Preclinical Evaluation of IRF5 Small Molecule Inhibitors with Potent Activity in Lupus-Relevant Systems

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INTRODUCTION

Transcription factor with essential role in immune regulation

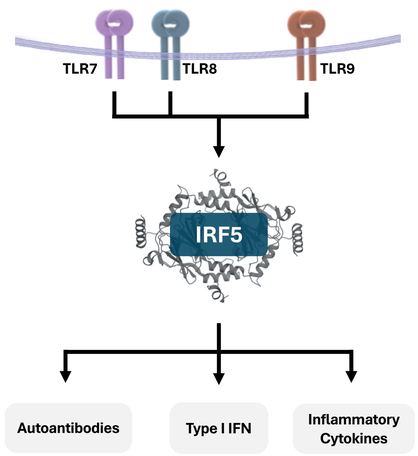
- Interferon regulatory factor 5 (IRF5) is a transcription factor downstream of Toll-like receptors 7, 8, and 9
- Primarily expressed in Dendritic cells, B cells, Monocytes, and Macrophages
- Activated by RNA/DNA-containing immune complexes
- Critical role in autoantibody production, type I interferon,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

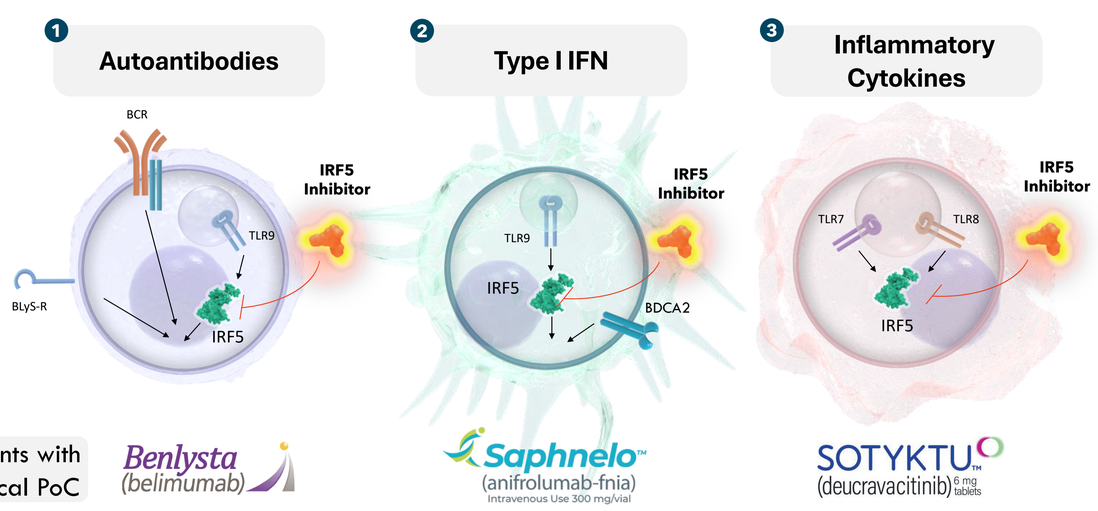
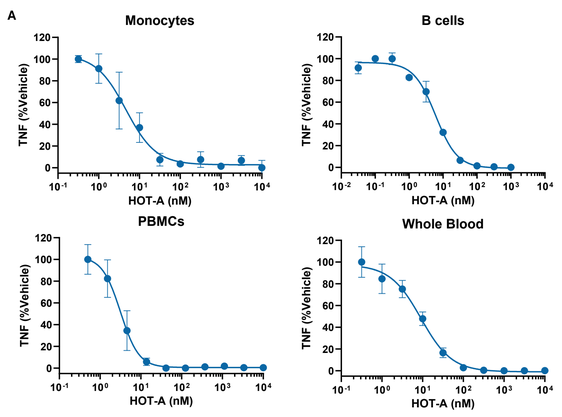


Figure 1. Smart Allostery Platform Enables Discovery of Potent and Selective IRF5 Inhibitors

