Discovery and development of odanacatib: A selective cathepsin K inhibitor for the treatment of osteoporosis

Shawn J. Stachel
On behalf of the odanacatib discovery and product development teams
Merck Research Laboratories

8th RSC-SCI Symposium on Proteinase Inhibitor Design

April 16, 2013
Acknowledgements

**Biology**
Christine Brideau
Wanda Cromlish
Sylvie Desmarais
Jean-Pierre Falgueyret
Louis-Jacques Fortin
Sonia Lamontagne
France Landry
Frederic Masse
Dave Percival
Denis Riendeau
Paul Tawa
Jennifer Wang

**Comp. Med.**
Stephanie Alleyn
Liette Belair
Joel Bosquet
Carl Brière
Nancy Kelly
Gayle Lapointe
Mira Lavoie
Sonia Lesvesque
Denis Normandin
Karen Ortega
Roberta Rasori
Josianne Rozon
Wayne Sturkenboom
Simon Wong

**Chemistry**
Kevin Bateman
Christopher Bayly
Cameron Black
Michael Boyd
Nathalie Chauret
Sheldon Crane
Stephen Day
Jacques Yves Gauthier
Robert Houle
Elise Isabel
C.K. Lau
Serge Leger
Tammy LeRiche
Jean-Francois Levesque
Chun-Sing Li
Christophe Mellon
Renata Oballa
Joel Robichaud
Bruno Roy
John Scheigetz
Carmai Seto
Michel Therien
Laird Trimble
Jean-Francois Truchon
Vouy-Linh Truong
Qingping Wang

**Process Chemistry**
Paul O’Shea
Dean Shear
Mirlinda Bibe
Jennifer Chilenski
Jimmy DaSilva
Rich Desmond
Paul Devine
Pete Dormer
Bruce Foster
Don Guathier
Danny Gauvreau
Francis Gosselin
Derek Henderson
Greg Hughes
John Leazer
Bill Leonard
John Limanto
Christian Nadeau
Thorsten Rosner
Kara Rubin
Ali Shafiee
Veena Upadhyay
Chris Welch

**Bone Biology (WP)**
Don Kimmel
Sevgi Rodan
Gideon Rodan
Le Duong
Pat Masarachia
Brenda Pennypacker
Maureen Pickarski
Gregg Wesolowski
Ya Zhuo

**Pharmaceutical R&D**
Cynthia Bazin
Sophie-Dorothee Clas
Elizabeth Kwong
Pauline Luk
Rafik Naccache
Wayne Parent
Suzanne Spagnoli
Dendi Susanto
Hongshi Yu

**Safety Assessment**
Laura Gumprecht
Cindy Fishman

**Pharmacology**
Sylvie Toulmond
Osteoporosis: “porous bones”
A progressive bone disease characterized by decreased bone density and mass. Leads to an increased risk of fracture

- Estimated 200 million women worldwide with osteoporosis.¹
- Only ~20% of osteoporosis patients are currently treated
- Hip fractures cause high morbidity and mortality²
  - ~20% die in the 1st year
  - ~33% are totally dependent or in a nursing home after one year

² International Osteoporosis Foundation statistics.
Bone Remodeling

Healthy: Bone resorption = formation
Osteoporosis: Bone resorption > formation
Osteoclast – The Target Cell for Cathepsin K Inhibition

Electron micrograph of an osteoclast on bone

Bone resorption by osteoclasts is the initial step in remodeling

Rodan & Duong Bone Key 2008, 5, 6
Cathepsin K Deficiency

- Discovered in 1995\(^1\)
  - Highly expressed in osteoclast
  - Cleaves at multiple sites in triple helical region of collagen type I and II

- Pycnodysostosis is a genetic disease associated with cathepsin K deficiency\(^2,3\)
  - Rare autosomal recessive skeletal dysplasia
  - ~150 cases reported worldwide
  - Short stature with high bone mass and increased fragility associated with high risk of fracture
  - Normal intelligence, sexual development and lifespan

- Cat K null mice\(^4\)
  - Osteopetrotic phenotype
  - Increased bone density
  - Otherwise healthy and fertile

---

1 Inaoka, et al, *BBRC* 1995, 206, 89
3 Saftig *PNAS* 1998, 95, 13453.
Cathepsin K as a Therapeutic Target

- Data suggestive that cathepsin K represents a promising target for treatment of osteoporosis
  - Human and mouse genetic data
  - Cathepsin K collagenase activity
  - Restricted cellular distribution

