A STAT3-Selective Targeted Protein Degrader Decreases The Immune-Suppressive Tumor Microenvironment And Drives Anti-Tumor Activity In Preclinical Models

LB-088

Fred Csibi, Bin Yang, Yogesh Chutake, Karen Yuan, Scott Rusin, Rahul Karnik, Veronica Campbell, Michele Mayo, Kirti Sharma, Haojing Rong, Henry Li, Sharon Townson, Hari Kamadurai, Mike Sintchak, Christine Loh, Alice McDonald, Anthony Slavin, Chris De Savi, Jared Gollob, Duncan Walker, Phillip Liu, Nan Ji and Nello Mainolfi. Kymera Therapeutics, Inc. 300 Technology Square, Cambridge, MA 02139. Contact: fcsibi@kymeratx.com

Introduction

- STAT3 is a transcription factor downstream of several signaling events including the IL-6/JAK pathway that has been implicated in multiple aspects of tumorigenesis.
- In addition to increasing cancer cell proliferation and survival, constitutively activated STAT3 is proposed to regulate cross-talk interactions between tumor, stroma and immune cells to promote immune evasion in the tumor microenvironment (TME).
- STAT3 activity in tumors promotes the production of immune-suppressive factors that activate STAT3 in diverse immune-cell subsets. Mechanistically, genetic studies support a direct role of activated STAT3 in regulating myeloid cell differentiation to contribute to an immuno-suppressed TME.
- STAT3 is therefore a highly attractive target for immuno-oncology. However, potent and selective agents that specifically and directly target STAT3 have remained elusive.
- Kymera Therapeutics is developing degraders of STAT3 with drug-like properties, represented here with KTX-201 and its related compound KTX-104. These compounds potently and selectively degrade STAT3 protein and reverse immuno-suppression in preclinical models.

Figure 1 - Overview of Targeted Protein Degradation

BROAD OPPORTUNITY
ONLY BINDING SITE REQUIRED

EFFICIENT
CATALYTIC

PROLONGED IMPACT
TARGET PROTEIN DEGRADATION

HITCHING A RIDE
UPS INTACT & FUNCTIONAL

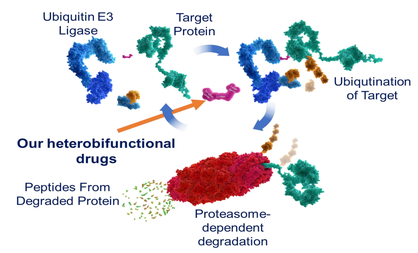
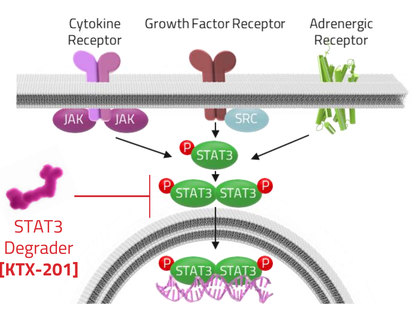


Figure 2- STAT3 Integrates Multiple Upstream Signaling Events To Regulate Tumor Intrinsic and Extrinsic Functions in The TME



- Hyperactivation of STAT3 promotes tumor-intrinsic gene expression programs involved with survival, proliferation, stemness and metastasis of tumor cells.
- Additionally, STAT3 promotes the differentiation and activity of immunosuppressive cells in the tumor microenvironment.
- STAT3 activation in endothelial cells contributes to angiogenesis.

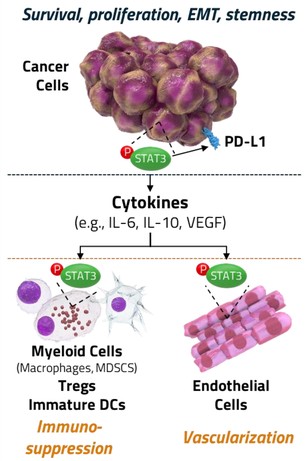
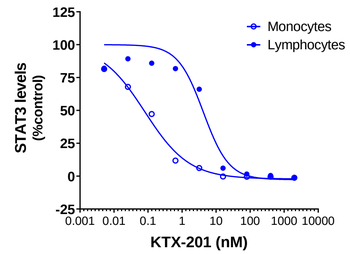


