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ORAL ABSTRACTS

604.MOLECULAR PHARMACOLOGY AND DRUG RESISTANCE: MYELOID NEOPLASMS

Targeting Mitochondrial Calcium Uptake to Eradicate Venetoclax-Resistant Acute Myeloid Leukemia Stem Cells

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Introduction

Previously, we demonstrated that human leukemia stem cells (LSCs) are preferentially reliant on oxidative phosphorylation (OXPHOS) for survival. Furthermore, inhibition of BCL2 with venetoclax (ven) acts to suppress OXPHOS, leading to the eradication of LSCs. While the activity of ven in targeting LSCs is well-established, nearly all patients eventually develop disease that is resistant to treatment. Thus, in the present study, we sought to better understand the biological properties of ven-resistant LSCs.

We reasoned that the expression of known BCL2 interacting proteins in ven-sensitive (ven-S) vs. ven-resistant (ven-R) would provide insights on resistance mechanisms. Initial findings showed that one of the most prevalent molecular features observable in ven resistant LSCs is up-regulation of calcium signaling. As calcium is a critical modulator of multiple enzymes involved in OXPHOS, we hypothesized that the maintenance of high mitochondrial calcium could contribute to circumventing the effects of ven treatment. Thus, the present study was designed to test the functional role of calcium in ven-R LSCs and to determine whether targeting mitochondrial calcium transport represents a therapeutic vulnerability.

Methods

CITE-Seq analysis was performed on 25 AML patient specimens and analyzed using TotalVI. All studies were conducted in primary human AML specimens with LSCs defined by a ROS-low phenotype. Mitochondrial calcium measurements were done using Rhod2-AM. OXPHOS activity was measured through seahorse and enzyme activity assays. Genetic perturbations were done using siRNA via electroporation and confirmed with western blot. LSC function was assessed by colony formation assays and PDX experiments. PDX experiments were also used to determine efficacy of tumor reduction in ven-R AML samples.

Results

To identify unique features of ven resistant LSCs, we performed single cell RNA-seq on known ven-S vs. ven-R specimens. As shown in Fig 1, resistant LSCs had higher expression of genes involved in calcium transport into the mitochondria, which was reflected by increased steady-state mitochondrial calcium levels compared to ven-S LSCs. Thus, we investigated targeting of the calcium uniporter, MCU, as a means to reduce mitochondrial calcium levels. Treatment of primary LSCs with pharmacological MCU inhibitors (Ru265, MCUi4), as well as genetic inhibition of MCU with siRNAs, led to decreased mitochondrial calcium levels, suppression of calcium dependent TCA cycle dehydrogenase activity, reduced OXPHOS activity, and impaired LSC function as measured by colony assays (CFU) and engraftment in immune deficient mice. Importantly, MCU inhibition did not significantly impair normal stem/progenitor cells.

To develop a clinically relevant system, we investigated bio-available agents known to inhibit MCU. Intriguingly, the only known FDA-approved direct MCU inhibitor is the chemotherapy agent, mitoxantrone (mitox). We performed a series of studies to assess the impact of mitox on ven-R LSCs. Our findings demonstrate that venetoclax resistant LSCs are exquisitely sensitive

to mitox. In CFU assays, doses as low 1.0nM show strong inhibitory activity. Further, treatment of immune deficient mice bearing human xenografts (Fig 2), showed significant reduction of bulk disease in primary mice, and strong suppression of LSCs in secondary transplants. Notably, DNA damage was not present in LSCs treated with mitox as measured by gamma H2AX staining. Additionally, parallel studies with the related agents, doxorubicin and etoposide, showed no inhibition of CFU, mitochondrial calcium, or OXPHOS activity. Lastly, no discernable effect on normal hematopoietic stem cell activity was evident.

Conclusions

Our findings suggest that ven-R LSCs have adapted to their unique cellular demands by increasing basal mitochondrial calcium levels to drive OXPHOS. Based on this finding, we propose that inhibition of mitochondrial calcium uptake impairs multiple enzymes required for OXPHOS, which in turns leads to eradication of LSCs. Importantly, we also show that the well-established chemotherapy agent mitoxantrone can effectively inhibit MCU at nanomolar concentrations and is a highly potent against ven-R LSCs. We are currently working to establish a clinical trial in which low-dose mitox will be deployed to augment ven-based therapy.

Disclosures Engel: *Syros Pharmaceuticals:* Research Funding; *Kura Oncology:* Research Funding; *Argenx:* Research Funding. **Ransom:** *Argenx:* Research Funding; *Syros Pharmaceuticals:* Research Funding; *Kura Oncology:* Research Funding. **Staggs:** *Kura Oncology:* Research Funding; *Syros Pharmaceuticals:* Research Funding; *Argenx:* Research Funding; *Elevate Bio:* Research Funding. **Smith:** *OncoVerity:* Current Employment, Current holder of *stock options* in a privately-held company; *AML JV:* Consultancy. **Jordan:** *AML JV:* Consultancy.

