Potent and Selective First-in-Class Oral IRF5 Degrader, KT-579, Inhibits Endosomal TLR-induced Responses in SLE Derived PBMCs and Significantly Reduces Disease Activity in the MRL.lpr Mouse Lupus Model

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BACKGROUND

IRF5 is a transcription factor and master regulator of pro-inflammatory immune responses activated downstream of specific pattern recognition receptors, like Toll-like receptors (TLRs). Downstream of TLR7, TLR8 and TLR9 activation, IRF5 can act as a central node and regulate genes for pro-inflammatory cytokines (TNFα, IL-6, IL-12, IL-23), Type I IFN, and cellular functions such as B cell activation and antibody secretion. IRF5 is constitutively expressed and activated in immune cell types such as dendritic cells, monocytes, macrophages, and B cells. Human genetic and functional studies have linked IRF5 dysregulation to the pathogenesis of multiple autoimmune diseases, including SLE and Sjögren's, and Irf5-deficient mice protect from lupus onset and severity. In SLE, endosomal TLRs recognize nuclear self-antigens, and can trigger IRF5 activation to drive the breakdown of immune tolerance via a cascade involving increased Type I IFN and pro-inflammatory cytokine production, and autoantibody production. Despite its strong mechanistic and genetic validation, IRF5 has historically remained undrugged likely due to its lack of catalytic activity, activation complexity and multiple functional isoforms. IRF5 is well suited for targeted protein degradation. KT-579, a potent, selective, oral IRF5 degrader, offers a novel approach to modulating immune responses driven by IRF5.

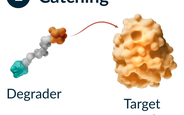
OBJECTIVES

To demonstrate potent *in vitro* activity of KT-579 induced IRF5 degradation in both human healthy or SLE-derived donor cells regardless of the presence of a common polymorphism. The rs2004640 T allele variant enables alternative splicing and alters IRF5 isoform expression and is a well established IRF5-associated risk variant in lupus[1]. In addition, to assess the *in vivo* therapeutic potential of the oral IRF5 degrader, KT-579, by evaluating its dose-dependent activity and immunomodulatory effects in the MRL.lpr mouse model of lupus and to compare the activity of KT-579 with targeted agents in clinical-stage testing or approved in SLE treatment.

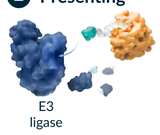
Targeted Protein Degradation: Achieving Biologics-like Activity with Oral Medicines

Targeted Protein Degradation (TPD) Mechanism of Action
Harnessing the E3 Ubiquitin Proteasome System

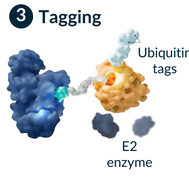
1 Catching — Hetero-bifunctional small molecules engage the target protein



2 Presenting — And recruits E3 ubiquitin ligase to bring target and E3 in close proximity



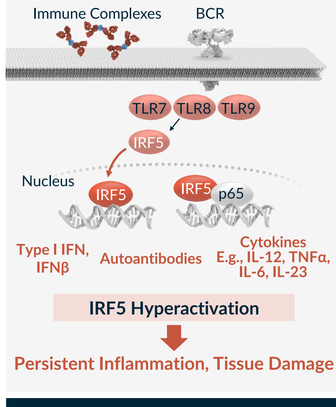
3 Tagging — E3 ligase places a chain of ubiquitin molecules on the recruited protein



4 Degrading — Ubiquitin tag is recognized by cell's recycling system — the proteasome — to selectively break down the protein

IRF5 MASTER REGULATOR

Chronic, Dysregulated Activation



IRF5 Hyperactivation

Persistent Inflammation, Tissue Damage

Target Biology and Rationale

- Chronic stimulation or dysregulated endosomal TLR signaling contributes to SLE pathogenesis
- IRF5 is primarily expressed in myeloid and B cells
- Downstream of endosomal TLRs, IRF5 regulates pro-inflammatory cytokines, Type I IFN and autoantibody production in a cell and activation-specific manner

