# KT-333, A First-in-Class STAT3 Degrader, Caused Strong STAT3 Degradation and Consistent Downstream Biology *In Vitro* and In *Vivo*

KYMERA

Yatao Shi, Eric Kuhn, Karen Yuan, Yogesh Chutake, Vaishali Dixit, Michele Mayo, Joyoti Dey, Bin Yang, Haojing Rong, Phillip CC Liu, Dirk M Walther, Chris De Savi and Kirti Sharma

## INTRODUCTION

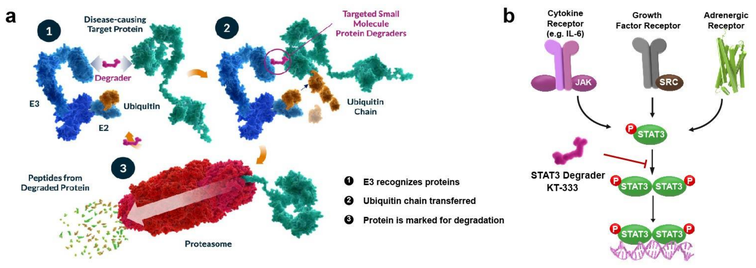
STAT3 is a crucial transcription factor regulating many downstream signaling pathways including the JAK-STAT and interleukin signaling pathways. Aberrant STAT3 activation contributes to the survival, proliferation, stemness and metastasis of tumor cells as well as the activation of immunosuppressive cells in the tumor microenvironment.

STAT3 cannot be targeted by traditional drug design approaches, and therefore has been historically considered an "Undruggable" target. Targeted protein degradation (TPD) is a novel therapeutic modality targeting "Undruggable" oncogenic proteins. Heterobifunctional degraders recruit endogenous E3 ligases to ubiquitinate substrate proteins, leading to their degradation by the proteosome.

Kymera Therapeutics has developed a highly selective STAT3 degrader, KT-333, for the treatment of heme malignancies. PK-PD/Efficacy studies with tumor xenografts demonstrated KT-333-driven STAT3 degradation and tumor regression. Quantitative MS-based proteomics was performed to understand the selectivity and mechanism of action (MoA) of KT-333 *in vitro* and *in vivo*.

## KT-333 AS A FIRST-IN-CLASS STAT3 DEGRADER

**Figure 1: Mechanism for targeted protein degradation (a). KT-333 causes STAT3 degradation and blocks STAT3 downstream biology (b).**

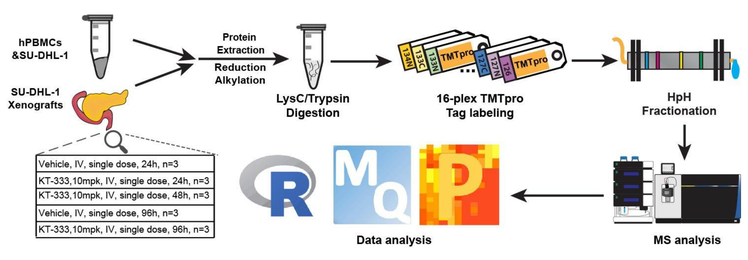



## EXPERIMENTS

***In Vivo* Efficacy Model**: Subcutaneous tumors were established in immunocompromised host strain mice using SU-DHL-1 ALK+ ALCL cell lines. KT-333 was formulated in buffered PBS and administered IV on either a QW or Q2W schedule. Tumor volumes were measured by caliper and bodyweight was taken twice a week.

**PKPD Study**: SU-DHL-1 tumor bearing animals were administered a single dose of KT-333, and plasma and tumors were harvested at 0, 6, 24, 48, 96, 168 and 240 hours. KT-333 drug levels was measured by LC-MS and tumors evaluated for STAT3 protein by a targeted MS assay.

