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POSTER ABSTRACTS

604.MOLECULAR PHARMACOLOGY AND DRUG RESISTANCE: MYELOID NEOPLASMS

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

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Introduction: Acute Myeloid leukemia (AML) is an aggressive hematological malignancy resulting from the transformation of immature myeloid progenitor cells followed by an uncontrolled clonal proliferation and accumulation of the transformed cells. AML is the most common myeloid malignancy in the elderly [1]. Venetoclax (Ven) is a selective inhibitor of the anti-apoptotic BCL2 protein. Ven is FDA approved in combination with hypomethylating agents (HMA's) or low dose cytarabine for the treatment of de-novo AML in patients > 75 years or those ineligible for standard induction therapies. Despite the recent FDA approval of HMA/Ven and other novel therapies, 5-year survival in newly diagnosed AML remain less than 30% and disease relapse is inevitable without a bridge to allogeneic stem cell transplantation [2].
Signal transducer and activator of transcription 3 (STAT3) belonging to the STAT family of transcription factors is well known to be inappropriately activated in several malignancies [3]. On stimulation by cytokines such as IL-6, STAT3 undergoes dimerization and phosphorylation. Phosphorylation at Tyr ^{705} leads to its translocation to the nucleus whereas phosphorylation at Ser ^{727} translocates it to the mitochondria [4]. Previous data from our lab demonstrated de-methylation and overexpression of STAT3 in MDS & AML stem cells, that is associated with an adverse prognosis [5]. 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* [5]. *While MCL1 is a well-known direct transcriptional target of STAT3, the role of STAT3 in venetoclax resistance (Ven-res) is unknown.*
Methods & Results: To understand the role of STAT3 in Venetoclax resistance (Ven-res), Ven-res AML cell lines (MOLM-13, MV-4-11) as well as a Ven-res large cell lymphoma cell line (SU-DHL-1) were generated, which exhibited increased levels of both total STAT3, phospho-STAT3 and its downstream effector, MCL1, when compared to their parental cell lines (Fig.

1A). Data from > 90 AML patients treated with prior Ven also showed high expression of total STAT3 along with enhanced phosphorylation for p ^{Ser727} and p $^{Tyr-705}$ STAT3, that strongly correlated with worse overall survival (OS) and reduced remission duration (RemDur) ($p<0.05$).

A highly specific potent heterobifunctional degrader of STAT3 (degrader) resulted in a dose dependent and selective degradation of STAT3 in both parental and Ven-res hematological malignancy cell lines. Treatment with degrader showed a significant (>60%) decrease in p $^{Tyr-705}$ as wellas p $^{Ser-727}$ STAT3 levels in MOLM-13 parental as well as MOLM-13 Ven-res cells. STAT3 degradation also led to induction of apoptosis in both parental and Ven-res AML cell lines ($p < 0.001$).

Importantly, colony assay of Ven-res AML patient samples showed effective degradation of STAT3 (>90%) together with the increased erythroid and myeloid differentiation (~ 2 fold) on treatment with degrader. Interestingly, no differentiation was observed in healthy samples, suggesting the specificity of the degrader to AML stem and progenitor cells. 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 degrader. This observation supports the clinical significance of our studies in AML patients with anemia. Although BH3 profiling of Ven-res MOLM-13 supports an increased dependency on MCL1, we have observed that treatment with degrader led to ~20% reduction in the MCL1 dependent mitochondrial depolarization.

Additionally, cell derived xenograft (CDX) models of Ven-res showed significant reduction of p $^{Tyr-705}$ STAT3(~60%), total STAT3 (>90%) and MCL1 (~70%), on treatment with STAT3 degrader - KT-333 (currently in an early phase clinical trial:NCT05225584), as early as week 2 (Fig 1B). Significant decrease in intracellular STAT3 in the spleen cells was also observed ($p < 0.05$), with ongoing survival analysis.

Conclusion: Our study suggests that targeting STAT3 and the downstream MCL1 represents a novel and effective strategy for Ven-resistant AML patients in clinic, with strong mechanistic rationales that can spur further clinical development of STAT3 degraders especially given the significant side effects of direct MCL1 inhibitors.

