HNE chemical probe
BAY-678
F. von Nussbaum, V. Li
August 6th 2015
Human Neutrophil Elastase, HNE (EC 3.4.21.37)
A Key Player in Inflammation (1/3)

**Structure**
- Ser protease (His-Asp-Ser)
- chymotrypsin family

**Function**
- broad substrate specificity
- Val-|-Xaa & Ala-|-Xaa

Classical Ser protease

Inflammation (ECM** & signalling
- host defense (bacteria)

Regulation
- by compartimentation
- by anti-proteases (α1PI)
Human Neutrophil Elastase, HNE (EC 3.4.21.37)
A Key Player in Inflammation (2/3)

**Structure**
- Ser protease (His-Asp-Ser)
- chymotrypsin family

**Function**
- broad substrate specificity
- Val-|-Xaa & Ala-|-Xaa

**Pharmacology**
- inflammation (ECM**) & signaling
- host defense (bacteria)

*Highly active enzyme ...*

*adapted from http://ckcsphysiology.wikispaces.com/
**extracellular matrix*
Human Neutrophil Elastase, HNE (EC 3.4.21.37)
A Key Player in Inflammation (3/3)

**Structure**
- Ser protease (His-Asp-Ser)
- chymotrypsin family

**Function**
- broad substrate specificity
- Val-|Xaa & Ala-|Xaa

**Pharmacology**
- inflammation (ECM**) & signaling
- host defense (bacteria)

**Regulation**
- by compartimentation
- by anti-proteases (α1PI***)

*Highly active enzyme that has to be regulated strictly*

*adapted from http://ckcsphysiology.wikispaces.com/
**extracellular matrix  ***α-1-proteinase inhibitor*
Elastase in Inflammation and Autoimmunity
The Elastase – Anti-Protease Balance is Disturbed

- Acute Lung Injury (ALI)
- Acute Respiratory Distress Syndrome (ARDS)
- Bronchiectasis (BE)
- Cystic Fibrosis (CF)
- Chronic Obstructive Pulmonary Disease (COPD)
- Non Small-Cell Lung Cancer (NSCLC)
- Pulmonary Hypertension (PH)
- Rheumatoid Arthritis (RA)
- Inflammatory Bowel Disease (IBD)
- Acute Myocardial Infarct (AMI)
- Graft versus Host Disease (GvHD)
- Systemic Inflammatory Response Syndrome (SIRS)
- Wound Healing disorders
- ...

Exciting target for ULTRA-DD collaboration
History of HNE Inhibitors: Biologicals
Selection of Inhibitors

**Loss of activity under oxidative stress conditions**

**Alpha-1 protease inhibitor: no inhibition of membrane bound HNE**

<table>
<thead>
<tr>
<th>Human alpha-1 protease inhibitor (AATD)</th>
<th>Rec. human elafin (Ph 2 postoperative inflam. complication)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Prolastin®</td>
<td>Elafin</td>
</tr>
<tr>
<td>Zemaira®</td>
<td></td>
</tr>
<tr>
<td>Aralast®</td>
<td></td>
</tr>
<tr>
<td>Talecris, 1987</td>
<td>Proteo Biotec, ongoing</td>
</tr>
<tr>
<td>CSL Behring, 2003</td>
<td></td>
</tr>
<tr>
<td>Baxter, 2003</td>
<td></td>
</tr>
</tbody>
</table>

- $K_i < 0.1 \text{ nM i.v.}$
- $K_i 0.08 \text{ nM i.v.}$
History of HNE Inhibitors: First SMOLs*
Suicide Inhibitors (a Selection of Inhibitors)

Mode-of-action: mechanism-based (covalent / reactive inhibitors)
multiple pharmacophores of electrophilic ketons, acylators, and transition state analogs

*small molecules

代谢性缺陷
→ prone to adverse effects

Elaspol® (ALI/SIRS, only Japan & South Korea)
Ono, 2002 & 2006

ONO 6818 (Ph1/2 COPD)
Ono, discontinued 2002

sivelestat

K_i 200 nM
i.v.

freselestat

K_i 12 nM
p.o.

