Potential and Selective Oral STAT6 Degraders Inhibit IL-4 and IL-13 Functions in Human Cells and Block TH2 Inflammation In Vivo in a Mouse Model

#Tu1488

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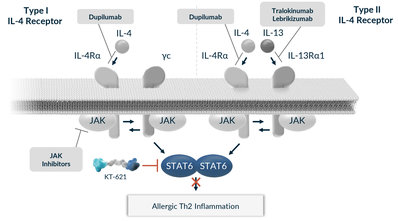
INTRODUCTION

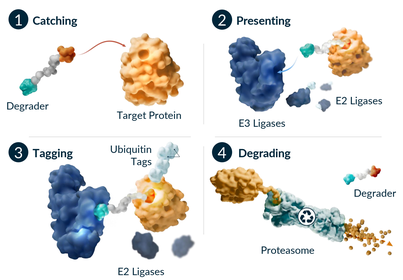
STAT6 is an undrugged essential transcription factor in the IL-4/IL-13 signaling pathways and the central driver of TH2 inflammation in allergic diseases. Multiple gain of function mutations of STAT6 were identified to cause severe allergic diseases in human. Dupilumab, an injectable monoclonal antibody that blocks IL-4/IL-13 signaling, is an approved therapy for multiple allergic diseases. STAT6 targeting is therefore supported by both human genetics and dupilumab's clinical pathway validation. STAT6 functions through protein-protein and protein-DNA interactions. For these reasons, it has been challenging to selectively and potently inhibit STAT6 with traditional small molecule inhibitors. It is, however, well suited for a targeted protein degradation approach, where a simple binding event is sufficient to drive degradation.

STAT6 Biology and Target Rationale



- STAT6 is the specific transcription factor required for IL-4 and IL-13 cytokine signaling.
- STAT6 regulated cytokines are clinically validated targets for allergic diseases.
- Gain of function (GOF) mutations of STAT6 cause severe allergic diseases in human.
- STAT6 KO mice develop normally, are viable and fertile.
- Dupilumab, an IL-4Rα monoclonal Ab has been approved in: Atopic dermatitis, Asthma, CRSwNP, Eosinophilic Esophagitis, Prurigo Nodularis, has positive Phase 3 data in COPD and is in development for multiple additional indications.
- STAT6 degradation can achieve dupilumab–like pathway inhibition.

Proteome Editing with Targeted Protein Degradation



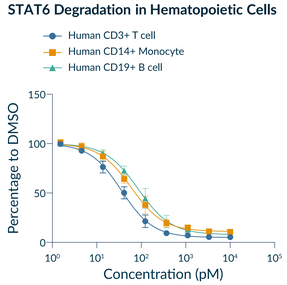
RESULTS

Figure 1. KT-621: A Highly Selective Picomolar Degrader of STAT6

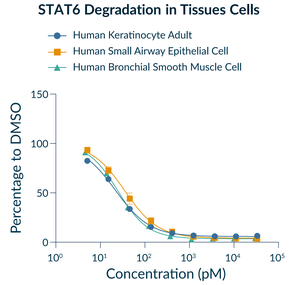
A

	Human Primary Cell Type	KT-621, DC_{50} (pM)
	Hematopoietic cell, all TH2 disease	
Blood	Human PBMC	13
Blood	Human CD3 T cell	36
Blood	Human CD14 monocyte	60
Blood	Human CD19 B cell	86
Blood	Human eosinophil	99
	Epithelial cell, AD, CPG, CU, asthma, COPD	
Skin	Human keratinocyte (adult)	22
Skin	Human keratinocyte (neonatal)	18
Lungs	Human bronchial tracheal epithelial cell	33
Lungs	Human small airway epithelial cells	35
	Smooth muscle cell, asthma, COPD, EoE	
Throat/Airway	Human bronchial smooth muscle cell	25
Throat/Airway	Human esophageal smooth muscle cell	33
	Endothelial cell, all TH2 disease	
Blood Vessels	Human vascular endothelial cell	46

