Fragment-based lead generation of reversible inhibitors for lysine-specific demethylases

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CSO Beactica AB

Drug Discovery 2015 Elrig
3 Sept 2015
Presentation overview

• Beactica Drug Discovery and Epigenetics

• Academia / CROs / Biotech and Pharma collaborations – finding an optimal blend

• Epigenetic programme KDM4C/JMJD2C (or KDM4X)

• Epigenetic programme LSD1/KDM1A
What is Beactica?

- Founded in 2006 by Dr Per Källblad and Prof. Helena Danielson based on research carried out at Uppsala University
- Molecular interactions, enzymology and drug design. Pioneered Fragment-Based Drug Discovery by SPR
- Dual business model – Contract Research Collaborations and internal programmes
- Headquarters in Uppsala, Sweden
Beactica’s Target Class Experience

**Enzymes**
- Kinases
- Proteases
- Polymerases
- Metalloenzymes
- Oxidoreductases
- Phosphodiesterases
- Histone demethylases

**Others**
- Ion channels
- Nuclear receptors
- Protein–protein interactions
- Antibodies

**Work in progress**
- GPCRs

Epigenetic Mechanisms – Covalent Modifications of Histones and DNA

### Lysine Demethylase (KDM) Family of Epigenetic Erasers

<table>
<thead>
<tr>
<th>Official Symbol</th>
<th>Other Aliases</th>
<th>Gene Location</th>
<th>Gene ID</th>
<th>Protein Domains</th>
<th>Histone Substrates</th>
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Arrowsmith et al. (2012)

*Nat Rev Drug Disc* 11, 384

Labbé et al. (2014)

Beactica - *Drug Discovery Pipeline*

**Novel potentially first-in-class small molecule therapeutics in areas of unmet medical need**

<table>
<thead>
<tr>
<th>Area (Mechanism)</th>
<th>Assay development</th>
<th>Hit identification</th>
<th>Fragm. Lead Generation</th>
<th>Lead Generation</th>
<th>Lead Optimization</th>
<th>Pre-Clinical Phase</th>
<th>Clinical Phase I-III</th>
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<td><strong>Exploratory projects</strong></td>
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Collaborating with Academia / CROs / Biotech and Pharma – searching for an optimal blend
Collaborations – A small biotech perspective

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<th>Organization</th>
<th>Advantages for small biotech</th>
<th>Advantages for partner</th>
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<tbody>
<tr>
<td>Academia</td>
<td>Expertize, capabilities and publications</td>
<td>Publications, industrial collaboration and visibility</td>
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<tr>
<td>Larger biotech / Pharma</td>
<td>Expertize, capabilities and funding</td>
<td>External innovation - access to unique technology / target / project / IP</td>
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<tr>
<td>CRO</td>
<td>“Anything” that pays... but expertise and commitment necessary to stay in business</td>
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Collaborations are based on personal trust, the organizations involved are secondary
Fragment-Based Drug Discovery Programme

KDM4C (or KDM4X)
KDM4C (JMJD2C) – An Epigenetic Oncology Target

Proposed Indications:

- Cancer (e.g. breast cancer, medulloblastoma)

Function:

- Regulates expression of tumour suppressor genes through demethylation of histones (H3K9, H3K36)

Clinical Trials:

- None currently ongoing.

Beactica’s Programme:

- Reversible binders based on 4 different chemical series
- One series not binding to the metal in the active site

Major challenge – Selectivity!
KDM4C - *Fragment screening campaign*

2150 Compound fragment library

- SPR fragment screen
- SPR $K_D$ determination
- SPR Competition with reference compounds
- DSF analysis of hits

63 Screening hits

- Ligand-based fragment lead generation

3 Fragment lead series
1 Back-up series
KDM4C – Series 1

- Monodentate non-ionic ligands
- Lowest $K_D$: 1.5 µM
- Highest LE: 0.81
- MW lowest $K_D$: 210 Da
- High ligand efficiency
- X-ray structure
Most literature inhibitors are chelators

- JIB-04
  - KDM4A = 0.44 µM
  - KDM4B = 0.43 µM
  - KDM4C = 1.1 µM
  - KDM4D = 0.3 µM
  - KDM4E = 0.34 µM

- Compound 7
  - KDM4E = 6.6 µM

- 2,4-PDCA
  - KDM4A = 0.6 µM
  - KDM4E = 1.1 µM

- GSK-J1
  - KDM4A = 0.050 µM
  - KDM4B = 0.073 µM
  - KDM4C = 0.034 µM
  - (not cell active)

Heinemann et al, Nature 2014
Thinnes et al, BioChemBiophysActa 2014
Labbe et al, AmJTranslRes 2014
Beactica Co-crystal Structures

- 3 chemical series (blue, green, red) in total 5 co-crystal structures
- Nickel (white) instead of Fe
- 2.4-2.8 Å resolutions
- No chelators (0-1 bonds to metal)

In collaboration with Saromics AB
KDM4C – Selectivity targets KDM4A/4B (and more)

- KDM4A (3RVH) in blue
- KDM4B (4LXL) in brown
- KDM4C (4XDO) in silver
- The largest differences ~7Å from the iron core