- Goal: To develop an orally active, selective and reversible cathepsin K inhibitor for treatment of osteoporosis
Substrate to Inhibitor: Warhead

- Use knowledge of mechanism to convert substrate into inhibitor:

\[
\begin{align*}
&\text{HS-Enz} \\
\xrightarrow{\text{R}^\text{N}} &\quad \text{HO-S-Enz} \\
\xleftarrow{\text{H}^-} &\quad \text{R}^\text{N} \text{R'}^- \quad \text{R}^- \text{N} \text{R'}^+ \\
\text{Tetrahedral intermediate} &\quad \text{Acyl enzyme intermediate}
\end{align*}
\]

\[\xrightarrow{\text{H}_2\text{O}} \quad \text{R}^- \text{O}^- + \text{Enz-SH} \]

- Replace scissile bond with an electrophile (warhead) that reacts with active site cysteine:

\[
\begin{align*}
&\text{HS-Enz} \\
\xrightarrow{\text{R}^\text{N}} &\quad \text{R}^- \text{N} \text{R'}^+ \\
\xleftarrow{\text{H}^+} &\quad \text{Enz-His} \\
\text{Irreversibly bound inhibitor}
\end{align*}
\]

Cathepsin K cleaves Type I collagen at multiple positions

Recognition sequence\(^1\): Gly-Leu-Lys—Gly-His

\[
P_3 \quad P_2 \quad P_1 \quad P_1' \quad P_2'
\]

- Recognition sequence combined with electrophilic warhead gives a mechanism based-inhibitor of cathepsin K\(^2,3\)

\(^1\)Atley, M.; Mort, J. S.; Lalumiere, M.; Eyre, D. R. *Bone* **2000**, 26, 241

\(^2\)McGrath, M. E.; Klaus, J. L; Barnes, M. G.; Brömme, D. *Nat. Struct. Biol.* **1997**, 4, 105

Opportunities for Inhibitor Design

- Co-crystal reveals key interactions with cathepsin K and opportunities for obtaining selectivity
  - $S_1$ pocket is a narrow groove on enzyme
  - $S_2$ pocket suggests accommodation of alternative hydrophobic groups
  - $S_3$ sub-site contains aspartic acid (Asp$^{61}$) that may be exploitable for selectivity
  - $P_2$-$P_1$ amide forms key interaction with enzyme backbone (Gly$^{66}$, Asn$^{158}$)
  - $P_2$-$P_3$ amide: NH hydrogen-bond to Gly$^{66}$, oxygen points towards solvent

McGrath, M. E.; Klaus, J. L; Barnes, M. G.; Brömme, D Nat. Struct. Biol. 1997, 4, 105
Reversible Inhibitors

- Irreversible inhibitors have potential liabilities
- Vinyl sulfone can be replaced by nitrile (electrophilic)
  - Adds cysteine reversibly to form thioimidate

Optimization to dipeptide nitrile

L-006235
Cat K IC$_{50}$ = 0.2 nM

Selective Inhibition of Cathepsin K is Important to Avoid Potential Off-target Activity

- Fifteen human cathepsins are present inside cells within acidic compartments called lysosomes.
- Potential consequences of off-target inhibition:
  - Cat L: cardiomyopathy, impaired neovascularization
  - Cat F: neuronal ceroid lipofuscinosis
  - Cat S: impaired immune response
- High selectivity for cathepsin K desired to avoid potential off-target effects.

L-006235: A Potent, Selective, Reversible Dipeptidic Nitrile Inhibitor of Cathepsin K

- >5000-fold selective in purified enzyme assays
- Active in rabbit and monkey models of osteoporosis

<table>
<thead>
<tr>
<th></th>
<th>Cat K</th>
<th>Cat B</th>
<th>Cat L</th>
<th>Cat S</th>
<th>Cat F</th>
</tr>
</thead>
<tbody>
<tr>
<td>IC₅₀ (nM)</td>
<td>0.2</td>
<td>1100</td>
<td>6300</td>
<td>47000</td>
<td>3000</td>
</tr>
</tbody>
</table>

L-006235 in Cathepsin K

Asn166
Cys25
Asp61
Gly66

2.9
3.0
Loss of Specificity of Cathepsin K Inhibition: Whole Cell Assay

Inhibition of Cathepsins, IC\textsubscript{50} (nM)

