STAT3 degraders inhibit Th17 development and cytokine production resulting in profound inhibition of collagen-induced autoimmune murine arthritis

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KYMERA

INVENTING NEW MEDICINES

WITH TARGETED PROTEIN DEGRADATION

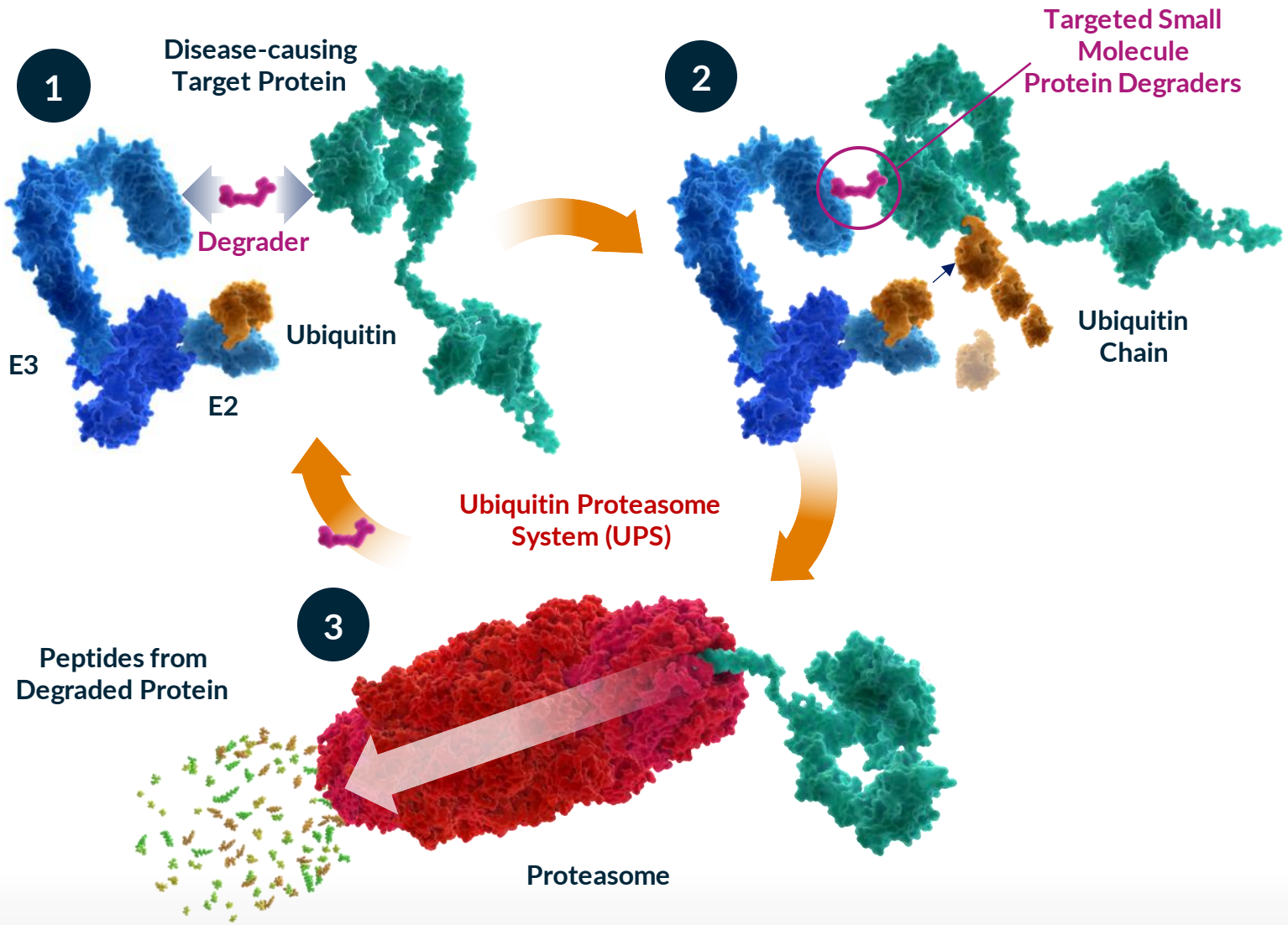
DISCLOSURES

Jeffrey Sullivan, Crystal Brown, Michele Mayo, Vaishali Dixit, Bradley Enerson, Haojing Rong, Bin Yang, Chris De Savi, Jared Gollob, Nello Mainolfi, Anthony Slavin are Kymera Therapeutics employees and equity owners.

Cedric Hubeau is a former Kymera Therapeutics employee.

