Preclinical and Translational Assessment of Small Molecule IRF5 Inhibitors in Lupus-Relevant Systems

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INTRODUCTION

Transcription factor with essential role in immune regulation

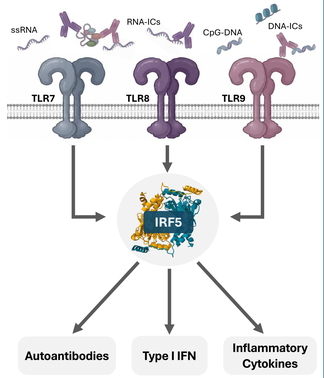
- IRF5 is a transcription factor downstream of Toll-like receptors 7, 8, and 9
- Primarily expressed in Dendritic cells, B cells, Monocytes, and Macrophages
- Critical role in autoantibody production, type I interferon, and proinflammatory cytokines

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 Pathways in Autoimmunity

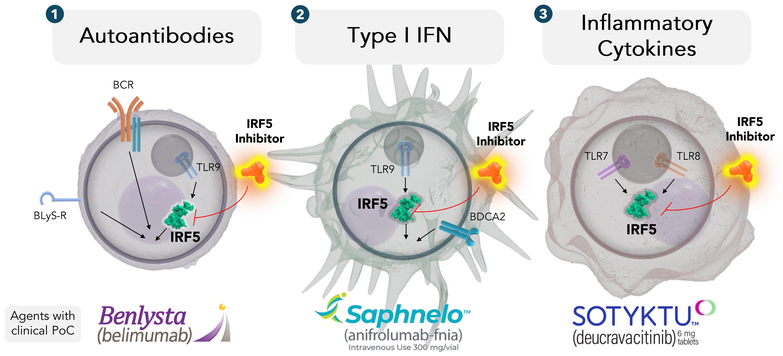
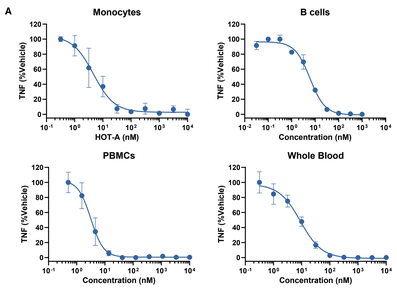


Figure 1. Smart Allostery Platform Enables Discovery of Potent and Selective IRF5 Inhibitors

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



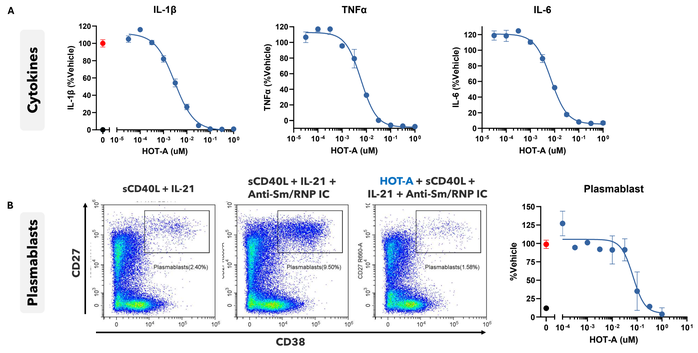
B

Assay	IC50 (nM)
Binding	4
THP-1	6
Monocytes	3
B cells	4
Neutrophils	3
PBMCs	5
Whole Blood	3

Compounds demonstrated exceptional selectivity over IRF3, TLR2/NFkB pathway, and a relevant panel of >400 kinases

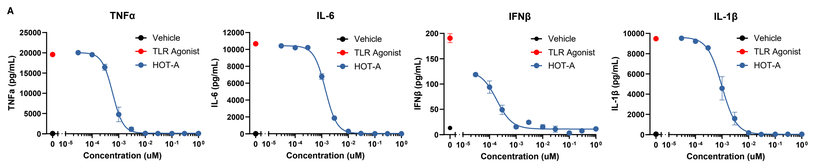
(A) Complete inhibition of TLR agonist-induced TNF production in human monocytes, B cells, PBMCs, and whole blood by the IRF5 inhibitor HOT-A. (B) Average IC50 values are shown for HOT compounds across multiple assays.

Figure 2. IRF5 Inhibition Blocks Cytokine Production and Plasmablast Differentiation Induced by Anti-Sm/RNP Immune Complexes in B Cells



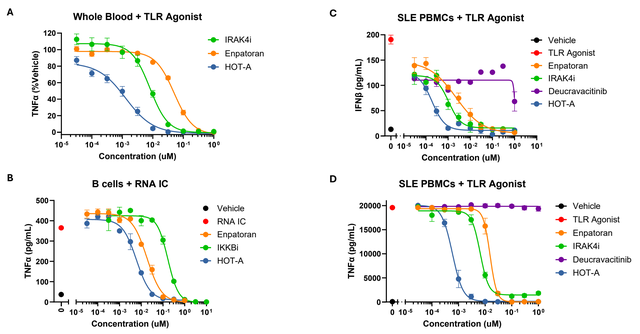
(A and B) Human B cells were pretreated with IRF5 inhibitors or vehicle before being cultured in the presence of soluble CD40L (sCD40L) and IL-21 and stimulated with Anti-Sm/RNP immune complexes. (A) Dose-dependent inhibition of cytokine production was observed after overnight incubation. (B) After 4 days, plasmablasts ($CD27^{+}CD38^{+}$) were assessed by flow cytometry. IRF5 inhibitor HOT-A prevented plasmablast differentiation in a dose-dependent manner.

