IRAK4 kinase inhibitors: The pharmacology of blocking Toll/IL1 signaling

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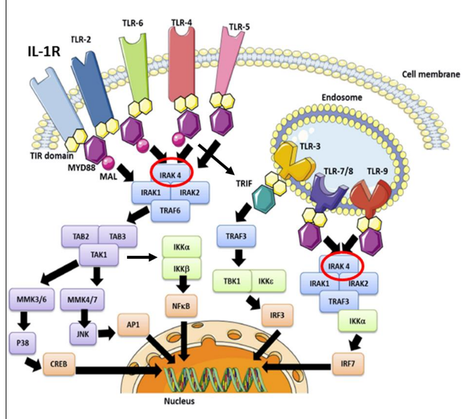
Objectives: IRAK4 is a central kinase in innate immune signalling that controls signaling from Toll-like receptors and IL1 family receptors which detect bacterial and viral pathogens and products of sterile inflammation to produce pro-inflammatory cytokines. We developed potent and highly selective inhibitors of IRAK4 with excellent cell potency and tested them in *in vitro* and *in vivo* efficacy for treatment of inflammatory disease. One of these compounds is currently in Phase 2 clinical trials.

Methods/Results: Inhibitors of IRAK4 show efficacy in mouse models of inflammatory disease and in primary human cells treated with disease-relevant inflammatory stimuli. In vitro experiments demonstrate that inhibition of IRAK4 kinase activity has little effect on the activation of the MAP-kinases and NFkB in primary human cells but profoundly affects the activation of the transcription factor IRF5. However, ablation of IRAK4 protein expression completely blocks activation of these processes demonstrating a kinase-independent scaffold activity essential for IL1 and TLR signaling. Furthermore, we show that while inhibition of IRAK4 has profound effects on TLR induced cytokine and interferon production in myeloid cells, it has only a modest effect on IL1 induced cytokine production in fibroblasts and epithelial cells.

Conclusions: These results demonstrate that there are separable scaffold and kinase dependent functions of IRAK4 and that there are differences in the role of IRAK4 kinase activity in TLR and IL1 signaling in different cell types. These data have implications for the use of IRAK4 inhibitors in the treatment of inflammatory disease and on the use of biomarkers to measure the inhibition of IRAK4.

Background



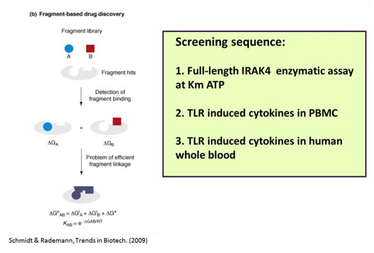
Adapted from Cognasse et al (2015) Frontiers in Immunology 6:1

Interleukin-1 Receptor Associated Kinase 4 (IRAK4) is a central regulator of innate immunty that interacts through the adaptor protein MyD88 to initiate TLR/IL1R signaling.

Humans and mice with IRAK4 deficiency are refractory to stimulation by IL1 and TLR ligands and are susceptible to bacterial infections.

TLR/IL1R pathways have been implicated in the pathogenesis of autoimmune and autoinflammatory diseases such as arthritis, SLE, psoriasis, and gout. Thus IRAK4 inhibitors may be effective treatments for these diseases.

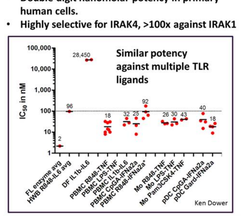
Fragment based drug discovery delivers potent and selective inhibitors of IRAK4 with in vivo efficacy

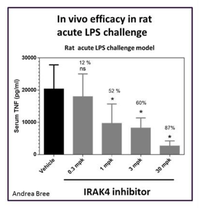


Screening sequence:

1. Full-length IRAK4 enzymatic assay at Km ATP
2. TLR induced cytokines in PBMC
3. TLR induced cytokines in human whole blood

- Single digit nanomolar potency against enzyme.
- Double digit nanomolar potency in primary human cells.
- Highly selective for IRAK4, >100x against IRAK1

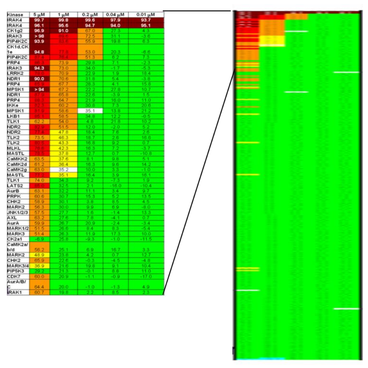




In cell selectivity assay (ActivX) of an IRAK4 inhibitor in THP1 cells

- Highly selective for IRAK4
- 95% occupancy of IRAK4 observed at 10 nM
- Only 50% occupancy of IRAK1 observed at 5 uM