Selective inhibitors will be challenging to develop, but may not be required in oncological indications

Pilka et al, Drug Discovery Today, 2015
KDM4X summary

- Fragment-based drug discovery programme, currently in fragment lead generation
- Multiple diverse series in development from fragment hits
  - Reversible catalytic site inhibitors
  - Focus on non-chelating, non-ionic series
  - Parallel SPR evaluation with KDM4A/B/C
- Beactica is searching for a partner to sponsor the research with a future option to licence the programmes

Crystal structure of Beactica compound bound to KDM4C
Fragment-Based Drug Discovery Programme

LSD1
LSD1 (KDM1A) – An Epigenetic Oncology Target

Proposed Indications:
- Cancer (e.g. AML, Bladder, Colorectal, Breast, Liver, Lung, Prostate, Glioblastoma), Alzheimer, Viral infection

Function:
- Regulates expression of disease-related genes through demethylation of histones (H3K4, H3K9)

Clinical Trials:
- Currently five ongoing studies, all in oncology with irreversible inhibitors

Beactica’s Programme:
- Reversible inhibitors based on 3–5 different chemical series
- Allosteric binders with potentially orthogonal modulation of enzyme activity
LSD1 – Enzyme structure

[Animation not available]

4KUM, Luka et al – LSD1 and CoREST

Stavropoulos et al, NatStructMolBiol 2006
LSD1 – Mechanism of H3K4 demethylation

Stavropoulos et al, NatStructMolBiol 2006
LSD1 – Function depending on protein complex context

Amente et al, BiochimBiophysActa 2013
LSD1 – An important part of the emerging epigenetic puzzle

Ooi et al, Nature Reviews Genetics 2007

Bhan et al, BiochimBiophysActa 2015

Pilotto et al. PNAS 2015
LSD1 – *Ongoing clinical trials*

- GlaxoSmithKline in Phase I/II with GSK2879552 (Relapsed/refractory AML patients)
- GlaxoSmithKline in Phase I/II with GSK2879552 (Relapsed/refractory SCLC patients)
- University of Miami in Phase I/II with tranylcypromine + ATRA (AML and MDS patients)
- Martin-Luther-Universität Halle-Wittenberg in Phase I/II with ATRA+tranylcypromine (Relapsed/refractory AML patients)
- Oryzon in Phase I with ORY-1001 (Relapsed/refractory AL)

*From Clinicaltrials.gov and Clinicaltrialsregister.eu the 17th of August, 2015*
**LSD1 - Ligand-based Fragment Lead generation**

1946 Compound fragment library

- SPR fragment screen
- SPR $K_D$ determination
- SPR Identification of binding site by competition with reference compounds
- DSF analysis of hits
- Inhibition analysis hits

12 screening hits

- Ligand-based Fragment lead generation

3 Fragment lead series
2 Back-up series
LSD1 – Prioritized series

Series 1
- Active site ligands
- Lowest $K_D$: 2.7 µM
- Highest LE: 0.34
- MW lowest $K_D$: 330 D

Series 3
- Active site ligands
- Lowest $K_D$: 380 nM
- Highest LE: 0.36
- MW lowest $K_D$: 370 D

Series 5 – Allosteric series
- Lowest $K_D$: 16 µM
- Highest LE: 0.38
- MW lowest $K_D$: 250 D
- Enzymatic inhibition does not correlate with affinity
LSD1 – Difficult to crystallize – Any answers from Thermodynamics?

Van’t Hoff analysis

Series 1

Series 3

Base  Neutral  Acid  Base  Neutral  Acid

Cmp01  Cmp02  Cmp03  Cmp04  Cmp05  Cmp06

kJ/mol

dH (kJ/mol)  TdS (kJ/mol)  dG (kJ/mol)
LSD1 - *Crystallography changes the game*

Catalytic site binders (Series 1 and 3)  
Allosteric site binders (Series 5)

Resolution 2.41-2.65 Å  
*In collaboration with Proteros GmbH*
LSD1 – Inhibitor Evaluation in Cells and In Vivo

Established link with Tim Somervaille at the Cancer Research UK Manchester Institute

- Haematologist with focus on leukaemia
- Cellular screening facility
- Validated LSD1 as candidate therapeutic target in AML

• Assays
  - Cell-based assays to evaluate LSD1 inhibitors as potential drugs against e.g. AML
  - Primary cell materials - evaluated *in vitro* or by xenograft
LSD1 and CoREST - *Allosteric binders*

- Beactica allosteric series binds at the hinge-region of the tower domain, essential for protein-complex formation

- Potential protein-protein interaction inhibitor of CoREST / ER / AR / PKC ...

- Major challenges
  - Evaluation with relevant assays
  - Choosing indication
**LSD1 - Summary**

- Fragment-based drug discovery programme, currently in lead generation
- Multiple diverse series in development from fragment hits
  - Validated in three different assays
  - Reversible interaction mechanism
  - Catalytic site inhibitors and allosteric binders
- Allosteric binders offer a potentially orthogonal mechanism of action
- Beactica is searching for a partner to sponsor the research with a future option to licence the programmes

[Structure not available]