Targeting STAT3 with Selective Protein Degraders for the Treatment of PTCL

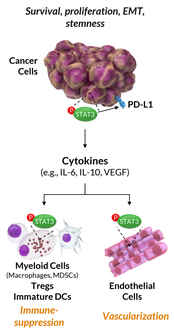
Phillip C.C. Liu[1], Bin Yang[1], Michele Mayo[1], Karen Yuan[1], Haojing Rong[1], Kirti Sharma[1], Rahul Karnik[1], Dirk Walther[1], Joyoti Dey[1], Yogesh Chutake[1], Yatao Shi[1], Giuseppina Astone[2], Kunal Shetty[3], Wing C. Chan[3], Giorgio Inghirami[2], Jared Gollob[1], Nello Manolfi[1], and Chris De Savi[1]

[1]Kymera Therapeutics, Watertown, MA; [2]Weill-Cornell Medical College, New York City, NY; [3]City of Hope National Medical Center, Duarte, CA

INTRODUCTION

The activity of Signal Transducer and Activator of Transcription 3 (STAT3) is dysregulated in many cancers including aggressive hematological malignancies with high unmet medical need. Aberrant activation of STAT3 can promote the establishment and progression of malignant cells through regulation of cell survival and proliferation pathways and suppression of anti-tumor immunity, also known as tumor intrinsic and tumor extrinsic mechanisms, respectively

Selective targeting of STAT3 has been challenging, but targeted protein degradation mediated by heterobifunctional small molecule degraders is a novel therapeutic modality to target difficult-to-drug oncogenic proteins. These molecules bind to both the target protein and an E3 ligase, enabling the formation of a ternary complex which leads to ubiquitination and proteasomal degradation of the target protein.



Tumor Intrinsic

- STAT3 promotes gene expression programs involved with survival, proliferation, stemness and metastasis of tumor cells
- In PTCL, activated STAT3 (phospho-STAT3) is detected in many subtypes due to: STAT3 mutation (ALK-ALCL, T-LGLL, NKTCL, AITL, PTCL-NOS), upstream kinase activation, ALK, JAK (ALK+ALCL, ALK-ALCL), and elevated inflammatory mediators (CTCL)[1-6]

Tumor Extrinsic

- STAT3 promotes the differentiation and activity of immunosuppressive and endothelial cells, resulting in an immunosuppressive tumor microenvironment
- Opportunities in multiple heme and solid tumor indications that are not responsive to immune checkpoint inhibitors

AIM

We have discovered a series of heterobifunctional STAT3 degraders that potently and selectively degrade STAT3. We evaluated the *in vitro* and *in vivo* activity and molecular mechanisms of degrading STAT3 in models representing different subtypes of mature T and NK cell lymphoma.

RESULTS

Figure 1: STAT3 Degraders are Highly Active against NK and T Cell Lymphoma Cell Lines Including Cells Expressing STAT3 Mutants

