Targeted STAT3 Degradation Leads to Remodeling of an Immunosuppressive Tumor Microenvironment and Subsequent Sensitization to Immune Checkpoint Inhibition

KYMERA

#603

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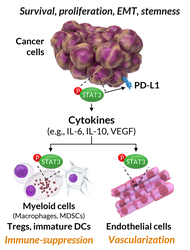
ABSTRACT

Signal Transducer and Activator of Transcription 3 (STAT3), a multifaceted transcription factor, is aberrantly activated across a variety of malignancies; however, its selective targeting has to-date remained a therapeutic challenge.

STAT3 plays a pivotal role in shaping the tumor immune landscape through cancer cell-intrinsic mechanisms, direct regulation of immune cell function and cancer cell- tumor microenvironment (TME) crosstalk, which collectively result in an immunosuppressive TME.

Targeted protein degradation represents a novel therapeutic modality enabling direct targeting of previously undruggable oncoproteins. Here, we investigated the immunomodulatory impact of STAT3 degradation on tumorigenesis in syngeneic mouse models representing cancers with heterogeneous immune milieus.

Tumor cell intrinsic and extrinsic functions of STAT3



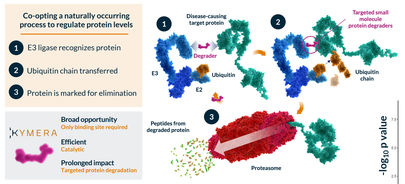
Tumor intrinsic

- 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
- Degradation of STAT3 provides therapeutic opportunities in multiple heme and solid tumor indications that are not responsive to immune checkpoint inhibitors

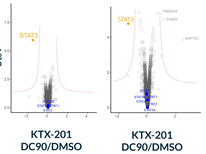
Figure 1: Targeted protein degradation of STAT3



KTX-201 is a potent and selective degrader of STAT3 as demonstrated in human immune cells and cancer cell lines

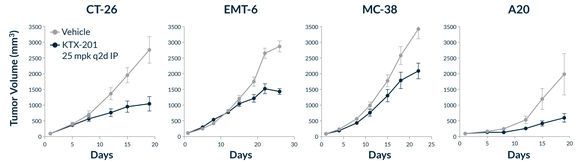
Selectivity analysis

Deep tandem mass tag proteomics (>10,000 proteins monitored)

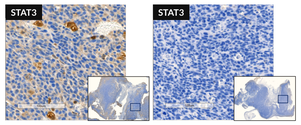


Cell Line	STAT3 DC_{50} (nM)
Monocyte	0.15
Lymphocyte	5.4
SU-DHL-1	11.7
SUP-M2	16.3

Figure 2: STAT3 degraders have *in vivo* anti-tumor activity in syngeneic mouse models of cancer



Representative IHC showing degradation of STAT3 in tumor and TME



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

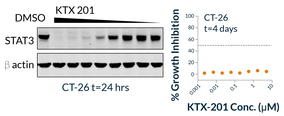
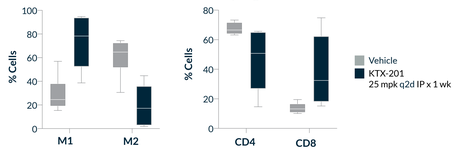
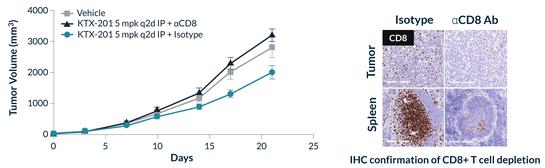


Figure 3: Anti-tumor activity of STAT3 degradation is driven by immune-directed mechanisms

KTX-201 treatment results in increased M1/M2 ratio of tumor-associated macrophages and CD8+ T cells



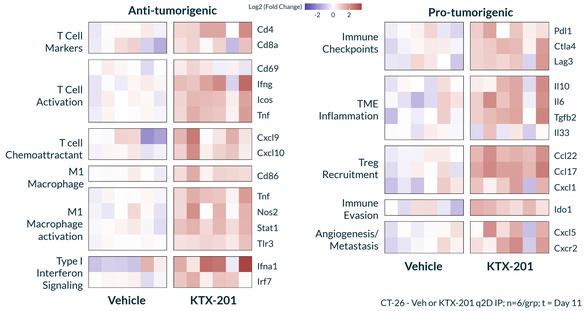
CD8+ T cell depletion abrogates KTX-201-mediated anti-tumor efficacy



IHC confirmation of CD8+ T cell depletion

Figure 4: STAT3 degradation-induced remodeling results in an immune-favorable TME

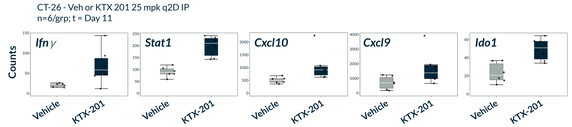
Transcriptomic analysis shows STAT3 degradation induces anti-tumorigenic changes as well as compensatory mechanisms in the TME of CT-26 tumors