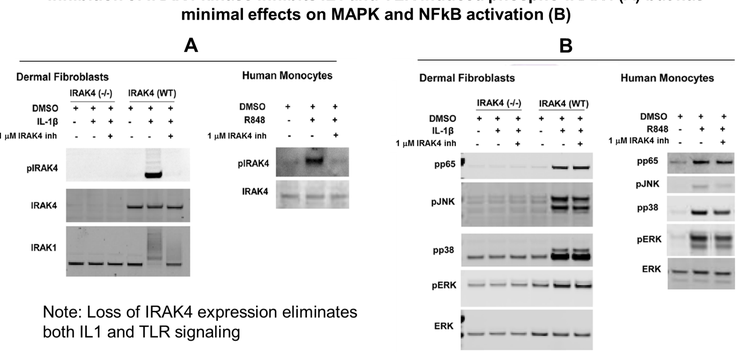
Efficacy is mostly driven by IRAK4 not IRAK1 inhibition



K. Lee et al J. Med Chem (accepted for publication)

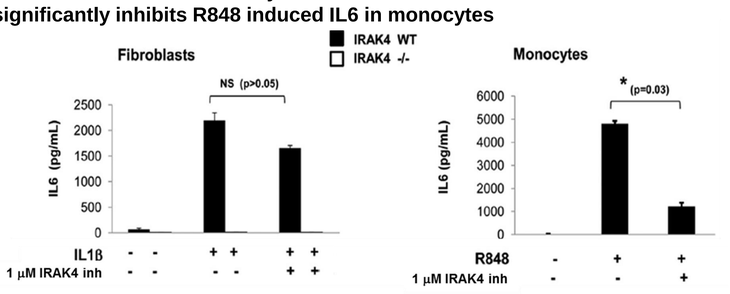
Effect of IRAK4 kinase on TLR and IL1 signaling in different cell types

Inhibition of IRAK4 kinase inhibits IL1 and TLR induced phospho-IRAK4 (A) but has minimal effects on MAPK and NFkB activation (B)



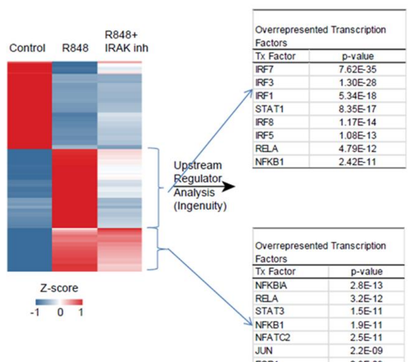
Note: Loss of IRAK4 expression eliminates both IL1 and TLR signaling

Inhibition of IRAK4 minimallly inhibits IL1 induced IL6 in fibroblasts but significantly inhibits R848 induced IL6 in monocytes



IRAK4 kinase regulates IRF but not NFkB dependent transcripts in R848 stimulated monocytes

Transcriptional analysis of IRAK4 regulated genes

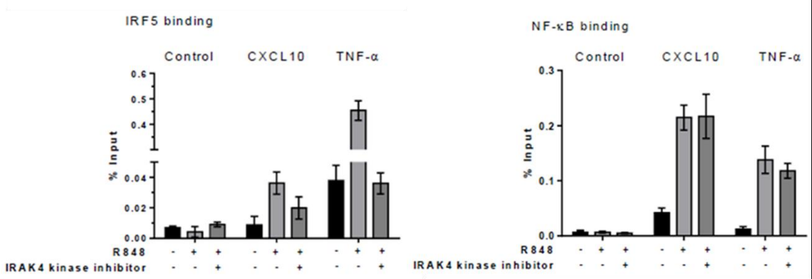


Overrepresented Transcription Factors	
Tx Factor	p-value
IRF7	7.62E-35
IRF3	1.30E-28
IRF1	5.34E-18
STAT1	8.35E-17
IRF8	1.17E-14
IRF5	1.08E-13
RELA	4.79E-12
NFKB1	2.42E-11

Overrepresented Transcription Factors	
Tx Factor	p-value
NFKBIA	2.8E-13
RELA	3.2E-12
STAT3	1.5E-11
NFKB1	1.9E-11
NFATC2	2.5E-11
JUN	2.2E-09
EGR1	2.9E-09
NFATC3	5.1E-09

