Potent and Selective Oral IRF5 Degrader, KT-579, Blocks Pro-Inflammatory Cytokines and Reduces Joint Swelling in Rodent Models of Rheumatoid Arthritis

#0930

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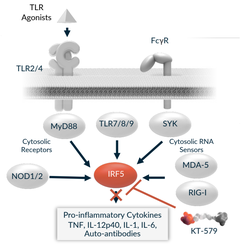
INTRODUCTION

IRF5, an undrugged transcription factor, is an autoimmune susceptibility gene that has been linked to RA and other autoimmune diseases including SLE, Sjögren's, and SSc. IRF5 is a regulator of immune responses activated downstream of pattern recognition receptors, like toll-like receptors (TLR). IRF5 regulates pro-inflammatory cytokines (TNFα, IL-6, IL-12, IL-23), autoantibody production and Type I IFN. IRF5 is selectively expressed and activated in specific cell types such as dendritic cells, monocytes, M1 macrophages, and B cells[1]. RA is a chronic disease where the target tissue is synovial joints. The joints are characterized by heavy leukocyte infiltration. Inflamed synovium contains a larger number of activated macrophages and T cells in addition to B cells and dendritic cells. Studies in IRF5 deficient mice show attenuated arthritis severity in models of RA[2,3]. Despite its strong mechanistic and genetic validation, IRF5 has historically remained undrugged likely due to its activation complexity and multiple functional isoforms. IRF5 is well suited for targeted protein degradation, where a single binding event drives activity. KT-579, an oral IRF5 degrader, has demonstrated potent and selective activity in preclinical *in vitro* myeloid assays and *in vivo* RA models, offering a new approach to modulating this key driver of immunity.

IRF5 Biology and Target Rationale

IRF5 Activates Pro-inflammatory Reponses Involved in RA



Target Biology and Rationale

- IRF5 regulates pro-inflammatory cytokines (TNF, IL-12, IL-6, etc.), Type I IFN, and autoantibody production in a cell and activation-specific manner
- Targeting IRF5 has the potential to block cell-specific immune dysregulation while sparing normal cell function
- Targeting myeloid IRF5 has the potential to restrict Th1/17 T cell responses

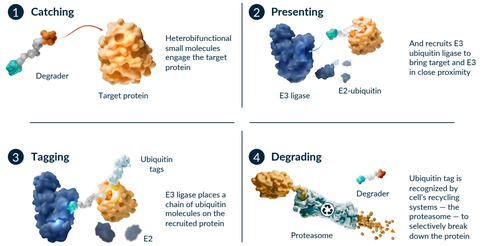
Genetically and Clinically Validated

- IRF5 functional risk variants associate with increased susceptibility to SLE, Sjögren's, RA, IBD, and SSc[1,2]
- IRF5 regulated pathways have been clinically validated by multiple drugs (i.e. TLR7/8, anti-IFN, -TNF, IL-6, IL-12, IL-23 Ab, B cell targeting agents)

IRF5 Degrader Advantage

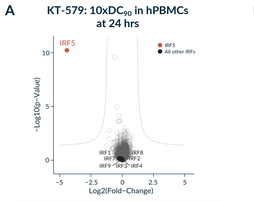
- Conventional approaches have failed to effectively and selectively block IRF5 due to multiple activation steps and IRF family member homology
- TPD allows for a single and specific binding event to drive depletion of the protein and disrupt all IRF5 signaling

Proteome Editing with Targeted Protein Degradation

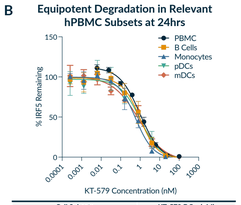


RESULTS

Figure 1. KT-579: An Exquisitely Selective and Picomolar Oral IRF5 Degrader



Selectivity Binding and Cellular Assays	KT-579
IRF3, 4, 6, 7, 8 SPR binding, Kd (nM)	>10,000
IRF3 Degradation DC_{50} (nM)	>10,000
IRF7 Degradation DC_{50} (nM)	>10,000

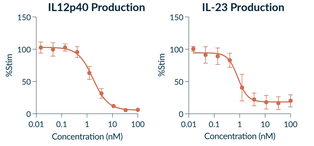


Cell Subsets	KT-579 DC_{50} (nM)
PBMC	0.8
CD19+ B cells	1.0
CD14+ Monocytes	0.6
HLA-DR+CD123+ pDCs	0.9
HLA-DR+CD11c mDCs	0.9

A) KT-579 selectively degrades IRF5 in the detectable proteome (>10,000 proteins). **B)** KT-579 potently degrades IRF5 in key functional cell types.

