STAT3 Degraders Inhibit Cellular Activation, Cytokine Production, and Th17 Development, Resulting in Profound Inhibition of Autoimmunity in the MOG-EAE Model of CNS Inflammation

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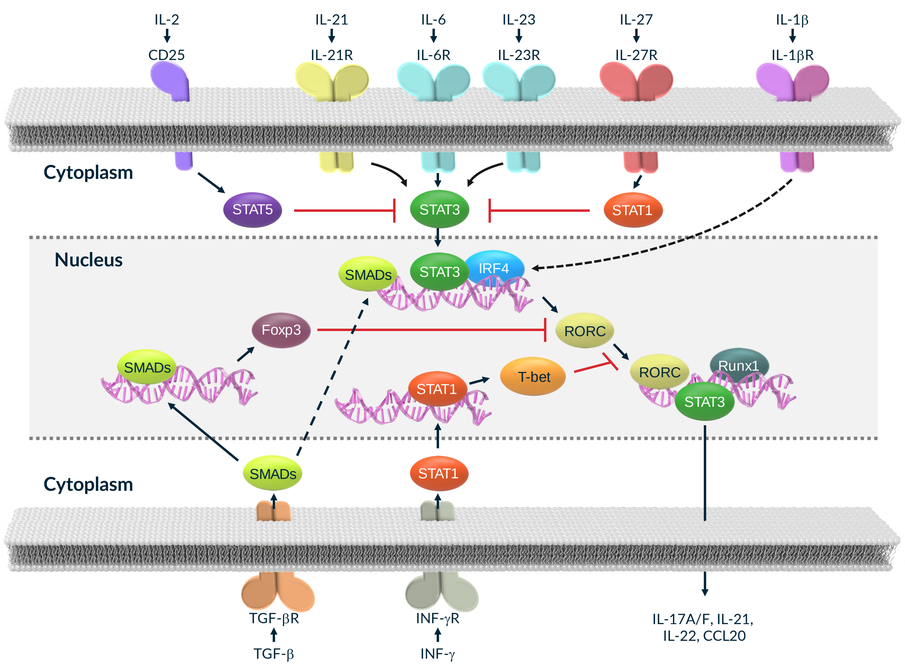
INTRODUCTION

Signal transducer and activator of transcription 3 (STAT3), an "undruggable" transcription factor activated by a variety of receptor- and non-receptor tyrosine kinases, plays a critical role in activation pathways triggered by cytokines, hormones, and growth factors (1), which makes it an attractive target for the treatment of autoimmune and autoinflammatory disorders. Kymera has developed heterobifunctional molecules that selectively target STAT3 for degradation by the ubiquitin-proteasome pathway.