Proteome Editing with Targeted Protein Degradation

A Nobel Prize (2004) Inspired Technology

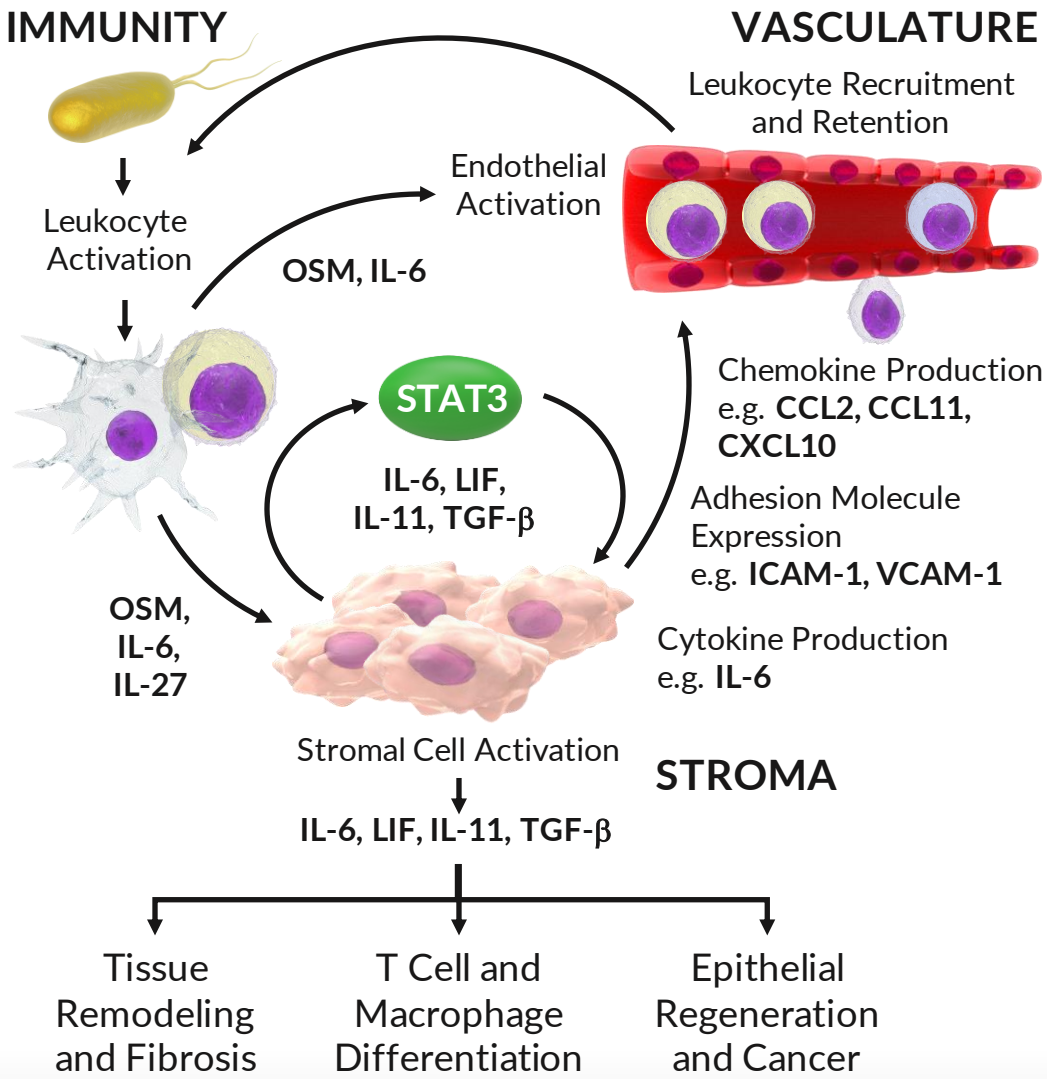


Expanded Opportunities

- Small molecule binds to E3 and target protein to affect its degradation
- Small Molecule only needs to "weakly" bind to protein:
  Not inhibit function
- Highly potent/catalytic:
  Small amount of drug needed
- Highly specific
- Genetic–like knock-down effects
- Advantage of small molecule development:
  Route of administration, manufacturing
- Agnostic to protein type and disease

