S) cKi
Pyrrolo[2,3-b]pyridines

H2L and Lead optimization provided *in vivo* active compounds

![Chemical structures](compound.png)

<table>
<thead>
<tr>
<th>LRRK2 G2019S cKi (nM)</th>
<th>13</th>
<th>2</th>
<th>0.49</th>
<th>0.3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ligand Efficiency /MPO</td>
<td>0.60/5.1</td>
<td>0.62/5.1</td>
<td>0.51/4.6</td>
<td>0.48/4.7</td>
</tr>
<tr>
<td><em>Cell (pSer-935) LRRK2 G2019S IC_{50} (nM)</em></td>
<td>-</td>
<td>70*</td>
<td>18</td>
<td>63</td>
</tr>
<tr>
<td>MDCK Papp ( (x10^{-6} \text{ cm/s}) ) /Efflux Ratio</td>
<td>2.92/0.77</td>
<td>8.79/0.59</td>
<td>8.79/0.59</td>
<td>10/3</td>
</tr>
<tr>
<td>Kinase Selectivity Other kinases Inhibited</td>
<td>Not tested</td>
<td>21&gt;95% inhib/453 @1( \mu )M (Kinomescan)</td>
<td>1&gt;90% inhib/73 @100nM (Millipore)</td>
<td>0&gt;90% inhib/75 @100nM (Millipore)</td>
</tr>
</tbody>
</table>

* In-vivo (pSer-935) Ic50 Mouse Brain = 1.3 nM
Target Engagement and Kinase Selectivity

**In Vivo LRRK2 inhibition pSer935 Autophosphorylation Assay**
- IC50 determination 1h post-dose
- Same free IC50 brain and kidney
- Pharmacodynamic marker for central/peripheral LRRK2 target engagement

**ActivX: Quantitative Measurement of Target Engagement**
- Profilation using the KiNativ™ Technology
- KiNativ™ is based on biotinylated acyl phosphates of ATP/ADP that irreversibly react with protein kinases on conserved lysine residues in the ATP-binding pocket
- Very good selectivity for LRRK2 in vivo
- Data for top 5-10 5 kinases shown
- In-line with high selectivity in full kinase panel screen

www.kinativ.com/technology.html
Structural biology

Binding mode and LRRK2 key residues

- Ala2016: Hydrophobic pocket below gate keeper
  - Ser147Ala Chk1 10-pt. mutant
- Leu1949: Hinge at gatekeeper +2
  - Tyr86Leu Chk1 10-pt. mutant
- Ser1954: Extension of Hinge
  - Glu91Ser Chk1 10-pt. mutant
**In vivo characterization**

Excellent PK/PD and dose response in mouse

- **25 mg/kg compound 22 mouse brain**
- **Relative LRRK2-pSer935 levels**
  - Exposure
  - Dose-response relationship in mouse brain and kidney (small insert)
  - LRRK2-pSer935 1 hrs after po dosing

[Graphs and data showing exposure and relative LRRK2-pSer935 levels]
LRRK2 – summary II

▶ Challenges up front solved/solvable
▶ Potent and selective high quality LRRK2 inhibitors (SBDD)
▶ Achieve central free exposure well above IC50
▶ target engagement demonstrated in Brain, Kidney and lungs
  ▶ ATP Probe-based chemoproteomics platform (ActivX Biosciences)
▶ Strong biology packages and understanding developed

▶ Many other obstacles along the way
▶ Almost all of the classical optimization road blocks
  ▶ BCRP in one series, pgp in another
  ▶ In vitro in vivo PK disconnect
▶ PET ligand not identified (yet)

▶ Still - Confident we can make a LRRK2 preclinical candidate
Tau tubulin kinase 1 – TTBK1

Biological rationale
★ TTBK1 linked to tau pathology and thus to AD
★ TTBK1 protein levels increased in AD post mortem brains
★ TTBK1 phosphorylates tau and leads to hyperphosphorylation
★ Based on genetic methods we assume that TTBK1 activity is responsible for tau hyperphosphorylation
★ Silencing of TTBK1 in primary neurons reduced okadaic acid induced tau hyper phosphorylation