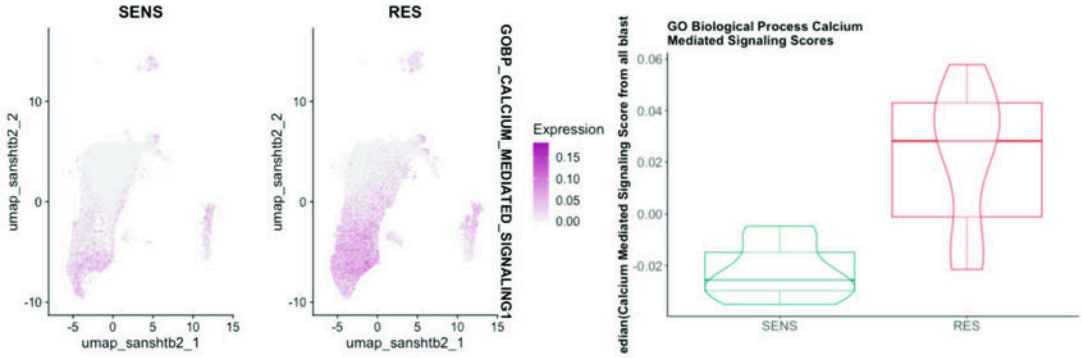

Figure 1. Cite-Seq Analysis of Venetoclax Sensitive versus Resistant Patient Specimens. Module scoring for the GOBP Calcium Mediated Signaling pathway across all clusters. The GOBP Calcium Mediated Signaling gene set was used to score cells object wide using Seurat's AddModuleScore function. The median score from monocytes, promyelocytes, and primitives together were calculated for each sample and plotted.

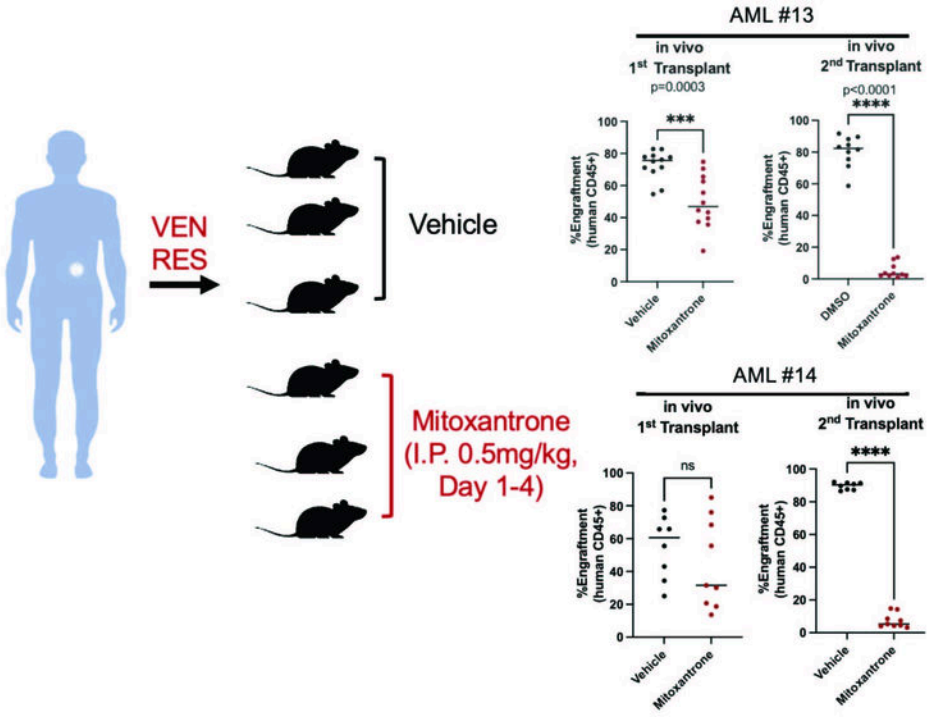

Figure 2. Tumor Burden and LSC Activity After Mitoxantrone Treatment in Murine PDX Models of Venetoclax Resistant AML. In vivo treatment of NSG-S mice with AML tumor burden. Mice were treated with either vehicle (PBS,i.p. 4 days) or mitoxantrone (0.5mg/kg,i.p. 4 days) upon at least 20% bone marrow tumor burden. Cells were then harvested after treatment regimen and transplanted into NSG-S mice for secondary transplants (1million cells per mouse per condition). Data are presented as mean values +/-SD. Significance was measured by two-tailed unpaired t-test. N=2 biological replicates with at least 8 technical replicates per condition.

Figure 1

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