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>Cell</th>
<th>Enzyme</th>
<th>Cell</th>
<th>Enzyme</th>
<th>Cell</th>
<th>Enzyme</th>
<th>Cell</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cathepsin K</td>
<td>L-006235</td>
<td>0.2</td>
<td>5</td>
<td>1100</td>
<td>17</td>
<td>6300</td>
<td>340</td>
</tr>
<tr>
<td>Cathepsin L</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cathepsin S</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>


---

- High selectivity profile of L-006235 is lost in whole cell assays
Hypothesis: Basic Inhibitors Accumulate in Acidic Lysosomes and Inhibit Off-Target Cathepsins

- Lysosomotropism: accumulation of lipophilic weak bases in acidic sub-cellular compartments

L-006,235

## Cat K Inhibitors in Whole Cell Assays

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>Cathepsin K</th>
<th>Cathepsin B</th>
<th>Cathepsin L</th>
<th>Cathepsin S</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>IC&lt;sub&gt;50&lt;/sub&gt; (nM)</td>
<td></td>
<td>IC&lt;sub&gt;50&lt;/sub&gt; (nM)</td>
<td></td>
</tr>
<tr>
<td>L-006235</td>
<td>0.2</td>
<td>1100</td>
<td>6300</td>
<td>47000</td>
</tr>
<tr>
<td>Cmpd 2</td>
<td>2.3</td>
<td>4200</td>
<td>24000</td>
<td>&gt;10000</td>
</tr>
<tr>
<td></td>
<td></td>
<td>17</td>
<td>340</td>
<td>790</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2900</td>
<td>&gt;10000</td>
<td>&gt;10000</td>
</tr>
</tbody>
</table>

- Non-basic analogs show reduced activity in cell-based assays
- Non-basic P<sub>3</sub> groups are generally less active

Why have a Basic Substituent?

- 10- to 100-fold increase in intrinsic potency
- Improved enzyme selectivity
- Enhanced pharmacokinetics (bioavailability, half-life)
- Liability of basic group:
  - Lysosomotropism leading to loss of functional selectivity
SAR Optimization Efforts

- Extensive SAR studies on dipeptide framework resulted in loss of selectivity and/or potency
- Abandoned dipeptide series of inhibitors
Amide Replacement

- P$_2$-P$_1$ amide forms key interaction with enzyme backbone (Gly$^{66}$, Asn$^{158}$) but, P$_2$-P$_3$ amide NH hydrogen-bond to Gly$^{66}$, oxygen points towards solvent

Amide bond can be replaced but loss in Cat K potency
- Selectivity can still be achieved without P$_2$-P$_3$ amide

J. Robichaud et al. *BMCL* 2004, 14, 4291
Further Optimization of Amide Replacement

- High selectivity can still be achieved without $P_2$-$P_3$ amide with addition of basic $P_3$ group

\[
\begin{align*}
\text{Cat K} & = \ 56 \text{ nM} \\
\text{Cat L} & = \ 498 \text{ nM (9x)} \\
\text{Cat S} & = \ 1578 \text{ nM (28x)} \\
\text{Cat B} & = >10000 \text{ nM (>180)} \\
\end{align*}
\]

\[
\begin{align*}
\text{Cat K} & = \ 11 \text{ nM} \\
\text{Cat L} & = \ 3950 \text{ nM (359x)} \\
\text{Cat S} & = \ 2010 \text{ nM (183x)} \\
\text{Cat B} & = \ 3725 \text{ nM (339x)} \\
\end{align*}
\]

J. Robichaud et al. *BMCL* 2004, 14, 4291
Additional of hydrogen bond donor is favorable for cat K potency.

Selectivity and potency can still be achieved without P$_2$-P$_3$ amide with addition of basic P$_3$ group

Cat K = 2 nM
Cat L = 108 nM (54x)
Cat S = 24 nM (12x)
Cat B = 26 nM (13x)

Cat K = 3 nM
Cat L = 2101 nM (700x)
Cat S = 158 nM (53x)
Cat B = 1812 nM (604x)

J. Robichaud et. al. *BMCL 2004, 14, 4291*
How can P2-amide be replaced while maintaining H-bond capability?

- H-bond donor requires non-basic nitrogen
  - Non-basic amine (pKa = 1.5) is not protonated at physiological pH
- Retains the H-bond donating properties of an amide bond

