STAT6: A Transcriptional Driver in Type 2 Immunity and a Rising Therapeutic Target

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Abstract

Signal Transducer and Activator of Transcription 6 (STAT6) is a pivotal transcription factor activated downstream of the interleukin-4 (IL-4) and interleukin-13 (IL-13) signaling pathways. It governs the expression of key genes involved in type 2 immune responses, including Th2 cell differentiation, IgE class switching, and M2 macrophage polarization. Dysregulated STAT6 activity has been implicated in a range of diseases, from asthma and atopic dermatitis to fibrosis and immune-evasive tumors.

With the clinical advancement of STAT6-targeting drugs like Kymera's KT-621 and Recludix's REX-8756, the demand for precise STAT6 binding profiling is rising—supporting target validation, mechanism studies, and therapeutic development.

Representative STAT6 Inhibitors:

STAT6-IN-3: A small-molecule inhibitor blocking STAT6 phosphorylation or DNA binding for Th2-driven diseases.

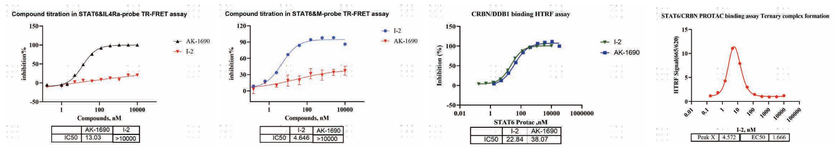
AK-1690: A highly selective STAT6 PROTAC degrader with a DC50 of 1 nM, developed by the University of Michigan/Shaomeng Wang group.

KT-621: An oral STAT6 PROTAC degrader with picomolar potency against IL-4/IL-13 signaling, developed by Kymera Therapeutics for atopic dermatitis and asthma.

I-2: The positive control in this article is molecule 2 from the Kymera Therapeutics patent (WO2025049820).

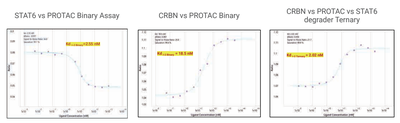
1 Binary/Ternary binder screening and orthogonal validation

A HTRF binding assay

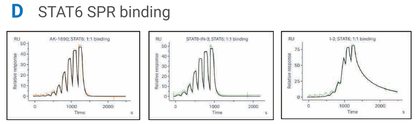


B Binary/Ternary complex formation

C Spectrum Shift (SpS) Assay



D STAT6 SPR binding

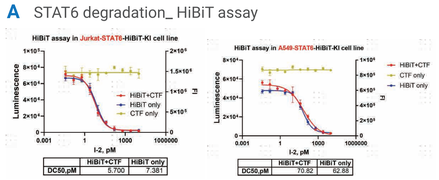


Immobilized ligand	Analyte	1:1 binding		kd (1/s)	KD (M)
		ka (1/Ms)			
STAT6	AK-1690	6.43E+05		2.53E-02	3.94E-08
STAT6	STAT6-IN-3	3.76e+06		1.30e-01	3.47e-08
STAT6	I-2	2.87E+06		2.56E-02	8.90E-09

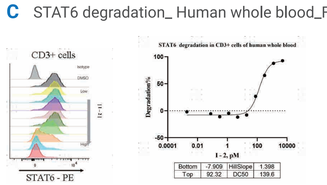
Figure 1. Assay portfolio for STAT6 binder screening and ternary complex profiling. (A) HTRF assay for STAT6 binding screen. The IL-4Ra-probe can screen for small molecules and PROTACs that target the IL-4Ra binding site, such as STAT6-INI3 and AK1690. The M-probe can screen for small molecules and PROTACs that bind to the allosteric site, such as the KT-621 series.(B) HTRF ternary complex profiling for STAT6 PROTAC (hook effect found). (C) Spectrum Shift (SpS) assay for cost-effective HTS of STAT6 binary and ternary binding. (G) SPR as the gold standard for STAT6 binder screening.