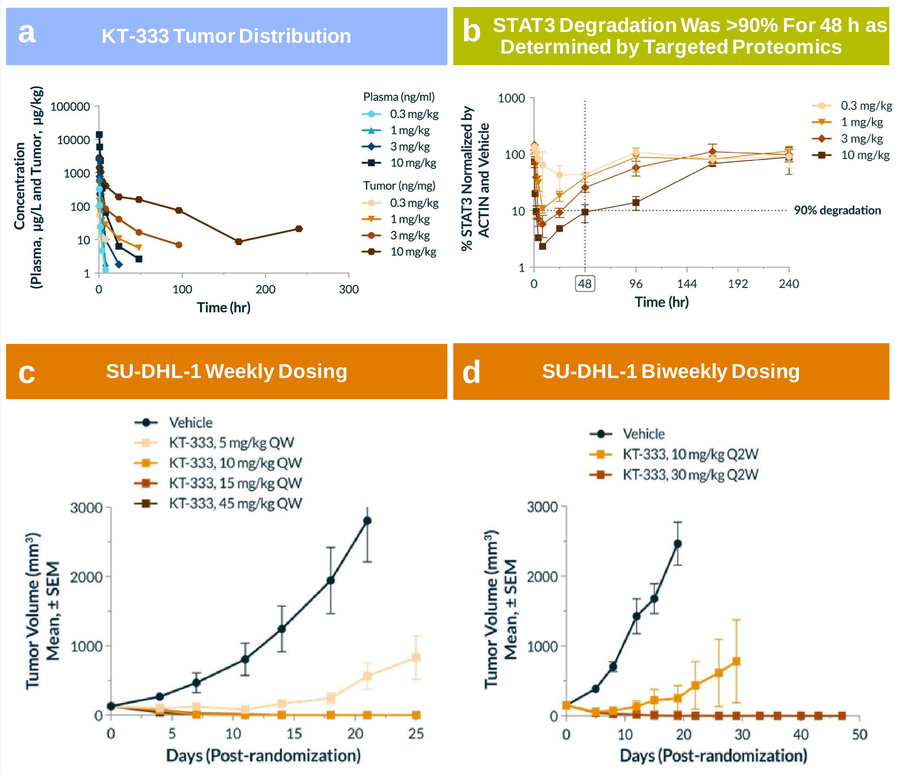
**Figure 2: General workflow of KT-333 *in vitro* and *in vivo* proteomics**




**Proteomics Study:** For selectivity analysis, Human Peripheral Blood Mononuclear Cells (hPBMCs) were treated with KT-333 at 300nM (10XDC95) for 24 hours. SU-DHL-1 cells were treated for 8h, 24h and 48h for selectivity and MoA analysis. Tumors from mice treated with vehicle for 24h and 96h as well as 10 mg per kg (mpk) KT-333, were harvested after 24h, 48h and 96 hours and selected for *in vivo* proteomics analysis. Cell pellets and tumors were processed as shown in **Figure 2**. 16-plex TMTpro tag was used for quantitative analysis. A proteome depth of >8,000 proteins was achieved in *in vitro* and *in vivo* proteomics experiments. Statistical analysis was carried out using the Limma statistical package. A weighted cutoff between statistical significance and fold-change was applied.

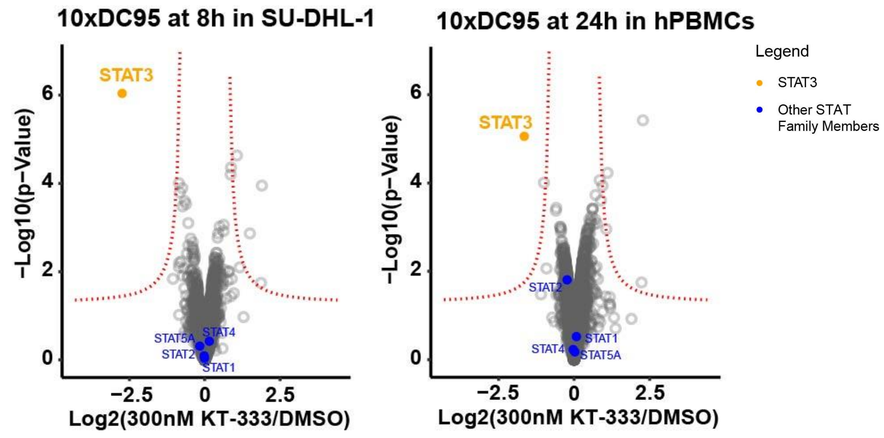
## *In Vivo* PKPD and Efficacy

**Figure 3: KT-333 distributed into tumors (a), induced strong STAT3 degradation (b) and resulted in deep and stable tumor growth inhibition (c, d).**




