E3 Pairing and Structural Mechanisms Underlying Anti-Tumor Activity of Clinical STAT3 Degrader KT-333

#LB037

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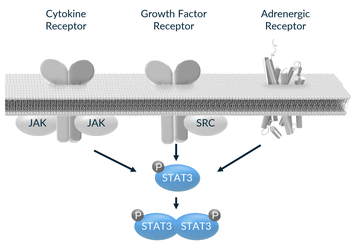
INTRODUCTION

STAT3 is an undrugged transcription factor activated through a variety of different cytokine and growth factor receptors via Janus kinases, as well as through oncogenic fusion proteins and mutations in STAT3 itself[1]. In certain malignant cells, STAT3 activation is amplified, leading to a dampened immune response, tumor progression, and metastasis. The role of STAT3 as a cancer driver and tumor microenvironment modulator has been validated in a multitude of studies, making it a strong candidate to target in the treatment of cancer[2-5]. We designed KT-333, a potent, highly selective, first-in-class, heterobifunctional degrader for the treatment of multiple STAT3-dependent pathologies, including hematological malignancies and solid tumors.

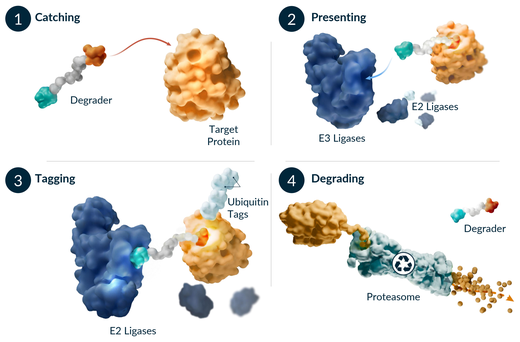
Based on the potential for STAT3 as a target for cancer therapeutics and limitations of prior approaches, we developed KT-333, a first-in-class, potent, highly selective, heterobifunctional STAT3 degrader currently in Phase 1 clinical trials. Here, based on STAT3 degradation by multiple E3s based degraders, structure of STAT3-KT333-VHL and a lysine site-resolved target ubiquitination model, we provide evidence for VHL as the ideal partner E3 for targeting STAT3 in cancer.

STAT3 Biology and Target Rationale



- STAT3 is a largely undrugged transcription factor activated through cytokine and growth factor receptors via JAKs and non-JAK mediated mechanisms
- STAT3 plays a role in tumor biology, evasion of immune surveillance and inflammation/fibrosis
- No known drugs selectively block STAT3 broadly across all relevant cell types or address both phosphorylation-dependent and – independent functions of STAT3

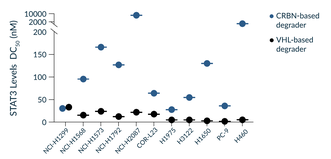
Proteome Editing with Targeted Protein Degradation[6]



RESULTS

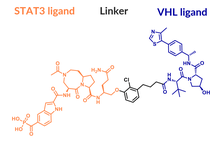
Figure 1: STAT3-E3 Pairing and Discovery of KT-333

A Increased & consistent better potency for VHL-based degraders compared with CRBN-based ones.



STAT3 Degradation after 24 hr Treatment

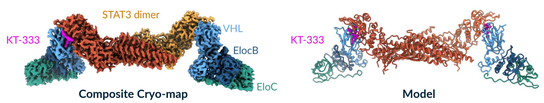
B Chemical structure of the heterobifunctional **VHL-based STAT3 degrader KT-333.**



STAT3 Degrader KT-333

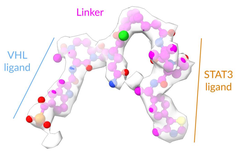
Figure 2: Cryo-EM Determined STAT3-KT-333-VHL Ternary Complex Structure Looks Native-like, Possibly Driven by the 'Bent' Linker-mediated Novel Pocket Between STAT3 and VHL

A The ternary complex forms a dimer through binding between the coiled coil and DNA binding domains on STAT3.



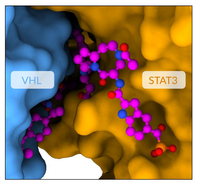
Composite Cryo-map | Model

B KT-333 adopts a bent conformation with its two heads recapitulating the binding modes of the ligands.



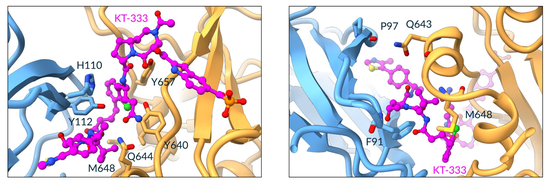
Structure and cryo-EM map of KT-333 in Ternary Complex

C The linker region of KT-333 is completely buried in a **novel pocket** between VHL and STAT3.