Bayer HealthCare
History of HNE Inhibitors: recent SMOLs
Reversible, Non-reactive Inhibitors (e.g. BAY-678)

Chemical Probe BAY-678

- Specific & selective
- Oral bioavailable
- Well tolerated & safe
Characterization of Chemical Probe BAY-678

Potency

see also von Nussbaum F, Li V et al. ChemMedChem 2015

High potency for a non-reactive, reversible protease inhibitor

(S)-enantiomer of BAY-678 is inactive (negative control)
### Characterization of Chemical Probe BAY-678

#### Selectivity

**Very high selectivity (>2,000 fold) versus similar serine proteases**

**Lower potency versus rodent neutrophil elastases**

---

#### Serine Protease IC₅₀ [µM] Table

<table>
<thead>
<tr>
<th>Serine Protease</th>
<th>IC₅₀ [µM]</th>
<th>Serine Protease</th>
<th>IC₅₀ [µM]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pancreas elastase</td>
<td>&gt; 30</td>
<td>Kallikrein-B1</td>
<td>&gt; 30</td>
</tr>
<tr>
<td>Cathepsin G</td>
<td>&gt; 30</td>
<td>Kallikrein-1</td>
<td>&gt; 30</td>
</tr>
<tr>
<td>Chymotrypsin</td>
<td>&gt; 30</td>
<td>Kallikrein-4</td>
<td>&gt; 30</td>
</tr>
<tr>
<td>Trypsin</td>
<td>&gt; 30</td>
<td>Kallikrein-5</td>
<td>&gt; 30</td>
</tr>
<tr>
<td>Chymase</td>
<td>&gt; 30</td>
<td>Kallikrein-7</td>
<td>&gt; 30</td>
</tr>
<tr>
<td>DPPII</td>
<td>&gt; 30</td>
<td>Kallikrein-12</td>
<td>&gt; 30</td>
</tr>
<tr>
<td>DPPIV</td>
<td>&gt; 30</td>
<td>FAP</td>
<td>&gt; 30</td>
</tr>
<tr>
<td>Urokinase</td>
<td>&gt; 30</td>
<td>FVIIa</td>
<td>&gt; 30</td>
</tr>
<tr>
<td>Thrombin</td>
<td>&gt; 30</td>
<td>FIXa</td>
<td>&gt; 30</td>
</tr>
<tr>
<td>FXa</td>
<td>&gt; 30</td>
<td>FXIa</td>
<td>&gt; 30</td>
</tr>
<tr>
<td>Plasmin</td>
<td>&gt; 30</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

---

**Characterization**

- **Ki** humanNE (HNE) 15 nM
- **Ki** ratNE (RNE) 600 nM
- **Ki** murineNE (MNE) 700 nM

---

**See also von Nussbaum F, Li V et al. ChemMedChem 2015**
Characterization of Chemical Probe BAY-678

Specificity (1/2) [See also Backup Slides]

see also von Nussbaum F, Li V et al. ChemMedChem 2015

LeadProfilingScreen @ MDS Pharma Services, Taiwan

Radioligand Binding Assays with 10 μM BAY-678:
Panel of 64 pharmacological relevant targets
(e.g. receptors & transporter)

\[ K_i \text{ humanNE} = 15 \text{ nM} \]

No significant effects against numerous pharmaceutical relevant targets
Characterization of Chemical Probe BAY-678
Specificity (2/2)

No significant effects against prominent kinase target class

Kinase Panel (in house)

Functional Kinase Activity with 20 µM* BAY-678:
Initial panel of 7 kinases
(incl. 4 tyrosine & 3 serine/threonine kinases)

\[ K_i \text{ (humanNEt)} = 15 \text{ nM} \]

* dose response, highest concentration used: 20 µM

Bayer HealthCare
Investigating the Binding Mode (X-Ray)
HNE Complex with Close Congener of BAY-678

see also Hansen et al. J. Mol. Biol. 2011

Elastase is clamped by combined S1/S2 pocket dive
Induced-Fit Binding Mode (X-Ray)
S2 Pocket Widens for Cyanophenyl Residue

see also Hansen et al. J. Mol. Biol. 2011

Significantly smaller S2 pocket in apo structure
Induced-Fit Binding Mode (X-Ray)

BAY-678

see also von Nussbaum F, Li V et al. ChemMedChem 2015

Crystal structure of HNE in complex with BAY-678: The protease is shown in a stick representation (white) with transparent Connolly-like surface; ligand 20 (purple) is shown as balls and sticks. Heteroatoms are colored as follows: oxygen, red; nitrogen, blue; fluorine, cyan.

RCSB Protein Data Bank (PDB) access code 5a0a
### Pharmacokinetics in Rats

**BAY-678 plasma concentration versus time**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>PK rat i.v.</td>
<td>(t_{1/2} = 1.3 \text{ h}, \text{CL}<em>{\text{matrix}} = 2.0 \text{ L/h*kg}, \text{AUC}</em>{\text{norm}} = 0.50 \text{ kg*h/L}, V_{ss} = 3.9 \text{ L/kg})</td>
</tr>
<tr>
<td>PK rat p.o.</td>
<td>(t_{1/2} = 1.3 \text{ h}, \text{AUC}_{\text{norm}} = 0.42 \text{ kg*h/L}, \text{BA} = 83%)</td>
</tr>
<tr>
<td>CYP inh.</td>
<td>CYP 2C9 12 (\mu\text{M}); other &gt; 50 (\mu\text{M})</td>
</tr>
<tr>
<td>(F_{U}) rat</td>
<td>~30%</td>
</tr>
<tr>
<td>Caco-2 cellperm.</td>
<td>(P_{\text{app, A-B}} = 228 \text{ nm/s}, P_{\text{app, B-A}} = 606 \text{ nm/s}, \text{Efflux ratio} = 2.7)</td>
</tr>
</tbody>
</table>

**Pharmacokinetics**

- BAY-678 is cell permeable and reveals an overall good pharmacokinetics.