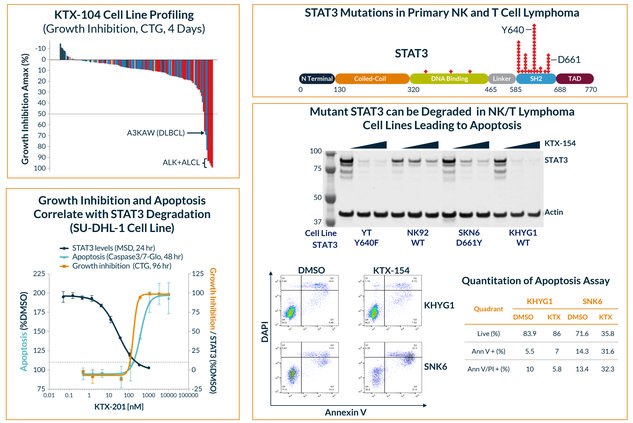


Figure 2: Activity Against STAT3-mutant ALK-negative ALCL Patient Derived Cells

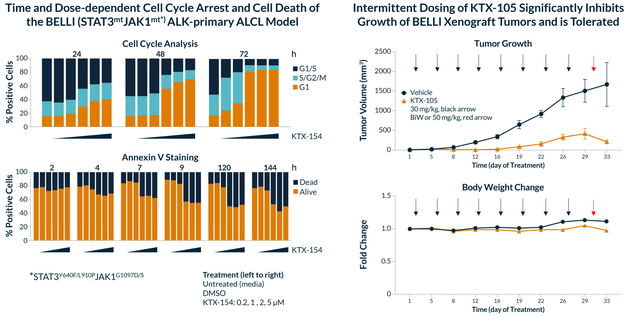
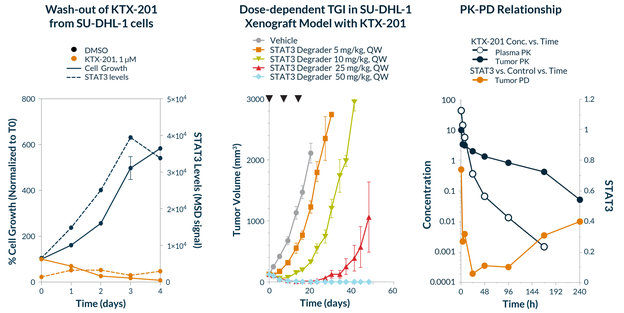
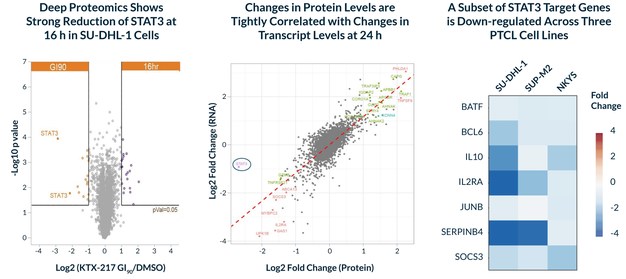


Figure 3: Anti-tumor Activity of STAT3 Degrader in SU-DHL-1 Model Can be Achieved with Intermittent (QW) Dosing



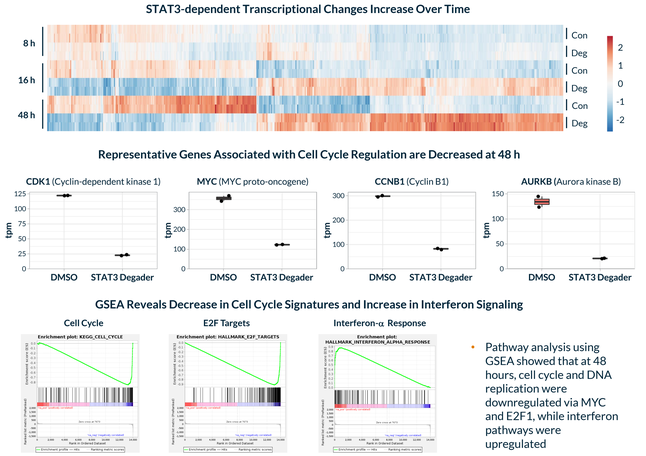
- Strong anti-tumor activity is observed when 90% or greater degradation of STAT3 is maintained for at least 48 h *in vitro* and *in vivo* in ALK+ALCL

Figure 4: Rapid and Profound Down-regulation of STAT3 and STAT3-Regulated Targets with Tight Correlation between mRNA and Protein Changes