2 Assessment of PROTAC-Mediated STAT6 Degradation

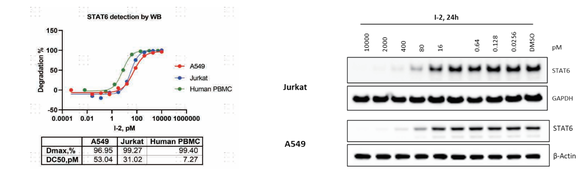
A STAT6 degradation_ HiBiT assay



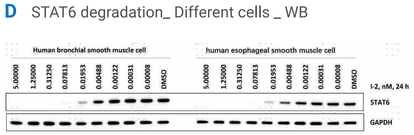
C STAT6 degradation_ Human whole blood_FACS



B STAT6 degradation_ Different cells _ WB



D STAT6 degradation_ Different cells _ WB



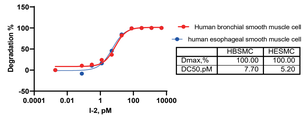
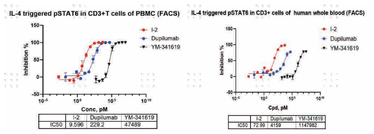


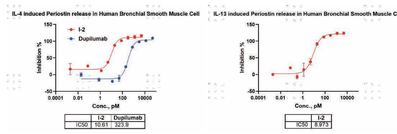
Figure 2. PROTAC-mediated STAT6 degradation assessed by HiBiT, WB, and FACS assays. (A) HiBiT-based STAT6 degradation, with degradation and viability measured in a single assay. (B) STAT6 Degradation evaluated by Western blot. (C) FACS-based STAT6 degradation in human T cells (whole blood sample). (D) Western blot analysis in human primary smooth muscle cells.

3 Functional Readouts in Primary Human Cells

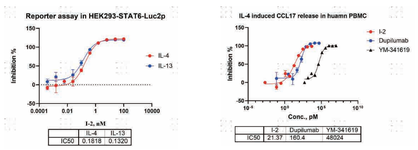
A STAT6 phosphorylation



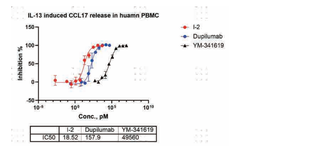
C Human Bronchial Smooth Muscle Cell _ Periostin



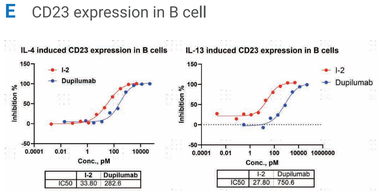
B Reporter assay



D CCL17 release in huamn PBMC



E CD23 expression in B cell

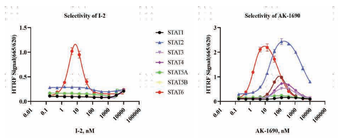


I-2 data summary		I-2	Dupilumab
Reporter	IL-4 induced STAT6 reporter	181.8 pM	103.8 pM
	IL-13 induced STAT6 reporter	132.0 pM	224.3 pM
TCR pathway	IL-4 induced CCL17 release	21.37pM	160.4 pM
	IL-13 induced CCL17 release	18.52 pM	157.9 pM
BCR pathway	IL-4 induced CD23 expression	33.80 pM	282.6 pM
	IL-13 induced CD23 expression	27.80	750.6 pM

Figure 3 . Functional assays in primary human cells. (A) FACS-based analysis of STAT6 phosphorylation following stimulation in T cells. (B) Luciferase reporter assay measuring STAT6 transcriptional activity. (C) Periostin release from human bronchial smooth muscle cells (HBSMC) upon stimulation. (D) TARC secretion from PBMCs upon stimulation. (E) CD23 expression in B cells under different stimulation conditions.