STAT3-VHL Interface

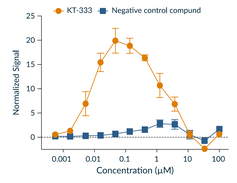
D The 752 Å2 **large novel pocket** is maintained by extensive interactions. The **linker-mediated interactions provide structural underpinnings of the stable ternary complex** beyond the expected protein-ligand binary interactions. This corroborates with observed strong cooperativity between STAT3 and VHL (α = 4.6). Hydrogen bonds are shown as dashed lines and residues participating in hydrogen bonding or hydrophobic interactions are shown as sticks.



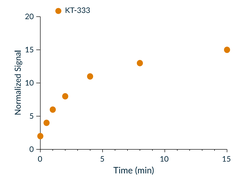
Two Views of KT-333 Interacting with VHL and STAT3

Figure 3: KT-333 Induces Selective, Rapid and Site-specific Ubiquitination of STAT3 by VHL

A KT-333 induces STAT3 ubiquitination by VHL in a **dose dependent manner**.



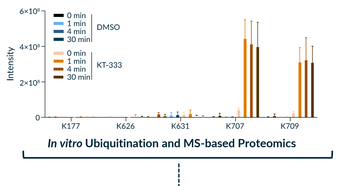
B KT-333 **induces rapid and saturable** addition of ubiquitin to STAT3 by the VHL-E3 complex.



In vitro Ubiquitination and Homogenous Time-resolved Fluorescence Energy Transfer Assay

STAT3 Degradation Assembly Model Corroborates with Site-specific Ubiquitination of the Most Proximal Lys Residues

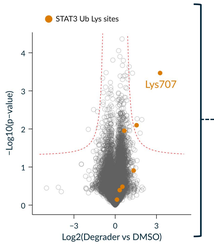
1 KT-333 induced **rapid** ubiquitination at **K707** & **K709**

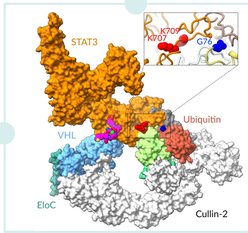


In vitro Ubiquitination and MS-based Proteomics

2 KT-333 induced **selective** STAT3 ubiquitination amongst ~20k Ub events on ~5k cellular proteins

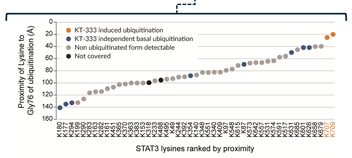
Global Ubiquitinomics





The model of degradation assembly demonstrated **proximity of key lysine residues** to C-terminal Gly76 residue of **ubiquitin**, a prerequisite for ubiquitination of STAT3.

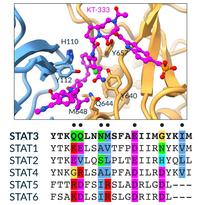
3 KT-333 induced STAT3 ubiquitination of the two lysine residues most **proximal** to Gly 76 of Ub



MD Simulation Based Proximity vs Proteomics-based Evidence of STAT3 Lys Site Ub

Figure 4: Selectivity of KT-333 for STAT3 Degradation

A STAT3 residues involved in the interfaces with VHL and the KT-333 linker are not conserved in other STAT family members, likely contributing to the specificity of KT-333 for STAT3.



B Selective STAT3 degradation amongst >8,000 robustly quantified proteins including all other six STAT family in SU-DHL-1, a clinically relevant ALCL cancer line

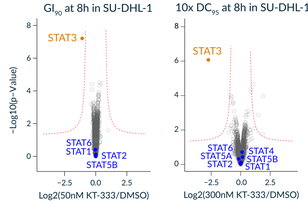
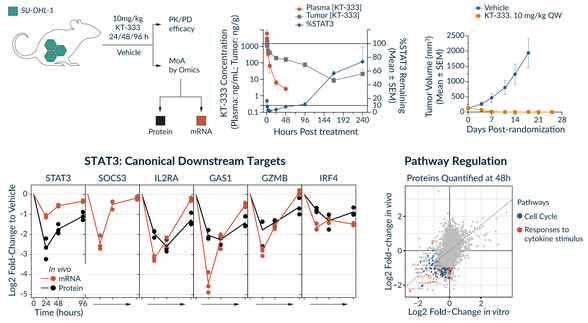
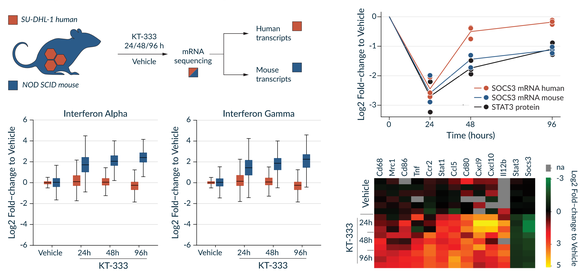


Figure 5: Antitumor Activity of KT-333 in SU-DHL-1, an ALK+ ALCL Tumor Model

1 **Tumor Intrinsic**: KT-333 mediated STAT3 degradation leads to a rapid reduction of canonical downstream targets causing cell cycle arrest and subsequent apoptosis as the main drivers of efficacy for KT-333.



