Targeting IRF5: Discovery and Preclinical Development of Selective Small Molecule Inhibitors

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INTRODUCTION

Transcription factor with essential role in immune regulation

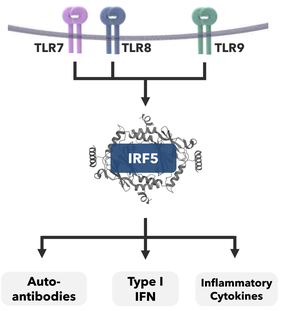
- Interferon regulatory factor 5 (IRF5) is a transcription factor downstream of Toll-like receptors 7, 8, and 9
- Primarily expressed in Dendritic cells, B cells, Monocytes, and Macrophages
- Activated by RNA/DNA-containing immune complexes
- Critical role in autoantibody production, type I interferon, and proinflammatory cytokines

Preclinical validation

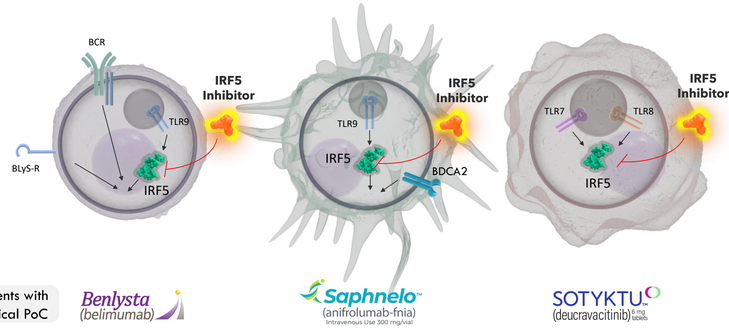
- IRF5-deficient and heterozygous mice exhibited strong disease protection in several models of SLE with reductions in type I interferon levels and autoantibodies

Genetic validation

- Genetic polymorphisms in IRF5 are associated with increased risk of SLE, Sjogren's syndrome, and other autoimmune diseases



IRF5: Master Transcriptional Regulator Impacting Three Clinically-Validated Mechanisms in Autoimmunity



RESULTS

Figure 1. Smart Allostery™ Platform and Proprietary IRF5 Monomeric Crystal Structure Enable Discovery of IRF5 Inhibitors

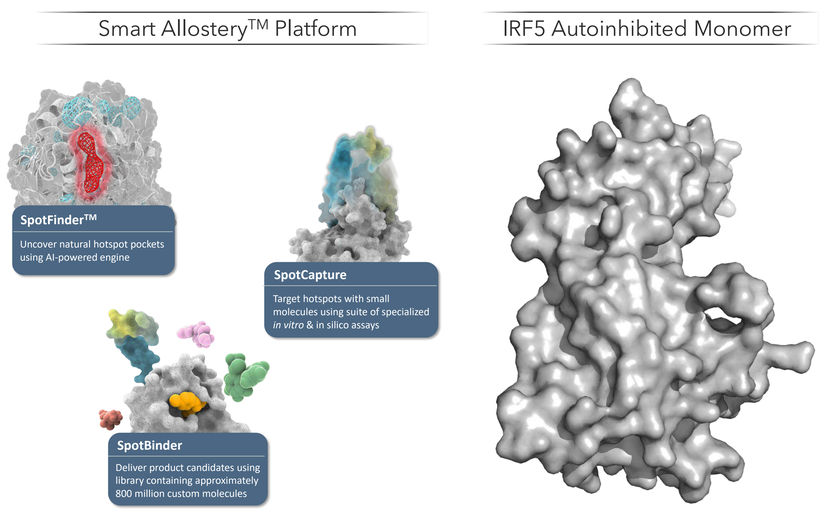
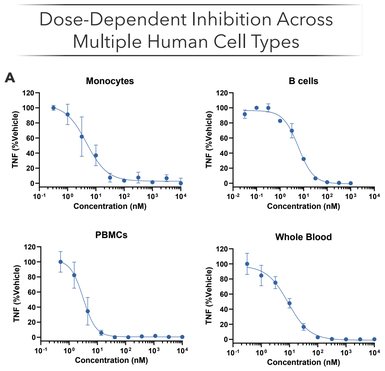


