Leveraging Pre-Clinical Animal Model of CTCL to Explore Therapeutic Potential of a Novel STAT3 Degrader

Ellie Ivanova[1], Cosmin Tegla[1,2], Ekaterina Novikova[1], Briana Mullins[1], Alberto Herrera[1], Jo-Ann Latkowski[2], Kenneth B. Hymes[2], Joyoti Dey[3], Bin Yang[3], Yatao Shi[3], Ashwin Gollerkeri[3], Jared Gollob[3], Sergei B. Koralov[1]

(1) NYU School of Medicine, Department of Pathology, New York NY (2) NYU Perlmutter Cancer Center, New York NY (3) Kymera Therapeutics, Watertown MA

NYU School of Medicine
NYU LANGONE MEDICAL CENTER

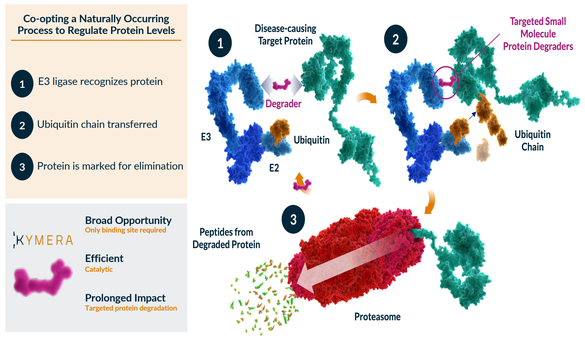
Abstract

STAT3 enhances pro-survival signaling essential for T cell expansion, but when this pathway is not appropriately regulated, aberrant STAT3 signaling contributes to T cell lymphomagenesis.

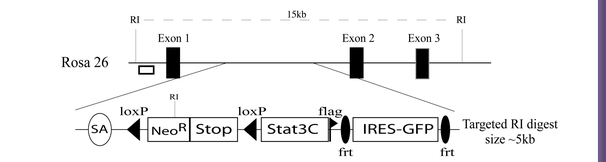
Cutaneous T cell lymphoma (CTCL) is a type of mature T cell lymphoma characterized by the accumulation of malignant T cells in the skin. Our analysis, along with other recently published sequencing studies, identified upregulation of the STAT3 cytokine signaling pathway as a key driver of CTCL pathogenesis. In normal processes, STAT3 activity is transient, but dysregulation of this pathway is observed in several human malignancies, including CTCL. Aberrant STAT3 activity in malignant cells promotes transcription of target genes that promote proliferation and survival of transformed T cells. Translocations resulting in STAT3 duplications, activating mutations in the SH2 domain of STAT3 and activating mutations in kinases upstream of STAT3 all contribute to hyperactivation of this JAK/STAT signaling pathway in CTCL. STAT3 signaling in bystander cells within the tumor microenvironment may further contribute to tumor progression, although the precise role of this signaling pathway in malignant disease is context dependent.

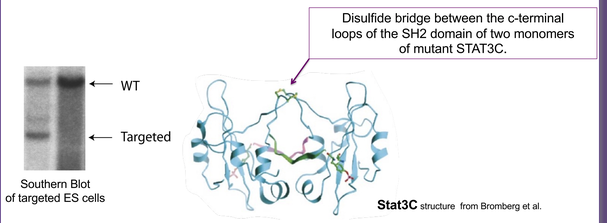
We have used conditional gene targeting to develop a fully penetrant STAT3-dependent small animal model of CTCL that recapitulates many key features of human disease. In this model, a constitutively active form of STAT3 is expressed exclusively in T lymphocytes. The autochthonous mouse model is characterized by progressive accumulation of malignant T cells in the skin and secondary lymphoid organs. Our molecular analysis of T cells from this mouse model revealed a transcriptional signature that largely mirrored the one found in malignant cells from patients. We have now taken advantage of this tractable animal model of CTCL to evaluate the therapeutic potential of a potent and selective E3 ubiquitin ligase-based novel STAT3 heterobifunctional degrader for targeting this difficult-to-treat hematologic malignancy. Weekly intravenous administration of the degrader was well tolerated and led to significant reduction of STAT3 levels in circulating and skin-resident T lymphocytes. Progression of disease in our preclinical model was notably blunted upon administration of the STAT3 degrader with dramatic reduction in T cell accumulation in the skin and lymph nodes. We will report on pre-clinical evaluation of the degrader and the impact on disease progression, T cell accumulation, activation and proliferation *in vivo* following weekly drug administration. Targeted protein degradation represents a novel therapeutic modality enabling direct targeting of previously undruggable oncoproteins such as STAT3. These data provide a rationale for selective STAT3 degradation as a therapeutic strategy for malignancies such as CTCL that are associated with constitutive activation of STAT3 signaling.