2 **Tumor Extrinsic**: KT-333 leads to induction of proinflammatory anti- tumorigenic transcriptional signatures in the SUDHL1 xenograft TME



METHODS

Cryogenic Electron Microscopy (cryo-EM)

We determined the structure of STAT3/KT-333/VHL ternary complex at 2.5 Å resolution . Ternary complex was isolated using size exclusion chromatography, applied to gold grids and plunged into liquid ethane using a Mark IV Vitrobot system. Data was acquired on a Titan Krios microscope and image analysis was done in cryosparc. A composite map consisting of STAT3-KT-333-VHL/EloB/EloC was generated using Phenix.

In vitro Ubiquitination Analysis

HTRF energy transfer assays detecting degrader induced ubiquitinated STAT3 were developed and used to follow KT-333 dose and time dependence. Next, we developed a parallel reaction monitoring targeted proteomics, an assay to monitor the kinetics of each peptide containing di-Gly remnant motif of ubiquitin on lysine residues of STAT3.

In vitro and *In vivo* Experiments

For degradation assays, cells were treated with KT-333 for 24 h, and total STAT3 protein assessed by western blotting. For *in-vitro* multi-omic MoA analysis, cells were treated with DMSO for 8h and 24h, or 50nM KT-333 in triplicates for 8h, 24h and 48h, respectively . SUDHL-1 subcutaneous tumors were established in immunocompromised host strain mice. After a single dose of KT-333, plasma and tumor were harvested at indicated time points. KT-333 drug levels was measured by LC-MS. Selected tumor samples were used for multi-omic MoA analysis.

Targeted proteomics

Tumor STAT3 protein levels were determined by a targeted proteomics assay using isotopically labeled peptide (TQIQSVEPYTK) unique for STAT3.

Global Proteomic Analysis

Ubiquitination: KT-333 induced ubiquitination of specific lysine residues in cells was analyzed by di-Gly peptide-based global ubiquitinomics.

Selectivity: SU-DHL-1 treated with KT-333 at GI 90 and 10x DC95 concentrations.

MoA: KT-333 treated SUDHL1 cells and tumor samples.

For all the above, Tandem Mass Tag (TMT) discovery proteomics was performed. Statistical analysis was carried out using the Limma statistical package.

Transcriptomic Analysis

Tumor: RNAseq was performed on KT-333 treated SU-DHL-1 cells or xenografts. Normalization and differential expression analyses were performed using sleuth.

TME: To interrogate the impact of STAT3 degradation on the host (mouse) immune TME, sequences were mapped separately to either the mouse or the human genome to enable quantification of species-specific transcripts and enrichment analysis.

CONCLUSIONS

KT-333 is a first-in-class drug candidate to address STAT3 driven pathology across broad indications. Our data provides precise structural and molecular mechanisms behind KT-333 mediated potent, selective and fast degradation of STAT3 by VHL.

- STAT3 degrader, KT-333, is designed and optimally paired with VHL resulting in a very stable *native-like* ternary complex, presumably driven by the 'bent' linker-mediated novel pocket between STAT3 and VHL.
- KT-333 dependent potent and selective STAT3 degradation shows consistent mechanism of action *in vitro* and *in vivo*.
  - Tumor intrinsic - Decreased STAT3 gene signatures and induction of growth arrest and cell death through cell autonomous mechanisms in models of hematologic malignancies.
  - Tumor extrinsic - Induction of proinflammatory anti- tumorigenic transcriptional signatures in the SUDHL1 xenograft tumor microenvironment
- The Phase 1 trial is ongoing (NCT05225584). The last clinical update on the program was shared at the American Society of Hematology 2023 Annual Meeting and available on Kymera's website.

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DISCLOSURES

Sharma, Fei, Shi, Browne, Walther, Daigle, Ramanathan, Yuan, Dey, Fasciano, Karnik, Kuhn, Breitkopf, Miller, Shaw, Mahasenan, Zhu, Yang are Kymera Therapeutics employees and equity owners. Huang, Rong, Liu, Mayo are former Kymera Therapeutics employees.

The safety and efficacy of this investigational agent have not been established. This agent is not approved by any regulatory body for any indication