Figure 2. Discovery of Potent IRF5 Inhibitors with Activity Demonstrated Across Multiple Human Cell Types



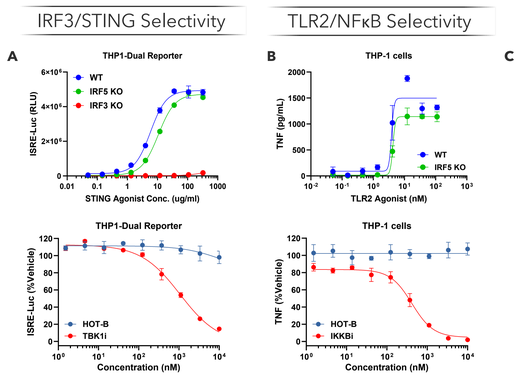
Single-Digit Nanomolar Potency Observed Across Multiple Cell Types and Whole Blood

B

Cells	IC50 (nM)
THP-1	5.0
Monocytes	3.0
B cells	4.1
PBMCs	5.1
Whole Blood	8.2

(A) Complete, dose-dependent inhibition of TLR agonist-induced TNF production in human monocytes, B cells, PBMCs, and whole blood by the IRF5 inhibitor HOT-A. (B) Average IC50 values are shown for HOT compounds across multiple human cell types.

Figure 3. Exceptional Selectivity Over IRF3, TLR2/NFκB Pathway, and Kinome Panel



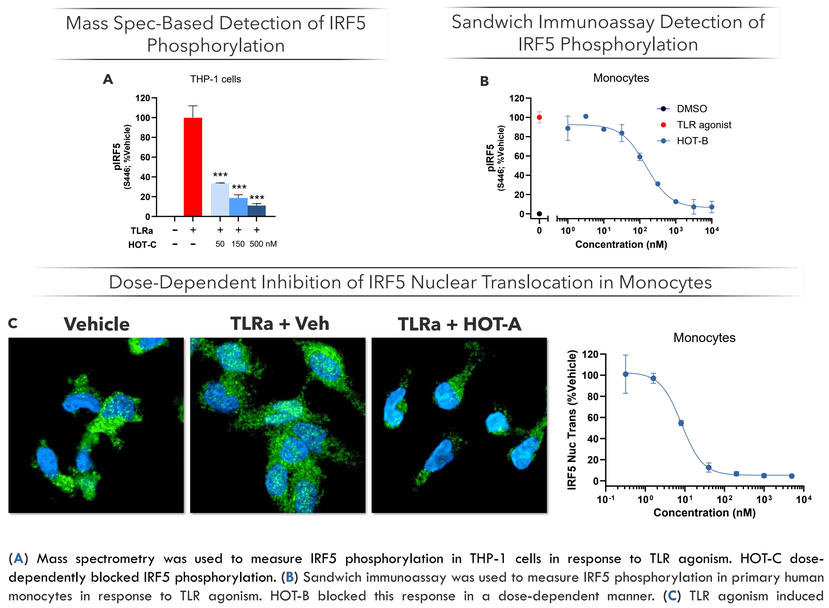
Kinome Panel Selectivity

C

Kinase	%Inhibition at 1uM
IRAK1	6.9
IRAK4	10.5
IKK-beta	7.1
IKK-epsilon	4.5
TBK1	4.4
JAK1	6.1
JAK2	1.2
JAK3	0