## *In Vitro* Proteome-wide Selectivity of KT-333

**Figure 4: KT-333 is highly selective for STAT3 in SU-DHL-1 cells and human PBMCs.**




## *In Vivo Vs. In Vitro* MoA and Biomarker ID

**Figure 5: *In vivo* proteomics confirmed significant STAT3 degradation with downstream signaling-related protein changes in 10 mpk KT-333-treated xenografts.**

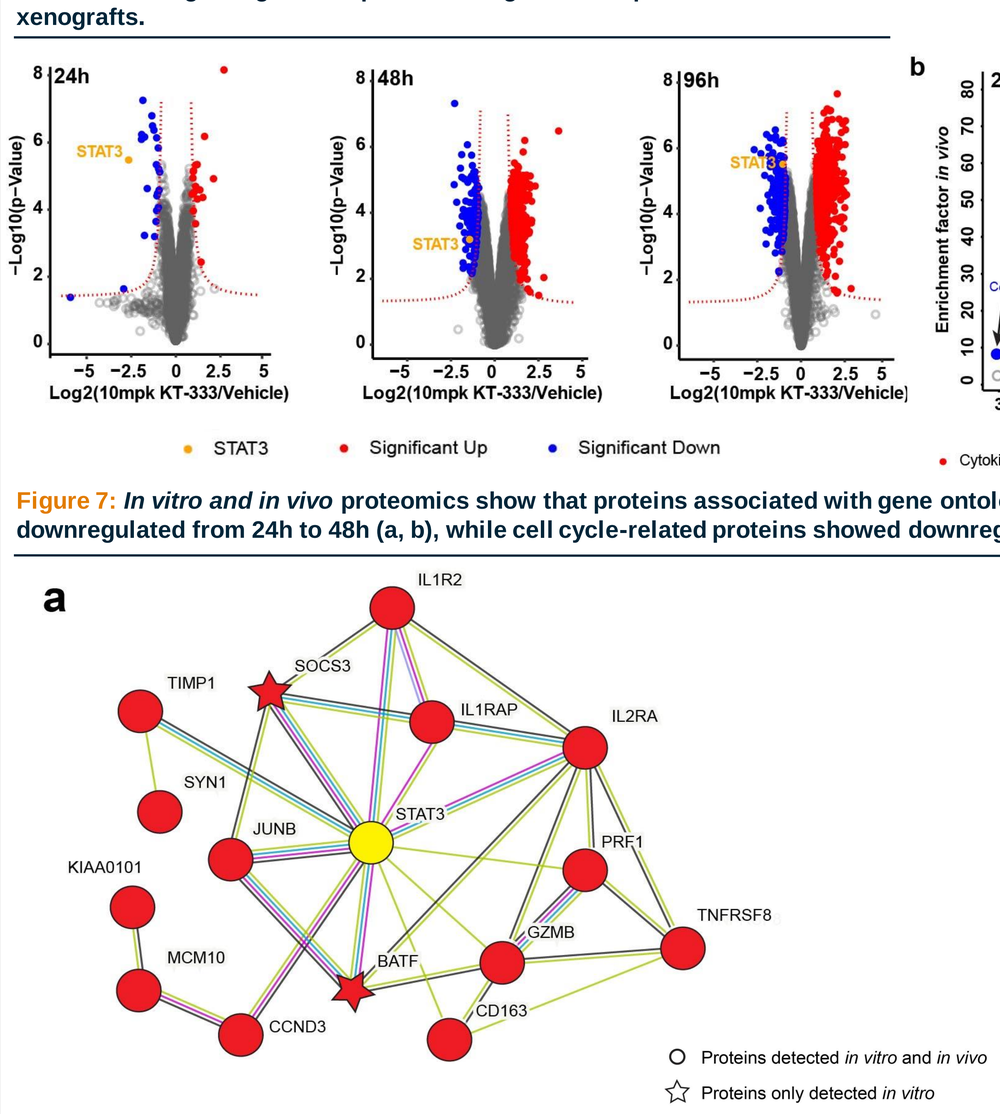



**Figure 6: Protein level changes (a) and enriched downregulated pathways (b) in response to KT-333 treatment show the same trend in cultured cells *(in vitro)* and in xenografts *(in vivo).***