Overview of STAT3 Biology

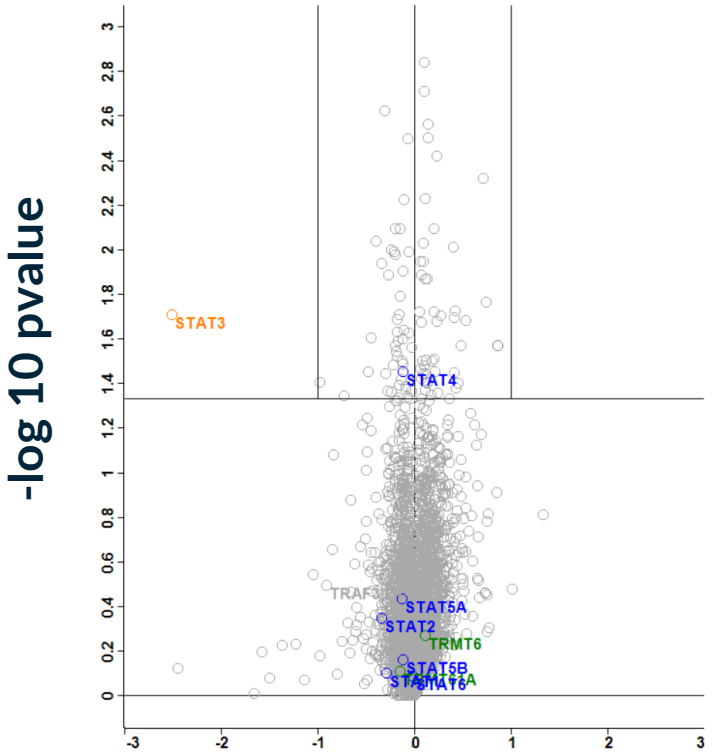
- STAT3 is an undruggable transcription factor
- STAT3 is activated by multiple tyrosine kinases and plays a critical role in the signaling of cytokines, hormones, and growth factors including **IL-6**, **IL-11**, **OSM**, **TGF-β**, **VEGF**
- STAT3 gain-of-function (GoF) mutations lead to a poly-autoimmunity reminiscent of conditions such as Systemic Sclerosis (SSc) and interstitial lung disease (ILD)
  - JAK inhibitors have shown activity in patients with STAT3 GoF mutations and multiple different autoimmune manifestation
- STAT3 signaling is required for Th17 differentiation in vitro and in vivo
- Increased STAT3 activation is associated with disease severity in chronic inflammation, including **SSc**, **RA**, **AS**, **MS**, **IBD**, **PsO**
- STAT3 activation is also implicated in conditions defined by intense stromal remodeling in the absence of overt inflammation, e.g. **IPF**, **PAH**, **NAFLD**, and **Diabetic Kidney Disease**



Adapted from West NT. *Front Immunol* 2019

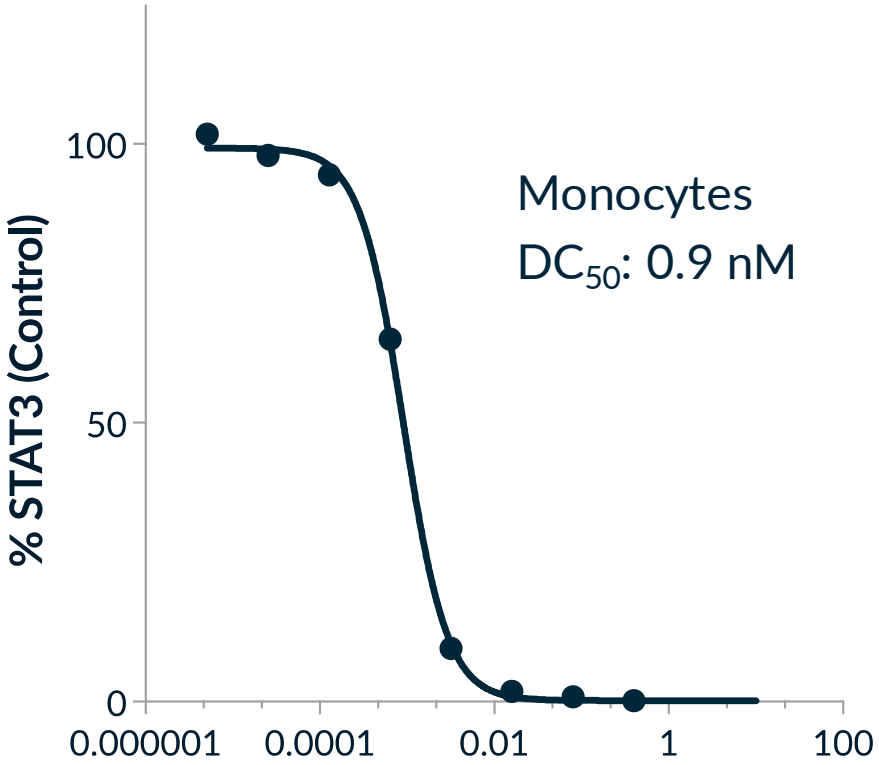
KTX-115 Selectively and Potently Degrades STAT3 in Human PBMC and Whole Blood

KTX-115 Selectively Degrades STAT3 in huPBMC (24 h Treatment)