4 Profiling STAT Family Selectivity and Cross-Species Specificity

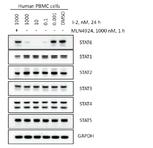
A STAT6 vs protac vs CRBN TR-FRET Ternary complex assay



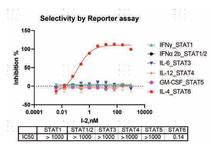
B SPR binary binding assay

	SPR assay_KD(nM)	
Target	I-2	AK-1690
STAT1	/	11400
STAT2	/	1820
STAT3	/	2920
STAT4	/	2360
STAT5A	/	6020
STAT5B	/	186
STAT6	7.97	33.7

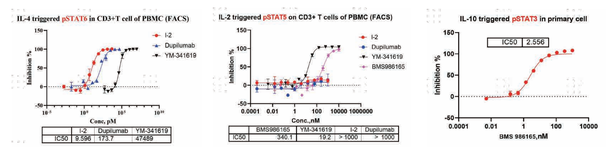
C WB analysis of STAT family selectivity



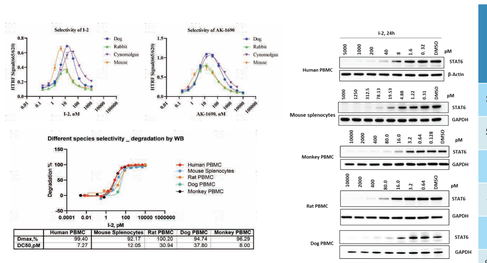
D Reporter assay



E STAT6 phosphorylation



E Different species specificity

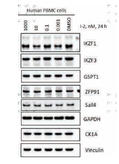


Immobilized ligand	TR-FRET Ternary complex assay EC50 (nM)		SPR binary binding assay KD (M)		Degradation DC50(pM)
	AK-1690	I-2	STAT6-IN-3	I-2	I-2
STAT6(Human)	1.52	2.19	3.47e-08	7.97e-09	7.27
STAT6(Mouse)	0.98	1.85	2.73e-08	5.88e-09	12.05
STAT6(Rat)	NA	NA	NA	NA	30.94
STAT6(Rabbit)	2.1	2.37	3.74e-08	2.08e-08	NA
STAT6(Dog)	3.15	3.82	2.98e-08	8.93e-09	37.80
STAT6(Monkey)	3.6	4.65	2.64e-08	9.19e-09	8.00

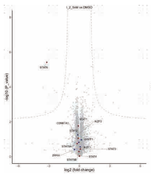
Figure 4. STAT family selectivity and cross-species specificity profiling. (A) HTRF evaluation of I-2–mediated STAT/CRBN ternary complex formation. (B) SPR analysis of STAT family selectivity. (C) Western blot–based assessment of STAT degradation selectivity. (D) Reporter assays across different STAT family members. (E) STAT phosphorylation measured by FACS, IFN-α_pSTAT1/2 and IL-12_pSTAT4 assays were ongoing. (F) Cross-species specificity evaluated by HTRF ternary complex formation, SPR binding, and degradation assays using several known STAT6 inhibitors/PROTACs.

5 Off-Target Profiling: Western, Proteomics, ICESTP Safety Panel & Hibit

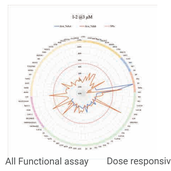
A Identification of off-target CRBN substrates by WB



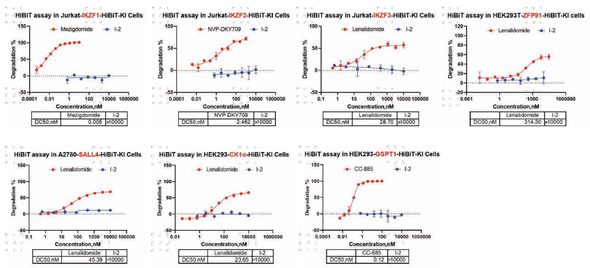
B DIA-based proteomics profiling



C ICESTP SAFETYPANEL® 77



D Identification of off-target CRBN substrates by Hibit



CRBN off-target	Status
HEK293-GSPT1-HiBiT-KI cell line	ready
Jurkat-IKZF1-HiBiT-KI cell line	ready
Jurkat-IKZF2-HiBiT-KI cell line	ready
Jurkat-IKZF3-HiBiT-KI cell line	ready
HEK293-CK1a-HiBiT-KI cell line	ready
A2780-SALL4-HiBiT-KI cell line	ready
HEK293-ZFP91-HiBiT-KI cell line	ready