![BAY-678 molecule](image)
Targeting Elastase in More Complex Settings
Model of the Kinetics of Free Extra-cellular Elastase, the Potential Driver of Inflammation and Autoimmunity

It’s all about concentration & timing
Investigating BAY-678 in More Complex Settings
Beyond the Interaction with the Isolated Target

**Investigating cellular action of elastase (not meaningful)**
No interference with intra-cellular elastase activity (host defense) expected as this would request a very high concentration of the inhibitor (target concentration in cells is millimolar!)

**Investigating extra-cellular action of elastase**
Efficacy of inhibitor treatment is assessed in *in vivo* models with out-of-balance elastase activity driving presumably the onset and progression of the disease

⇒ *In vivo / ex vivo efficacy assessment of BAY-678*
In vivo / ex vivo Efficacy Assessment of BAY-678
Per oral Administration of Inhibitor (1/2)

Efficacy demonstrated in **acute in vivo** models
- Protease-induced acute lung injury (ALI) in mice
  (lung hemorrhage, neutrophil count in lung lavage)
- Thread infarct model in rats
  (infarct size, cardiac function)

Efficacy demonstrated in **sub-chronic in vivo** models
- Cigarette smoke-induced lung injury in guinea pigs
  (inflammatory cell count in lung lavage)

→ **Anti-inflammatory mode-of-action of BAY-678**
In vivo / ex vivo Efficacy Assessment of BAY-678
Per oral Administration of Inhibitor (2/2)

Efficacy demonstrated in chronic in vivo models

- Protease-induced lung emphysema in mice
  (lung compliance and alveolar morphometry)

- Hypoxia-induced pulmonary arterial hypertension (PAH) in mice and rats
  (heart hemodynamics & hypertrophy, biomarker, pulmonary artery muscularisation)

- Monocrotaline (MCT)-induced PAH in rats
  (heart hemodynamics & hypertrophy, biomarker)

Anti-remodeling mode-of-action of BAY-678

* chronic obstructive pulmonary disease
BAY-678 in Protease-induced ALI Mice Models

Scheme of HNE-induced and PPE*-induced ALI Scenario

see also von Nussbaum F, Li V et al. Chem. Med. Chem. 2015

* porcine pancreas elastase
BAY-678 in Protease-induced ALI Mice Models

Lung Hemorrhage Data

see also von Nussbaum F, Li V et al. Chem. Med. Chem. 2015

Hitting the (exogenous) HNE target in the lung after oral administration

Reduction of (endogenous) MNE driven lung inflammation

* biochemical in vitro assay
BAY-678 fulfills all SGC chemical probe criteria.

We consider BAY-678 as an attractive & novel SGC probe for HNE
## HNE Inhibitor Programs

### Acknowledgements

- Adrian Tersteegen
- Armin Kern
- **Barbara-Albrecht**
- Christopher Kallus
- Dagmar Karthaus
- Daniel Meibom
- Dieter Lang
- Dirk Meurer
- Dirk Schneider
- Frank Kramer
- **Franz von Nussbaum**
- Günter Benz
- Herbert Himmel
- **Heike Gielen-Härtwig**
- Holger Paulsen
- Hubert Trübel
- Ines Bohlinger
- Jens Ergüden
- Jens Schamberger
- Joachim Mittendorf
- Jörg Keldenich
- Johannes Nagelschmitz
- Julia Freundlieb
- Karl-Heinz Schlemmer
- Katja Zimmermann
- Kevin Nash
- **Klemens Lustig**
- Lars Bärfacker
- Leila Telan
- Martin Radtke
- **Martina Delbeck**
- **Martina Schäfer**
- **Mary Fitzgerald**
- Michael Gerisch
- Michael Hoffmann
- Raimund Kast
- Rolf Grosser
- Rolf Henning
- **Sina Micus**
- Sonja Anlauf
- Stefan Golz
- Stefan Schäfer
- Susanne Wegener
- Swen Allerheiligen
- Thomas Kuhlmann
- Ullrich Rosentreter
- Ursula Krenz
- Uwe Münster
- Volker Geiss
- **Volkhart Li**
- Walter Kroh

Bayer HealthCare
Thank you!

Science For A Better Life