Figure 3. Potent Inhibition of Cytokines in SLE PBMCs with TLR Stimulation



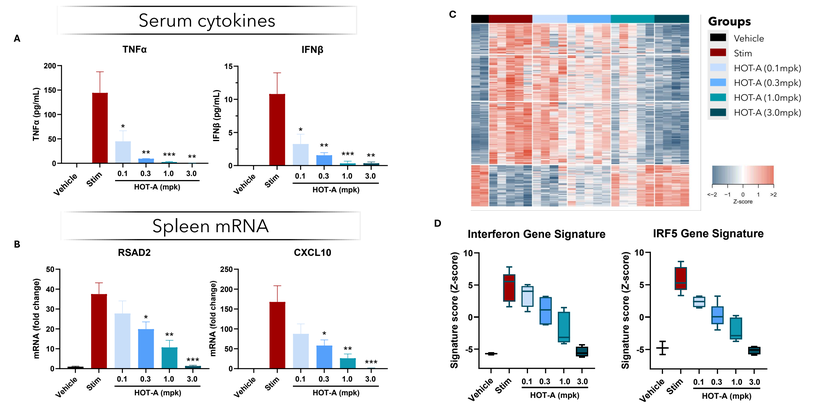
(A) SLE PBMCs were pretreated with a titration of HOT-A before stimulation with a TLR agonist. HOT-A blocked the production of various cytokines in a dose-dependent manner.

Figure 4. IRF5 Inhibition Demonstrates Excellent Potency And Outperforms Relevant Inhibitors



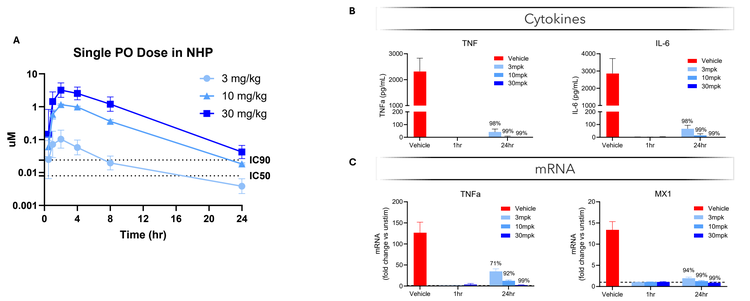
(A) Whole blood was stimulated with a TLR agonist and TNF was measured after overnight incubation. (B) Human B cells were stimulated with Anti-Sm/RNP immune complexes (RNA IC) and TNF was measured after overnight incubation. (C and D) SLE PBMCs were stimulated with a TLR agonist and (C) IFNβ and (D) TNF were measured after overnight incubation.

Figure 5. Oral Dosing of HOT-A Blocks Cytokine and mRNA Responses Driven by TLR Stimulation in a Dose-Dependent Manner



(A -D) Humanized NOG-EXL mice were dosed orally with HOT-A before challenging the mice with a TLR agonist. Doses are listed in mg/kg (mpk) (A) Serum cytokines were observed 3h post-stimulation. (B) Spleens were isolated 3h post-stimulation and mRNA was measured by qPCR. (C) RNA sequencing was also performed on spleen RNA. A heatmap is shown of genes significantly modulated by TLR agonism. (D) Type I interferon and IRF5 gene signature scores were assessed from the RNAseq data.

Figure 6. Oral Dosing in Cyno Provides Complete Inhibition of the IRF5 Pathway for 24h at Lowest Dose



(A) PK profile of HOT-A following oral dosing in Cynomolgus monkeys. Dotted lines to indicate IC50/IC90 values are based on Cyno whole blood potency. (B and C) At 1h and 24h post-dose, blood was removed and stimulated with a TLR agonist for 4h. (B) Plasma cytokine levels were assessed by MSD. (C) mRNA levels were assessed by qPCR.

CONCLUSIONS

- Potent and selective inhibition of a previously undruggable transcription factor with a traditional small molecule
- Complete inhibition of B cell cytokine production and plasmablast differentiation induced by immune complexes
- Outperforms relevant inhibitors in activated SLE PBMCs
- Excellent in vivo potency and inhibition of IFN and IRF5 gene signatures; 24h complete inhibition observed in Cyno at lowest tested dose
- Potential to significantly impact a broad range of autoimmune diseases