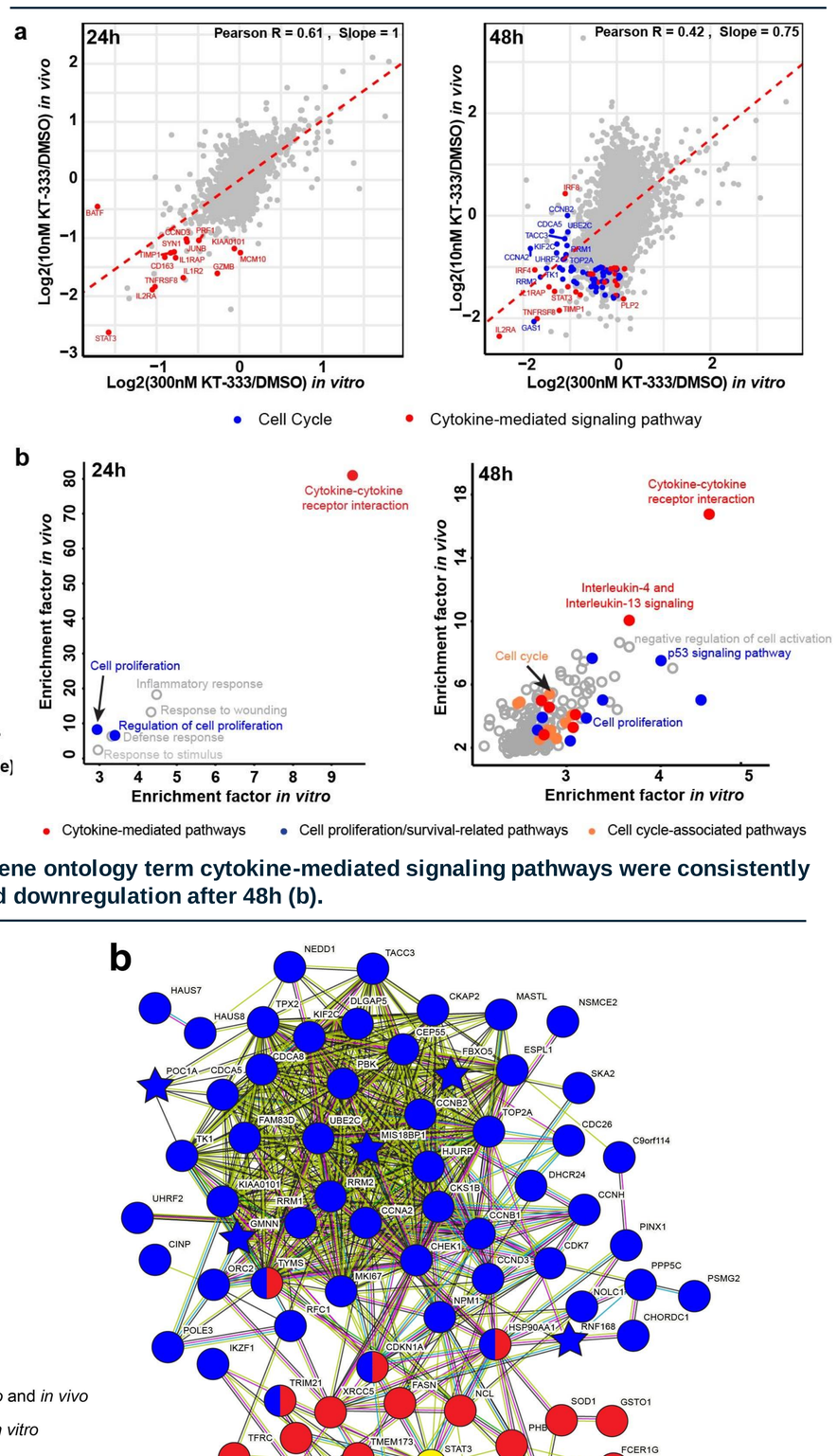
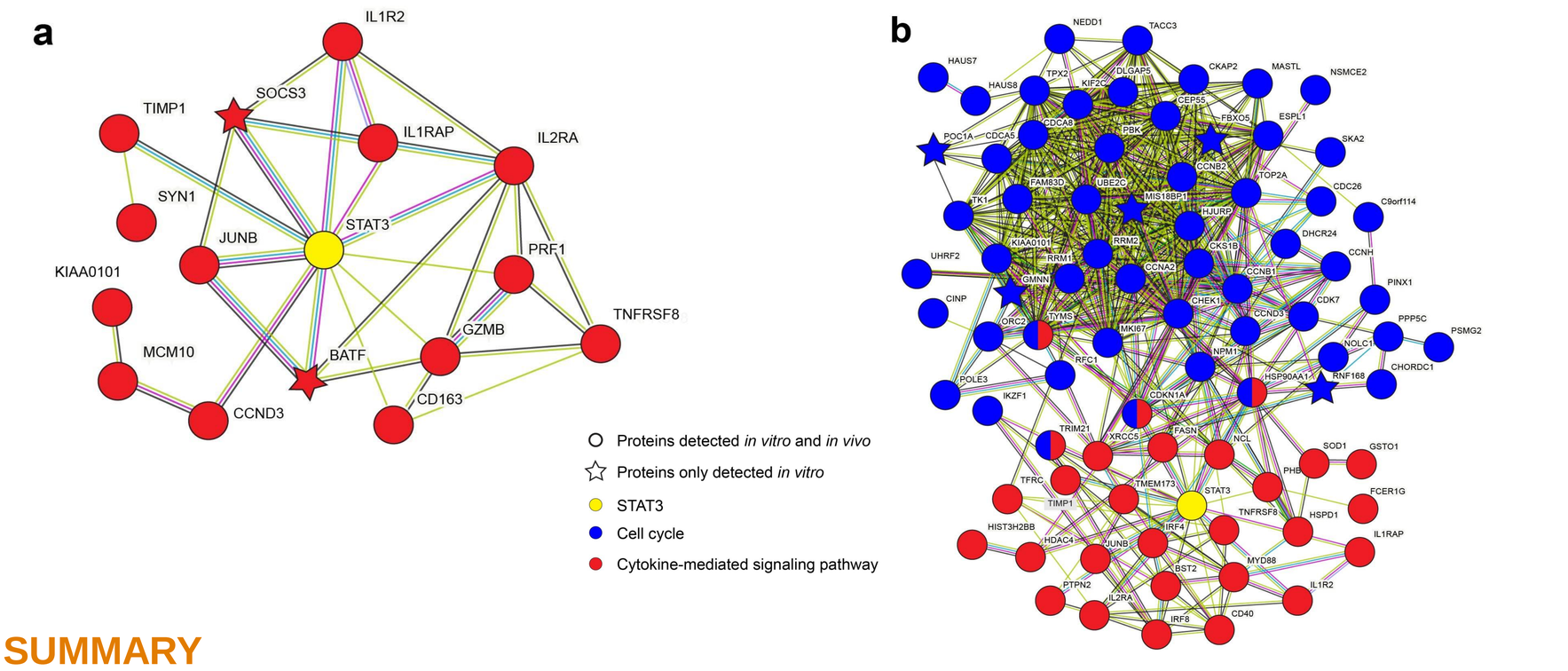



**Figure 7: *In vitro and in vivo* proteomics show that proteins associated with gene ontology term cytokine-mediated signaling pathways were consistently downregulated from 24h to 48h (a, b), while cell cycle-related proteins showed downregulation after 48h (b).**




## SUMMARY

- KT-333 is a first-in-class, heterobifunctional small molecular degrader that potently and selectively degrades STAT3 protein in immune cells and tumor cells.
- Targeted proteomics showed >90% degradation of STAT3 by KT-333 in tumor cells within 48h. KT-333 treatment induced tumor cell death and growth arrest in xenografts.
- Pathway analyses revealed down-regulation of cytokine-mediated signaling pathways at 24h and 48h, while cell cycle signatures were downregulated from 48h onwards, indicating that cell cycle arrest and subsequent apoptosis are the main drivers of efficacy for KT-333.
- The consistent protein and pathway changes *in vitro* and *in vivo* demonstrate that the MoA of KT-333 observed *in vitro* translates well into *in vivo* tumor models.

Kymera Therapeutics • 200 Arsenal Yards Blvd., Suite 230, Watertown, MA 02472, USA

Contact: Yatao Shi / yshi@kymeratx.com

20th Discovery on Target Conference • Oct 18-20, 2022 • Boston, MA