A STAT3 Degrader Demonstrates Pre-Clinical Efficacy in Venetoclax Resistant Acute Myeloid Leukemia

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INTRODUCTION

- Acute Myeloid Leukemia (AML) is the most common myeloid malignancy in the elderly [1].
- AML is an aggressive hematological malignancy caused by transformation of immature myeloid stem & progenitor cells.
- Venetoclax (Ven), a selective inhibitor of the anti-apoptotic BCL2 protein, is FDA approved for AML.
- Despite available therapies, survival of AML patients is dismal.
- Aberrant activation of transcription factor- STAT3 is implicated in several hematological malignancies [2].
- Previous data from our lab demonstrated de-methylation and overexpression of STAT3 in MDS & AML stem cells is associated with an adverse prognosis [3].
- We have also reported that STAT3 controls several important leukemic drivers such as the anti-apoptotic protein myeloid cell leukemia-1 (MCL1).

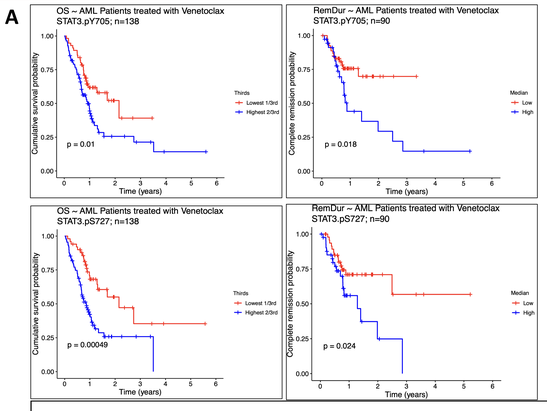
➢ ***MCL1 overexpression is the central mechanism of resistance to BCL2 inhibition (Ven) in AML*** **[3].**

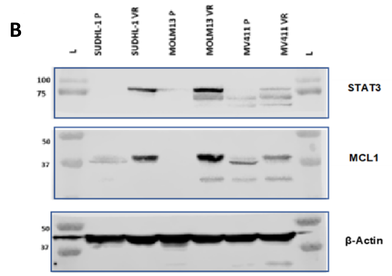
➢ ***While MCL1 is a well-known direct transcriptional target of STAT3, the role of STAT3 in venetoclax resistance (Ven-res) is unknown.***

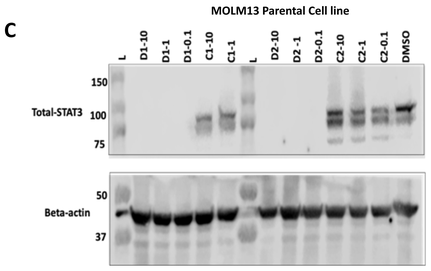
AIM

The AIM of our study is to understand the role of STAT3 in Venetoclax resistance (Ven Res/VR) and the therapeutic implications of a novel STAT3 degrader in Acute Myeloid Leukemia

RESULTS







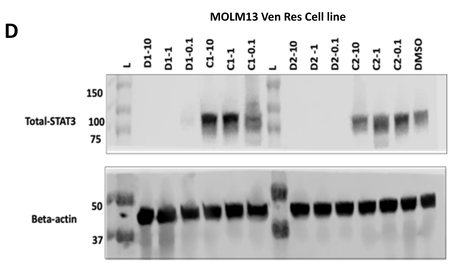


Figure 1: Elevated STAT3 in Venetoclax Resistance can be specifically targeted using STAT3 degrader in vitro.
A) Phospho proteomic analysis shows worse OS & RemDur for high phospho-STAT3 expression post Ven Rx; for STAT3pY705 and STAT3pS727.
B) Western blot showing increase in total STAT3 and MCL1 in Ven resistant cell lines (denoted as VR), as compared to parental (P) cell lines.
C,D) Effective degradation of STAT3 observed in MOLM13 Parental and MOLM13 Ven Res cells, respectively, on treatment with two STAT3 Degraders (D1: KTX-201 ,D2: KTX-105) at 0.1, 1 and 10μM doses for 24 hours, with no effect on STAT3 levels when treated with structural controls (C1, C2) or DMSO.