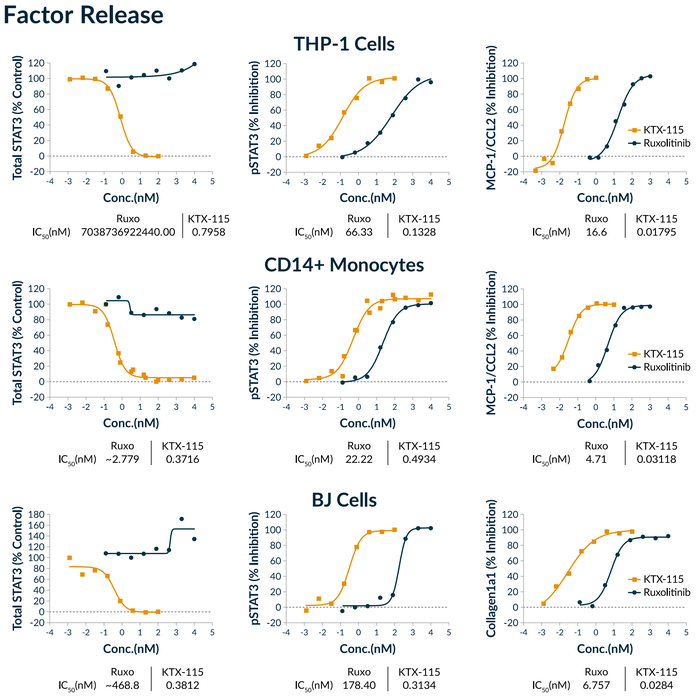
METHODS

All assays were conducted by pre-incubating cells with a STAT3 degrader, KTX-115, for up to 24h before stimulation. THP-1 cells were stimulated for 24h with LPS from E. coli (0111:B4 from Sigma). Primary CD14+ monocytes were stimulated for 24h with human IL-6 (R&D Systems). BJ cells were stimulated for 72h with human IL-6/IL-6R (R&D Systems). For STAT3/pSTAT3 detection, the same stimuli were applied for 15 min. Th17 development was induced using isolated CD4+ naïve T cells cultured for 7 days with a cocktail of anti-CD3/anti-CD28 beads supplemented with human IL-2, TGF-β1, IL-6, IL-23, IL-21, IL-1β, anti-IL-4 and anti-IFNγ. Th17 cells were defined as CD4+CD25-RORγt+CXCR6+CD45RA- whereas regulatory T cells (Treg) were defined as CD4+CD25+CD127lowFOXP3+CD45RA- in flow cytometric assays. Murine experimental autoimmune encephalomyelitis was induced by immunizing animals with MOG peptide emulsified in Complete Freund Adjuvant, followed by Pertussis Toxin injection. The animals were then treated with a STAT3 degrader or an alternative anti-inflammatory drug on day 3 or day 13. MOG restimulation assay was performed by collecting draining lymph node cells from MOG/CFA immunized mice (day 11) and in vitro stimulation with MOG peptide for 72h.

RESULTS AND CONCLUSIONS

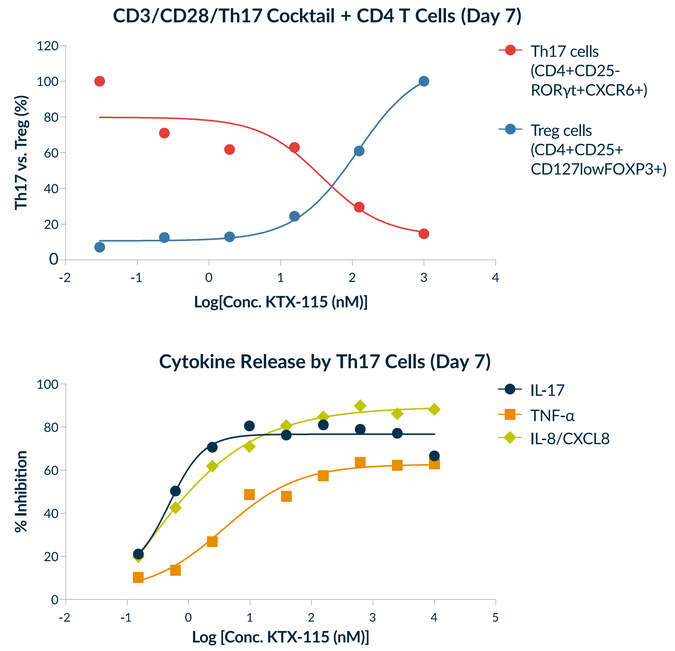
These degraders have broad and potent activity in-vitro against TLR and cytokine-induced activation of immune and stromal cells and attendant release of MCP-1 (CCL2) and collagen1a1. STAT3 degradation in CD4+ T cells potently inhibited Th17 development, decreasing IL-17, IL-22, IL-8/CXCL8, and TNFα production, with concomitant increase in Treg numbers, that was superior to JAK1/2 kinase inhibition. STAT3 degradation was subsequently evaluated in-vivo in murine models of autoimmune disease. In the Th17-driven inflammatory model of MOG-induced Experimental Autoimmune Encephalomyelitis (EAE), dose-dependent decrease of incidence, disease onset, clinical scores, and histopathology were observed in comparison to a S1P1 inverse agonist or steroid treatment. Ex-vivo MOG-stimulated cytokine release by leukocytes isolated from draining lymph node was also robustly inhibited. These data demonstrate the broad activity of STAT3 degradation in alleviating autoimmune inflammation in systems relevant to human disease.

Figure 1: STAT3 Degradation Leads to pSTAT3 Inhibition and Provides Potent Inhibition of Inflammatory/fibrotic Factor Release



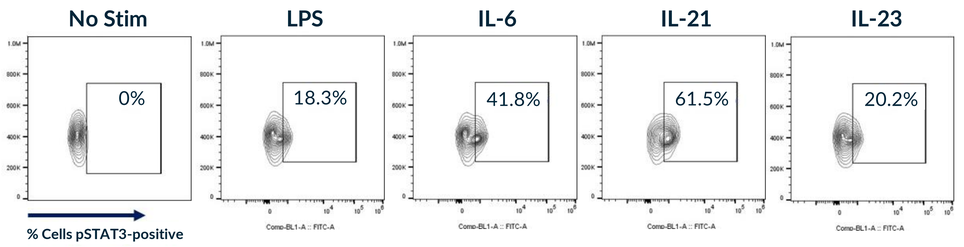
Only STAT3 degraders remove STAT3 from cells. This leads to enhanced inhibition of STAT3 activation (pSTAT3) and inflammatory factor release compared to a JAK1/2 inhibitor.