STAT6 Degradation in Hematopoietic Cells

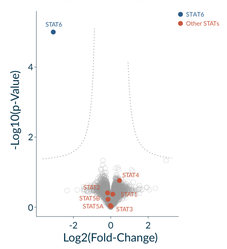


STAT6 Degradation in Tissues Cells



B

KT-621 Degradation Selectivity
Human PBMC at 100 x DC90



KT-621 Function Selectivity

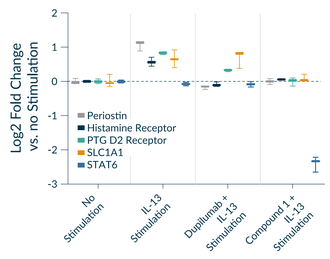
STAT Assays	KT-621, DC_{50} (pM)
IFN-α induced pSTAT1	>1000
IFN-α induced pSTAT2	>1000
IL-10 induced pSTAT3	>1000
IL-12 induced pSTAT4	>1000
IL-2 induced pSTAT5	>1000
IL-3 induced pSTAT6	0.042

A) Consistent degradation by KT-621 across all disease relevant cell types evaluated. **B**) Complete STAT6 degradation and function selectivity of KT-621 against all other STATs.

Figure 2: KT-621 Fully Blocks IL-4/13 Pathways, More Potently than Dupilumab

A

Rescue of IL-13 Effects in Esophageal Smooth Muscle Cells by STAT6 Degrader or Dupilumab

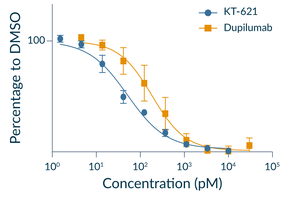


B

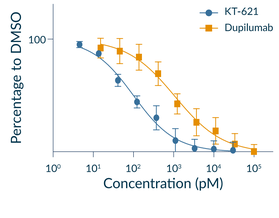
		Cellular Functional Assay	KT-621 IC_{50} (pM)	Dupilumab IC_{50} (pM)
TARC	Serum Th2 biomarker, chemoattractant for Th2 cell	IL-4 TARC release in human PBMC	62	194
TARC	Serum Th2 biomarker, chemoattractant for Th2 cell	IL-13 TARC release in human PBMC	43	113
CD23	B cell activation marker, correlates with IgE class switch	IL-4 CD23 expression in human CD19 B cell	125	354
CD23	B cell activation marker, correlates with IgE class switch	IL-13 CD23 expression in human CD19 B cell	98	1070
PERIOSTIN	Serum Th2 biomarker and ECM protein associated with tissue remodeling in atopic diseases	IL-13 Periostin release in human bronchial smooth muscle cell	24	637
PERIOSTIN	Serum Th2 biomarker and ECM protein associated with tissue remodeling in atopic diseases	IL-13 Periostin release in human esophageal smooth muscle cell	39	431

C

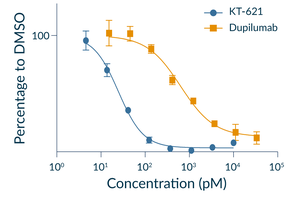
IL-4 Induced TARC Release in Human PBMC



IL-13 Induced CD23 Expression in Human CD19 B Cell



IL-13 Induced Periostin Release in Human Bronchial Smooth Muscle Cell

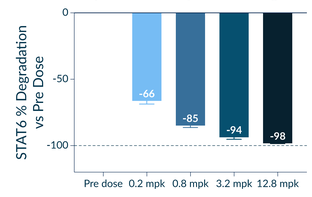


A) STAT6 tool degrader Compound 1 (100 nM) fully blocks IL-13 induced gene expression in esophageal smooth muscle cells vs partial inhibition by dupilumab (300 nM). **B**, **C**) KT-621 fully blocks key IL-4/13 functional readouts in human cells with lower IC_{50}s vs. dupilumab.

Figure 3: KT-621 Potently Degrades STAT6 Across Multiple Preclinical Species with Low Oral Doses

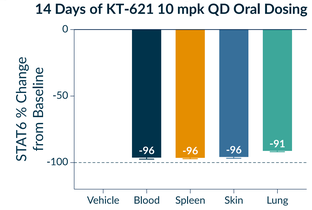
A

STAT6 Degradation Dog Blood Post 7 Days of KT-621 QD Oral Dosing



B

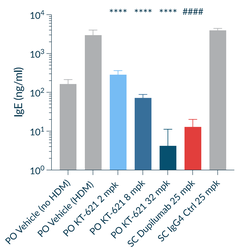
STAT6 Degradation Dog NHP Tissues Post 14 Days of KT-621 10 mpk QD Oral Dosing



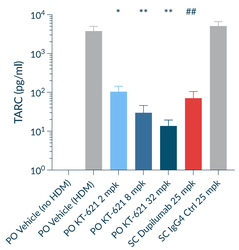
A) KT-621 achieves dose dependent deep degradation in dogs with low oral doses. STAT6 levels determined at 24 hours post the Day 7 dosing in PBMC isolated from the whole blood. **B**) KT-621 degrades STAT6 in disease relevant tissues in NHP. STAT6 levels determined at 24 hours post the Day 14 dosing in PBMC isolated from the whole blood or the indicated tissues.