Tools compound requirements
★ Cell permeable
★ Non-toxic
★ IC50 < 1 µM
★ Selectivity (selected tau kinases)

Concerns up front
★ Brain exposure
★ Selectivity
★ Km for ATP – large cell shift

Objective: identify small molecule inhibitor for further target validation
Hitfinding approached

Fragment screening provided best starting points (MTS hits rate 0.3%, 3 compound classes)

**Fragment Library**
(1800 compounds)

**HCS** → **TINS**

“Visual” analysis
Fragment hits

100 Selected

**15-20 Defined Structures**
At least 6 different binding modes

Crystalization of “best” hits for X-Ray

Representative binding modes

IC50: 10-50 uM
LE: 0.4 – 0.5
4-Azol-pyridines – H2L

Hit to Tool compound and Binding modes

The “selectivity pocket” is an induced fit.

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<th>0.008 μM</th>
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<tr>
<td>Selectivity</td>
<td>&gt;20 folds</td>
</tr>
<tr>
<td>A-B</td>
<td>4.1</td>
</tr>
<tr>
<td>B-A</td>
<td>4.8</td>
</tr>
<tr>
<td>Sol (HPLC)</td>
<td>39</td>
</tr>
<tr>
<td>Cytotox</td>
<td>Low</td>
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No in vivo effect
ATP-competitive inhibitors of TTBK1 - *Druggability*?

**cell shift – in vitro biochemical vs cell based assays**

- **TTBK1:** $K_m,ATP = 10 \text{ uM}$ (purified kinase domain, generic substrate)
- $K_m,ATP$ for TTBK1 is in the low-end of ‘normal’ range for kinases
- Low $K_m,ATP$ of kinases calls for high-potency ATP-competitive inhibitors

**How potent?**

- Assumption: >90% inhibition at 100 nM compound in cells
- $[ATP]_{intracellular} = 1 \text{ mM}$ and $K_m,ATP = 10 \text{ uM}$
- Calculated $K_i$ of 0.1 nM

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**Millipore kinase panel**

*Distribution of $K_m,ATP$ value across 270 wild-type kinases*
TTBK1 summary

- TTBK1 is ligandable
- Several different chemotypes and binding modes
- Able to identify good \textit{in vitro} tool compound
- No \textit{in vivo} effect due to large cell shift
- Difficult to find \textit{pM} compounds from current chemistry

Future directions
- Explore allosteric inhibition

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Summary

- Know your advantages and limitation
- Keep focus and specialization (in CNS)
- Collaboration to leverage competencies

- Fragments as starting points
- Control your molecular foot print

- Target engagement early on
- Push molecules forward to learn
Acknowledgement

LRRK2:
Garrick P. Smith (Chemistry Lead and PL)
Kenneth V. Christensen (Biology Lead)
Douglas S. Williamson (Vernalis)
Many many more

TTBK1:
Mauro Marigo (Chemistry lead)
Christiane Volbracht (Biology lead and PL)
Many many more
PROGRESS IN MIND

Improved medical treatment
- Scientific cooperation and partnerships
- Funding independent research
- Scientific education

Broader acceptance of patients
- Disease awareness campaigns and films
- Access to health activities and donations
- Community partnerships

Help patients regain possibilities
- Patient support programmes
- Patient safety information
- Social media interaction

More help for affected families
- Family support programmes
- Educational films and events
- Fundraising and donations
THE LUNDBECK FOUNDATION
AND THE LUNDBECK INSTITUTE

The Lundbeck Foundation

The Lundbeck Foundation is the largest shareholder of Lundbeck owning approximately 70% of the company. The Foundation annually grants around DKK 500 million to support research within medical and natural sciences including the world’s biggest neuroscience research prize of EUR 1 million, The Brain Prize.

The Lundbeck Institute

The Lundbeck Institute provides healthcare professionals with education in the treatment of psychiatric and neurological disorders. The activities are non-product related and build upon objective and evidence-based knowledge. More than 100,000 healthcare professionals have benefited from the educational activities.