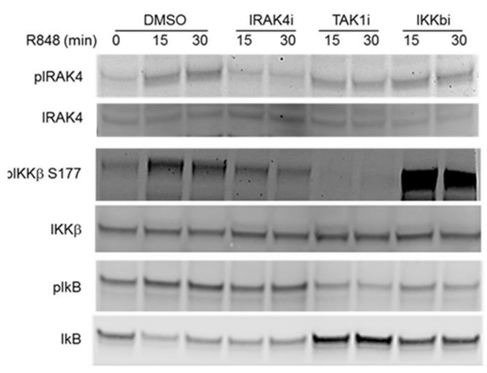
IRAK4 inhibition blocks IRF5, but not NFkB, binding to cytokine promoters

ChIP analysis

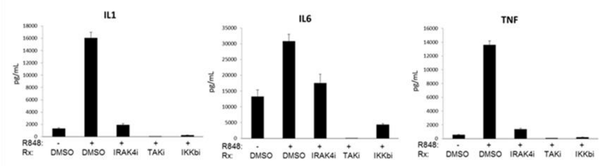


IRAK4 kinase controls IRF5 translocation through TAK1 and IKKβ

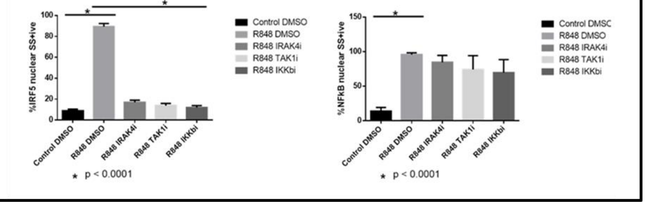
Inhibition of IRAK4 and TAK1 blocks R848 induced IKKβ phosphorylation in monocytes



Inhibition of IRAK4, TAK1, and IKKβ blocks R848 induced cytokines in monocytes

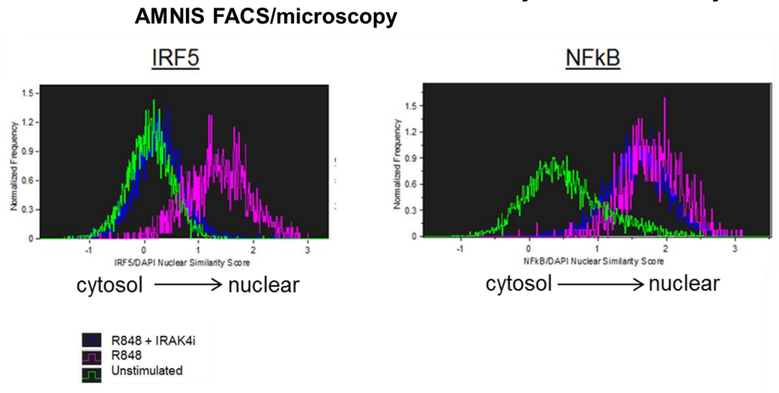


Inhibition of IRAK4, TAK1, and IKKβ blocks R848 induced IRF5, but not NFkB, nuclear translocation in monocytes

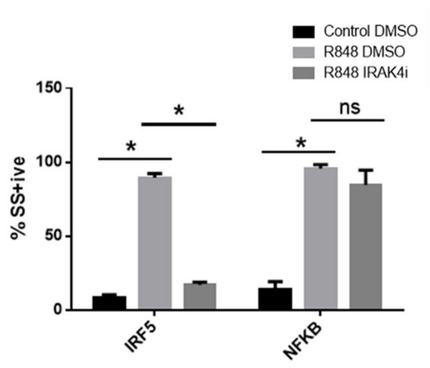


Inhibition of IRAK4 kinase blocks IRF5 but not NFkB nuclear translocation in R848 treated human monocytes

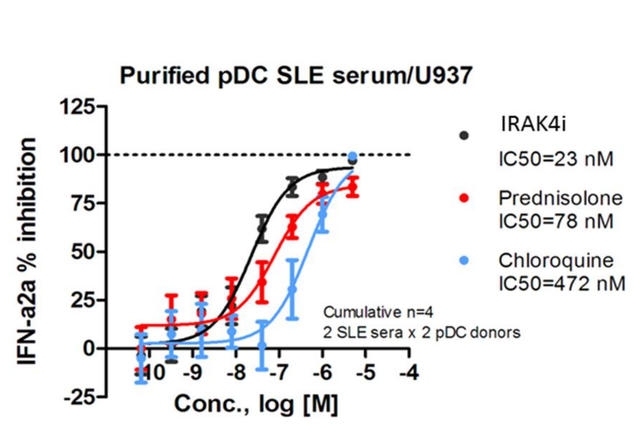
Nuclear localization in CD14+ monocytes determined by AMNIS FACS/microscopy