M. Zanda et. al ChemMedChem 2007, 2,1693
<table>
<thead>
<tr>
<th></th>
<th>Cat K</th>
<th>Cat K</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>IC&lt;sub&gt;50&lt;/sub&gt; (nM)</td>
<td>IC&lt;sub&gt;50&lt;/sub&gt; (nM)</td>
</tr>
<tr>
<td><img src="image1" alt="Cyclohexyl" /></td>
<td>73</td>
<td>51</td>
</tr>
<tr>
<td><img src="image2" alt="Leucine" /></td>
<td>3575</td>
<td>4</td>
</tr>
</tbody>
</table>

- Trifluoroethylamine is an effective amide isostere
- Cyclohexyl ring in P<sub>2</sub> is much less potent than the isobutyl side-chain
- Modeling suggests loss of potency in cyclohexyl analog is a result of steric interactions between P<sub>2</sub> and the CF<sub>3</sub> group

**CF₃ Amide Replacement**

- Phenyl piperazine has greater potency and selectivity than thiazole piperazine
- Can this exquisite potency/selectivity advantage be extended to non-basic inhibitors?

<table>
<thead>
<tr>
<th></th>
<th>Cat K</th>
<th>Cat B</th>
<th>Cat L</th>
<th>Cat S</th>
</tr>
</thead>
<tbody>
<tr>
<td>L-006235</td>
<td>0.2</td>
<td>1100</td>
<td>6300</td>
<td>47000</td>
</tr>
<tr>
<td>3</td>
<td>0.03</td>
<td>3315</td>
<td>26</td>
<td>848</td>
</tr>
<tr>
<td>4</td>
<td>0.005</td>
<td>1110</td>
<td>47</td>
<td>451</td>
</tr>
</tbody>
</table>

Non-basic derivatives in this series are potent and selective
L-873724 stands out as best compound in this series

C. S. Li et al. BMCL 1985, 16, 1985
Why are Trifluoroethylamines so Potent?

X-ray structure of Cat K Complex

S3, Tyr 67, Gly 66, S2

H$_2$NO$_2$S
L-873724 has Similar Potency in Whole Cells and Purified Cathepsins

- Trifluoroethylamine isostere gave enough potency to remove the basic P₃
- Selectivity profile of L-873724 is maintained in whole cell assays

Inhibition of Cathepsins, IC₅₀ (nM)

<table>
<thead>
<tr>
<th></th>
<th>Cathepsin B</th>
<th></th>
<th>Cathepsin L</th>
<th></th>
<th>Cathepsin S</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Enzyme</td>
<td>Cell</td>
<td>Enzyme</td>
<td>Cell</td>
<td>Enzyme</td>
</tr>
<tr>
<td>L-006235</td>
<td>1100</td>
<td>17</td>
<td>6300</td>
<td>340</td>
<td>47000</td>
</tr>
<tr>
<td>L-873724</td>
<td>5240</td>
<td>4800</td>
<td>264</td>
<td>1220</td>
<td>178</td>
</tr>
</tbody>
</table>

C. S. Li et al. *BMCL* 1985, 16, 1985
L-872724 is extensively metabolized in human hepatocytes (56% metabolism)
Modifications to Reduce Metabolism

Modify P₂ to block metabolism

Add substituent to block amide cleavage and lactonization
L-873,724 has similar potency in whole cells and purified cathepsins.

- Modifications result in odanacatib which maintains excellent selectivity and activity in both enzymatic and cell-based assays.

<table>
<thead>
<tr>
<th></th>
<th>Cathepsin K</th>
<th>Cathepsin B</th>
<th>Cathepsin L</th>
<th>Cathepsin S</th>
</tr>
</thead>
<tbody>
<tr>
<td>Enzyme Cell</td>
<td>Enzyme Cell</td>
<td>Enzyme Cell</td>
<td>Enzyme Cell</td>
<td>Enzyme Cell</td>
</tr>
<tr>
<td>L-873724</td>
<td>0.2 3</td>
<td>5240 4800</td>
<td>264 1220</td>
<td>178 94</td>
</tr>
<tr>
<td>Odanacatib</td>
<td>0.2 5</td>
<td>1034 1050</td>
<td>2995 4843</td>
<td>60 45</td>
</tr>
</tbody>
</table>

J. Gauthier et. al. *BMCL* **2008**, 18, 923
Odanacatib has Improved Pharmacokinetics in Preclinical Species

- Modification of L-873724 to block sites of oxidative metabolism provides a superior pharmacokinetic profile.
- High recovery (>99%) after incubation with dog and human hepatocytes

