A First-in-Class STAT3 Degrader KT-333 in Development for Treatment of Hematologic Cancers

#1865

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INTRODUCTION

Signal Transducer and Activator of Transcription 3 (STAT3) plays important roles in the transduction of signals from growth factors and cytokines in both normal and malignant cells. Upon activation, STAT3 controls expression of genes that regulate cell growth, survival, differentiation, stemness and cell-cell interactions[1]. Aberrant activation of STAT3 has been observed in many cancers including lymphomas and leukemias through activating mutations, hyper-signaling through upstream regulators or loss of negative feedback regulation[2-4]. Additionally, STAT3-mediated cross-talk in the tumor microenvironment results in suppression of immune surveillance compromising anti-tumor immunity[5].

STAT3 has been historically considered "undruggable". Heterobifunctional degraders that recruit endogenous E3 ligases to ubiquitinate substrate proteins leading to their degradation by the proteosome have emerged as a novel therapeutic modality with great potentials to drug undrugged protein targets[6]. Here we introduce a first-in-class, potent and selective STAT3 degrader KT-333 that is being developed for the treatment of hematologic malignancies and solid tumors.

Tumor Cell Intrinsic and Extrinsic Functions of STAT3

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

Mechanism for Targeted Protein Degradation

Co-opting a Naturally Occurring Process to Regulate Protein Levels

1. E3 ligase recognizes protein
2. Ubiquitin chain transferred
3. Protein is marked for elimination

KYMERA

Broad Opportunity
Only binding site required

Efficient
Catalytic

Prolonged Impact
Targeted protein degradation

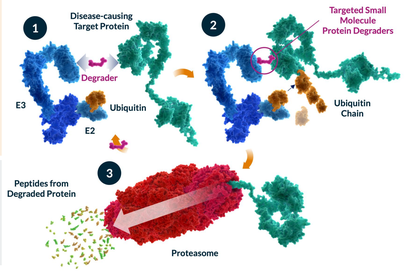
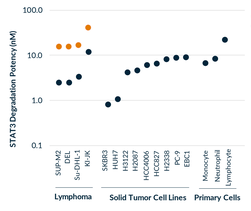
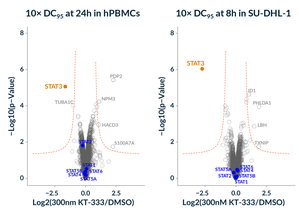


Figure 1: KT-333 is a Potent and Selective Degrader of STAT3 that Induces Growth Inhibition and Apoptosis of Lymphoma Cells

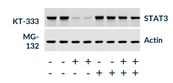
KT-333 degrades STAT3 across many cell types



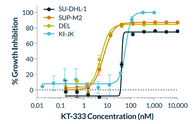
KT-333 is highly selective for STAT3 in PBMC and SU-DHL-1 cells



Degradation activity of KT-333 is proteasome dependent



KT-333 inhibits growth of ALK+ ALCL cell lines



KT-333 induces apoptosis

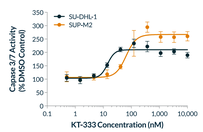
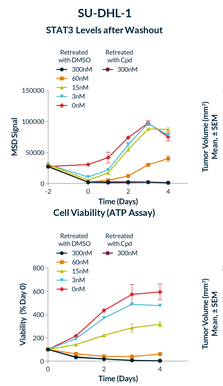


Figure 2: KT-333 Induces Growth Arrest and Cell Death After Transient STAT3 Degradation *in vitro* and with Intermittent Dosing *in vivo*

Wash out shows ~48 h of STAT3 iDC_{90} coverage leads to rreversible growth inhibition



Deep and durable tumor growth inhibition can be achieved with weekly or bi-weekly dosing