Dose-Dependent Inhibition Across Multiple Human Cell Types

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



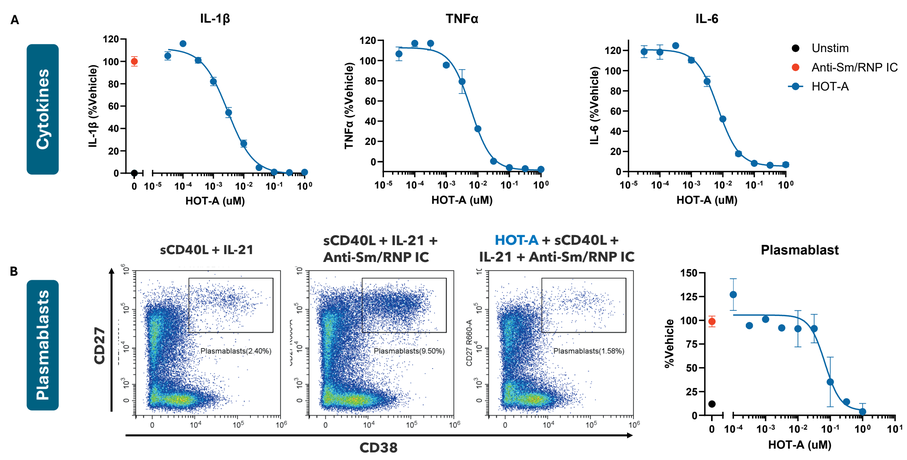
B

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

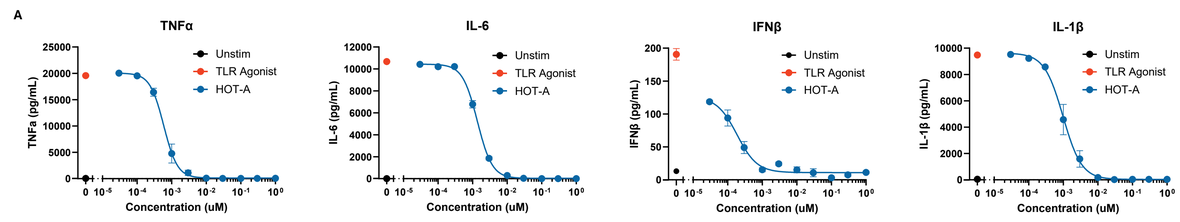
(**A**) Complete, dose-dependent inhibition of TLR agonist-induced TNF production in human monocytes, B cells, PBMCs, and whole blood by the IRF5 inhibitor HOT-A. (**B**) Average IC50 and Kd values are shown for HOT-A across multiple human cell types and recombinant IRF5.

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



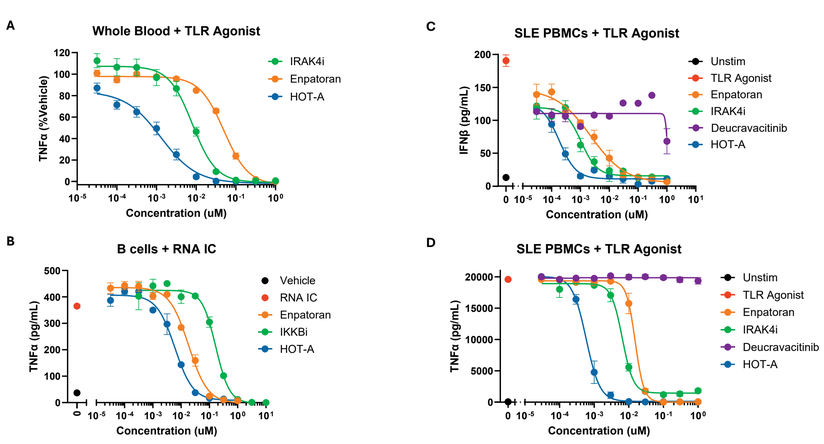
(**A and B**) Human B cells were pretreated with IRF5 inhibitors or vehicle before being cultured in the presence of soluble CD40L (sCD40L) and IL-21 and stimulated with Anti-Sm/RNP immune complexes. (**A**) Dose-dependent inhibition of cytokine production was observed after overnight incubation. (**B**) 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