Genetically and Clinically Validated

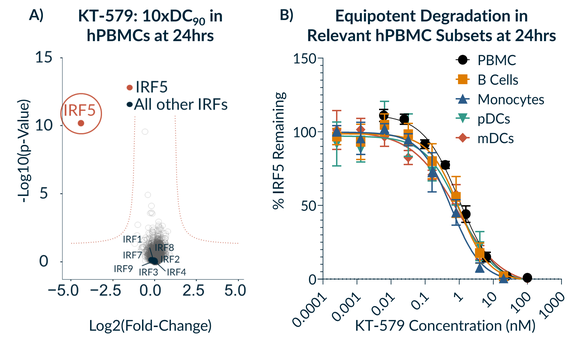
- IRF5 risk variants associate with increased susceptibility to SLE, Sjögren's, RA, SSc, IBD

Degrader Approach: KT-579

- TPD allows for a single and specific binding event to drive depletion of the protein and disrupt all IRF5 signaling

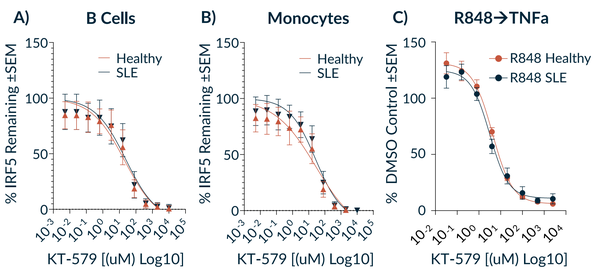
RESULTS

Figure 1. An Exquisitely Selective and Potent Oral IRF5 Degrader



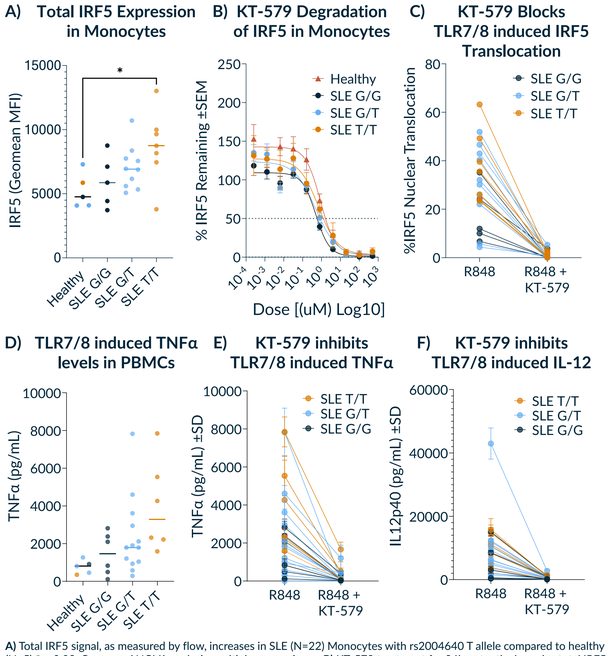
A) KT-579 selectively degrades IRF5 in the detectable proteome (>10,000 proteins). **B)** KT-579 potently degrades IRF5 in key functional cell types.

Figure 2. KT-579 Potent Activity in Human Whole Blood Assays



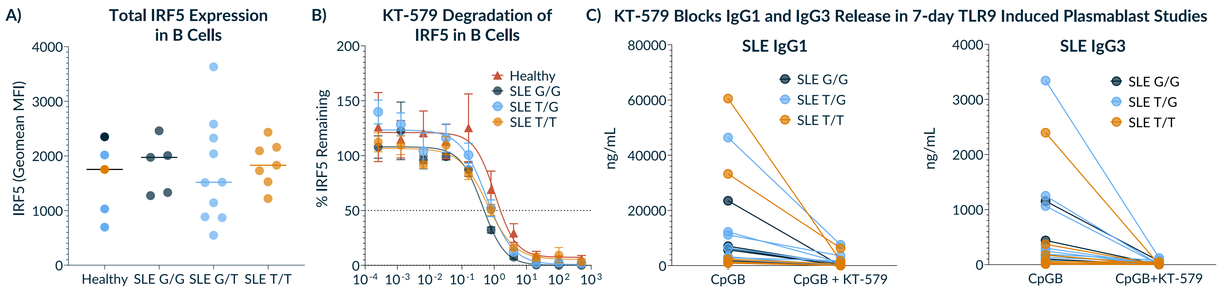
A) & **B)** Healthy or SLE Whole blood (N=6 donors, each) incubated with KT-579 for 24h induces IRF5 degradation in immune subsets. **C)** KT-579 treatment for 24h, followed by 24h of R848 (TLR7/8) stimulation leads to robust TNFa inhibition (N=10 healthy, N=15 SLE).