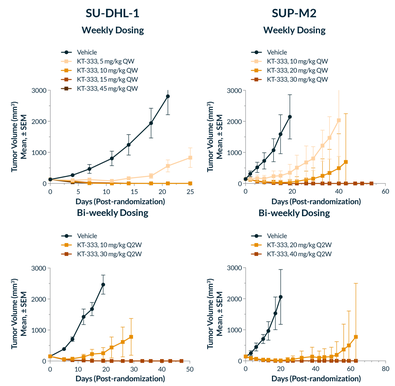
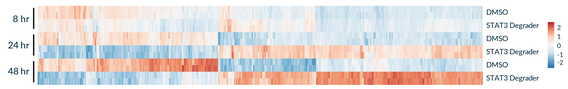
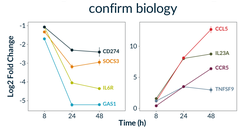


Figure 3: KT-333 Modulates STAT3-dependent Pathways

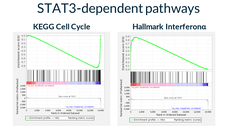
STAT3-dependent transcriptional changes increase over time



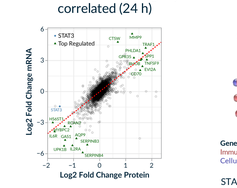
Changes in canonical STAT3 targets confirm biology



GSEA reveals significantly enriched STAT3-dependent pathways

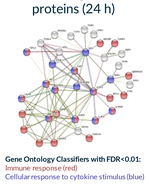


Changes in protein and transcript levels are tightly correlated (24 h)

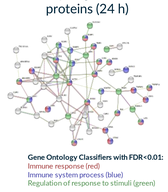


STAT3 shows strong decrease at protein but small changes in transcript levels.

Down-regulated proteins (24 h)



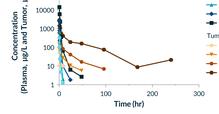
Up-regulated proteins (24 h)



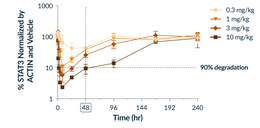
STAT3 degradation significantly modulates proteins that are involved in pathways downstream of STAT3 including immune response and response to stimuli /cytokine stimulus.

Figure 4: Integrated PK-PD-Efficacy Modeling

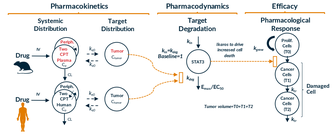
KT-333 distributed into tumor



Durable TGI achieved when STAT3 degradation is >90% for 48h in SU-DHL-1 model



Semi-mechanistic PK-PD/Efficacy model was built to simulate human dose resulting in tumor regression and substantial STAT3 degradation



Simulated Human PD and Efficacy profile indicates that human dose of >1 mg/kg given weekly will result in tumor growth inhibition

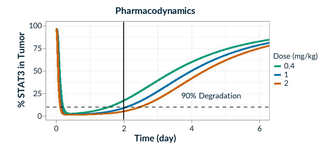
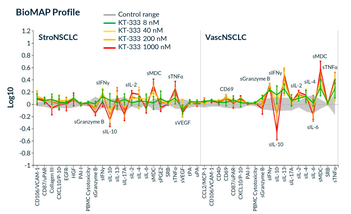
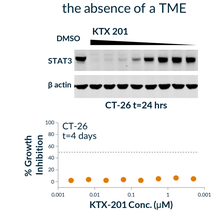


Figure 5: STAT3-degradation Positively Modulates Anti-tumor Immunity

KT-333 reduces mediators of immune suppression in *ex vivo* co-culture models of the TME



STAT3 is potently degraded *in vitro* but cell viability is not impacted in the absence of a TME



Combination activity of STAT3 degrader with anti-mouse PD-1 in CT-26 syngeneic xenograft model

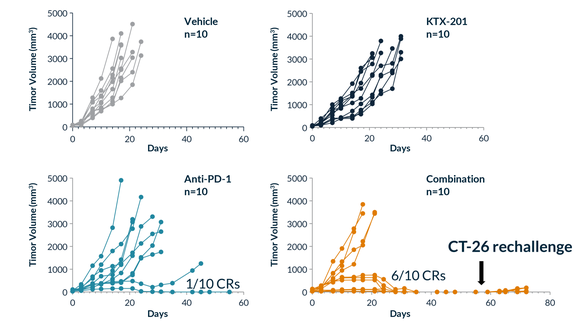


Figure 6: PK and *in vitro* Safety Pharmacology of KT-333 Support Clinical Development

