SELECTIVE STAT3 DEGRADERS DISSECT PERIPHERAL T-CELL LYMPHOMAS VULNERABILITIES EMPOWERING PERSONALIZED REGIMENS

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Peripheral T-cell lymphomas (PTCLs)

• Peripheral T-cell lymphomas (PTCLs) comprise a heterogeneous group of aggressive malignancies that are derived from mature T-cells.

• Recurrent aberrations and the deregulated activation of distinct signaling pathways have been mapped and linked to selective subtypes. The JAK/STAT signaling pathway’s deregulated activation plays a pathogenetic role in several PTCL, including ALCL subtypes.

• Somatic activating mutation of JAK1/STAT3 (~50% ALK- ALCLs) drives constitutive activation of STAT3 pathway (Crescenzo R et al, 2015).

• STATs regulate the differentiation/phenotype, survival and cell-growth, metabolism, and drug resistance of T-cell lymphomas as well as host immunosuppressive microenvironments.
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.
Patient-Derived-Tumor-Xenograft (PDTX) models

- Resemble the pathophysiology of human tumors
- Reproduce somehow the tumor heterogeneity
- Grow within a host environment
Patient-Derived-Tumor-Xenograft (PDTX) derivative models

- **PDTX**
  - High Density T-Cell Culture
  - 2D Co-Culture with Stromal Cells
  - Interleukin Supplementation

Graph showing clonal evolution with markers for different stages and cell lines.

- **IL2 PDTX** - Deep sequencing clonal evolution

Legend:
- **Cell line**
- **PDX**
- **ALK- ALCL**
- **ALK+ ALCL**
- **BIA ALCL**
- **PTCL-NOS**
STAT-degraders lead to the rapid and effective down-regulation of STAT3

Flow cytometry determination of p-STAT3 in ALK- and ALK+ ALCL cells exposed to 1µM of KTX-115 degrader

Loss of STAT3 protein expression in ALK- and ALK+ ALCL cells exposed to KTX-154 or KTX-200 degrader (144 h)

Cytosolic, mitochondrial and nuclear STAT3 downregulation in ALK+ ALCL (L82) exposed to 1µM STAT3 degraders

C= cytoplasmic
N= Nuclear
Protein degradation efficiency of different STAT3 degraders

BELLi
(ALK- ALCL: mut Jak1 and Stat3)

IL89
(BIA ALCL: mut Jak1)

IL2
(PTCL: mut Jak1)

IC = Inactive Control
Effect of STAT3 degraders KTX-154 and KTX-105 on the growth of the BELLI (STAT3\textsuperscript{mt}JAK1\textsuperscript{mt*}) ALK- primary ALCL model in vitro and in vivo

Time and dose-dependent cell cycle arrest and cell death of the BELLI model

- **Cell Cycle Analysis**
  - % Positive Cells
  - h: 24, 48, 72
  - G0/S, S/G2/M, G1
  - KTX-154

- **Annexin V Staining**
  - % Positive Cells
  - h: 24, 48, 72, 96, 120, 144
  - Dead, Alive
  - KTX-154

*TSTAT3\textsuperscript{Y640F/L910P}, JAK1\textsuperscript{G1097D/S}*

Treatment (left to right)
- Untreated (media)
- DMSO
- KTX-154: 0.2, 1, 2, 5 \(\mu\)M

Intermittent dosing of KTX-105 significantly inhibits growth of BELLI xenograft tumors and is tolerated

- **Tumor Growth**
  - Tumor Volume (mm\(^3\))
  - Time (day of Treatment)
  - Vehicle, KTX-105 (30 mg/kg, black arrow, 50 mg/kg, red arrow)

- **Body Weight Change**
  - Fold Change
  - Time (day of Treatment)
ALCL cells treated with specific STAT3-degraders undergo shared transcriptional changes

Deep proteomics shows strong reduction of STAT3 in SU-DHL-1 Cells treated with KTX-217 (at 16 h)

Overlapping genes down-regulated in STAT3-degrader (BELLI, IL89 and L82)

Heatmap of the overlapping upregulated genes (201) and downregulated (206) genes in STAT3-treated cells (L82)

KTX-154: BELL, L82
KTX-200: IL89
Individual ALCL cell lines display unique transcriptomic changes after STAT3 loss

Genes which at one or more time point (24h, 48h, 72h, 96h, 120h) showed a treatment-specific effect

KTX-154: BELL, L82
KTX-200: IL89
Pathway activity modulation by STAT3 degrader identifies selective changes in BELL1
Hallmark pathways in ALK- ALCL (BELLI)
Enriched pathways STAT3 regulated genes

BELLi 24h

Enriched GO pathways in down-regulated genes in BELLi 24h

L82 24h

Enriched GO pathways in down-regulated genes in L82 24h
Gene modulation trajectories after STAT3 loss in BELL1

- **ITGAL**
  - DMSO: Increasing trend
  - KTX-154: Increasing trend

- **ITGA3**
  - DMSO: Decreasing trend
  - KTX-154: Decreasing trend

- **BIRC3**
  - DMSO: Decreasing trend
  - KTX-154: Decreasing trend

- **TNC**
  - DMSO: Decreasing trend
  - KTX-154: Decreasing trend

- **HSPA5**
  - DMSO: Peak at 24H48H72H96H120H
  - KTX-154: Low count

- **SLC2A3**
  - DMSO: Peak at 24H48H72H96H120H
  - KTX-154: Low count

- **MYBPC2**
  - DMSO: Decreasing trend
  - KTX-154: Decreasing trend

- **ICAM1**
  - DMSO: Decreasing trend
  - KTX-154: Decreasing trend
Mapping of STAT3 DNA binding in PTCL/ALCL cells

Percentage of share and private DNA binding sites corresponding to coding and non-coding region in 6 different ALCL models

ChIP-seq STAT3 Untreated

Peak Annotation

nPeaks=12558

- Promoter (<1kb) (15.6%)
- Promoter (1-2kb) (1.51%)
- 5' UTR (1.6%)
- 3' UTR (1.46%)
- 1st Exon (0.74%)
- Other Exon (2.19%)
- 1st Intron (16.54%)
- Other Intron (24.28%)
- Downstream (<=3kb) (0.73%)
- Distal Intergenic (35.35%)

Differential genes

RNA-seq

qvalue < 0.05
abs(log2FC) > 1

n=346

n=491

N=837 point = differential gene

- 488 diff. genes are associated to ~4100 loops
Loss of STAT3 leads to significant chromatin changes
RNA-seq - Differential expression by loop group (gene centric)

downregulated genes are associated to loops with decreased interactivity

upregulated genes are associated to loops with increased interactivity
STAT3 is enriched in loops with decreased interactivity (loop centric)

Loops with decreased interactivity are associated to downregulated genes.

Loops with increased interactivity are associated to upregulated genes.
Conclusions

• Heterobifunctional STAT3 degraders can potently and selectively target STAT3 leading to growth arrest in primary ALCL cell models.
• STAT3 degraders are powerful tools to define the STAT3 pathogenetic mechanisms and dissect genes/pathways to be targeted for T-cell lymphoma eradication.
• STAT3 degraders will provide the mean to define STAT3 tumor addiction of PTCL and other human cancers
• STAT3 degraders represent powerful tools to dissect the mechanisms of STAT3-mediated gene transcription
• The pre-clinal data generated in PDTX models will provide the rationale for testing STAT3 degraders in the clinic for the treatment of aggressive malignancies including PTCL/ALCL.
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