Figure 3- KTX-201 Is a Potent and Selective STAT3 Degrader in Human Immune Cells

Fig.3A- Degradation of STAT3 in immune cells

PBMCs, flow cytometry, 24hr

STAT3 Alexa Fluor™ 647 staining:
- Lymphocytes: CD8/CD4/CD19/CD56 (T/B/NK cells)
- Monocytes: CD14



Immune cells	STAT3 DC_{50} (nM)	STAT3 DC_{90} (nM)
Monocytes	0.15	1.25
Lymphocytes	5.4	14

Fig. 3B- Selectivity in PBMCs

Deep Tandem Mass Tag Proteomics, 8hr (>10,000 proteins monitored)

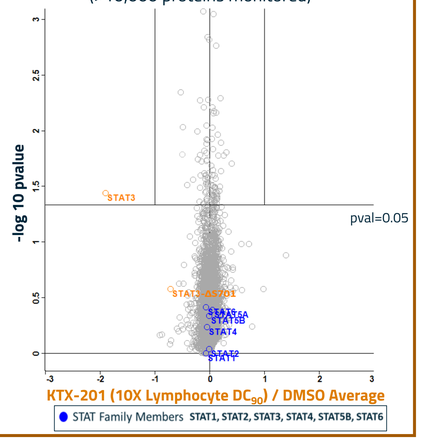
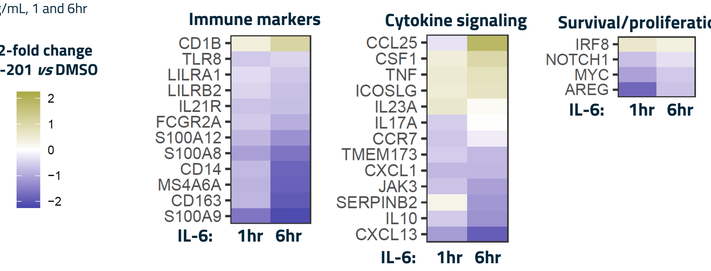


Figure 4- STAT3 Degradation Downregulates Genes Involved with Immune Suppression in Both Immune and Tumor Cells

Fig. 4A- STAT3 degradation inhibits IL-6-dependent gene expression changes in human PBMCs

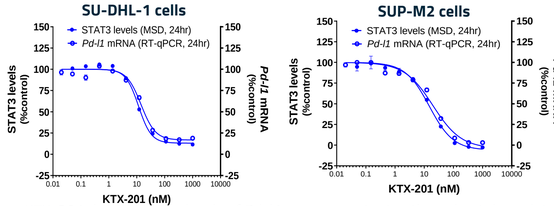
(Nanostring analysis)

- Human PBMCs (2 donors)
- KTX-201, 400nM pre-treatment 18hr
- IL-6 10ng/mL, 1 and 6hr



- KTX-201 decreases genes involved with immunosuppressive myeloid cells (e.g., *CD163*, a M2 macrophage marker) and changes in cytokine signaling (e.g, decrease in *IL-10* and *IL-17A* and increase in *TNF*) as well as genes involved with survival and proliferation (e.g., decrease in *Myc*)

Fig. 4B- STAT3 degradation downregulates *Pd-l1* mRNA in lymphoma (ALK+ ALCL)



Cell line	STAT3, DC_{50} (nM)	*Pd-l1* mRNA IC_{50} (nM)
SU-DHL-1	11.7	14.3
SUP-M2	16.3	19.8

- KTX-201 decreases the levels of Pd-l1 gene consistent with a robust degradation of STAT3 in Anaplastic Large T Cell Lymphoma cells

Figure 5- STAT3 Degradation Reverses Immuno-Suppression in a Co-Culture Model of the Tumor Microenvironment in NSCLC

Fig. 5A- Co-culture model of human TME

BioMAP® NSCLC Oncology System (Eurofins Discovery)