Disclosures Dey: *Kymera Therapeutics:* Current Employment, Divested equity in a private or publicly-traded company in the past 24 months. **Chutake:** *Kymera Therapeutics Inc.:* Current Employment, Divested equity in a private or publicly-traded company in the past 24 months, Patents & Royalties: Patent Application. **Mantzaris:** *Kite, a Gilead company:* Honoraria. **Choudhary:** *Curis:* Current Employment, Current equity holder in publicly-traded company, Current holder of *stock options* in a privately-held company. **Verma:** *Eli Lilly:* Research Funding; *Novartis:* Other: Scientific Advisor; *Bakx:* Current equity holder in private company, Other: Scientific Advisor; *Acceleron:* Other: Scientific Advisor; *BMS:* Research Funding; *Curis:* Research Funding; *Incyte:* Research Funding; *Medpacto:* Research Funding; *Stelexis:* Current equity holder in private company, Honoraria, Other: Scientific Advisor; *Celgene:* Other: Scientific Advisor; *GSK:* Research Funding; *Prelude:* Research Funding; *Janssen:* Honoraria; *Throws Exception:* Current equity holder in private company. **Gavathiotis:** *Guidepoint, Boehringer Ingelheim:* Consultancy; *BAKX Therapeutics, Life Biosciences, Stelexis Therapeutics:* Current equity holder in private company; *Albert Einstein College of Medicine:* Current Employment, Patents & Royalties. **Konopleva:** *AbbVie, Forty Seven, Precision Biosciences, Gilead Sciences, Genentech, Janssen, Sanofi, MEI Pharma, Daiichi Sankyo Pharmaceutical, AstraZeneca Co., Menarini.:* Consultancy; *Reata Pharmaceuticals.:* Current holder of *stock options* in a privately-held company, Patents & Royalties; *Abbvie, Allogene Therapeutics, Cellectis, Forty Seven, Gilead Sciences, Genentech, Sanofi, MEI Pharma, Rafael Pharmaceuticals, Daiichi Sankyo Pharmaceutical, AstraZeneca Co., Menarini, Precision BioSciences.:* Research Funding. **Gollob:** *Kymera Therapeutics:* Current Employment. **Shastri:** *Rigel Pharmaceuticals:* Honoraria; *Kymera Therapeutics:* Honoraria, Research Funding; *Gilead Sciences:* Honoraria; *Janssen Pharmaceuticals, Inc.:* Consultancy, Honoraria.

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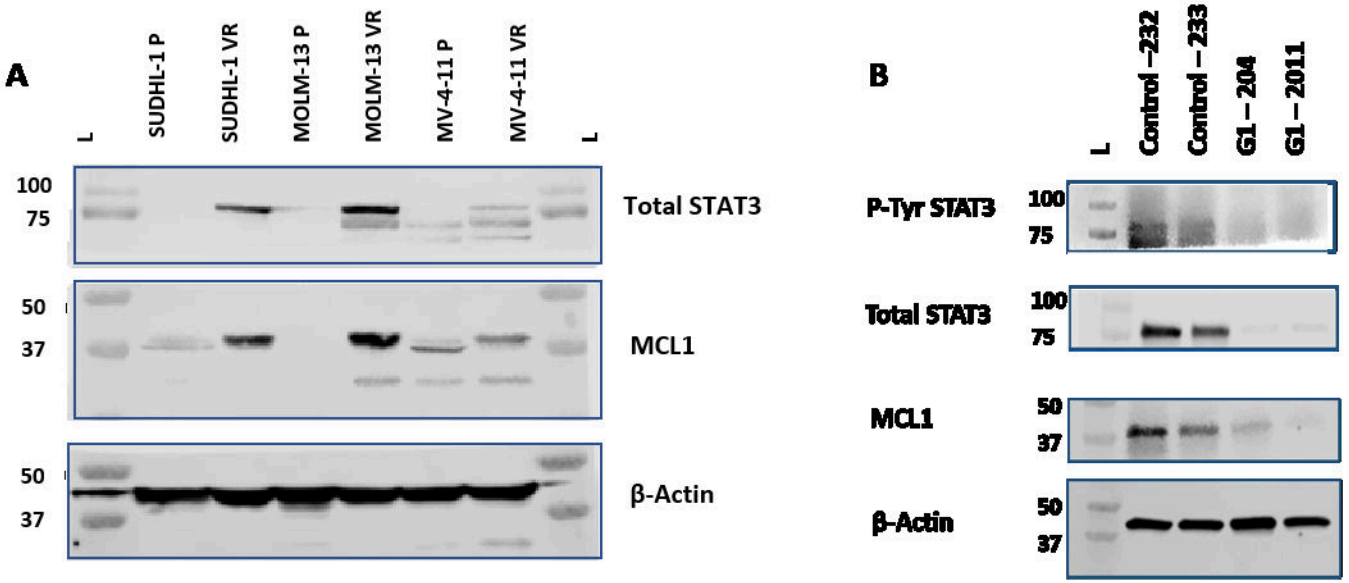

Figure 1: A) Western blot showing increase in total STAT3 and MCL1 in Ven resistant cell lines (denoted as VR), as compared to parental (P) cell lines. B) Western Blot showing significant reduction in p-Tyr-705 STAT3, total STAT3 and MCL1 protein level in murine model of Ven-res CDX post two-week treatment of KT-333; G1 represents KT-333 treated mice vs vehicle treated controls.

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Figure 1