Highly selective and reversible STAT6 inhibition demonstrates potential for differentiated efficacy and safety profile in type 2 allergic inflammation

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Background

Biologics targeting the cytokines IL-4 and IL-13 have achieved clinically meaningful efficacy in type 2 (allergic) inflammatory and pruritic diseases. Binding of IL-4 or IL-13 to their cognate receptors triggers the intracellular JAK-STAT signaling cascade to propagate the inflammatory response. However, the clinical utility of small molecule JAK inhibitors is confounded by their on-target safety signals, including dysregulated hematologic homeostasis (e.g., anemia, thrombocytopenia). Selective STAT6 inhibition represents a potential opportunity to achieve biologic-like efficacy, while avoiding broad JAK-mediated immune suppression.

Src Homology 2 (SH2) domains are highly conserved protein domains. The STAT6 SH2 domain exclusively mediates binding to the IL-4/IL-13 cytokine receptors and triggers downstream transcriptional activity **(Figure 1)**, thereby representing an attractive therapeutic target.

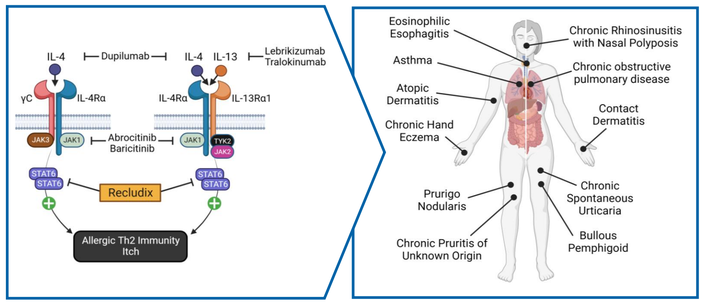


Figure 1. IL-4/IL-13 activity is dependent on downstream STAT6 intracellular signaling. Clinical trials targeting the IL-4/IL-13 pathway have reported significant efficacy in multiple human autoimmune and inflammatory diseases.

Methods & Technology

The Recludix Pharma SH2 domain discovery platform integrates custom and proprietary DNA-encoded libraries (DEL), structure-based design enabled by rapid STAT SH2 domain crystallography, and a biochemical assay panel to assess selectivity and potency **(Figure 2)**.

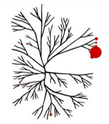
Figure 2. Integrated SH2 domain drug discovery and chemical optimization platform

Proprietary DEL libraries identify novel hits which feed into crystallography for rapid hit optimization using structure-based drug design, *in silico* optimization, and a suite of biochemical and biophysical assays to monitor potency and physicochemical properties. Discovery of potent and selective STAT6 inhibitors exemplifies the value of this integrated approach to target previously undruggable targets.

Data Results

Recludix has identified highly potent STAT6 SH2 domain inhibitors, exemplified here by STAT6i that demonstrates excellent selectivity across a panel covering ~75% of human SH2 domains; no hits outside of the STAT family with affinity <10 μM were observed **(Table 1)**.

Table 1. Discovery of novel, potent, and selective STAT6 inhibitors

	STAT6i
Biochemical Potency (STAT6 K_D)	0.025 nM
Cellular Potency (pSTAT6 IC_{50} in human PBMCs)	0.88 nM
Cellular Selectivity (PBMCs)	>1,000X vs. STAT1/2/3/4/5
Cellular Cytotoxicity (CTG IC_{50} in Jurkat cells)	>10,000 nM
SH2 Family Selectivity	

Primary human PBMCs were stimulated with cytokines resulting in specific phosphoSTAT (pSTAT) isoform activation. STAT6i exhibits highly selective, sub-nanomolar potency for inhibiting STAT6 activation driven by IL-4 **(Table 1)**.

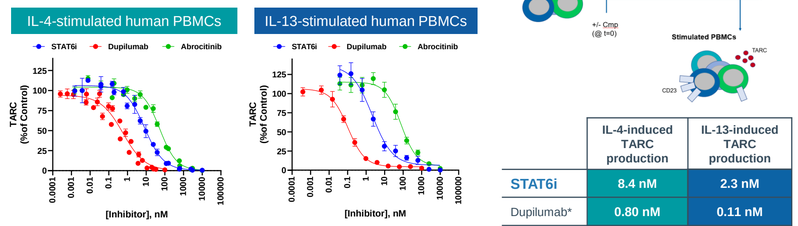
STAT6i phenocopies dupilumab and differentiates from JAK inhibitors by selectively blocking T helper cell differentiation into the type 2 (Th2) subtype without impacting hematologic homeostasis **(Table 2.** STAT6i also inhibits other downstream products of STAT6 transcriptional activity as demonstrated by the disruption of TARC production, a known biomarker for type 2 inflammatory diseases, following stimulation of human PBMCs with IL-4 or IL-13 **(Figure 3)**.