KT-333 is predicted to be low clearance, low volume of distribution in humans with good solubility to enable intravenous administration

Physical and DMPK properties	KT-333
LogD	-1.4
Solubility at pH 7.4 (mg/mL)	303
Solubility in PBS pH 7.4 (mg/mL)	103
HLM (µL/min/mg)	1.3
H-PPB (Fu)	11%
CYP3A4 / 2C9 / 2C19 / 2D6 inhibition (IC_{50})	35/ > 50/ > 50/ > 50 µM
Mouse/Rat/Dog/Monkey Cl (mL/min/kg)	8.4 / 24 / 3.6 / 2.5
Mouse/Rat/Dog/Monkey Vdss (L/kg)	0.4 / 1.1 / 0.3 / 0.2
hERG (µM)	> 10

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. Viability was assessed using cells treated with degraders for 4 days and assayed by CTG assay. To block proteasome-mediated degradation, A549 cells were treated with the proteasome inhibitor (MG-132, 1 µM) for 1 hr prior to addition of KT-333 (DC_{90}) and incubated for an additional 4 h. Apoptosis was measured using the Caspase 3/7 Glo assay.

In vivo Experiments

Subcutaneous tumors were established in immunocompromised host strain mice using SU-DHL-1 or SUP-M2 human ALK+ ALCL cell lines. CT-26 syngeneic tumors were established as subcutaneous xenografts in C57BL/6 mice. STAT3 degraders were formulated in buffered PBS and administered IV on either a QW or BiW schedule.

Tumor volumes were measured by caliper and body weight was taken twice a week. For PK-PD assessment, SU-DHL-1 tumor bearing animals were administered a single dose of KT-333, and plasma and tumor were harvested at 0, 6, 24, 48, 96, 168 and 240 hours. KT-333 drug levels was measured by LC-MS and tumors evaluated STAT3 protein by a targeted MS assay.

Transcriptomic Analysis

RNAseq was performed on SU-DHL-1 and SUP-M2 cells treated with KT-333 for 8, 24 or 48 h. Pathway analysis was performed by Gene Set Enrichment Analysis (GSEA). RT-PCR assays were used to confirm response to KT-333 for a set of genes that showed significant changes.

Proteomic Analysis

Tandem Mass Tag discovery proteomics was performed on SU-DHL-1 and human Peripheral Blood Mononuclear Cells treated with KT-333 at 300 nM (equivalent to 10X DC_{95} in SU-DHL-1) to a depth of >8,000 proteins. Statistical analysis was carried out using the Limma statistical package. A weighted cutoff between statistical significance and fold-change was applied.

CONCLUSIONS

- KT-333 is a first-in-class clinical candidate that potently and selectively degrades STAT3, a previously undrugged transcription factor, in both tumor cells and immune cells
- STAT3 degradation results in decreased STAT3 gene signatures and induces growth arrest and cell death through cell autonomous mechanisms in models of hematologic malignancies
- ALK+ ALCL tumors could be eradicated by intermittent dosing schedules that achieve 90% or greater STAT3 degradation for approximately 48 hours during the dosing interval
- Degradation of STAT3 in cells of the tumor microenvironment could positively modulate anti-tumor immunity and suggest opportunities for combining with immuno-oncology therapies
- KT-333 exhibits PK properties suitable for intermittent, iv dosing regimens in the clinic
- KT-333 first-in-human study in patients with lymphomas will be initiated in 4Q21

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DISCLOSURES

Liu, Dixit, Mayo, Dey, Yuan, Karnik, Walther, Shi, Shaw, Breitkopf, Chutake, Sharma, Rong, Yang, Gollerkeri, Gollob and and De Savi are Kymera Therapeutics employees and equity owners.