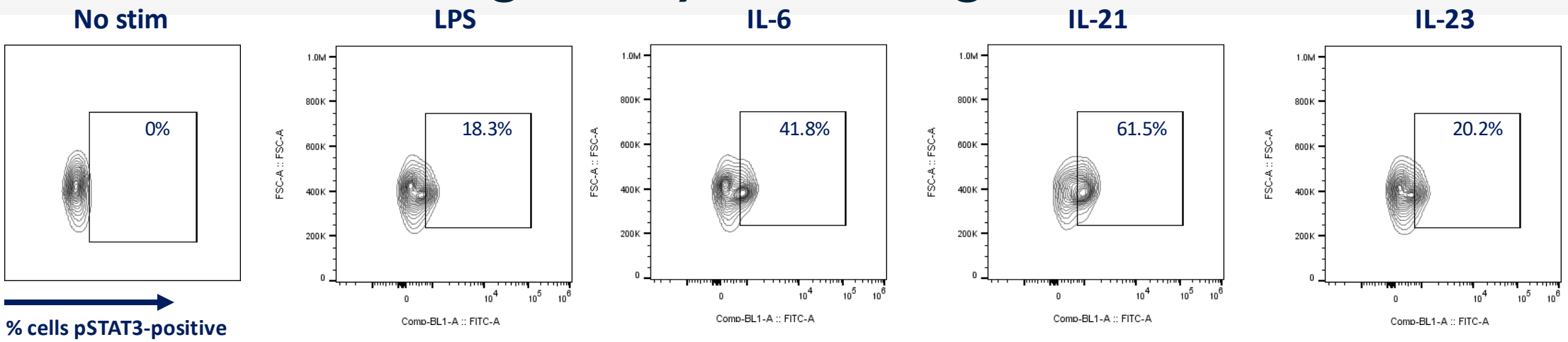
KTX-115 25 nM (~DC_{95}) / DMSO Average

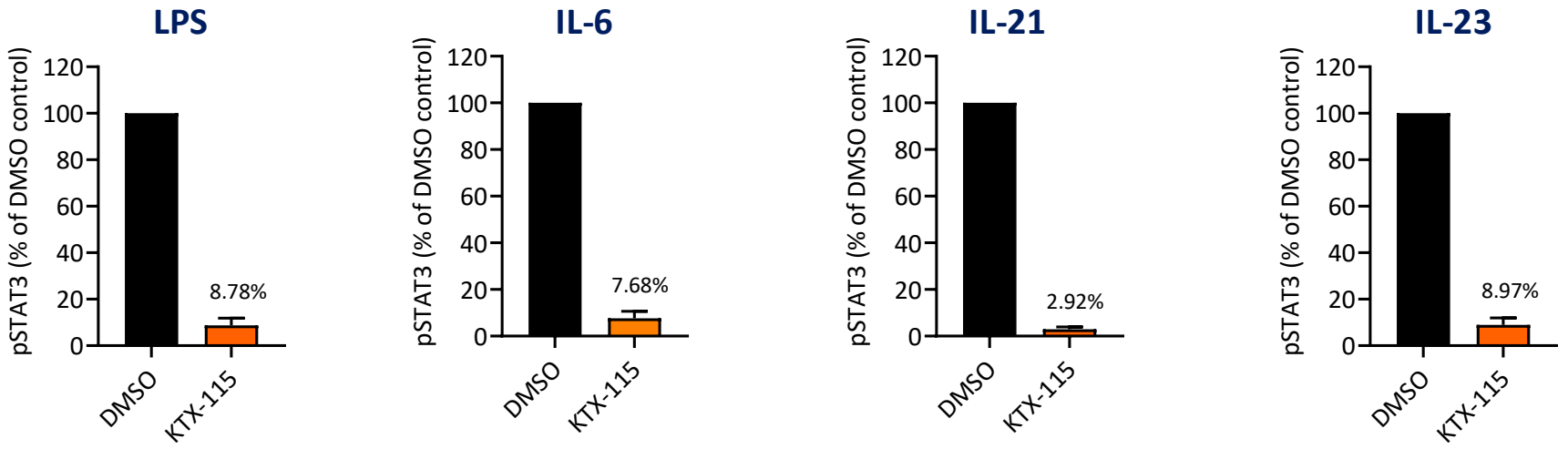
KTX-115 Potently Degrades STAT3 Human Whole Blood (24 h Treatment)



KTX-115 Concentration (FACS)

PBMC STAT3 Phosphorylation via Multiple Inflammatory Stimuli is Abrogated by STAT3 degradation





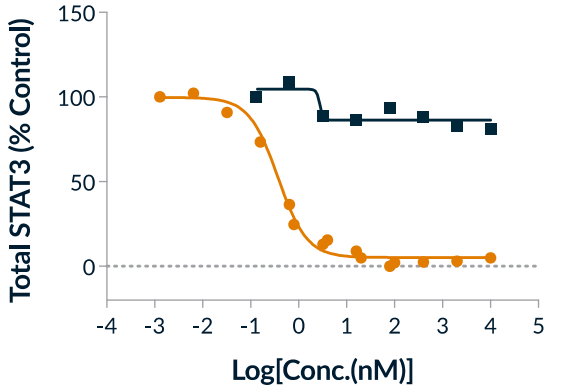
- huPBMC were treated overnight with KTX-115 before stimulation for 30 min. with various stimuli
- Percentages of pSTAT3 positive cells as well as pSTAT3 inhibition (IC_{50}) were estimated for each stimuli

