Novel Small Molecule Inhibitors of Interleukin-1 Receptor Associated Kinase-4 Are Effective in a Preclinical Model of Arthritis

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Disclosure
All authors were employees of Ligand Pharmaceuticals at the time this research was conducted

Interleukin-1 Receptor Associated Kinase-4 (IRAK4)
• IRAK4 is a serine/threonine protein kinase (TLK family)
• A key signaling component downstream of TIR family (IL-1R, IL-18R, Toll-like receptors)
• Important for innate immunity and inflammation

IRAK4 Inhibitor Binds to ATP Pocket
• Kinase inhibition assay used to identify hits in a high throughput screen of over 5 million compounds
• X-ray crystallography confirms binding

Lead Compound LG0224912 is a Potent IRAK4 Inhibitor

IC₅₀ = 0.7 nM
• Inhibition of kinase activity determined in IMAP fluorescence polarization assays
• Lead compound LG0224912 is a very potent inhibitor that has been extensively characterized

LG0224912 Has Favorable Kinase Selectivity
S(IC₅₀ off-target / IC₅₀ primary target) <10
Karaman et al., Nature Biotechnology, 26:1, 2008
Dendrogram adapted from: Manning et al., Science, 298, 2002 and Cell Signaling Technology www.cellsignal.com
LG0224912 Inhibits IRAK1 Degradation

- IL-1β binding to the IL-1 receptor results in the formation of a complex including MYD88, IRAK1, and IRAK4
- IRAK4 subsequently phosphorylates IRAK1, leading to rapid IRAK1 ubiquitination and degradation

LG0224912 Suppresses IL1β Signaling in Cell Models

- Cells stimulated overnight with IL-1β
- IL-6 secretion measured by ELISA

LG0224912 Suppresses TLR4 Signaling in Cell Models

- Cells stimulated with LPS (TLR4 ligand)
- ICAM-1 (CD54) presentation measured by flow cytometry
- IL-6 secretion measured by ELISA

Inflammatory Pathway Selectivity of LG0224912

- Mouse model used to assess IRAK4 inhibition of IL-1β pathway
- Injection of IL-1β results in increased production of IL-6

LG0224912 Selectively Inhibits the IL-1β Pathway

- Inhibition of downstream phosphorylation induced by IL-1β, TNFα, or IFNγ measured by flow cytometry in THP-1 cells
- LG0224912 is >100-fold selective vs. the two IRAK4 independent pathways
Summary

- We have identified novel, potent IRAK4 inhibitors
- IRAK4 inhibitors suppress IL1-β and TLR4 signaling in cell based models
- Oral administration of IRAK4 inhibitors is effective in the mouse collagen induced arthritis model
- IRAK4 inhibition may be a therapeutic option for multiple inflammatory conditions including:
  - RA
  - SLE
  - IBD
  - Gout
  - Asthma / Allergic Rhinitis

LG0224912 Inhibits the IL-1β Pathway In Vivo

- IL-1β increases serum levels of IL-6 and TNFα in female BALB/c mice
- Oral administration of LG0224912 inhibits the increase in IL-6 and TNFα

LG0224912 Reduces Inflammation in Collagen Induced Arthritis Model

- Oral administration of LG0224912 initiated after clinical signs of disease onset observed in male DBA/1J mice
- LG0224912 inhibits inflammation and erythema associated with collagen induced arthritis

Thank you