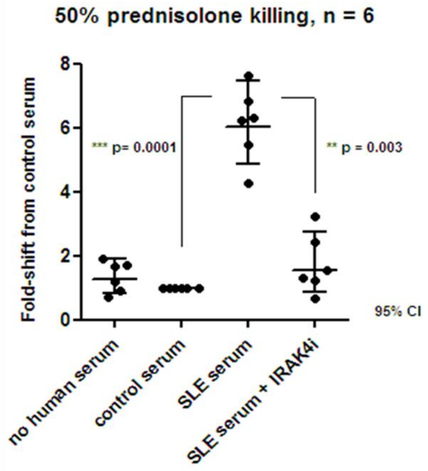


Quantitation of R848 induced nuclear translocation



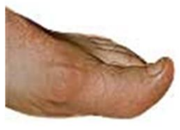
Efficacy in lupus relevant assays: IRAK4 inhibitors block SLE serum induced IFNa and reverse steroid insensitivity in human plasmacytoid dendritic cells (pDCs)



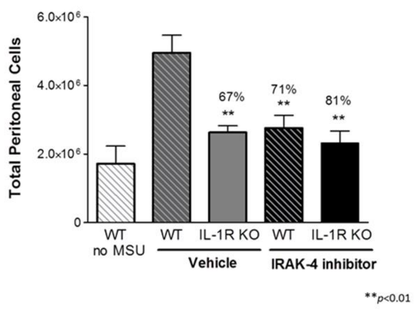


Efficacy of IRAK4 inhibitor in multiple inflammatory disease models

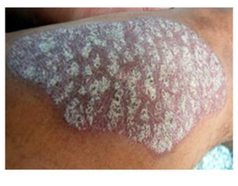
Gout



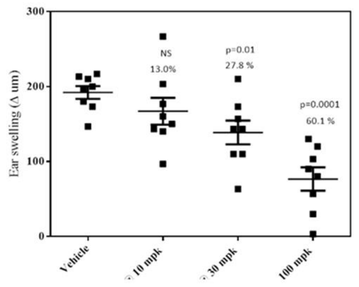
Reduction in MSU induced peritonitis gout model



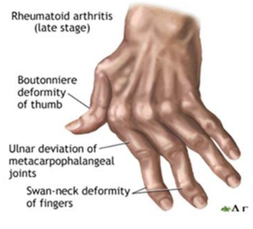
Psoriasis



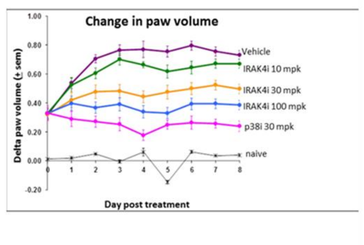
Reduction in imiquimod induced ear swelling model of psoriasis



Arthritis



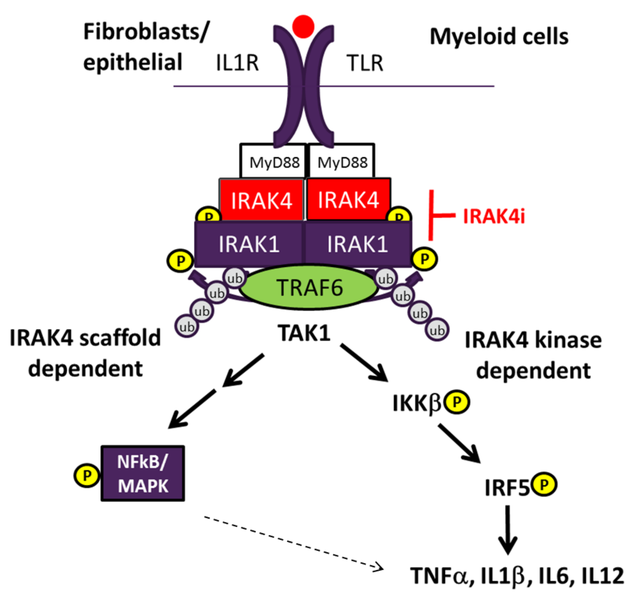
Reduction in collagen induced paw swelling arthritis model



Conclusions:

1. Potent and selective inhibitors of IRAK4 are effective in animal models of inflammation.
2. IRAK4 inhibitors block SLE induced IFNa and reverse steroid resistance in human cells *in vitro*.
3. Inhibition of IRAK4 kinase has greater effects on TLR than IL1 induced cytokines.
4. IRAK4 kinase activity minimally controls NFkB or MAPK activation in TLR/IL1R signaling.
5. IRAK4 activates IRF5 through IKKβ to control inflammatory cytokines in TLR signaling.

Model of IRAK4 signaling



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