Stimulus	No stim	LPS	IL-6	IL-21	IL-23
pSTAT3 IC_{50}, nM [Flow]	-	6.91	11.27	4.37	9.52
STAT3 DC_{50}, nM [Flow]	n/a	19.79	29.26	16.62	23.02

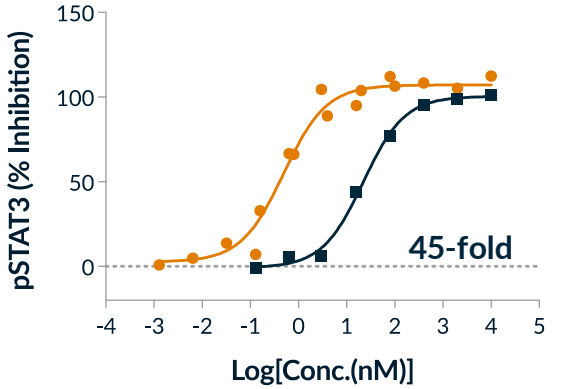
STAT3 Degradation Abrogates STAT3 Phosphorylation and MCP-1/CCL2 Release by Human Monocytes More Potently than JAK Inhibition

- Primary human monocytes or THP-1 monocytes were pre-treated with KTX-115 (20h) or Ruxolitinib (30 min) and then stimulated with rhIL-6 or LPS for 24h before collecting supernatants for MCP-1/CCL2 detection
- For STAT3/pSTAT3 evaluation, cell lysates were collected 30 min. post-stimulation

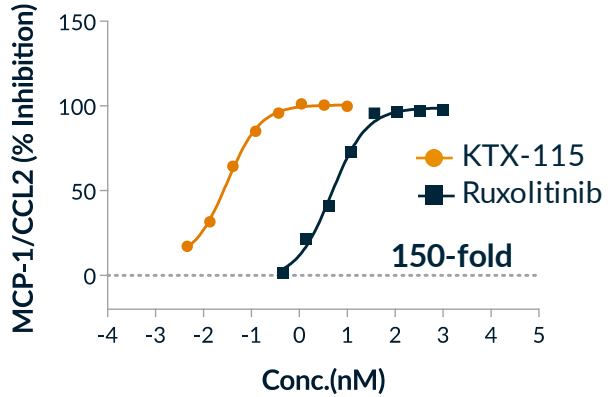
IL-6 stim (CD14+ Monocytes)



HTRF	Ruxolitinib	KTX-115
DC_{50} (nM)	n/a	0.37

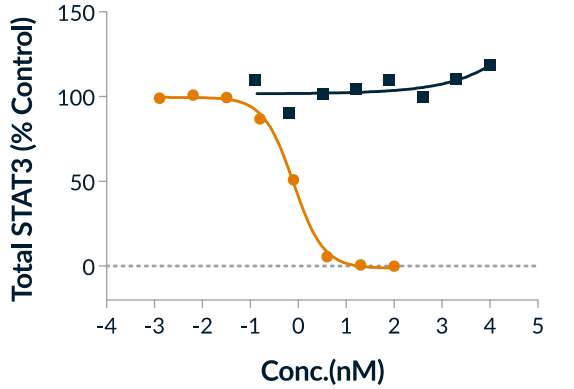


HTRF	Ruxolitinib	KTX-115
IC_{50} (nM)	22.22	0.49

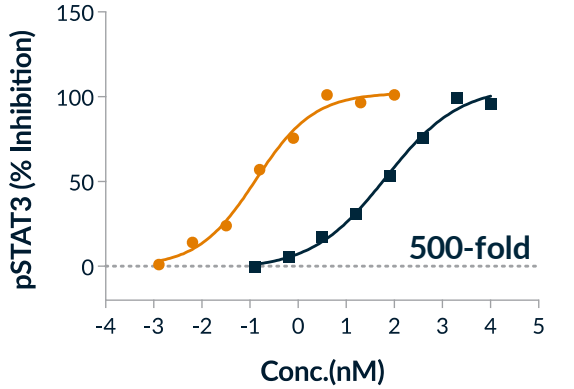


ELISA	Ruxolitinib	KTX-115
IC_{50} (nM)	4.70	0.03

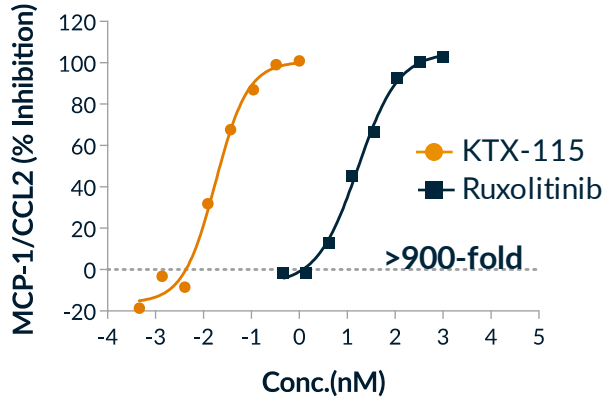
LPS stim (THP-1 Cells)