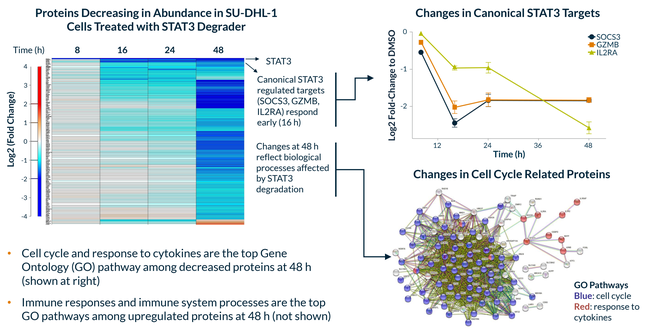
- At 8 h, only STAT3 is significantly down-regulated
- At 16 h, 16 proteins are significantly down-regulated with STAT3 showing greatest decrease in abundance
- STAT3 (circled) shows significant decrease in protein levels in SU-DHL-1 cells but minimal change in RNA consistent with degrader mechanism while most other genes/proteins have highly correlated changes
- At 48 h, all 3 cell lines show down-regulation of direct STAT3 targets

Figure 5: Time-Dependent Changes in Transcription in SU-DHL-1 Cells Upon STAT3 Degradation



- Pathway analysis using GSEA showed that at 48 hours, cell cycle and DNA replication were downregulated via MYC and E2F1, while interferon pathways were upregulated

Figure 6: Temporal Changes in Proteins Upon STAT3 Degradation



- Cell cycle and response to cytokines are the top Gene Ontology (GO) pathway among decreased proteins at 48 h (shown at right)
- Immune responses and immune system processes are the top GO pathways among upregulated proteins at 48 h (not shown)

METHODS

***In vitro* Assays:** All cell lines were cultured according to recommended procedures unless otherwise noted. For degradation assays, cells were treated with compounds for 24 h, and total STAT3 protein assessed by western blotting or MSD assay.

Viability was assessed using cells treated with degraders for 4 days and assayed by CTG (SU-DHL-1) or MTT (KHYG1, SNK6) assay. For KHYG1 and SNK6 assays, cells were cultured in low IL-2 (50 ng/mL). Cell cycle and cell death analysis was performed by flow cytometry using cells stained with propidium iodide (PI) or PI and AnnexinV (BELLI) or DAPI/Annexin V(KHYG1, SNK6).

***In vivo* Experiments:** Subcutaneous xenografted tumors were established in immune compromised mice. STAT3 degraders were formulated in buffered PBS and administered by iv infusion. For PK/PD study, a single administration of STAT3 degrader was administered on day 0. Plasma and tumor levels of drug were measured by MS, and tumor STAT3 levels measured by western blotting.

Transcriptomic and Proteomic Analysis: RNAseq was performed on cells treated with STAT3 degraders to identify differentially expressed genes. Pathway analysis was performed using Gene Set Enrichment Analysis (GSEA).

Proteomics was conducted using Tandem Mass Tag. Protein association was evaluated by STRING analysis with Gene Ontology (GO) classifiers.

CONCLUSIONS

- Kymera has discovered a series of potent and selective STAT3 degraders.
- STAT3 degraders show activity against both wild-type and clinically-relevant mutant forms of STAT3 resulting in growth arrest and increased cell death of ALK+ALCL as well as STAT3-mutant NK lymphoma and ALK-ALCL cell lines *in vitro* and *in vivo*.
- Transcriptomic and proteomic analyses of STAT3 degrader-treated cells reveal tightly correlated changes in proximal STAT3-dependent genes and proteins in ALK+ALCL cell lines.
- Subset of transcriptional responses to STAT3 degrader is conserved between ALK+ ALCL and STAT3-mutant NK and T cell lymphoma cell lines.
- Pathway analyses confirm down-regulation of STAT3-regulated processes including cytokine responses at 16-24 h and consistent down-regulation of cell cycle signatures and up-regulation of immune pathways at 48 h suggesting modulation of tumor cell-intrinsic processes and the potential to regulate cell-cell interactions in the tumor microenvironment.
- Additional *in vivo* models of PTCL and related heme malignancies are being investigated, but these data illustrate the therapeutic potential of STAT3 degraders for the treatment of cancer with aberrant STAT3 activation.

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