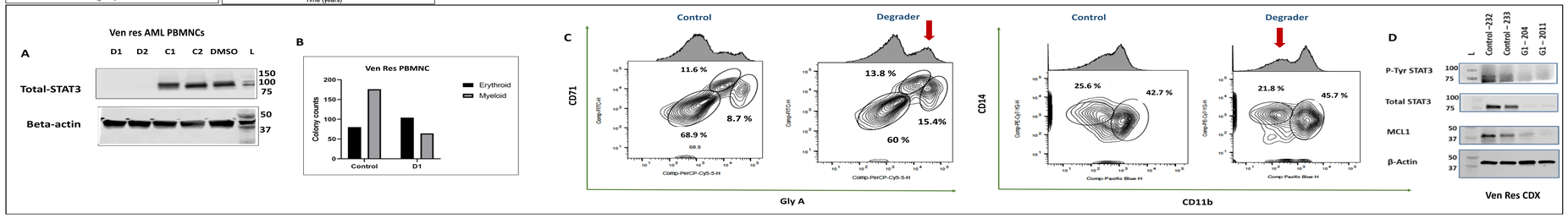


Figure 2: STAT3 Degrader shows promising findings in Venetoclax resistant AML Patient Samples and CDX models
A) Treatment of VR AML patient PBMNCs with 10μM STAT3 Degraders (D1: KTX-201 , D2: KTX-105) for 24 hours shows effective and specific degradation of STAT3 (>90%), with no effect on STAT3 levels when treated with structural controls (C1, C2) or DMSO.
B) In patients with Ven-res AML, erythroid colony counts were seen to increase (~1.5 fold) with a concomitant decrease in myeloid colony counts (>2.5 fold), on treatment with D1: KTX-201, as observed in colony assay.
C) Colony assay FACS of Venetoclax resistant PBMNCs in presence of KTX-201 shows increased erythroid and myeloid differentiation (with distinct expansion of mature cell populations).
D) Western Blot showing significant reduction in p-Tyr-705 STAT3, total STAT3 and MCL1 protein level in murine model of Ven-res CDX post two-week treatment of the in vivo STAT3 degrader- KT-333; G1 represents KT-333 treated mice vs vehicle treated controls.

METHOD

Phospho-proteomic analysis

- Performed on > 90 AML patients treated with prior Ven to look for effect of activated STAT3 on OS and RemDur.

Generation of Venetoclax Resistant Cell lines

- Ven-res AML cell lines (MOLM-13, MV-4-11) as well as Ven-res large cell lymphoma cell line (SU-DHL-1) were generated

Treatment with STAT3 Degrader in vitro

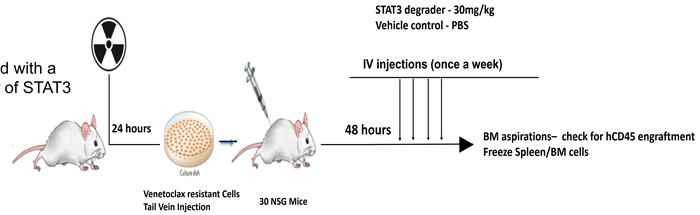
- Parental and Ven Resistant Cell lines were treated with a highly specific potent heterobifunctional degrader of STAT3 (degrader) to check for functional effect of STAT3 degradation using
  - ➢ Proliferation Assays
  - ➢ Western Blot
  - ➢ Apoptosis Assay

Primary AML patient samples

➢ Colony Assay and FACS for cell differentiation analysis post STAT3 degrader treatment was performed.

Murine Model

➢ CDX model of Ven Res strategy:



CONCLUSIONS

- STAT3 and MCL1 are overexpressed in Ven Res cell lines and murine models of VR-AML.
- Novel and clinically relevant STAT3 degrader demonstrated significant activity in Ven Res AML patient samples as well as Ven Res murine model.
- Increase in myeloid and erythroid differentiation of the HSPCs in Ven Res increases the clinical application of the STAT3 degrader.
- The degradation of previously undruggable transcription factors such as STAT3 is a promising therapeutic strategy for patients with Ven Res AML, a large unmet clinical need.
- Currently, phase 1 clinical trial of STAT3 degrader KT-333 (NCT05225584) is in progress.

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ACKNOWLEDGEMENTS

NIH NATIONAL CANCER INSTITUTE

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