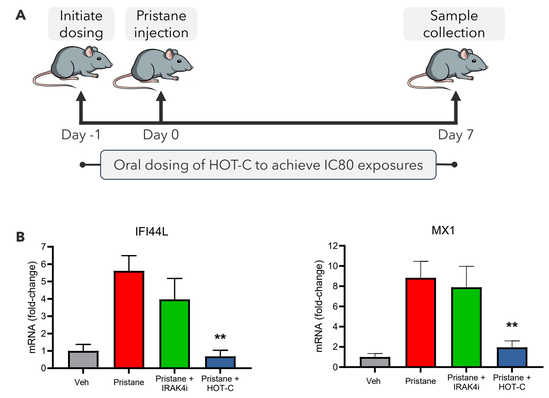
(A) IRF3 and not IRF5 is critical for ISRE reporter activity after STING agonism in THP1-Dual cells. HOT-B was selective in this assay whereas a TBK1 inhibitor blocked reporter activity. (B) IRF5 is not required for TNF production in THP-1 cells after TLR2 stimulation. HOT-B demonstrated complete selectivity in this assay whereas an IKKβ inhibitor was active. (C) HOT-C was tested at 1 uM for functional inhibition of >200 kinases; the percent inhibition of relevant kinases are shown in the table.

Figure 4. Complete, Dose-Dependent Inhibition of IRF5 Phosphorylation and Nuclear Translocation



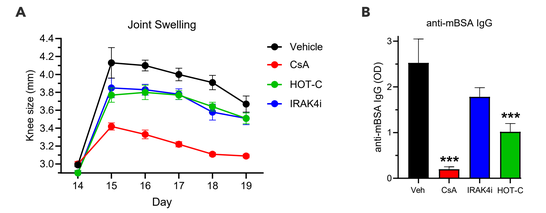
(A) Mass spectrometry was used to measure IRF5 phosphorylation in THP-1 cells in response to TLR agonism. HOT-C dose-dependently blocked IRF5 phosphorylation. (B) Sandwich immunoassay was used to measure IRF5 phosphorylation in primary human monocytes in response to TLR agonism. HOT-B blocked this response in a dose-dependent manner. (C) TLR agonism induced significant IRF5 nuclear translocation in human monocytes that was blocked by HOT-A in a dose-dependent manner.

Figure 5. Inhibition of Type I Interferon-Induced Genes in the Short-Term Model of Pristane-Induced Lupus



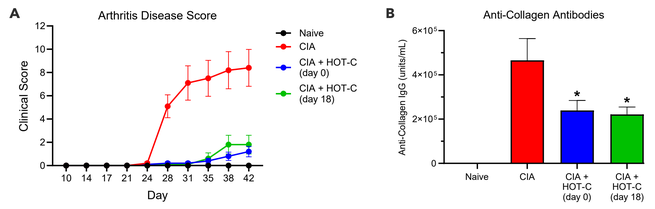
(A) Short-term pristane-induced lupus model timeline. BALB/c mice were orally dosed each day with HOT-C (IC80 coverage) or an IRAK4 inhibitor starting on day -1 followed by an IP injection of pristane on day 0. On day 7, peritoneal lavage was collected, and mRNA levels were assessed by qPCR. (B) mRNA levels of interferon-induced genes are shown.

Figure 6. IRF5 Inhibition was Efficacious in the mBSA Antigen-Induced Arthritis Model



(A) mBSA antigen-induced arthritis model was performed in C57BL/6 mice beginning with an immunization on day 0 followed by a challenge injection into the knee on day 14. Compounds were dosed daily to achieve IC50 coverage beginning 2 hours before the initial vaccination. Cyclosporin A (CsA) was used as a model control compound. Knee swelling was measured daily along with (B) anti-mBSA specific IgG antibodies at the end of the study.

Figure 7. IRF5 Inhibition Reduced Joint Swelling and Antigen-Specific Antibodies in the Collagen-Induced Arthritis Model



(A) Collagen-induced arthritis model in DBA/1 mice. Mice received daily oral doses of HOT-C beginning either on day 0 (prophylactic regimen) or day 18 (semi-therapeutic regimen) with terminal exposures reaching IC90 levels. Clinical score is shown over the course of the study. (B) Anti-bovine type II collagen IgG antibody levels were measured at the end of the study.

CONCLUSIONS

- Potent and Selective Inhibition of a Previously Undruggable Transcription Factor
- Complete Inhibition of IRF5 Phosphorylation and Nuclear Translocation
- Significant Activity In Vivo with Effects on Clinically-Validated Mechanisms
- Potential to Significantly Impact a Broad Range of Autoimmune Diseases