**Pharmacokinetics**

<table>
<thead>
<tr>
<th></th>
<th>Rat</th>
<th>Dog</th>
<th>Rhesus Monkey</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Cl (ml/min/kg)</td>
<td>t1/2 (h)</td>
<td>Cl (ml/min/kg)</td>
</tr>
<tr>
<td>L-873724</td>
<td>6.3</td>
<td>2.7</td>
<td>1.12</td>
</tr>
<tr>
<td>Odanacatib</td>
<td>2</td>
<td>6</td>
<td>0.13</td>
</tr>
</tbody>
</table>

J. Gauthier et. al, *BMCL* 2008, 18, 923
Mean Concentration Profile of Odanacatib in Women Administered Once-Weekly Doses for 3 Weeks

- Human half-life similar to dog; human $t_{1/2} = 60-70$ h
- At therapeutic exposures, odanacatib maintains selectivity over other cathepsins

Effect of Odanacatib on Bone Resorption After 3 Weeks of Once-Weekly Dosing

S-CTx Mean Percent Change (95%CI) from Baseline

- Placebo
- 50 mg
- 5 mg
- 100 mg
- 25 mg

N = 9-12/group

Selectivity of Other Cathepsin K Inhibitors

- Relacatib has low selectivity towards related cathepsins (L, V, B, S) in enzymatic assays
  - Relacatib clinical development was halted after Phase I studies
- Balicatib is very selective towards related cathepsins in enzymatic assays
  - Development of balicatib was discontinued during Phase IIb due to morphea-like skin changes
  - Cathepsin B, K, L, S and V are all expressed in human skin and have collagenase activity
  - Postulate that morphea is due to lysosomotropic-based loss of selectivity

Brömme, D. *Expert Opin. Investig. Drugs.* 2009, 18, 585
Desmarais, S. et al *Molecular Pharmacology* 2008, 73, 147
Odanacatib has Similar Potency in Whole Cells and Purified Cathepsins

<table>
<thead>
<tr>
<th></th>
<th>Cathepsin K</th>
<th>Cathepsin B</th>
<th>Cathepsin L</th>
<th>Cathepsin S</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Enzyme</td>
<td>Cell</td>
<td>Enzyme</td>
<td>Cell</td>
</tr>
<tr>
<td>Odanacatib</td>
<td>0.2</td>
<td>5</td>
<td>1034</td>
<td>1050</td>
</tr>
<tr>
<td>Balicatib</td>
<td>0.6</td>
<td>22</td>
<td>4800</td>
<td>61</td>
</tr>
</tbody>
</table>

The low level of off-target activity of odanacatib in enzyme assays is maintained in whole cell assays.
Effect of Cat K Inhibitors on Collagen Accumulation in Dermal Fibroblasts

- Develop model of intracellular collagen accumulation in primary human dermal fibroblasts

- Odanacatib has a minimal effect on intracellular collagen accumulation compared to balicatib and the pan-cathepsin inhibitor relacatib

J. Gauthier et al. *BMCL* 2008, 18, 923
Bisphosphonates and denosumab reduce the number of osteoclasts
- Fewer resorption pits, reduced new bone formation

Odanacatib reduces the activity of Cat K in the osteoclast
- Same number of, but shallower resorption pits
- Allows new bone formation

Leung et al. *Bone* 2011, 49, 623
Odanacatib inhibits bone resorption (uNTx) while exhibiting less reduction of bone formation (BSAP) than observed with alendronate.

**Historical Comparison to Alendronate**

- **Odanacatib Phase IIB**
- **Year**
- **Geometric Mean Percent Change from Baseline (±SE)**
- **Urinary NTx**
- **Serum BSAP**

1 Binkley et al., ASBMR 2011
2 Merck data on file
Odanacatib: Phase IIb Study 5-Year Data
Bone Mineral Density (DXA)

- Odanacatib progressively increases BMD over 5 years at lumbar spine, femoral neck and total hip

**Historical Comparison to Alendronate**

**Odanacatib Phase IIb**

- Lumbar Spine
- Femoral Neck
- Total Hip

**Alendronate Phase III**
Early inhibitors were found to accumulate in lysosomes thereby lowering their selectivity towards related cathepsins in functional assays.

Optimized leads produced selective non-basic inhibitors.

Optimized non-basic leads reduced metabolism and improved pharmacokinetic properties.

In post-menopausal women, odanacatib treatment:
- Demonstrates less suppression of markers of bone formation than has been observed with alendronate*
- Progressively increases BMD over 5 years at lumbar spine, femoral neck, and total hip

* Historical comparison