HTRF	Ruxolitinib	KTX-115
DC_{50} (nM)	n/a	0.80

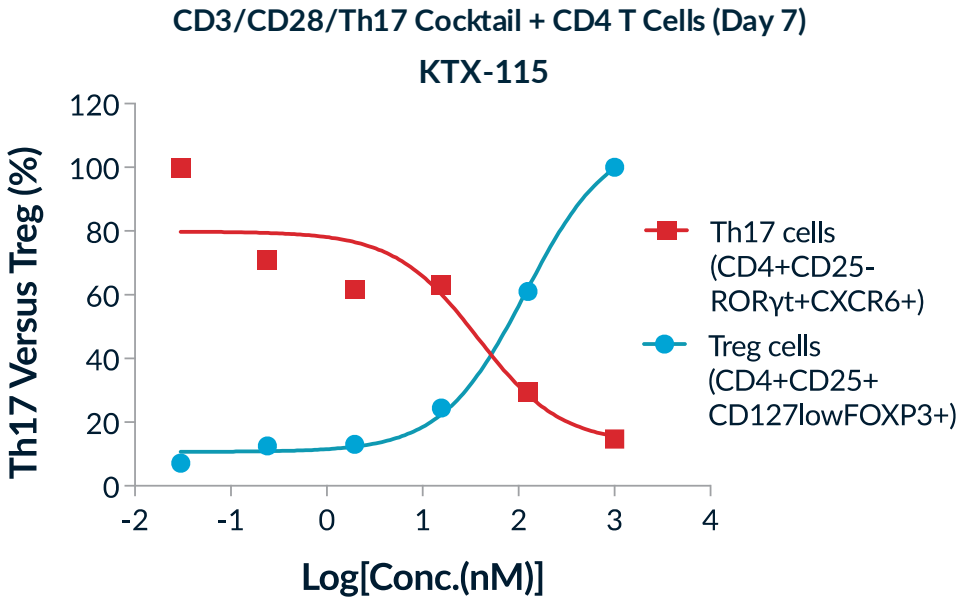


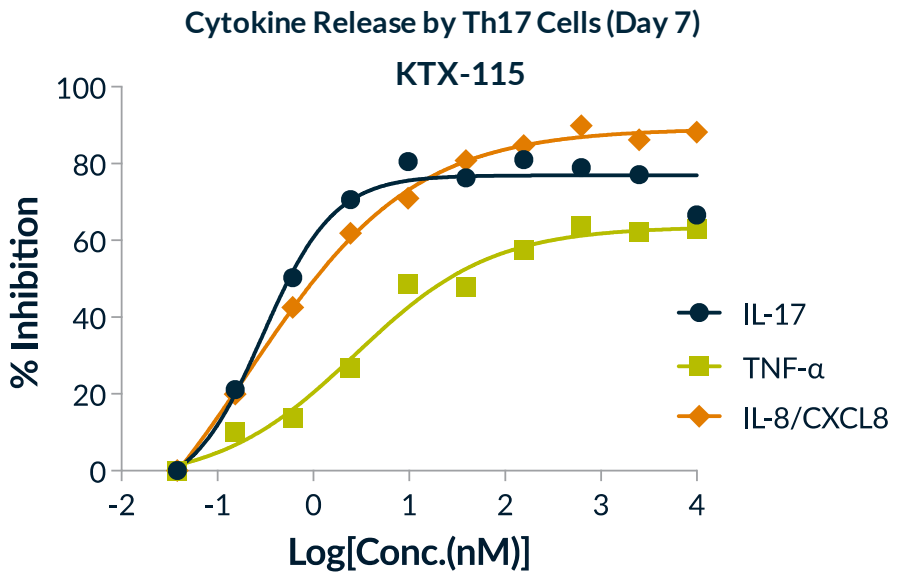
HTRF	Ruxolitinib	KTX-115
IC_{50} (nM)	66.33	0.13