Table 2. STAT6 inhibition phenocopies dupilumab and differentiates from JAK inhibitors in functional T helper differentiation and hematologic homeostasis assays. Average IC_{50} values are summarized for each assay.

		T cell function				Hematologic homeostasis	
		General Adaptive Immune response	Defense against viruses and bacteria	Defense against extracellular pathogens	Defense against parasites and mediation of antibody responses	Erythropoiesis	Thrombopoiesis
		T Cell Activation (CD25)	Th1 Cell Function (IFNγ)	Th17 Cell Function (IL-17A)	Th2 Cell Function (IL-5)	EPO-Induced STAT5-Driven Transcription	TPO-Induced STAT5-Driven Transcription
STAT6 Inhibitors	**STAT6i**	**>10,000 nM**	**>8,900 nM**	**>10,000 nM**	**20 nM**	**>10,000 nM**	**>10,000 nM**
IL-4/IL-13 Antagonist	Dupilumab*	>10,000 nM	>1,000 nM	>1,000 nM	**21 nM**	>1,000 nM	>1,000 nM
JAK Inhibitors	Abrocitinib	1,300 nM	900 nM	81 nM	**81 nM**	3,200 nM	2,800 nM
	Upadacitinib	39 nM	35 nM	8.0 nM	**4.5 nM**	69 nM	20 nM
	Baricitinib	110 nM	210 nM	15 nM	**15 nM**	56 nM	42 nM

Selectivity relative to Th2 inhibition: >30X | 10 - 30X | <10X

*Recludix Pharma data on file, April 2022



	IL-4-induced TARC production	IL-13-induced TARC production
STAT6i	**8.4 nM**	**2.3 nM**
Dupilumab*	**0.80 nM**	**0.11 nM**
Abrocitinib	**51 nM**	**100 nM**

*Recludix Pharma data on file, April 2022

Figure 3. STAT6i blocks cytokine stimulated production of type 2 relevant TARC biomarker. Average IC_{50} values are summarized in the table for each assay.

Following single or multiple intraperitoneal (i.p.) doses in mice, STAT6i demonstrates dose-dependent and durable target modulation of IL-4-induced pSTAT6 signaling in PBMCs. This pharmacodynamic response is achieved without degradation of STAT6 protein **(Figure 4)**.

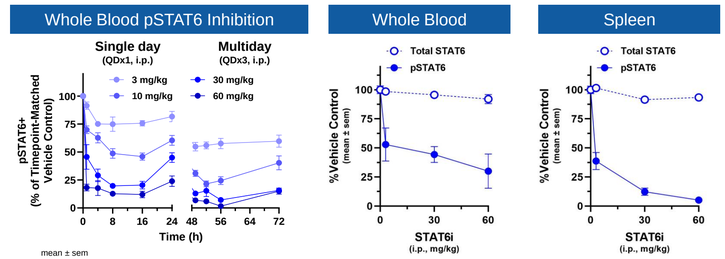


Figure 4. Recludix STAT6i achieves dose-dependent and durable target modulation *in vivo* without requiring degradation of STAT6 protein.

This durable *in vivo* target modulation by STAT6i translates to significant dose-dependent reduction of airway inflammation that is on par with anti-IL-4/IL-13 treatment in a mouse ovalbumin (OVA) model of asthma in both prophylactic and therapeutic dosing paradigms **(Figure 5)**.

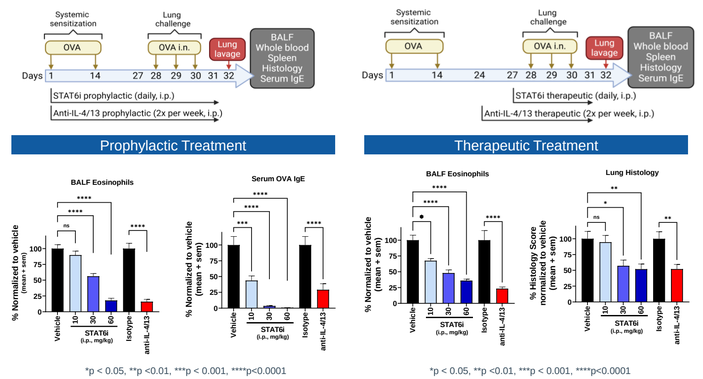


Figure 5. STAT6i reduces lung inflammation in a mouse ovalbumin (OVA) asthma model.

Conclusions

IL-4 and IL-13 require downstream STAT6 signaling to mediate type 2 (allergic) inflammation.

Recludix Pharma has generated potent, selective, and reversible small molecule STAT6 inhibitors.

Selective STAT6 targeting has the potential to combine the efficacy of biologics with the convenience of oral administration, while avoiding known safety concerns observed with JAK inhibitors.

STAT6 inhibition may represent a compelling future therapeutic opportunity for the treatment of asthma, COPD, prurigo nodularis, and other IL-4/IL-13-dependent diseases.

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