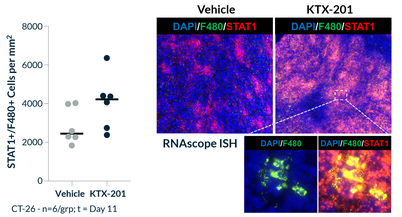
CT-26 - Veh or KTX-201 q2D IP; n=6/grp; t = Day 11

Figure 5: STAT3 degradation enriches for an IFNγ-dependent gene signature predictive of sensitivity to anti-PD-1[1,2]

CT-26 - Veh or KTX 201 25 mpk q2D IP n=6/grp; t = Day 11



Increased *STAT1*+ TAMs in KTX-201-treated TME support IFN-primed M1 macrophage polarization



CT-26 - n=6/grp; t = Day 11

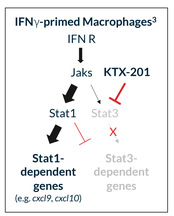
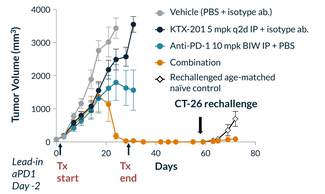
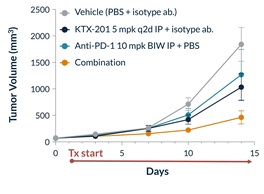


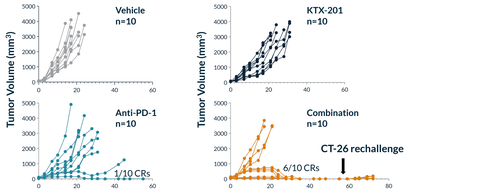
Figure 6: STAT3 degradation enhances anti-PD-1 responses in mouse models of solid and hematologic cancers

KTX-201 synergizes with anti-PD-1 leading to 60% complete response and development of long-term immunological memory in CT-26 tumors

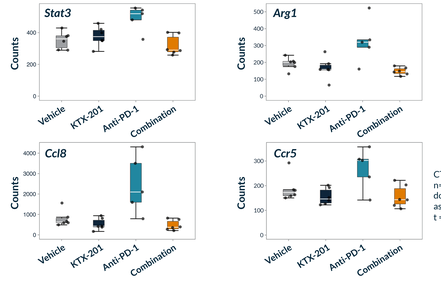


KTX-201 + anti-PD-1 trend towards a combination effect in A20 mouse lymphoma





Transcriptomic analyses of CT-26 tumors show upregulation of *STAT3* and other immunosuppressive genes with anti-PD-1 monotherapy, which are neutralized when combined with KTX-201



CT-26 n=6/grp; dose/schedule as above t = Day 11

METHODS

In vitro Assays

All cell lines were cultured according to recommended procedures. For degradation assays, cells were treated with compounds for 24 h, and total STAT3 protein was assessed by western blotting. For DC50 determination, STAT3 levels were determined by MSD assay in cancer cell lines and by flow cytometry in human PBMCs. Viability was assessed by CTG assay using cells treated with KTX-201 for 4 days.

Proteomic Analysis

Human PBMCs and SU-DHL-1 cells were lyzed, digested and desalted, labeled with TMTpro 16plex, fractionated into 24 fractions on an Agilent off-line HPLC with an Extend300 C18 column and analyzed on Thermo Scientific Q Exactive HF-X Hybrid Quadrupole-Orbitrap Mass Spectrometer.

In vivo Experiments

Subcutaneous tumors were established in immunocompetent host mice strain using CT-26, A20, MC-38 or EMT-6 cell lines (ATCC). KTX-201 was formulated in buffered PBS and administered IP. In the CT-26 combination study, a single lead-in dose of anti-PD-1 was administered 2 days prior to administration of KTX-201 in animals with palpable tumors. Plots show mean tumor volume with error bars representing SEM. Immunophenotyping was carried out using FACS on day 7 after treatment. Tissue STAT3, CD8 levels were evaluated using immunohistochemistry. Anti-CD8 or isotype antibodies were administered 200 µg IP BIW for T cell depletion experiments.

Transcriptomic Analysis

Nanostring nCounter PanCancer IO360 panel was used for gene expression analysis using CT-26 tumors collected on Day 11 post treatment in respective cohorts. Normalization and differential expression was performed with DESeq2.[5]

SUMMARY

- Kymera's potent and selective STAT3 degraders have anti-tumor activity in models of solid and hematologic malignancies that are poorly sensitive to immunotherapies.
- STAT3 degradation results in a remodeled TME that is predictive of favorable responses to checkpoint inhibitors.
- STAT3 degrader is highly synergistic with anti-PD-1 in the CT-26 model leading to durable anti-tumor responses and development of long-term immunological memory.
- KT-333, a first-in-class selective STAT3 degrader, is expected to clear IND by end of 2021 and to be in Phase 1 clinical trial in liquid and solid tumors shortly thereafter.

REFERENCES

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2. Proia *et al.* Clin Can Res 2020
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4. Hu *et al.*. J Immunol 2005
5. Love *et al.* Genome Biology 2015

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