Figure 3. KT-579 Potent Activity in Human SLE-derived Monocytes and PBMCs Regardless of Common rs2004640 G→T Variant



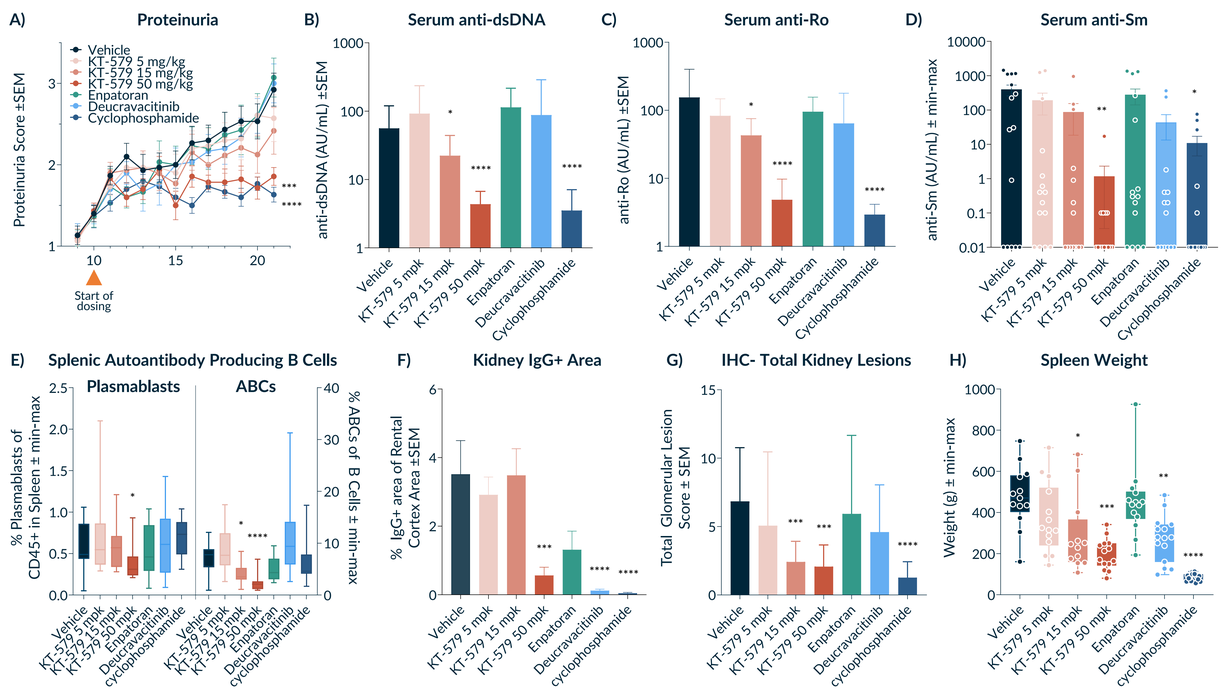
A) Total IRF5 signal, as measured by flow, increases in SLE (N=22) Monocytes with rs2004640 T allele compared to healthy (N=5) *p=0.02, One-way ANOVA analysis, multiple comparisons. **B)** KT-579 treatment for 24h potently degrades total IRF5 in both healthy and SLE monocytes across rs2004640 genotypes. **C)** KT-579 at 100nM blocks TLR7/8 induced nuclear translocation. **D)** Varying levels of TLR7/8 induced TNFα concentrations at 24h in human SLE PBMCs (N=25) are **E-F)** inhibited by KT-579 treatment in addition to blocking IL-12 release.