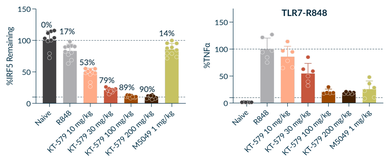
Figure 2. KT-579 Potently Inhibits Production of Key Pro-Inflammatory Cytokines and Type I IFN in Human Primary Cellular Assays

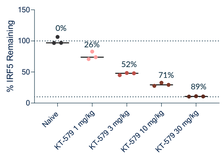
Biology	Receptor	Stim	Cell	Cytokine Readout	KT-579 DC_{50} (nM)
Th1 IFN Production	TLR8	ssRNA40	Monocytes	IFNβ	0.3
	TRL7/8	R848	Monocytes	IFNβ	0.4
	TLR9	ODN2006	PBMC	IFNβ	0.8
Pro-inflammatory Cytokine Production	TLR7/8	R848	PBMC	TNFα	1.5
	TLR7/8	R848	B cells	TNFα	0.4
	TLR8	ssRNA40	Monocytes	TNFα	1.3
	TL7/8	R848	Monocytes	IL-12p40	0.5
	TLR9	ODN2006	PBMC	IL-12p40	1.8
	TLR7/8	R848	Monocytes	IL-1β	0.15
	TLR4	LPS	PBMC	IL-23	1.1



IRF5 degradation potently inhibits proinflammatory cytokines (TNFα, IL-12, IL-23, IL-1) and Type I IFN (IFNβ) downstream of TLR4, TLR7, TLR8, and TLR9 activation. Primary cells were incubated with compound for 24h prior to TLR stimulation.

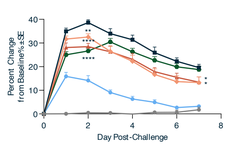
Figure 3. Orally Dosed KT-579 Effectively Degrades IRF5 in Rodent Tissues and Blocks Cytokine Induction

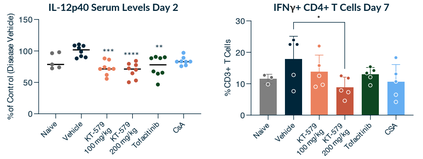




A) KT-579 induced on average a max of 90% IRF5 degradation in mouse spleen tissue. KT-579 blocks TLR7 induced cytokines, including TNFα (shown), IL-6, IL-12p40, IFNβ (not shown), as effectively as M5049 (TLR7/8 SMI). **B)** KT-579 induces dose-dependent degradation in rat spleen.

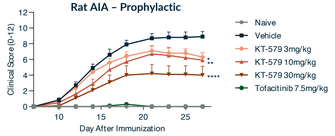
Figure 4. KT-579 Reduces Joint Swelling in a Mouse Model of RA

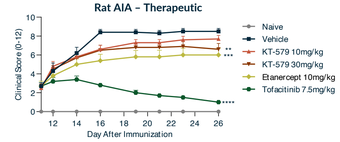




The mouse antigen induced arthritis (AIA) model was established by sensitization with methylated bovine serum albumin (mBSA). 14 days later, mice were challenged with mBSA (Day 0) that induced rapid inflammation and swelling at the site of injection. Prophylactic daily oral dosing of KT-579 results in significant reduction of **A)** joint swelling at Day 2 at peak inflammation and at Day 7 at resolution and **B)** circulating pro-inflammatory cytokines (IL-12p40, shown, Day 2) and pathogenic infiltrating CD4 IFNγ+ T cells (Day 7). ANOVA, *<0.05, **<0.005, ***<0.001, ****<.0001.

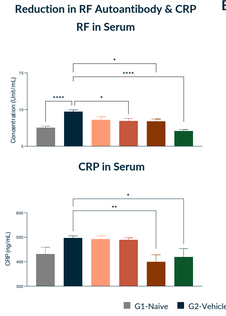
Figure 5. KT-579 Demonstrates Dose-Dependent Activity in the AIA Rat Model

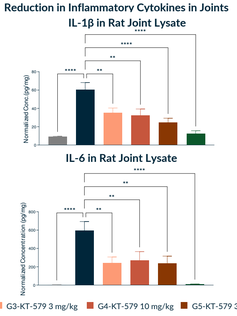


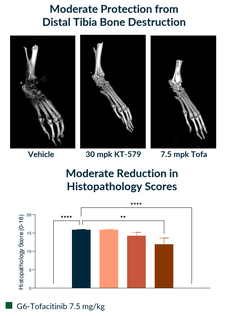


Daily oral dosing of KT-579, results in a significant dose-dependent reduction in clinical scores in the rat model of Adjuvant-induced arthritis (AIA) in both **A)** prophylactic and **B)** therapeutic dosing regimens. The rat AIA model was established by sensitization with Freund's Complete Adjuvant (CFA) and heat-killed Mycobacterium tuberculosis (Mtb) on Day 0. Prophylactic dosing started on Day -3; therapeutic dosing started on Day 11. Samples for analysis were collected on Day 26. ANOVA, *<0.05, **<0.005, ***<0.001, ****<.0001.