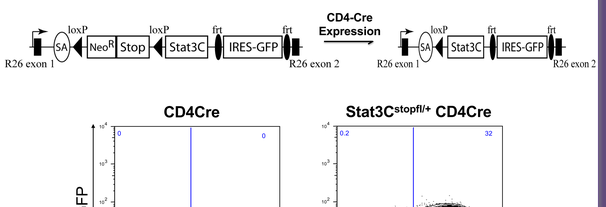
Mechanism for Targeted Protein Degradation

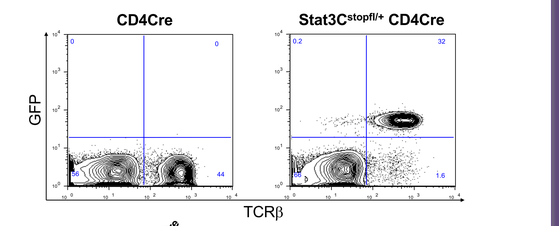


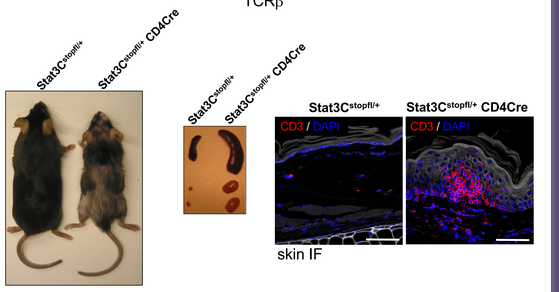
Mouse Model of CTCL







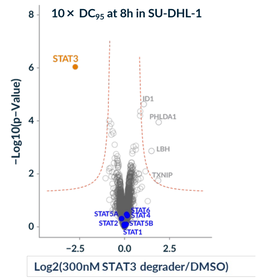




Our analysis, along with that from other groups, identified upregulation of the STAT3 cytokine signaling pathway as one of the key drivers of CTCL. To further explore the role of STAT3 signaling in lymphomagenesis we took advantage of conditional gene targeting to generate a mouse model in which expression of a hyperactive STAT3 mutant protein (STAT3C) is induced in a tissue-specific manner via Cre-mediated deletion of an upstream stop cassette. The mutation in the SH2 domain of STAT3 mimics the activating mutations found in some CTCL patients. The R26STAT3C^{stopfl} allele has an IRES-GFP sequence to track the cells in which the floxed stop cassette is deleted. To examine the role of STAT3 in the transformation of T lymphocytes we used CD4Cre, a T cell–specific Cre line. Young CD4Cre R26STAT3C$^{stopfl/+}$ mice are indistinguishable from wild-type littermates. However, by 6-8 months of age >90% of CD4Cre R26STAT3C$^{stopfl/+}$ mice develop a disease highly reminiscent of CTCL: (i) these animals have severe inflammation and scaling of the skin, (ii) striking expansion of activated T cells in peripheral lymph nodes and (iii) characteristic T cell infiltration in the epithelium.

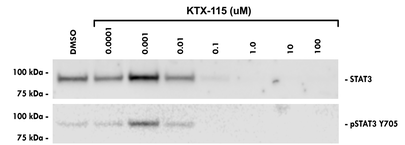
High selectivity of the STAT3 degrader

Kymera degrader is highly selective for STAT3 in a non-Hodgkin SU-DHL-1 cell line, with proteomics analysis revealing selective targeting of STAT3 and no impact on other STATs:

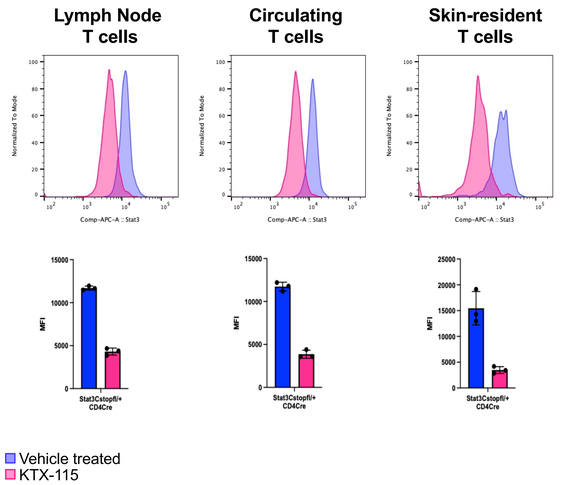


Deep tandem mass tag proteomics (>10,000 proteins monitored)

Targeting STAT3 signaling in murine model of CTCL

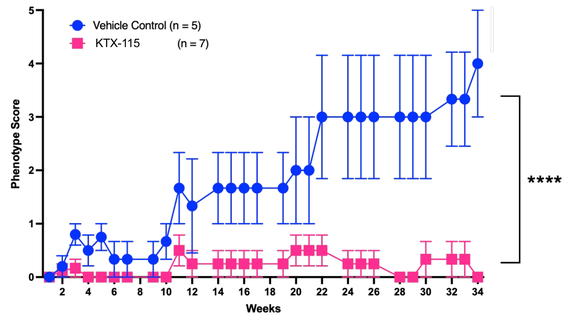


Degradation of STAT3 by degrader, KTX-115 in stimulated T cells isolated from the animal model.

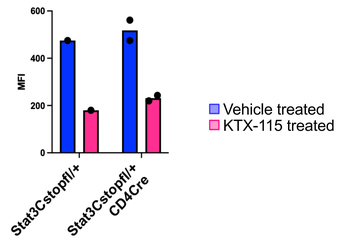


Efficacy of STAT3 inhibition in T cells *in vivo*. Mean fluorescent intensity of total STAT3 labeling on *ex-vivo* isolated T lymphocytes from lymph nodes, circulation and skin of STAT3C$^{stopfl/+}$ CD4Cre mice treated with STAT3 degrader shows 3-4x reduction in STAT3 protein levels after a single treatment with the STAT3 degrader. Cells from animals treated 48hrs prior to SACing were analyzed by intracellular flow cytometry. STAT3 staining for CD4+ TCRβ+ lymphocytes shown. Lymphocytes from skin were isolated following liberase-based digestion protocol.

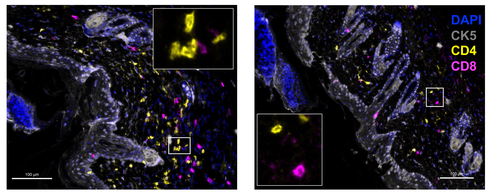
Pre-clinical efficacy studies in a murine model of CTCL



Dramatic amelioration of disease in animals treated with STAT3 degrader starting at ~3mo of age 1x week IV KTX-115 with drug holiday every ~4 weeks (QW x4 weeks, 1 week off). Phenotype scale ranges from (0) no phenotype to moribund (5). The mice on the lower end of the scale display dry skin and a range of hair loss, while on the more severe end of the scale animals develop obvious sores and lesions.



Evaluation of STAT3 degradation in skin-resident T lymphocyte from pre-clinical animal model of CTCL. Flowcytometric analysis for CD4 T lymphocytes isolated from skin of animals treated with QW x 4 weeks/1 week off schedule. Treated CD4Cre STAT3C$^{stopfl/+}$ mice and control littermates were sacrificed 48hrs following the last dose.



Immunofluorescent imaging of untreated (left) and treated (right) dorsal skin from STAT3$^{stopfl/+}$ CD4Cre mice. Inset shows the morphology of the T lymphocytes.

Conclusions

STAT3 hyperactivation is sufficient to drive T cell expansion and accumulation of malignant T cells in the skin of CD4Cre STAT3C$^{stopfl/+}$ animals, demonstrating the critical role of JAK/STAT signaling in T cell lymphoma pathogenesis. A STAT3-specific degrader demonstrated exquisite specificity for STAT3 in cell lines and in *ex-vivo* lymphocytes. Treatment of CD4Cre STAT3C$^{stopfl/+}$ mice results in dramatic amelioration of disease and prevented development of characteristic skin pathology. The STAT3 degrader KT-333 is under development for STAT3-dependent T cell malignancies including CTCL and is currently in a Phase 1 clinical study.