Figure 4. KT-579 Potently Degrades IRF5 in Human SLE-derived B Cells and Inhibits Secretion of Pathogenic IgG Subtypes in 7-day Plasmablast Studies



A) Total IRF5 levels, as measured by flow, in healthy and SLE B cells across rs2004640 genotypes (N=22). **B)** KT-579 treatment for 24h led to potent degradation in heathy and SLE B cells across all donors. **C)** SLE PBMCs were cultured with TLR9 agonist +/- KT-579 at 100nM for 7 days. On day 7, TLR9 induced secreted levels of IgG1 and IgG3 were measured and inhibited by KT-579 across donors (n=20).

Figure 5. Orally Administered KT-579 Leads to Dose-dependent Reduction of Disease Activity and Human Relevant Disease Biomarkers in the MRL.lpr Mouse Model of Lupus



In the MRPL.lupus model, KT-579 daily oral treatment led to dose-dependent reduction of **A)** proteinuria and **B-D)** serum autoantibodies better than targeted comparators tested. **E-G)** KT-579 significantly reduced splenic autoantibody producing B cells, kidney IgG deposition and kidney disease progression as measured by total kidney lesions. **H)** KT-579 reduced splenomegaly as measured by reduction in spleen weight. Statistical Methods: Proteinuria: Student's T-test at Final Measurement- ***p≤0.001, ****p≤0.0001; Serum Autoantibodies, Spleen Weight, Plasmablast and ABC, Kidney IgG+ Area and Total Kidney Lesions: Kruskal-Wallis with Uncorrected Dunn's test- *p≤0.05, **p≤0.005, ***p≤0.0005, ****p≤0.0001

METHODS

Whole blood or PBMCs from healthy or SLE donors were cultured with KT-579 for 24h in the presence or absence of TLR7/8 activation to evaluate KT-579 selectivity by global proteomics, potency to degrade IRF5 in immune subsets by flow cytometry, and functional activity via cytokine release. Donor whole blood was collected in PAXgene DNA tubes and genomic DNA extracted using the PAXgene blood DNA kit. SNP genotyping was done with Taqman qPCR for rs2004640 G/T alleles. SLE PBMC were cultured for 7-days with CpGB and KT-579 to evaluate impact on TLR9 induced plasmablast differentiation and IgG release. IgG subtyping was performed by immunoassay. In the MRL.lpr mouse lupus model, treatment for all groups (N=15) was initiated when mice reached 10 weeks of age. KT-579 (5, 15, or 50mg/kg), Deucravacitinib (30mg/kg), and Enpatoran (1mg/kg) were administered orally via gavage. Cyclophosphamide (50mg/kg) was administered i.p. and anti-IFNAR 20-25mg/kg (5A3) was administered s.c. Comparator doses were selected to cover reported efficacious exposures[2, 3]. Cyclophosphamide was used as a positive control. Proteinuria scores were recorded throughout the study to monitor disease activity. All other disease endpoints and biomarker data were generated from terminal study collection. Data are graphed as mean +/- error unless otherwise noted.

CONCLUSIONS

- KT-579 was highly selective for IRF5 and demonstrated equivalent *in vitro* cellular potency across SLE donor cells independent of baseline IRF5 levels or the presence of the common IRF5 rs2004640 T risk allele associated with increased SLE risk.
- Oral treatment with KT-579 led to dose-dependent reduction in disease severity and human disease-relevant biomarkers in the MRL.lpr mouse model of lupus, with activity superior to targeted comparators tested.
- These findings support KT-579's potential as a first-in-class oral approach for lupus and other autoimmune diseases. A Phase 1 healthy volunteer clinical trial is ongoing, with data expected in 2H26.

REFERENCES & DISCLOSURES

1. F. Y. K. Demirci, et al. Association of a common interferon regulatory factor 5 (IRF5) variant with increased risk of systemic lupus erythematosus (SLE). Annals of Human Genetics 2006. (PMID: 17166181)
2. Burke, J.R., et al. Autoimmune pathways in mice and humans are blocked by pharmacological stabilization of the TYK2 psuedokinase domain. Science Transl. Medicine 2019 (PMID: 31341059)
3. Vlach J, et al. Discovery of M5049: A Novel Selective Toll-Like Receptor 7/8 Inhibitor for Treatment of Autoimmunity. J Pharmacol Exp Ther. 2021 Mar;376(3):397-409. doi: 10.1124/jpet.120.000275. Epub 2020 Dec 16. PMID: 33328334.

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