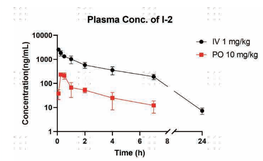
Figure 5. Off-target profiling of STAT6 degraders (A) I-2 did not show off-target degradation of common CRBN substrates. (B) DIA-based proteomics profiling of I-2 off-targets. (C) ICE-STP safety panel revealed off-target effects of I-2 on LCK, A2a, PR, RARα and NR1/NR2B.

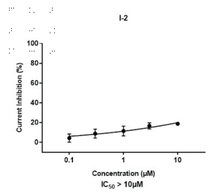
6 Developability: ADME, PK & hERG

A PK parameters

PK parameters	IV 1mg/kg	PO 10mg/kg
t1/2(h)	3.58±0.104	2.40±0.429
Tmax(h)	0.0833±0.00	0.333±0.144
C0/Cmax(ng/mL)	3030±145	249±39.2
AUClast(h*ng/mL)	5838±1395	344±126
AUCInf(h*ng/mL)	5876±1404	389±157
AUC_%Extrap_obs(%)	0.645±0.104	10.8±3.31
MRTInf_obs(h)	3.81±0.247	2.75±0.563
AUClast/D(h*kg*ng/mL/mg)	5838±1395	34.4±12.6
Vd(L/kg)	0.920±0.263	/
CL_obs(mL/min/kg)	2.96±0.760	/
F(%)	/	0.662±0.268

B Cardio-one®---hERG study





C In vitro ADME assay

Parameters		ICE data
Kinetic solubility (FaSSGF/FaSSIF/ FeSSIF，PBS pH= 7.4)	Solubility (μM)	FaSSGF(pH=1.2): 31.99 μM FaSSIF(pH=6.5): 19.64 μM FeSSIF(pH=5.0): 161.29 μM PBS(pH= 7.4): 0.58 μM
LogD	1-Octanol/PBS pH 7.4	3.98
LM Stability (Human/Mouse)	t1/2 (min)	Human:16.55; Rat:40.02; Mouse: 78.82 Dog: 25.24; Monkey: 2.72
	CLint (μL/min/mg protein)	Human:83.76; Rat: 34.63; Mouse: 17.58; Dog: 54.92 Monkey: 509.76
Hepatocyte Stability (Human/Mouse)	t1/2 (min)	Human: 119.64; Mouse: 64.42
	CLint (μL/min/106 cells)	Human: 11.58; Mouse: 21.51
Plasma Protein Binding (human)	fu%	fu%：7.91%（Human）
Plasma Stability(~120min) (Human/Rat/Mouse/Dog/Monkey)	t1/2 (min)	Human > 372.67 min; Rat: 215.24 min Mouse: 239.90 min; Dog: 209.96 min Monkey: 351.64 min
CYP Inhibition (1A2/2C9/2C19/2D6/3A4M/3A4T)	@5uM Inhibition%	9.19%/18.86% /14.15%/8.25%/ 38.82%/42.06%
Caco-2 permeability	Papp/ER(1E-6 cm/s)	Papp A-B: 5.42; Papp B-A: 11.96 ER: 2.21

Figure 6. Developability Profiling of STAT6 Degraders. (A) Pharmacokinetic profile of the STAT6 degrader following IV and PO administration. (B) hERG current measured by manual patch clamp. I-2 showed no significant inhibition of hERG current. (C) In vitro ADME properties of the STAT6 Degraders.

Summary

ICE Biosciences' discovery platform integrates HTS-compatible screening, orthogonal biochemical and cellular assays, and mechanistic studies. Supported by a broad suite of established assays and customizable modules, it generates high-quality activity and safety data to advance drug discovery pipelines. Incorporating human primary cell assays, PK/PD, and safety profiling ensures translational relevance, accelerating the development of next-generation STAT6 therapeutics with clinically meaningful insights.