ELISA	Ruxolitinib	KTX-115
IC_{50} (nM)	16.63	0.018

STAT3 Degradation Inhibits CD4+ Th17 Development and Related Cytokine Production

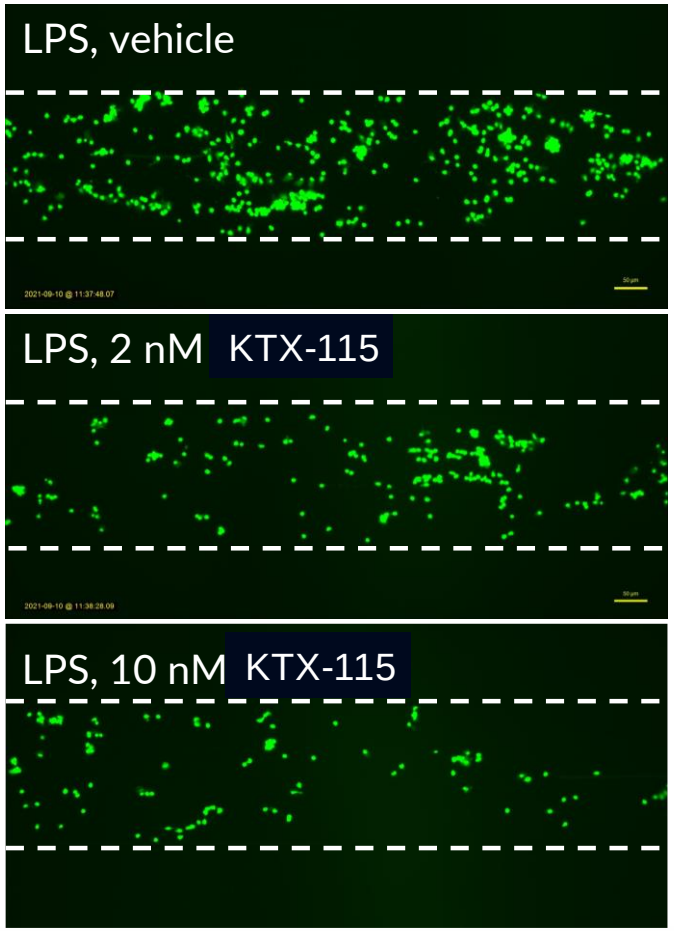




- CD4+ naïve T cells isolated from huPBMC were treated overnight with KTX-115 before activation with aCD3/CD28 coated beads and cultured with a pro-Th17 cocktail of cytokines and antibodies
- Ratios of Th17 cells vs. Treg cells as well as cytokines in supernatants were estimated after 7 days of cell culture

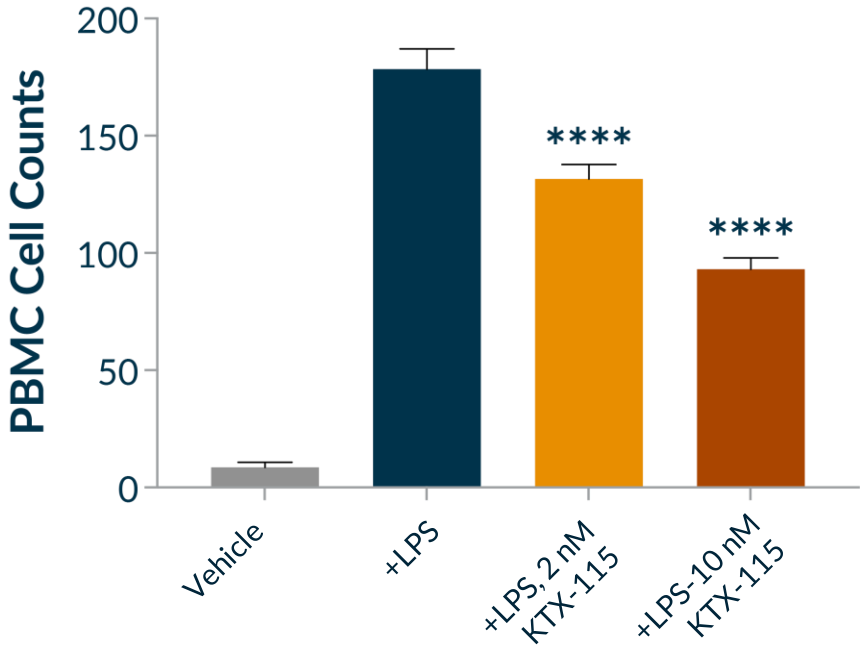
STAT3 Degradation Modulates Inflammation-induced Adhesion of Blood Leukocytes to the Endothelium

Blood Cells Adhering to Endothelial Cells in Shear Flow



Distler/UZH-UCJM collaboration

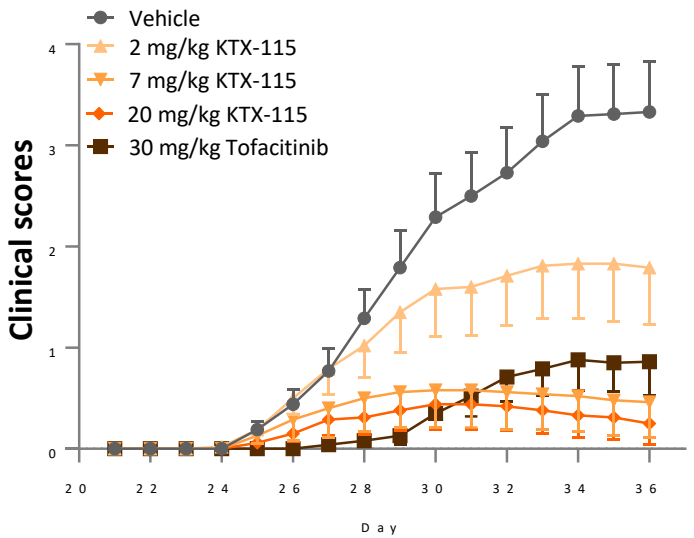
LPS-induced PBMC Adhesion (24h)