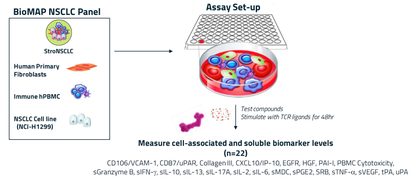


Fig. 5B- STAT3 degraders decreases STAT3 in H1299 cells without affecting cell growth

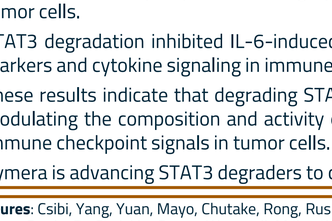
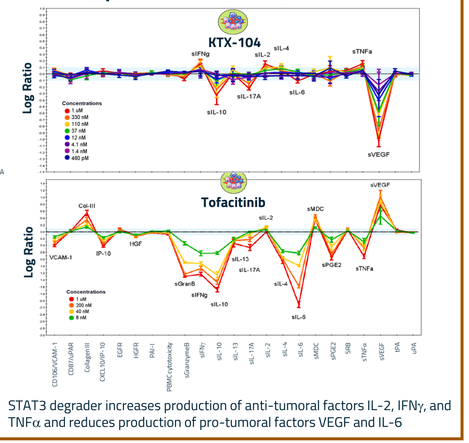


Fig. 5C- STAT3 degrader reverses immuno-suppression with a profile distinct from JAK inhibitor



STAT3 degrader increases production of anti-tumoral factors IL-2, IFNγ, and TNFα and reduces production of pro-tumoral factors VEGF and IL-6

Figure 6- STAT3 Degrader Enhances Anti-Tumor Immunity in CT26 Colorectal Tumors

Fig. 6A- STAT3 degraders shows anti-tumor activity at well tolerated doses in the syngeneic CT26 model

In vitro CT26 DC_{50} (WB) at 24h = 14 nM

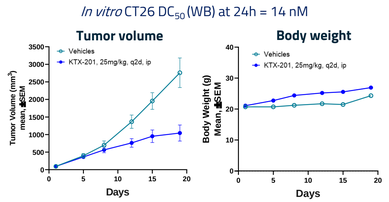


Fig. 6B- Strategy for Pharmacodynamic Immunophenotyping

KTX-201, 25mg/kg, 3 doses every 2 days. Tumor collection 24hr post last dose

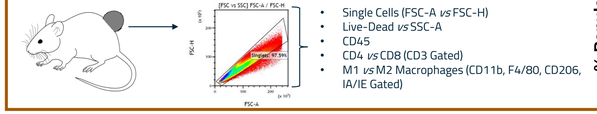
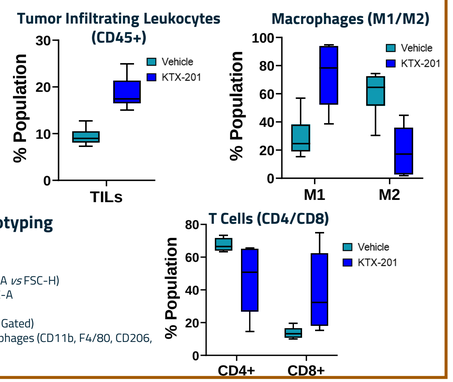


Fig. 6C- STAT3 degrader increases anti-tumor (M1, CD8) and decreases immuno-suppressive (M2) infiltrates



Conclusions

- Kymera has developed potent and highly selective STAT3 degraders with activity in immune and tumor cells.
- STAT3 degradation inhibited IL-6-induced changes in gene expression involved with myeloid cell markers and cytokine signaling in immune cells and downregulated *Pd-l1* mRNA in tumor cells.
- These results indicate that degrading STAT3 has the potential to reverse immune suppression by modulating the composition and activity of immune cells in the TME and directly downregulating immune checkpoint signals in tumor cells.
- Kymera is advancing STAT3 degraders to clinical development in both liquid and solid tumors.

Disclosures: Csibi, Yang, Yuan, Mayo, Chutake, Rong, Rusin, Karnik, Sharma, Campbell, Li, Townson, Kamadurai, Sintchak, McDonald, Slavin, De Savi, Liu, Gollob, Walker, Ji, Mainolfi. *Kymera Therapeutics:* Employment, Equity Ownership.