Figure 2: STAT3 Degradation Promotes the Rebalancing of Th17 vs. Treg Cell Population



STAT3 degradation blocks Th17 cell development and cytokine release in inflammatory conditions, while promoting Treg cell expansion in an *in vitro* co-culture system.

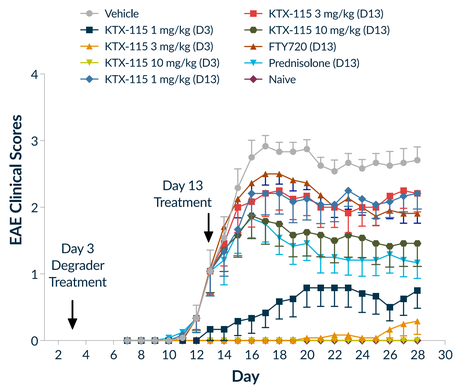
Figure 3: STAT3 Degradation Inhibits pSTAT3 Activation in Human PBMC Upon Stimulation with Various Pro-inflammatory Factors



Stimulus	No stim	LPS	IL-6	IL-21	IL-23
pSTAT3 IC_{50} (nM)	-	6.91	11.27	4.37	9.52

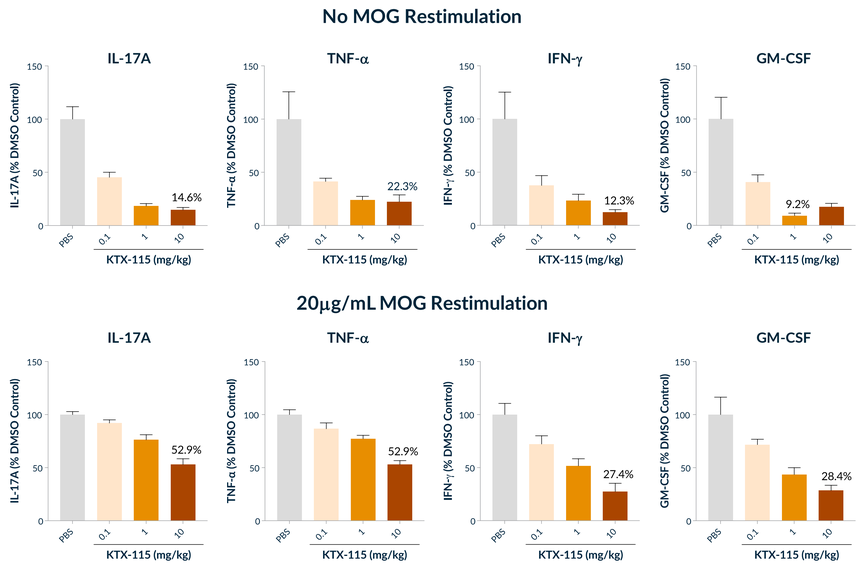
STAT3 degraders potently block STAT3 activation induced by various inflammatory stimuli in PBMC, suggesting broad applicability to complex immune responses in human disease.

Figure 4: STAT3 Degradation Blocks Disease Induction and Diminishes Ongoing Disease in Murine EAE



Treatment	EAE Incidence (%)	Median Day of Onset	End Score (+/-SD)
Vehicle	100.0%	13.0	2.71 +/- 0.69
1mg/kg KTX-115	66.7%	23.0	0.75 +/- 0.92
3mg/kg KTX-115	16.7%	>28.0*	0.29 +/- 0.69
10mg/kg KTX-115	0.0%	>28.0*	0.00 +/- 0.00

Figure 5: STAT3 Degradation Inhibits Th17 Cytokine Release by DLN Cells Even When Restimulated by their Cognate Antigen (MOG)



STAT3 degraders dose-dependently inhibit MOG-EAE induction, preventively. They can also outperform a S1P receptor agonist in therapeutic mode. *In vitro* restimulation of T cell collected from MOG/CFA immunized mice shows that STAT3 degraders robustly abrogate Th17 reactivation, e.g. during disease flares.

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References:
1. Maddur MS, et al. *Am J Pathol*, 2012

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