**** p<0.0001

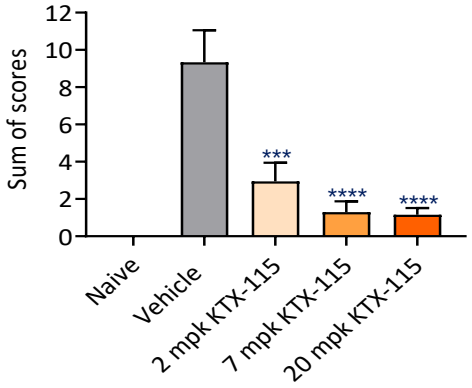
STAT3 Degradation Prevents Collagen Induced Arthritis

Collagen-induced Arthritis (BIW)

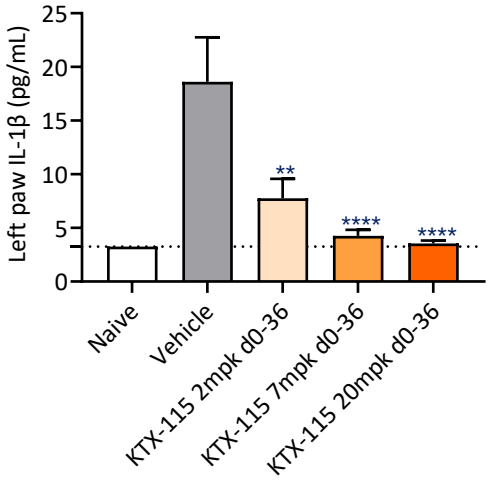


STAT3 Degradation	Vehicle	2 mg/kg KTX-115	7 mg/kg KTX-115	20 mg/kg KTX-115	30 mg/kg Tofa
Whole blood cells	0%	50%	66%	90%	n/a
Left hind paw	0%	58%	60%	62%	n/a

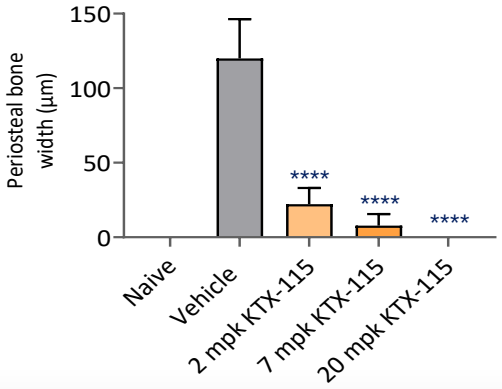
Pathology Score



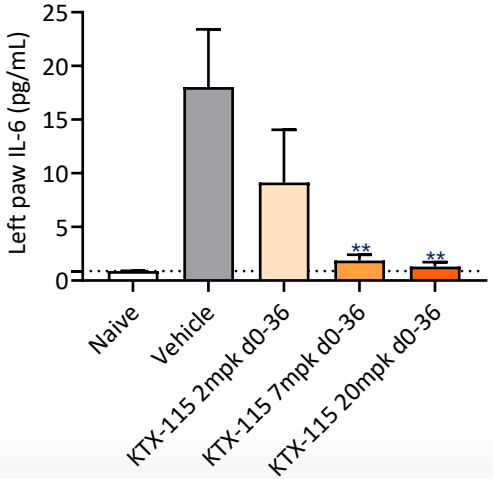
Left fore paw IL-1β



Periosteal Bone Growth



Left fore paw IL-6



Animals dosed with KT-6995 (Day0-36) Q2W, IP: Tofa (D0-36) QD
** p<0.01, *** p<001, **** p<0.0001

Summary

- Kymera has developed highly potent and selective degraders of STAT3
- STAT3 degradation abrogates activation by multiple inflammatory stimuli in PBMC, monocytes, and CD4+ T cells
- Even limited degradation of STAT3 results in significant inhibition of cytokines involved in inflammation in several cell types (PBMC, monocytes, and T cells)
- STAT3 degradation reduces leukocyte recruitment in shear flow
- Robust, dose-dependent inhibition of mouse arthritis (CIA)
- Additional data can be observed in POS0479: STAT3 degraders protect from immunofibrotic changes in preclinical models

Thank you

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