Figure 4: KT-621 Has Comparable *in vivo* Efficacy to IL-4Rα Saturating Dose of Dupilumab in the Intranasal HDM Asthma Model

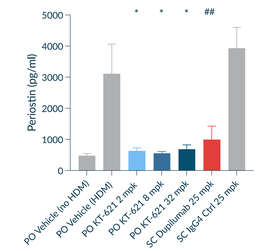
Total Serum IgE



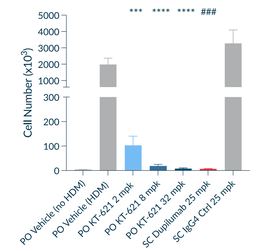
TARC in Bronchoalveolar Lavage



Periostin in Bronchoalveolar Lavage



Eosinophil in Bronchoalveolar Lavage



A lung inflammation model induced by intranasal house dust mite administration with dominant Th2 inflammation in the IL4/IL4RA humanized mice (Le Floc'h et al. Allergy. 2020). KT-621 dosed QD orally for 31 days. 2/8/32 mpk doses showed 72/85/91% STAT6 degradation respectively in mouse spleen. Dupilumab dosed 9 times subcutaneously, 25 mpk BIW (IL-4Rα saturating dose), effect equivalent to 300 mg every other week in human.

METHODS

We developed highly potent and selective oral STAT6 degraders and assessed their functions in disease-relevant human primary immune and tissue cells in vitro. We also compared the efficacy of our STAT6 degraders to dupilumab in vivo in an intranasal house dust mite (HDM) induced lung inflammation model in a hIL4/hIL4RA humanized mice.

Our STAT6 degraders potently and selectively degraded and depleted STAT6 in various disease relevant human primary cells including lymphocytes, myeloid cells, lung epithelial cells, bronchial smooth muscle cells, and vascular endothelial cells. As a result of STAT6 degradation, our degraders fully blocked various IL-4/IL-13 functions in these cells with picomolar potencies comparable or superior to dupilumab and did not degrade or inhibit any other STAT transcription factors. Specifically in esophageal smooth muscle cells, global proteomics demonstrated that our STAT6 degraders fully inhibited IL-13 induced expression of effector genes including periostin (POSTN), histamine H1 receptor (HRH1), and prostaglandin D2 receptor 2 (PTGDR2) that contribute to enhanced contractile response of esophageal muscle cells. Our STAT6 degraders showed potent STAT6 degradation and IL-4/IL-13 functional inhibition in human whole blood, were orally bioavailable in multiple preclinical species, and were able to deplete STAT6 in vivo. In an HDM induced asthma mouse model, orally administered STAT6 degrader KT-621 demonstrated robust degradation and reduced TH2 inflammation comparable to an IL-4Rα saturating dose of dupilumab.

CONCLUSIONS

STAT6 degradation is a potential novel oral approach for blocking the IL-4/IL-13 signaling pathways.

STAT6 degrader KT-621 has best-in-pathway potential for allergic diseases with a dupilumab-like activity profile and oral bioavailability

- KT-621 is a pM STAT6 degrader with exquisite selectivity developed with the targeted protein degradation platform at Kymera.
- STAT6 degradation by KT-621 fully blocks IL-4/13 in key human TH2 cellular assays with picomolar IC_{50}s lower than dupilumab.
- STAT6 degradation fully blocks IL-13 induced gene transcription in human primary esophageal smooth muscle cells, a key cell type in the pathophysiology of eosinophilic esophagitis.
- KT-621 is orally bioavailable and can fully degrade STAT6 in vivo with low oral doses.
- KT-621 is well tolerated in multiple preclinical species.
- STAT6 degradation by KT-621 robustly inhibits TH2 inflammation in vivo in the HDM asthma model comparable to the IL-4Rα monoclonal antibody dupilumab.

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DISCLOSURES

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