Figure 6. KT-579 Reduces Inflammatory Biomarkers and Progression of Bone Destruction in the Prophylactic AIA Rat Model



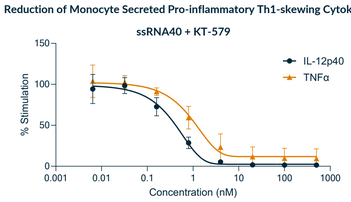


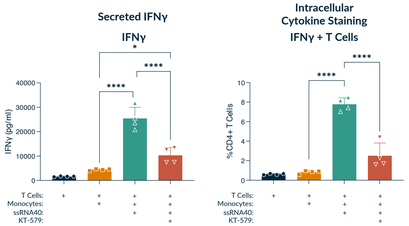


G1-Naive ■ G2-Vehicle ■ G3-KT-579 3 mg/kg ■ G4-KT-579 10 mg/kg ■ G5-KT-579 30 mg/kg ■ G6-Tofacitinib 7.5 mg/kg

Prophylactic dosing of KT-579 results in significant reduction of **A)** circulating (serum) levels of inflammatory biomarkers, **B)** local joint inflammatory cytokines and **C)** leads to moderate protection against bone destruction in the rat AIA model. Samples for analysis were collected on Day 26. ANOVA, *<0.05, **<0.005, ****<.0001.

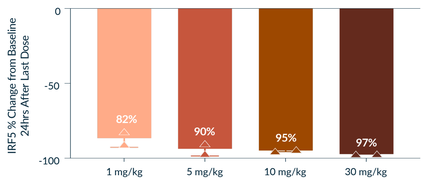
Figure 7. IRF5 Degradation in Monocytes Leads to Significant Reduction in Th1 Polarization *In Vitro*





KT-579 leads to significantly decreased Th1 polarization in monocyte-T cell co-culture experiments. **A)** KT-579 potently inhibits inflammatory, T cell-skewing cytokines in monocytes stimulated with ssRNA40 for 24 hours. Pre-treated monocytes were then co-cultured 1:2 with CD4+ T cells. **B)** Significant decrease of Th1 polarization as measured by soluble IFN-γ and immunophenotyping of IFN-γ+ T cells 48 hours post co-culturing. ANOVA, *<0.05, ****<.0001.

Figure 8. KT-579 Potently Degrades at Low Oral Doses in NHP with an Excellent Safety Profile



KT-579 potently degrades IRF5 across multiple preclinical species with low oral doses. IRF5 levels were measured in isolated PBMCs from blood. KT-579 completed non-GLP toxicity studies in Monkey and rodents with no adverse effects at up to 200-fold predicted human efficacious exposure.

METHODS

- *In vitro* cell cultures for PBMCs, monocytes, B cells, and dendritic cells were performed by treating cells with compound for 24 hours prior to collection for selectivity and potency assessment or stimulation with TLR agonists for functional studies.
- Monocyte:CD4+ co-culture systems were established with paired donor (autologous) CD14+ monocytes and naïve CD4+ T cells. KT-579 was first added to monocytes 24 hours prior to TLR8 stimulation (ssRNA40) for an additional 24 hours. Monocytes then underwent a washout before being co-cultured with CD4+ T cells in a 1:2 ratio in the presence of bound anti-CD3 and soluble anti-CD28. Cocultures were assessed for T cell polarization after 48 hours for cytokine production and immunophenotyping of CD4+ T cells.
- *In vivo* studies: IRF5 degradation and inhibition on cytokine production were confirmed in mouse spleen following 4 days of oral dosing and TLR7 stimulation with R848. Similarly, IRF5 degradation was confirmed at multiple doses in rat spleen following 7 days of oral dosing. Mouse antigen induced arthritis (AIA) model was established by sensitization with methylated bovine serum albumin (mBSA). 14 days later, mice were challenged with mBSA (Day 0) that induced rapid inflammation and swelling at the site of injection. KT-579 was administered prophylactically starting at Day -17 (3 days prior to initial challenge). Paw swelling, serum cytokine analysis, and joint flow cytometry for T cell populations were assessed at Day 2 and Day 7. The rat AIA model was established by sensitization with Freund's Complete Adjuvant (CFA) and heat-killed Mycobacterium tuberculosis (Mtb) on Day 0. Prophylactic dosing started on Day -3; therapeutic dosing started on Day 11 (compared to disease induction, Day 0).
- One- and Two-Way ANOVA used for statistical analysis.

CONCLUSIONS

We report here the first selective, potent, oral IRF5 degrader that can sufficiently deplete IRF5 and impact myeloid cell effector function by potently inhibiting pro-inflammatory cytokines found to be critical in amplifying inflammatory responses, including cytokines that promote Th1 and Th17 T cell responses, and can reduce joint swelling in a mouse model of RA. Additionally, prophylactic dosing of KT-579 demonstrates activity in a rat model of RA by potently reducing inflammatory biomarker production and cytokines and reducing bone destruction. *In vivo* KT-579 demonstrated promising preclinical safety profile in rodents and monkeys achieving >90% degradation. These findings support KT-579 as a novel therapeutic agent with the potential to be a first-in-class oral therapy for the treatment of RA and other autoimmune diseases. Phase I clinical testing expected in early 2026.

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DISCLOSURES

This study was funded by Kymera Therapeutics. Camire, Zhang, Massa, Leedberg, Corcoran, Lurier, Carroll, Ho, Chen, Enerson, Mehovic, Zhao, Howarth, Breitkopf, Martinez, Ford, Fei, Sathappa, Williams, Weiss, Shabbir, Mainolfi, Campbell are Kymera Therapeutics employees and equity owners.