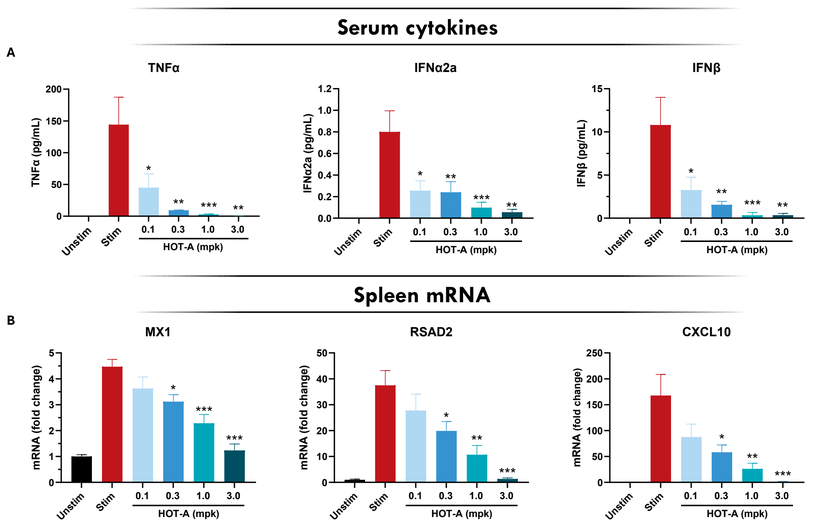
(**A**) SLE PBMCs were pretreated with a titration of HOT-A before stimulation with a TLR agonist. HOT-A blocked the production of various cytokines in a dose-dependent manner

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



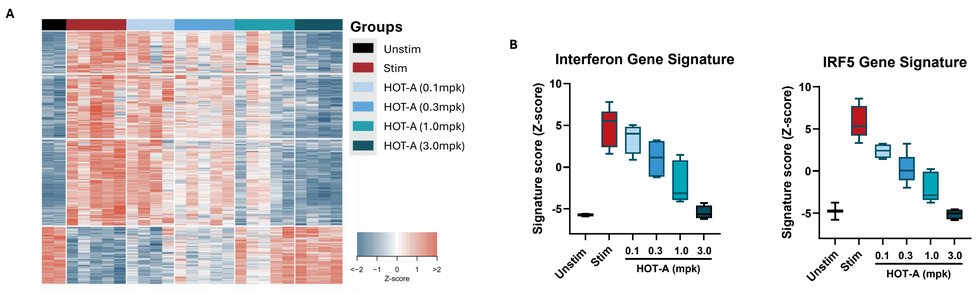
(**A**) Whole blood was stimulated with a TLR agonist and TNF was measured after overnight incubation. (**B**) Human B cells were stimulated with Anti-Sm/RNP immune complexes (RNA IC) and TNF was measured after overnight incubation. (**C and D**) SLE PBMCs were stimulated with a TLR agonist and (**C**) 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 and B**) 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**) Dose-dependent inhibition of serum cytokines were observed 3h post-stimulation. (**B**) Spleens were isolated 3h post-stimulation and dose-dependent inhibition of mRNA was observed by qPCR.

Figure 6. Dose-Dependent Inhibition of Interferon and IRF5 Gene Signatures with HOT-A in Mice Stimulated with TLR Agonist



(**A and B**) Spleen mRNA obtained in Figure 5 was assessed by RNA sequencing. (**A**) A heatmap is shown of genes significantly modulated by TLR agonism. Dose-dependent inhibition of these gene changes were observed by HOT-A. (**B**) Type I interferon and IRF5 gene signature scores were assessed from the RNAseq data. TLR agonist increased these signatures which were blocked by HOT-A in a dose-dependent manner.

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
- Potential to significantly impact a broad range of autoimmune diseases